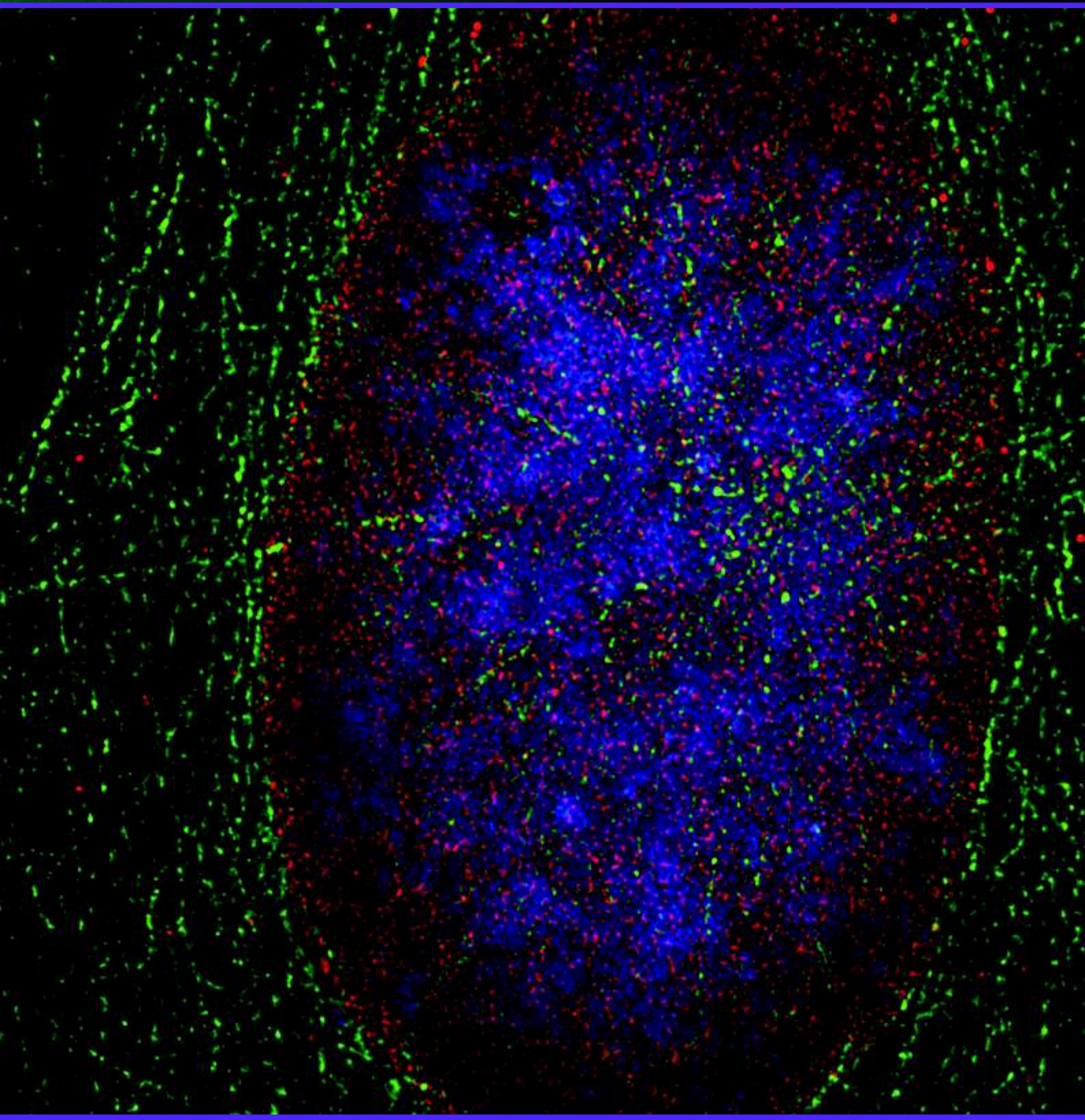
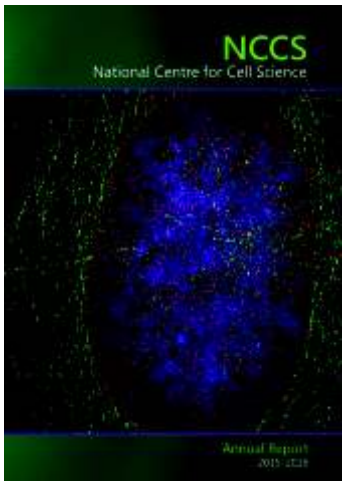
NCCS

National Centre for Cell Science



Annual Report

2015-2016



Cover page image

Super-resolution microscopy of a HeLa cell displaying the nucleus (blue), microtubules (green) and nuclear pore complexes (red).

(Image courtesy of Dr. Jomon Joseph & Mr. Deepak Khuperkar)

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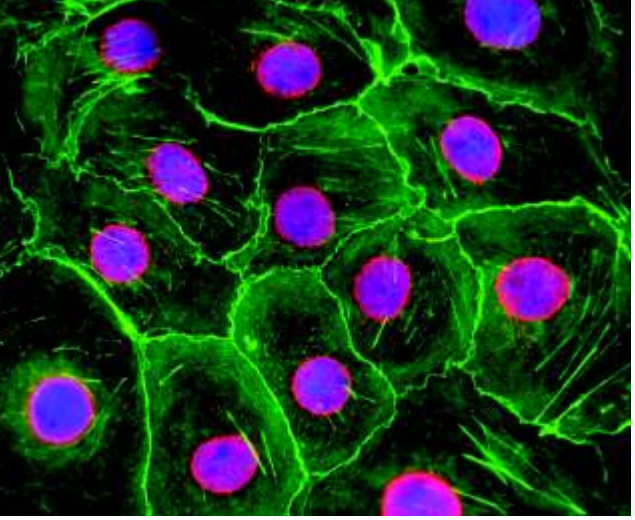
Annual Report 2015 - 2016



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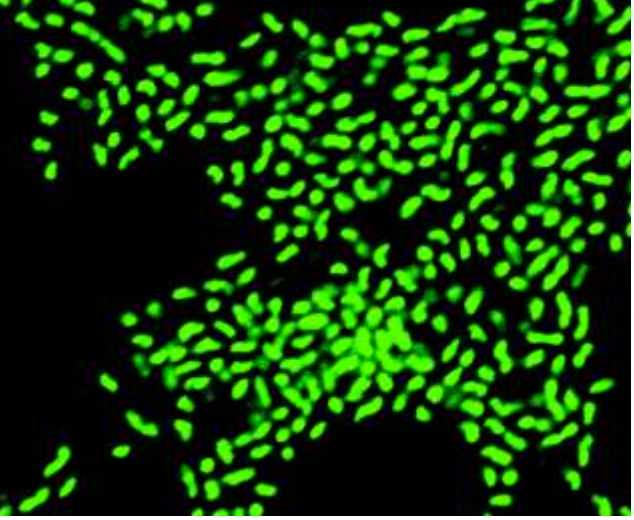


Mandate of NCCS

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

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

- Research and development.

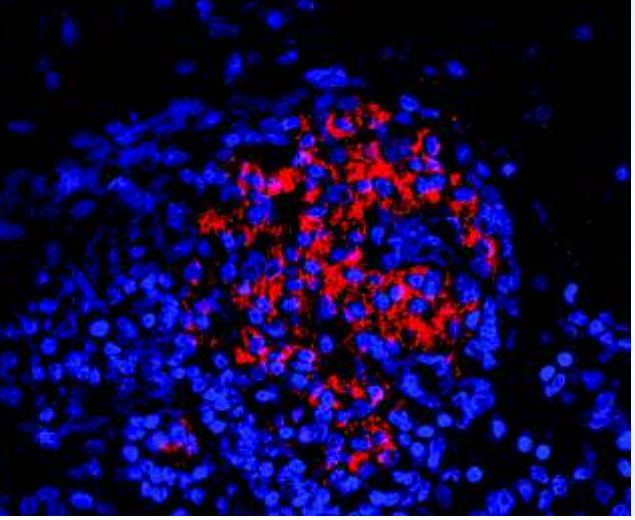
- To establish and conduct postgraduate courses, workshops, seminars, symposia and training programs in the related fields.

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

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

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

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



Summary of NCCS Activities for the Unacquainted

NCCS carries out research in cell biology, which involves the study of cells, the 'basic unit of life'. The bodies of all animals, including humans, are composed of trillions of different types of microscopic cells. These cells, in turn, are composed of a variety of molecules, including DNA, RNA, proteins, and several others, which determine the properties and biological activities of the cell. Cellular activities are also influenced by other determinants, including interactions of the cells with the environment and molecules outside the cell, with each other, and with microorganisms that they encounter. All these molecules, interactions and other factors 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 to 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 help in the development of improved methods and treatment regimens / therapeutics to diagnose, manage and cure these diseases. Our studies hold special relevance for the latter 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 in the past year are described in the research reports of the individual scientists. 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 free cell lines to cell biologists from academic and research institutions across the country. Therefore, a significant proportion of cell lines-based research in India is dependent on the cell repository at NCCS, and is also supported by the training and guidance provided by NCCS to develop the skills required to handle cell lines.

The Microbial Culture Collection (MCC) of NCCS plays a big role in preserving the nation's microbial biodiversity, by serving as a national depository for microorganisms. It has successfully undertaken the enormous task of obtaining several different microorganisms from a variety of environments across India, preserving them in the laboratory in the form of 'cultures', and characterizing them to identify them and to explore their potential for application in biotechnology. The MCC is the largest individual collection of microorganisms in the world and is instrumental in India being internationally ranked as the country with the third 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, MCC has been recognized by the World Intellectual Property Organization (WIPO) in Switzerland, as an International Depository Authority (IDA) for the deposit of microorganisms to fulfill the requirements of the patent procedure in 55 countries.

From the Director's Desk

It is my pleasure to present the Annual Report of the National Centre for Cell Science (NCCS), Pune, for the year 2015-16. We are proud to have completed yet another fruitful year of frontline research and service through the supply of cell lines and human resource development. During 2015-16, the national cell repository at NCCS supplied five thousand one hundred cell lines to four hundred research institutions in India. During this year, we had thirty four research scholars join us for the Ph.D. programme and twenty five of our research scholars registered as Ph.D. students with the University. We had 113 research scholars registered for a Ph.D., as on 31st March, 2016. Thirty four students submitted their theses to the University and six students were awarded the Ph.D. degree during the said year. 34 project trainees & 21 summer trainees also received training at NCCS under the mentorship of our faculty.

NCCS has been at the forefront of cancer research in the country for several years. Dr. Sharmila Bapat's team, one of the research groups studying the biology of cancer at NCCS, has recently been successful in developing a novel method to address the issue of diverse cell composition of tumours. Tumors are known to harbor cellular, molecular and functional intra-tumoral heterogeneity, which confounds our understanding of the perturbation(s) of basic biological processes in the context of cancer. This issue was addressed by Dr. Bapat and her group by using a flow cytometry-based approach for the deconstruction of a tumor into discrete tumor fractions, which relies on resolution of the cancer stem cell (CSC)-based hierarchy, genetic instability and differential cell cycling fractions. Since these are universal principles, this approach is applicable across different cancer / cell types. Further integration of this real-time definition of cellular heterogeneity with molecular profiling can lead to the assignment of cell-specific functional context(s) within tumors, as against derivation of biological functions based on averaged tumor data. Thus, gene expression studies across sorted cell fractions led to the establishment of a unique association of the surface marker CD53 with regenerative potential in CSCs as well as progenitors within the tumor regenerative hierarchy. Another interesting finding of their research was the association of cytoskeletal remodeling, and epithelial to mesenchymal transition (EMT) with aneuploid fractions under conditions of stress, which emphasizes the contribution of genetic heterogeneity and Darwinian principles of selection during tumor metastases. Tumor deconstruction was also demonstrated to be a convenient tool to overcome limitations of the currently used simplistic drug screening

strategies in which consideration of cell line-based cytotoxicity and/or in vivo tumor regression as end-points of drug efficacy fail to address residual regenerative potential following therapeutic regimes. This is important since at a clinical level, tumor heterogeneity represents a major therapeutic hurdle in targeting heterogeneous cell fractions that drive recalcitrant disease. Dr. Bapat and her group showed that applying the same principles of deconstruction improved the identification of cellular and molecular drug targets, and that this information could thus be used to achieve complete tumor regression. These developments could thereby provide a convenient tool for drug screening and repositioning, as well as for predicting responses to therapeutics, and for formulating novel drug combinations.

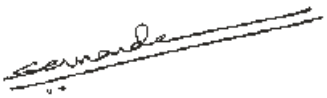
The scientists at NCCS have also been studying communicable diseases, such as those caused by viruses. Dr. Debashis Mitra and his group has been working on different aspects of the HIV virus, related to host-virus interactions, immune response and drug discovery. The primary objective of their research is to understand the virus and its interactions with the host cell better, which could lead to improved antiviral strategies. This group has recently elucidated the role of the HSP70-binding protein, HspBP1, a co-chaperone molecule of HSP70, in HIV-1 pathogenesis. Their finding revealed that HspBP1 inhibits HIV-1 gene expression and viral replication by interacting with NF-κB enhancer sequences in the LTR promoter. They also determined that HspBP1 competes with p65 of the NF-κB heterodimer for recruitment on the κB enhancer site. To summarize, HspBP1 appears to inhibit HIV-1 gene expression and replication by restricting p65 from binding to the NF-κB enhancer sequence on the viral promoter. The identification of this novel role of HspBP1 as a host intrinsic inhibitor of HIV-1, which negatively regulates HIV-1 gene-expression and replication, could be a promising lead towards identifying new anti-HIV therapeutic strategies. As is evident from the extensive research being done worldwide, this is the need of the hour since the current therapeutic strategy involving the use of a combination of anti-retrovirals has proven useful in controlling the virus but not in eradicating it from patients. Further, with the ultimate objective of identifying novel cellular targets for the inhibition of HIV-1, Dr. Mitra and his group have also screened a library of pharmacologically-active bio-molecules known to target cellular pathways, to identify novel anti-HIV molecules. They have been successful in identifying several promising bioactive molecules and further characterization of a few of these molecules and their targets is currently in progress.

Dr. Arvind Sahu and his group study other factors that play a role in protection from viral infections, such as host factors, especially the complement cascade of the immune system. These studies assume importance when we take into consideration that the pandemic influenza A(H1N1) 2009 virus (Swine Flu virus) is now circulating seasonally and causing a significant disease burden worldwide. Their data demonstrate that the presence of an intact complement is essential for clearing the pandemic influenza virus infection, wherein locally-

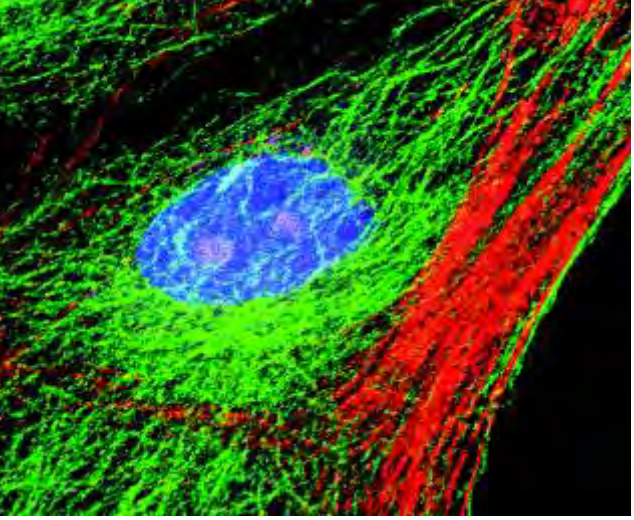
synthesized complement plays a major role. Further, their results reveal that activation of the classical as well as alternative pathways of complement system is a requisite for efficient neutralization of the virus as well as optimum generation of C3a and C5a (complement fragments generated after complement activation), which is necessary for boosting the protective immune responses. Their findings thus reveal that deficiencies of components of the classical and alternative pathways enhance susceptibility to and the severity of a pandemic influenza virus infection.

Osteoimmunology is another area of research at NCCS. Dr. Mohan Wani and his research group study the role of interleukin-3 (IL-3) in the pathophysiology of bone and cartilage remodelling. Their recent findings revealed for the first time that IL-3 plays a chondroprotective role in osteoarthritis (OA). OA is a chronic disease of articular joints that leads to degeneration of both cartilage and subchondral bone. This group discovered that IL-3, a cytokine secreted by activated T cells, up-regulates the expression of chondrocyte genes Sox9 and col2a, which are important for matrix synthesis, and down-regulates the expression of matrix metalloproteinases, MMP-3 and MMP13, under inflammatory conditions in both mouse and human chondrocytes. Interestingly, they also found that IL-3 reduces the degeneration of articular cartilage and subchondral bone microarchitecture in mouse model of human osteoarthritis. Further, they found that IL-3 decreased IL-1β-induced matrix degradation in micromass pellet cultures of human mesenchymal stem cells. These studies have thrown light on the therapeutic potential of IL-3 in the amelioration of articular cartilage and subchondral bone microarchitecture degeneration associated with OA.

We aspire to expand our horizons to encompass more extensive academic and research collaborations with organizations within and outside India, and partnerships with the industry. NCCS has initiated steps towards achieving these goals by signing MoUs with neighbouring institutions like IISER-Pune, the S. P. Pune University, a university overseas and some private companies. Furthermore, drawing strength from the rich research and academic ecosystem already available in Pune, we also propose to establish a Pune Biotechnology Cluster (Pune BioCluster) in association with IISER-Pune, with the aim of seamlessly integrating the high quality work being done at various organizations in Pune in the field of biology. We strongly believe that the Pune BioCluster would augment inter-institutional interactions and synergize research-&-teaching activities, proving beneficial to many in academia and the industry.



Shekhar C. Mande
Director



Human Resource Development

During the year 2015-16, thirty four research scholars joined NCCS for the Ph.D. programme, under the guidance of different faculty members. Twenty five research scholars registered as Ph.D. students with the University during this year, taking the total number of registered Ph.D. students to 113 as on 31st March, 2016. Thirty four students submitted their theses to the University and six students were awarded the Ph.D. degree during the said year.

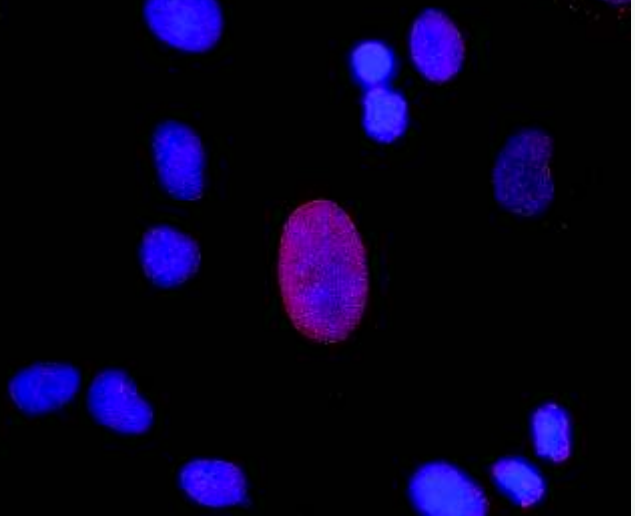
NCCS also conducts training programmes for students every year, as given below:

(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 2015-16 is as follows:

Project trainees	:	34
Summer trainees	:	21



Cell Repository

NCCS serves as a national cell bank for animal cell lines. The repository manages the expansion, cryopreservation and distribution of cell lines to research and academic institutions throughout the country. In the year 2015-16, five thousand one hundred cell lines were supplied to four hundred research institutions.

Scientists

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Dr. Rahul Patil

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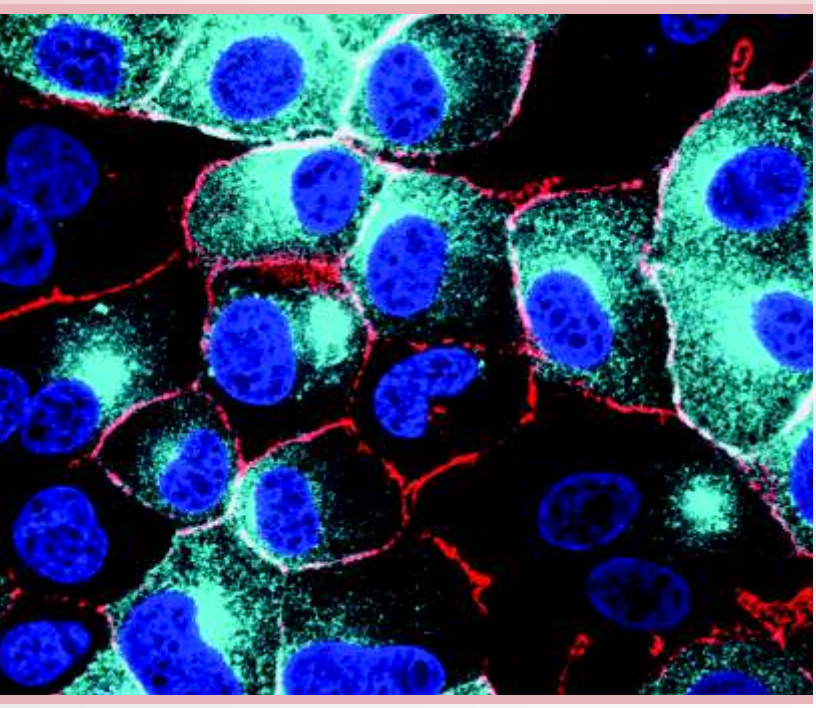
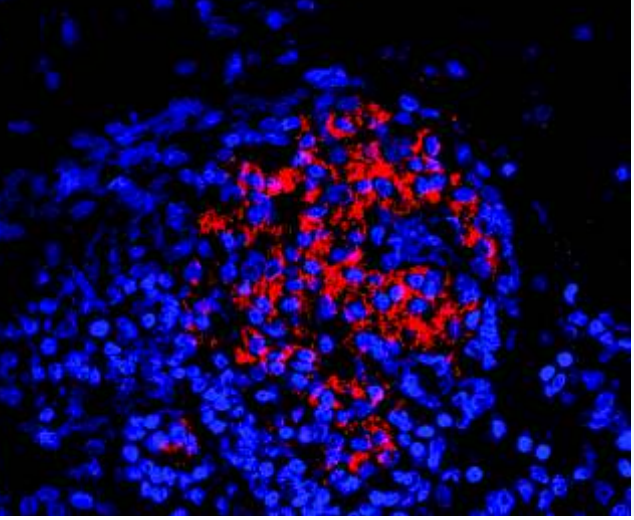


Research Reports



Index of Research Reports

Sharmila Bapat

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Elucidation of molecular and functional heterogeneity through differential expression networks analyses of discrete tumor subsets

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Background

Intratumour heterogeneity (ITH) represents coexistence of molecular and cellular diversity within a tumor, and is often overlooked in studying tumor models as a homogeneous system. We are trying to understand ITH through resolution of cancer stem cell (CSC) hierarchies using label-chase (PKH) and fluorescence-activated cell sorting-based sorting of tumors (Fig.1a-left panel). PKH^{hi} cells were established to be CSCs, PKH^{lo} as progenitors while PKH^{neg} cells contribute to the differentiated bulk of the tumor. Further probing for variability based on the cellular DNA content (also indicative of genetic instability) identified two additional tumor fractions representing euploid and aneuploid cells (Fig. 1b-left panel). Each of five fractions is associated with definitive spatio-temporal functionalities within the tumor including dormancy and regeneration that could contribute to drug resistance and long-term tumor survival. It further becomes imperitive to understand the molecular networks that are involved in the generation, maintenance and functionality of such diverse cellular identities.

Aims and Objectives

- Identification of molecular crosstalk between tumor subpopulations and its correlation with tumor dormancy.
- Evaluation of the behavior of aneuploid cells under drug induced stress.

Work Achieved

Microarray-based expression profiling and class comparison of expression datasets from each tumor fraction indicated association of definitive expression

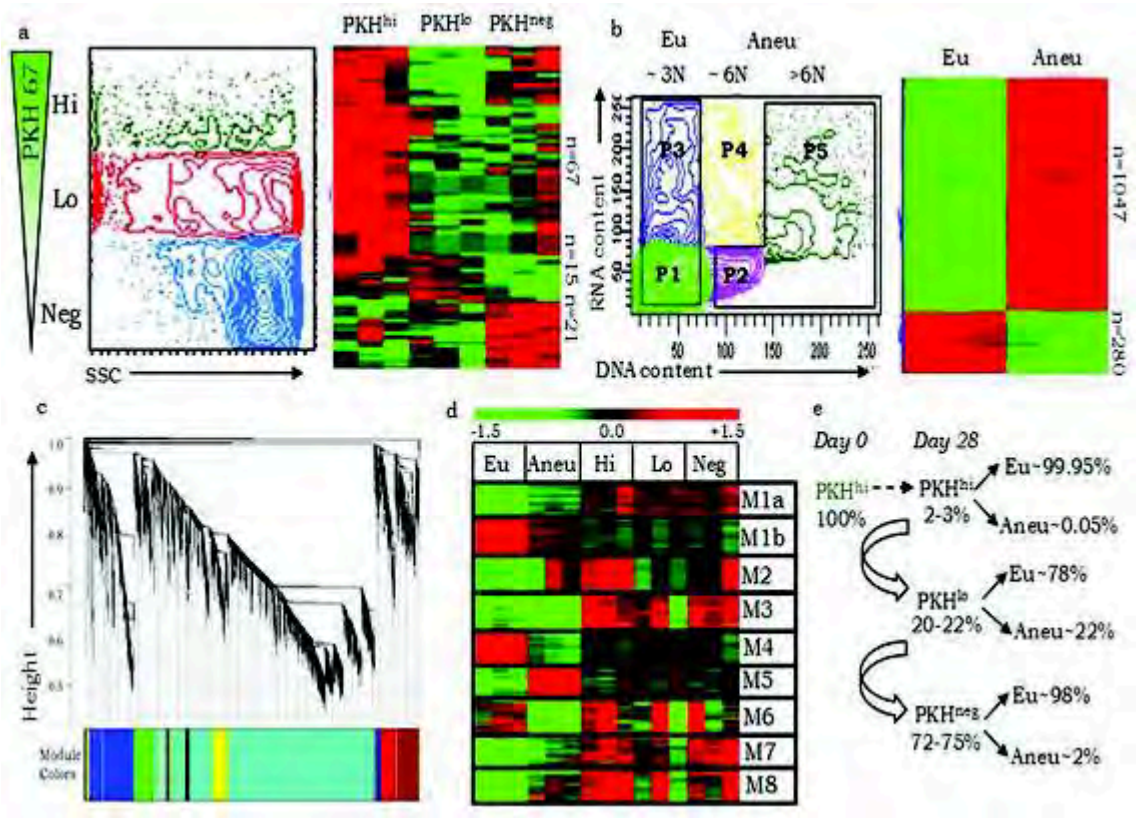

Fig. 1: **(a,b)** left panels - Representative FACs profiles for PKH derived cancer stem cell (CSC) hierarchy and Hoechst PyroninY-based DNA content analysis respectively, where P1- EuG0, P2-AneuG0, P3-EuG1, P4- (EuSG2M + AneuG1), P5-AneuSG2M, denoting sorted euploid (P1+P3) and aneuploid (P2 + P5) fractions; **(a,b)** -right panels - Representative heat maps of differential genes obtained through class comparison across various PKH- and ploidy- based fractions respectively, red and green colors correspond to up-regulated and down-regulated genes; **(c)** WGCNA-based cluster dendrogram identifies modules of correlated genes in the differential expression data; **(d)** Heatmap of top 50 highly correlated genes from each WGCNA module; **(e)** Flow-chart depicting frequency of fraction turnover in xenografts from Day 0 to Day 28 based on our earlier studies.

patterns (Figs. 1a, b - right panels). 5216 differentially expressed genes were identified across the five cell fractions that could be clustered in 8 discrete modules using Weighted Gene Correlation Network Analysis (WGCNA; Fig. 1c). Unsupervised, Principal Component Analysis of the top, most significant 50 WGCNA module genes (n=439) distinctly segregated euploid from other data; aneuploid datasets exhibited low distance similarity with two PKH^{lo} datasets, while data of the PKH^{hi} fractions clustered closely with that of PKH^{neg}. Most importantly, WGCNA modules were differentially enriched across various cell fractions (Fig. 1d) that assigns a distinct molecular expression profile to each fraction. Based on our earlier studies we could eliminate the more unlikely associations (Fig. 1e). The most significant association identified was between PKH^{hi} and PKH^{neg} fractions that constitute non-cycling states, while dormant aneuploid cells appeared to be confined to the PKH^{lo} fraction (Fig. 1e).

One of the likely associations identified included enrichment of module M2 expression in the PKH^{hi} fraction, and an anti-correlative association of M5 and M6 module genes in euploid and aneuploid cells respectively. To further establish unequivocal associations, gene expression intensities of M2 and M5 module genes were compared across tumor fractions. Distinct up -regulation

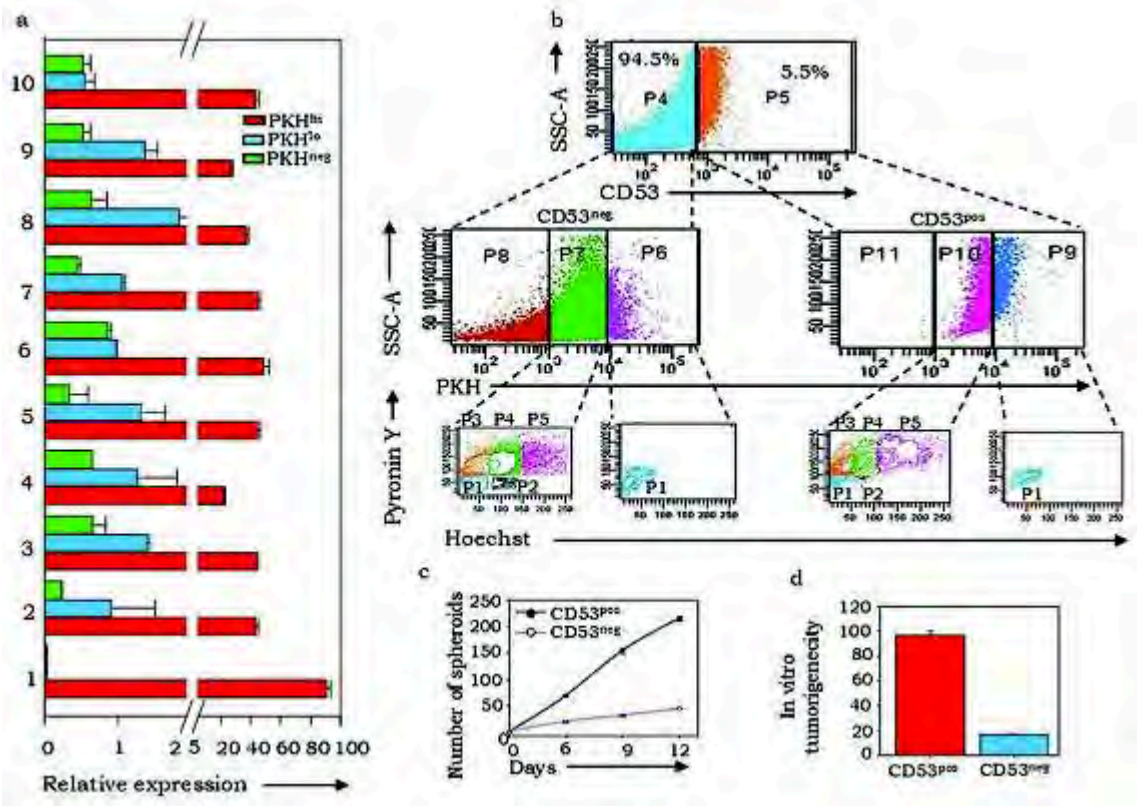

Fig. 2: **(a)** Real time PCR quantification of M2 genes (1-CD53, 2-CD84, 3- KRT1, 4- CCL18, 5-CXCL6, 6-MMP26, 7- S1007A, 8- SKT3B, 9- PRPY1, 10- TRAT1) in A4 tumor sorted PKHhi, PKHlo, PKHneg cells; **(b)** Representative FACS profile of CD53 expression in A4 xenograft tumor (upper panel) and across PKH based CSC hierarchy (middle panel) and Hoechst Pyronin Y based cell cycle profiling of PKHhi and PKHlo fraction across CD53 expression where fraction P1-P5 are same as mentioned in Fig. 1a; **(c)** Graphical representation of functional potential of sorted populations ($CD53^{neg}$; $CD53^{pos}$) in assays for: spheroid formation; **(d)** anchorage-independent clonogenecity.

affirmed association of M2 and M5 genes with PKH^{hi} and aneuploid fractions respectively. Literature-based functional annotation assigned further relevance to expression of some of the M2 genes in the PKH^{hi}/CSC fraction through their known involvement in stem cell functioning and differentiation, cell survival, cell adhesion and migration. Specifically, network analyses of these genes revealed the cell surface tetraspanin molecule CD53 to be a major hub with several interacting partners. In validating expression of some of the M2 genes, CD53 exhibited a striking association with PKH^{hi}, and also with euploid and G2M-growth arrested aneuploid PKH^{lo} fractions (Fig. 2 a, b). CD53 was also expressed in xenografts derived from other serous ovarian cancer cell lines including CAOV3, OVMZ6, OVCAR3 and OV90 that suggests a pan-ovarian cancer association. In further probing for functional correlates of this expression, we observed differences in regenerative potential of CD53 expressing- ($CD53^{pos}$) vs. CD53 lacking- ($CD53^{neg}$) cells when assayed for spheroid formation, *in vitro* tumorigenecity and *in vivo* limiting dilution assays for tumorigenecity potential (Figs. 2c, 2d). Taken together, these data suggest an association with regenerative functions within a tumor, and a liklihood of CD53 being useful as a putative marker for tumor initiating/regenerative potential.

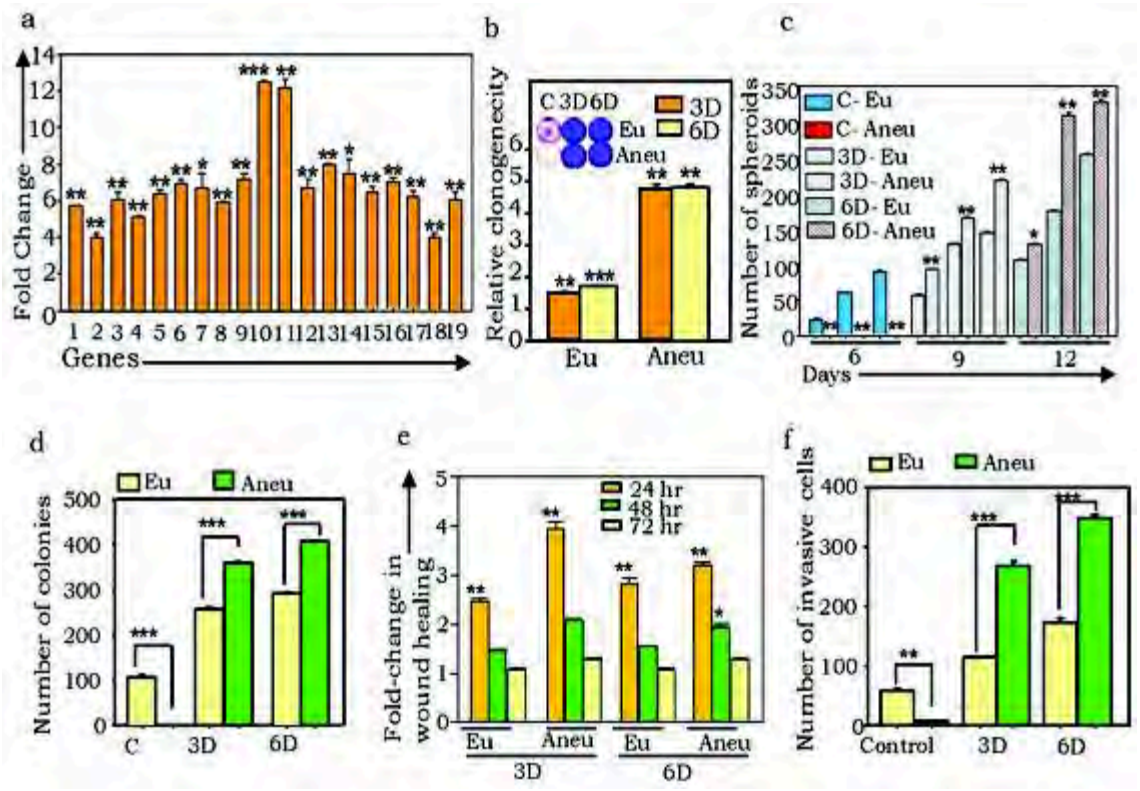

Fig. 3: **(a)** Real time PCR quantification of expression of M5 genes (1- DST, 2- VAMP3, 3- PKM2, 4- F2RL2, 5- ASPH, 6- MCM4, 7- PLEC1, 8- SNRPB, 9- ARHGDIA, 10- MSN, 11- FLOT2, 12- PRICKLE1, 13- RDH10, 14- ABL2, 15- WWTR1, 16- BRCC2, 17-MYO1E, 18-PSCD3, 19- F3) in aneuploid fraction of A4 naïve (untreated) tumor, fold-change gene expression is calculated with respect to that in the euploid fraction; **(b)** Anchorage dependent clonogenecity of tumor sorted fractions - inset shows crystal violet stained images, **(c)** Quantification of spheroid formation potential of sorted euploid and aneuploid cells (Eu and Aneu; blue and red color patterns respectively) in untreated tumors (C) or exposed to either 3 or 6 doses of paclitaxel (3D and 6D respectively); **(d)** *In vitro* tumorigenecity or similarly sorted populations; **(e)** Fold-change wound healing/ migratory efficiency cells; **(f)** Quantification of matrigel invasion assay.

WGCNA also established an association between module M5 expression and aneuploidy (Fig. 1c). Further detailed literature annotation assigned several functions including migration, invasion and metastases through EMT, change in morphology through cytoskeletal remodeling, enzymatic penetration of basal lamina, cell cycle, immune evasion to overcome host resistances and re-acquisition of 'stemness' features to ensure long-term cell survival and regeneration. Gene-gene interaction network analyses identified cytoskeletal remodeling genes as a major hub with several interacting partners. Validation of core hub genes affirmed the association with aneuploid over euploid cells (Fig. 3a). We had earlier observed that the aneuploid fraction in naïve, untreated tumors is growth-arrested and exposure to stress and selective pressure triggers cell cycling. Such aneuploid cells have potential to generate parallel drug resistant hierarchies within the same tumor; this emphasized microenvironmental stress to be essential in predicting functional correlates of aneuploid cells. We further examined and compared the functional dynamics of aneuploid cells from naïve tumors with those exposed to either three or six doses of paclitaxel. Such drug-induced stress not only enhanced frequency of aneuploidy within tumors, but significantly contributed to enhanced *in vitro* regenerative potential of these cells as assayed by colony and spheroid forming assays (Figs. 3b, 3d). Profiling of M5 genes in paclitaxel treated tumors also

identified their upregulation in a dose-dependent manner suggesting contributions to tumor cell survival. Most strikingly, expression of moesin and flotillin (known to contribute to centrosomal abnormilities, cytoskeletal remodelling and migration) were further enriched. This led us to hypothesize that aneuploid cells in naïve tumors are 'poised' for metastases, which could be triggered by stress. On testing this conjecture through functional wound healing (migration) and matrigel invasion assays, aneuploid cells from paclitaxel treated xenografts indeed validated this predicted function (Figs. 3e, 3f).

Future Research Plans

Our study underscores the need to focus on study of tumor cell fraction specific capabilities important from the point of regeneration, treatment resistance and disease progression that together are determinants of tumor survival. The gene correlation analyses and functional validation in this study can be further extrapolated in identifying novel markers associated with tumor recurrence. Designing of optimum treatment to target the aneuploid population would lead to improvement of present day therapy.

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Cancer, chemotherapy, and metabolic disorders

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Background

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

The effectiveness of chemotherapy has suffered due to the lack of specificity, rapid drug metabolism , intrinsic and acquired drug resistance, induction of side effects due to high dosage leading to transient decline in the quality of life of the patients. Moreover, the mechanisms of chemotherapeutic drugs mediated cell killing have not been completely understood. These limitations could be partly overcome by a low dose treatment of drugs in combination with other compounds/drugs, which show additive or synergistic cytotoxic effects, together with a better understanding of chemotherapeutic drugs-specific propagation of bystander cells killing.

Owing to the heterogeneous nature and scanty vascularization, the access of anticancer regimen to all strata of the tumor is one of the major challenges in cancer therapy. Of late, the phenomenon of bystander effect (BE), which refers to transmission of death signals from the drug-exposed cells to the unexposed cells, is being explored to improve the therapeutic response. Although BE has

been well documented in radiotherapy and experimental approaches of gene therapy, very limited information is available with respect to conventional chemotherapeutic drugs. We have previously demonstrated the chemotherapy-induced bystander killing in breast cancer cells and hepatocellular carcinoma cells. Recently, other groups also have demonstrated occurrence of chemotherapy induced BE in breast cancer and lung cancer, which is in agreement with our studies. BE has been shown to be dependent on cell type and class of drugs, and the role of tumor microenvironment in response to chemotherapeutic drug-induced BE is poorly understood.

Cervical cancer is one of the most common solid tumors. Mitomycin C (MMC), a DNA alkylating agent, has been widely used in this malignancy as a constituent of combination therapy. From the pharmacological point of view, MMC is effective at relatively low dose with minimal organ-associated toxicity and it has been shown to activate innate immunity. However, therapeutic efficacy of MMC principally depends on other drug types in combination therapy. Therefore, a well-designed strategy that could enhance the efficacy of MMC is desirable. MMC has been demonstrated to induce BE in hepatocellular carcinoma, but not in cervical cancer cells. Although the precise mechanisms of bystander killing remain elusive, we have previously reported the involvement of death ligands, which was later supported by other studies. The ability of cancer cells to escape programmed cell death plays a critical role in survival of cancer cells and tumor progression. Despite the presence of cellular apoptotic factors, cancer cells reprogram their molecular events and signaling to evade apoptosis. Moreover, it has been reported that exposure to proteasomal inhibitor inhibits the growth of various cancer cells and sensitize them to death ligand mediated death by stabilizing death receptors. Considering these notions, we speculated that non-functionality of death receptors could be one of the possible factors associated with defective BE in cervical cancer. We, therefore, hypothesized that treating cervical cancer cells with combination of MMC and proteasomal inhibitor could elicit BE, thereby may significantly improve the therapeutic outcome.

Till date, studies explicate cancer cells exposed to chemotherapy as the effector cells in inducing bystander mediated killing. However, due to the heterogeneous nature of cellular population in tumor, other cellular components are also likely to play a key role in inducing BE. Tumor microenvironment consists of a heterogeneous mass of malignant as well as non-malignant cells. The non-malignant cells include endothelial, fibroblast and immune cells those establish multitude of interactions among themselves and also with malignant cells. Macrophages are the most abundant immune

cells present in tumors, also termed as tumor-associated macrophages (TAMs). TAMs are differentiated monocytes which infiltrate the tumor microenvironment, and are exposed to chemotherapeutic regimen. Studies have demonstrated that TAMs could account for approximately more than 60% of tumor mass in some cancers. TAMs exposed to radiations and chemotherapy have been shown to play a significant role in inducing BE. Studies support the notion that targeting TAMs could improve the therapeutic index of various drugs. Under chemotherapy, increased recruitment of macrophages with enhanced expression of tumoricidal factors like perforin and granzyme, death ligands, or ROS has been reported in tumors. Therefore, we speculated that BE could further be amplified by infiltrating macrophages resulting in enhanced therapeutic efficacy of anticancer regimens. In the present study, we evaluated combination effect of MMC and MG132 in enhancing bystander killing of cancer cells in vitro and in vivo, in part, through involvement of cancer cells and TAMs. Herein, we demonstrate that stabilization of Fas on cervical cancer cells facilitates dramatic reduction in tumor progression as a consequence of increase in apoptosis. This study could be helpful in designing novel therapeutic strategies in treating cancer by involving proteasomal inhibitors in combination with chemotherapeutic drugs those specifically activate death receptor mediated killing.

Aims and Objectives

- To study the potential implication of MMC in combination with the proteosonal inhibitor on human cervical cancer cells.
- To evaluate the role of tumor associated macrophages in this combination regimen.

Work Achieved

MMC induces FasL expression in effector cells

We have previously demonstrated that MMC induces bystander killing in hepatocellular carcinoma cells. However, MMC failed to promote bystander killing in cervical cancer cells under identical experimental conditions (Figure 1ai and aii). This led us to further investigate the ability of MMC to differentially promote bystander response in other cancer cells types. We examined whether MMC could induce expression of death ligands in cervical cancer (HeLa and SiHa) cells. After 24 h of treatment with MMC, FasL was increased in dose-dependent manner at transcriptional as well as protein levels (Figure 1b and c).. As FasL is a secretory as well as membrane bound protein, we checked the expression of FasL on the cell surface following MMC treatment. Flow cytometric analysis confirmed that MMC induced FasL expression on

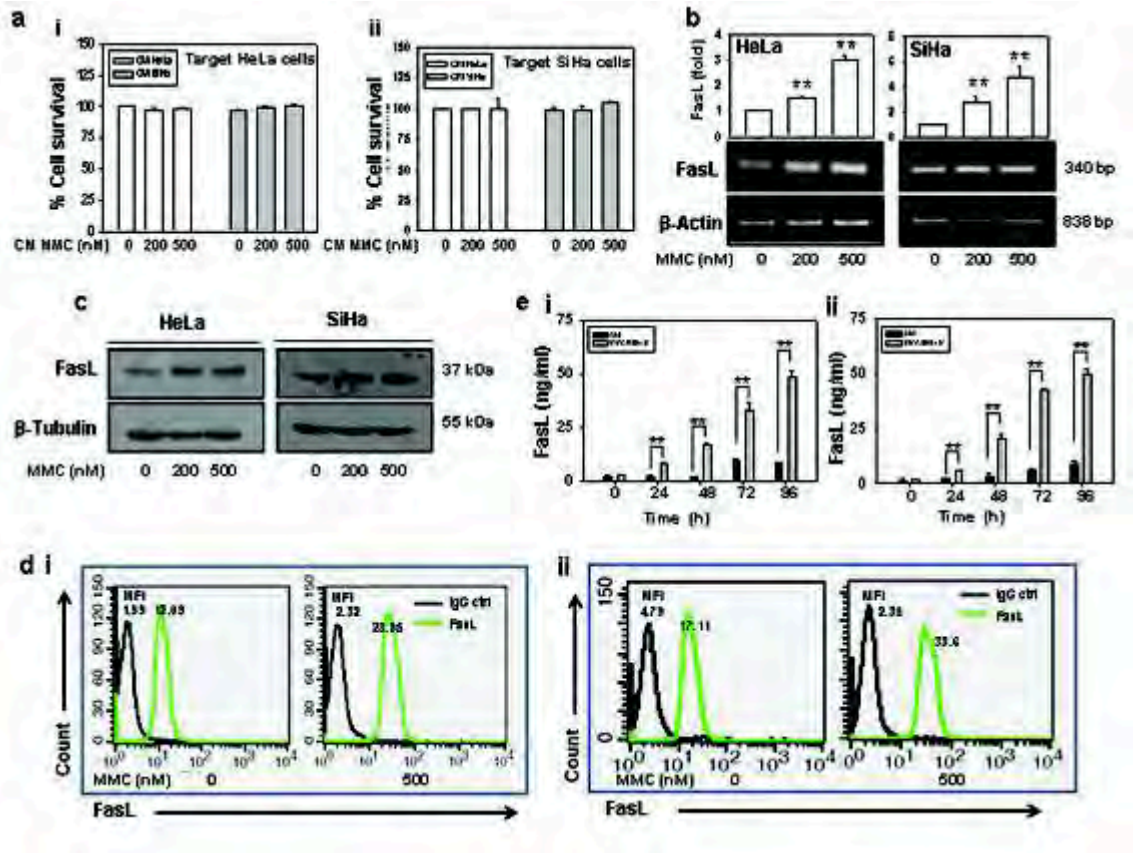


Fig. 1: MMC induces expression of death ligands in cervical cancer cells. (a) Bystander killing in CM transfer experiment. The effector cells (HeLa and SiHa) were treated with indicated concentrations of MMC for 24 h, and then CM medium was collected after 48 h. Target HeLa (i) and SiHa (ii) cells were incubated with the respective CM for 24 h. Cell survival was evaluated by MTT assay. (b) Semi-quantitative RT-PCR for FasL mRNA. HeLa and SiHa cells were treated with indicated concentrations of MMC for 24 h, and were processed for RT-PCR. β-actin was used as a loading control. Data are mean ± S.D., and are representative of three independent experiments. (c) Western blot analysis of FasL. HeLa and SiHa cells were treated with indicated concentrations of MMC for 24 h, and cell lysates were subjected to SDS-PAGE and probed for protein levels of FasL. (d) Flow cytometric analysis of FasL expression. HeLa (i) and SiHa (ii) cells were treated with MMC as described above. Untreated or MMC-treated cells were probed with FasL primary antibody or IgG control (1:100), and further with PE-conjugated secondary antibody (1:200). Cells were then washed with PBS, and

membrane of HeLa and SiHa cells (Figure 1di and dii). Consistent with these observations, secretory FasL (sFasL) level was also increased in the conditioned

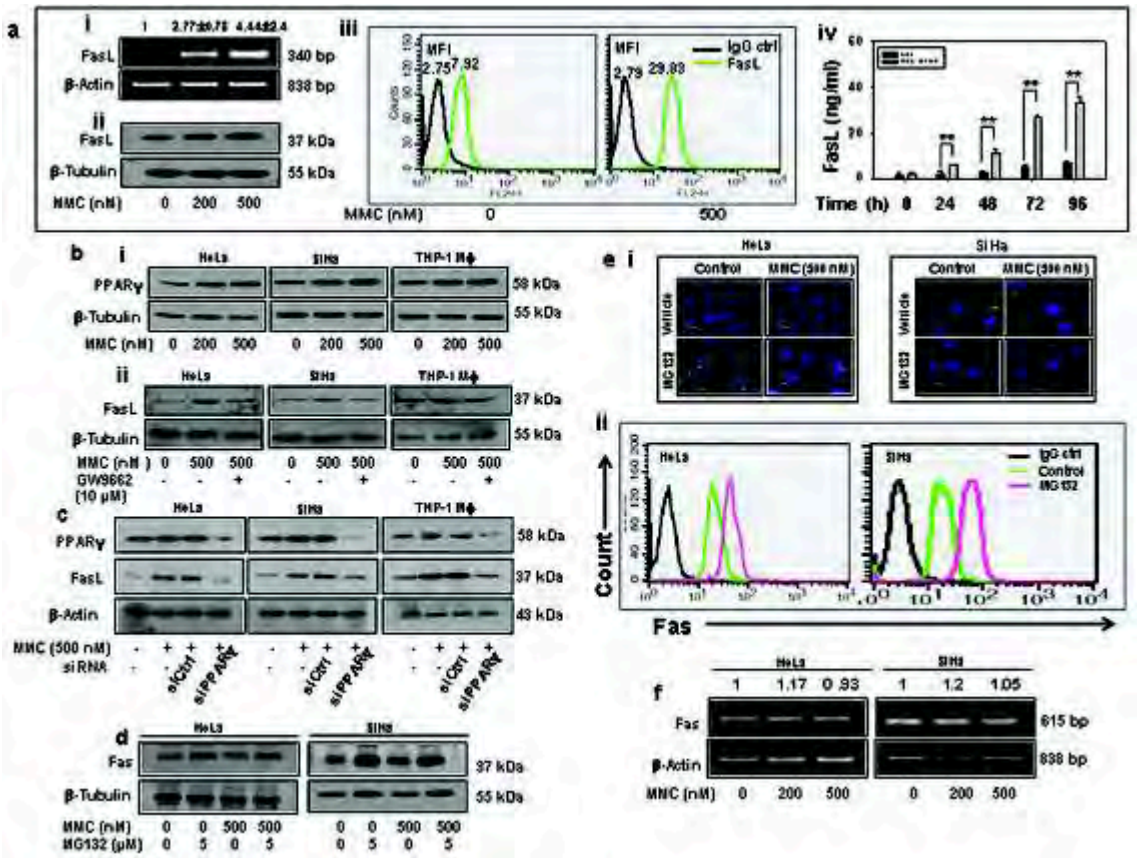
FasL expression was analyzed by flow cytometry. (e) Sandwich ELISA for quantitation of sFasL from MMC treated HeLa (i) and SiHa (ii) cells at the indicated time points. Data are mean ± S.D., and are representative of three independent experiments (**p < 0.01 when compared to their respective controls).

found that MG132 enhanced Fas expression in HeLa and SiHa cells (Figure 2d). Enhanced localization of Fas to the plasma membrane was observed by confocal microscopy (Figure 2ei) and FACS analysis (Figure 2eii)., which was sustained up to 24 h even after the withdrawal of MG132 However, MMC treatment did not affect the expression of Fas at protein and mRNA level (Figure 2d and f). We therefore evaluated the combination effect of MMC and MG132 on HeLa and SiHa cells. In cell survival assay, it was found that MMC treatment, when combined with MG132, diminished the survival in a dose-dependent manner as compared to either treatment alone in HeLa and SiHa cells (Figure 3ai and bi). Under identical experimental set up, we also observed significant increase in annexin V-FITC positive cells in combination treatment as compared to either agent alone (Figure 3 aii and bii).

Fig. 2: MMC induces death ligand expression via PPARγ and proteasomal inhibition increases level of death receptors in cervical cancer cells. (a) Analysis of expression of FasL in MMC-treated THP-1 MΦ. (i) semi-quantitative RT-PCR for FasL mRNA. THP-1 MΦ were treated with indicated concentrations of MMC, and processed for RT-PCR. β-actin was used as a loading control. Data are mean ± S.D., and are representative of three independent experiments. (ii) THP-1 MΦ were treated with indicated concentrations of MMC, and whole cell lysate were subjected to Western blotting for

Co-administration of MG132 and MMC inhibits xenografted tumor progression in mice

To ascertain our findings in vivo, we performed experiments in NOD/SCID mice bearing HeLa xenografted tumor. When tumor volume reached to approximately 100 mm3, mice were divided into four groups (Figure 3a). Mice were treated with MG132 and MMC. Tumor progression was monitored and



FasL. (iii) Flow cytometric analysis of FasL expression. THP-1 MΦ were treated with MMC as described above. Untreated or MMC treated cells were probed with FasL primary antibody or IgG control (1:100), and further with PE-conjugated secondary antibody (1:200). Cells were then washed with PBS, and FasL expression was analyzed by flow cytometry. (iv) Sandwich ELISA for quantitation of sFasL from untreated and MMC treated THP-1 MΦ at indicated time points. (b) Analysis of involvement of PPARγ in regulation of FasL expression. (i) Western blot analysis of PPARγ in MMC-treated cells. HeLa, SiHa and THP-1 MΦ were plated in 35 mm culture dishes. After 24 h, MMC treatment was given at indicated concentrations, and cells were further incubated for 24 h. Cell lysates were then subjected to SDS-PAGE and Western blotting for PPARγ. (ii) Effect of PPARγ inhibition on FasL expression. HeLa, SiHa and THP-1 MΦ were plated in 35 mm culture dishes. After 24 h, cells were pretreated with GW9662 (10 μM) for 2 h. Thereafter, MMC (500 nM) treatment was given and cells were incubated for 22 h. Whole cell lysates were subjected to Western blotting for FasL. (c) Effect of knock down of PPARγ on MMC-induced expression of FasL. HeLa, SiHa and THP-1 MΦ were transfected with control siRNA or PPARγ siRNA for 15 h, and allowed to grow for a further 15 h. Control siRNA and PPARγ siRNA-transfected blot analysis of PPARγ and FasL. (d) MG132 induced expression of Fas. HeLa and SiHa cells treated with MMC and/or MG132 for 24 h. Western blot analysis of whole cell lysates subjected to SDS-PAGE and probed for Fas. (e) Analysis of expression and localization of Fas in MG132 treated cervical cancer cells. (i) Immunofluorescence staining of HeLa and SiHa cells. Cells were treated with MMC and/or MG132 for 24 h, washed twice, then fixed and permeabilized with 4% paraformaldehyde and 1% Triton X-100 respectively, and blocked with 5% FBS. Cells were further incubated with anti-Fas primary antibodies (1:100) for 2 h and subsequently stained with FITC-conjugated secondary antibodies (1:200) for 1 h. (ii) Flow cytometric analysis of Fas expression in cervical cancer cells. HeLa and SiHa cells were treated with MG132 for 24 h. Untreated or MG132 treated cells were probed with primary antibody against Fas or IgG control (1:100) for 1 h, and further with PE-conjugated secondary antibody (1:200) for 30 min. Cells were then washed with PBS, and Fas expression was analyzed by flow cytometry. (f) Semi-quantitative RT-PCR for Fas mRNA in MMC-treated cervical cancer cells. HeLa and SiHa cells were treated with indicated concentrations of MMC for 24 h, and were processed for RT-PCR. β-actin was used as a loading control. d cells were exposed to MMC for 24 h, and cells were harvested for Western

followed up during the course of experiment. We observed that combination treatment of MG132 and MMC diminished the tumor progression as compared

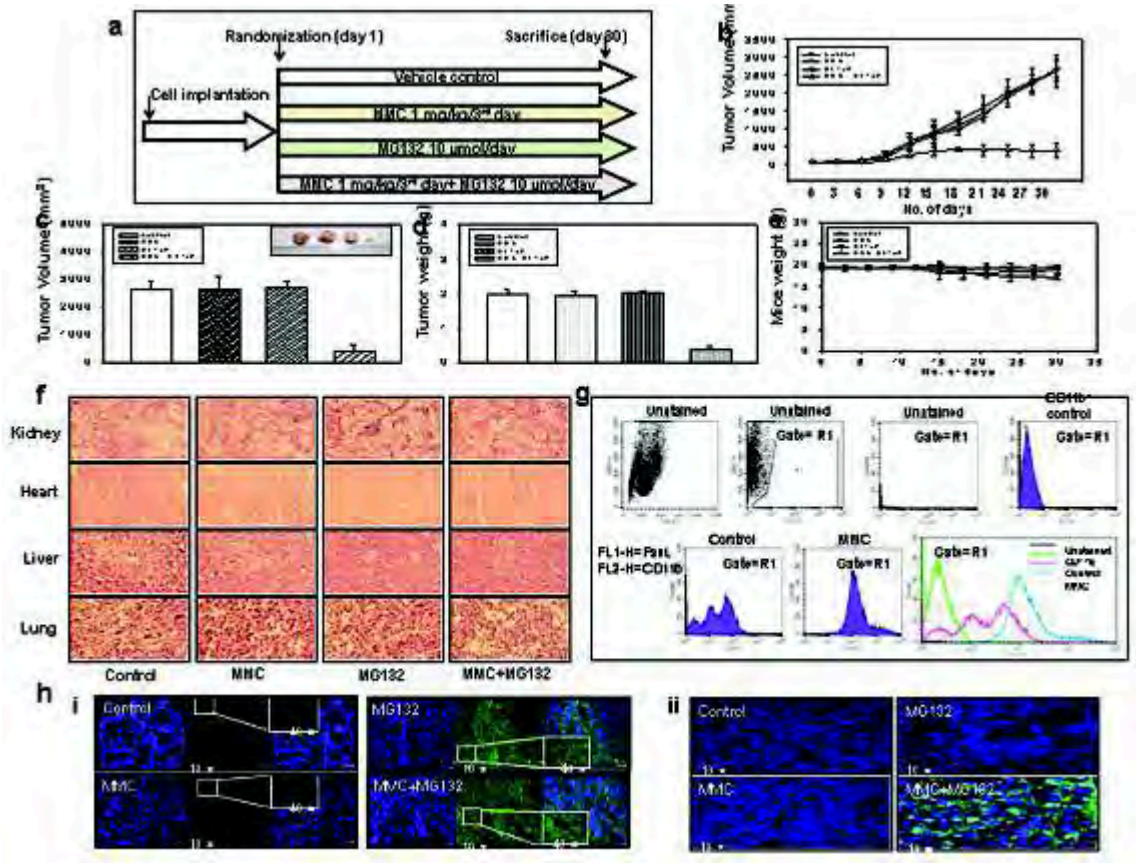


Fig. 3: The combination treatment of MMC and proteasomal inhibitor reduces tumor progression in HeLa cells xenografted mouse tumor model. (a) Experimental layout of in vivo study. HeLa cells (1 × 106 in 100 µl PBS) were injected on the right flank of the mice to form tumors. Tumor bearing mice were treated with combination of MMC (1 mg/kg/every 3rd day) and MG132 (10 µM/kg/day) as described in Materials and Methods. Control mice were administered with equal volume of vehicle on the same treatment day. (b) Tumor progression after drug administration in control and treated mice. (c and d) Bar graph showing tumor volume and tumor weight in mice at the end of the experiment. (e) Changes in body weight in mice during the course of the experiment. (f) Histopathological analysis of major vital organs collected from experimental mice. Kidney, heart, liver and lungs were fixed in 4% formaldehyde. The tissues sections were stained with hematoxylin and eosin (H&E) (magnification, 400×). (g) TAMs were isolated from tumor as described in Materials and Methods. Cells were analyzed for FasL expression by flow cytometry.

Cells were dually stained with CD11b (1:100) and FasL (1:100), and CD11b positive cells were gated to analyze FasL expression. (h) Representative images of immunostained section analysis of Fas (i) TUNEL assay (ii) in tumor tissues of different treatment groups (Magnification, 40× with inset at 400×).

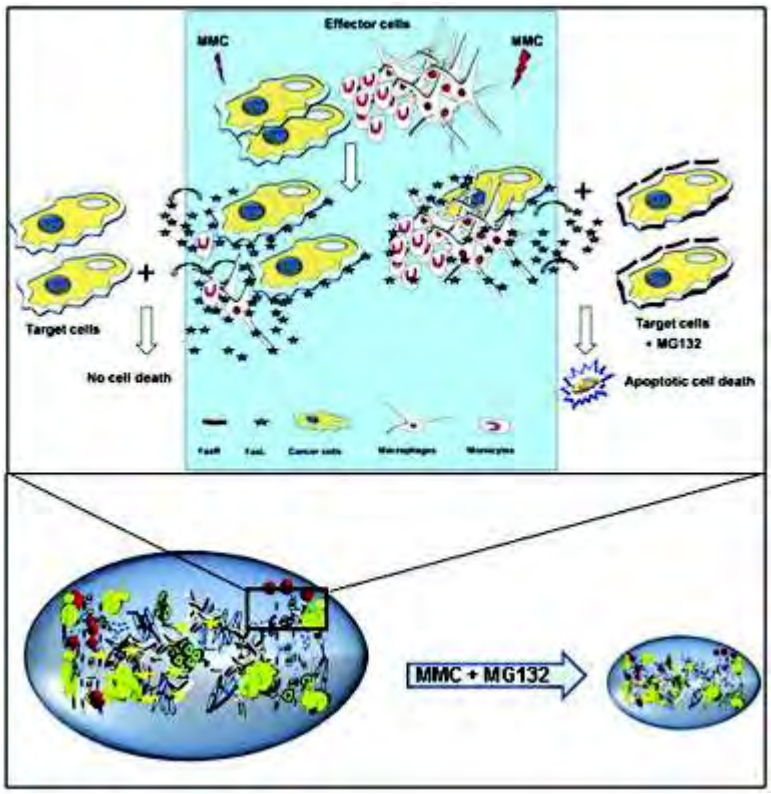


Fig. 4: Proposed model for bystander effect. MMC induces expression of membrane bound and secretory forms of death ligands (FasL) in cancer cells as well as macrophages. Restoration of Fas by inhibiting proteasomal degradation facilitates bystander killing of tumor cells and, thus effectively retarding the tumor progression

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Understanding newer functions of tumor suppressor, SMAR1

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Background

The nuclear matrix provides structural framework to the nucleus tethering several proteins which are important for many processes like transcription, splicing, DNA repair etc. The nuclear chromatin is organized in loops by the nuclear matrix thus modulating the chromatin architechture. SMAR1 is a nuclear matrix binding protein and belongs to a family of BEN domain proteins. This BEN domain is crucial for DNA binding and protein binding function of this protein. Earlier studies from our lab have shown that SMAR1 is a chromatin modifier which recruits HDAC1 to the promoter and brings about modulation of the activity of promoter like that of Cyclin D1. SMAR1 was also reported to regulate apoptosis and survival by regulating the expression of Bax and Puma. Recently SMAR1`s role as a stress response protein was well elucidated wherein SMAR1 was reported to modulate the acetylation status of Ku70 by interacting with HDAC6 (Chaudhary et. al. 2014, Cell death and Disease). Additionally SMAR1 was reported to negatively regulate the alternative splicing by modulating the acetylation status of Sam68 by recruiting HDAC6 (Nakka et. al. 2015, PNAS). Recently we also showed that the switch between effector T cells and regulatory T cells is governed 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 Treg and Th17 phenotype (Mirlekar et.al. 2015 Mucosal Immunology). The ChIP-seq analysis predicted a plethora of SMAR1 gene targets, to which SMAR1 can bind in the presence and absence of p53. A significant number of genes, however, favor the binding of SMAR1 irrespective of p53 status. (Mathai et. al., 2016)

Aims and Objectives

- Role of SMAR1 in Wnt signaling pathway
- Metabolic regulation of epigenetic changes in tumor suppressor gene SMAR1
- Proteomic profiling of SMAR1 regulated genes and their implication in tumorigenesis and antigen processing and presentation
- Mechanism of action of sulphorophane and its derivatives on SMAR1 mediated alternative splicing

Work Achieved

Role of SMAR1 in Wnt signaling pathway

In cancer cells there is aberrant activation of signaling pathways which help the cancer cells maintain their cancerous phenotype and their proliferation potential. One such signaling cascade is Wnt signaling. Few tumor suppressors that negatively regulate Wnt signaling have been reported so far in mammals. TCF4/LEF1 can act both as an activator or repressor depending on their binding to β-catenin. Studies have reported that when HDACs bind to LEF1 there is repression of the gene thus regulating the down-stream targets. Active Wnt signalling / β-catenin pathway results in breast, colon, prostrate and liver cancers. Tumor suppressors like GSK 3β, Adenomatos Polypopsis Coli (APC), Axin, etc. check β-catenin ubiquitin degradations mediated by β-Trcp1. Factors like Wnt ligands, DKK, CDC20, APC/C, mutation in β-catenin degradations sites stabilize β-catenin expressions resulting in poor prognosis. For the first time SMAR1 (Scaffold Matrix Attachment Binding Protein 1), a tumor suppressor has been shown to regulate β-catenin negatively through transcriptional repression. Reduced expression of SMAR1 through ubiquitin degradation in colon cancers upon active Wnt 3a signalling is the precursor for enhanced β-catenin stability.

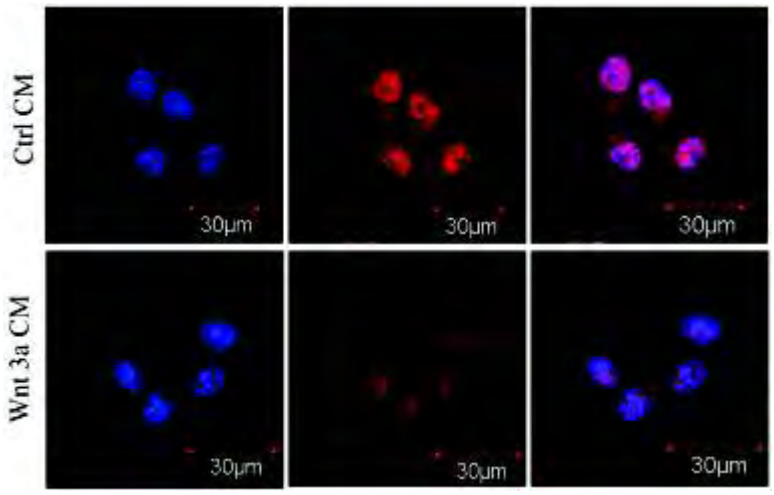

Fig. 1: Immunoflurescence for SMAR1 protein after treating HCT116 cells with Ctrl CM or Wnt 3a CM.

Our studies have revealed that SMAR1 is a negative regulator of β-catenin and may prevent the Wnt signaling activation. We found that SMAR1 negatively regulates β-catenin and thus prevent activation of Wnt signaling. Our findings also show that a Wnt 3a activation results in the down-regulation of SMAR1 and over-expression of SMAR1 was found to revert the effect. CDC20 has been reported to be stabilized upon Wnt 3a activation and is also responsible for SMAR1 degradation. CDC20 recruits E3 ligase to SMAR1 and results in SMAR1 degradation leading to its instability. During active Wnt signaling CDC20 gets stabilized and results in SMAR1 degradation. We found that blocking the ubiquitin sites in SMAR1 using peptides (AT-01C) prevented its degradation that attenuated β-catenin expression. This segment of peptide is derived from MPT63, a secretory protein of Mycobacterium tuberculosis. Isothermal titration calorimetry and Autodock experiments suggested that AT-01C interacts with SMAR1. Hence, prevention of SMAR1 degradation using small molecule compounds or peptides can serve as a potential therapeutics in cancer. Since SMAR1 suppresses β-catenin, we checked LEF1/TCF4 promoter binding of β-catenin..

We are reporting for the first time that SMAR1, a tumor suppressor protein regulates β-catenin at the transcriptional level. Most of the reports have shown to regulate β-catenin either by degradation or by preventing its binding to TCF4/LEF1. Inhibition of Wnt signaling pathway is an important event in controlling some of the cancers where Wnt/ β-catenin is very active.

Metabolic regulation of epigenetic changes in tumor suppressor SMAR1

Rapidly proliferating cells show significant increase in glycolysis known as the "Warburg effect". The role of SMAR1 as a stress response protein in repair of DNA damage was already reported from our lab. A rapidly proliferating cancer cell has to circumvent many stresses in order to survive and continue proliferation one of which is the metabolic stress. A cancer cell has high energy requirements because of their highly proliferative nature thus it was interesting to check the effect of glucose deprivation on levels of SMAR1.

Epigenetic regulation of a gene is primarily obtained by methylation of the DNA stretch and also the methylation and acetylation of histones. Methylation of cytosine residue is mainly observed where a methyl group gets attached to cytosine. This is brought about by the DNMTs; mainly Dnmt1 which is a maintenance methyl transferase, Dnmt3a and Dnmt3b which are the de-novo DNMTs. Upon methylation several methyl binding proteins like MeCP2, Sin3a etc. come and bind to the methylated cytosine moieties on the DNA.

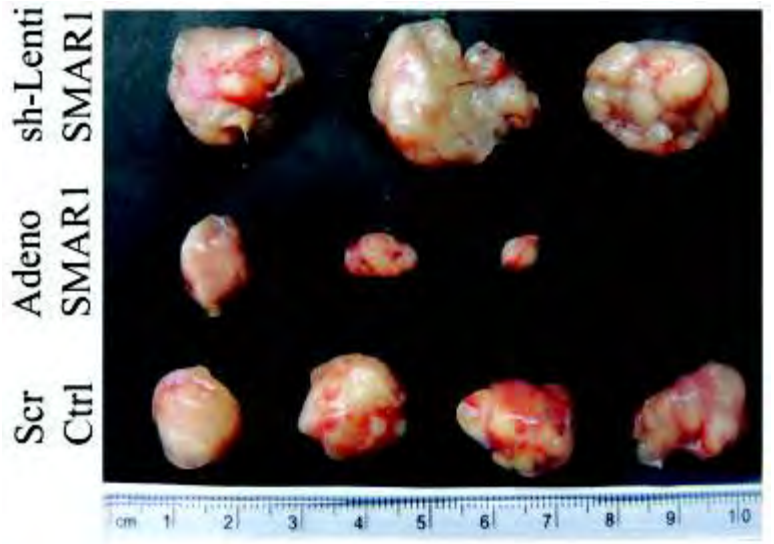

Fig. 2: Mouse xenograft model of stable HCT116 cells using Adeno-SMAR1, Scrambled

Methylation of the promoter region causes two major events; firstly it recruits HDACs which de-acetylates the histone. This causes the second change i.e, change in the chromatin conformation which makes the DNA inaccessible to the RNA polymerase machinery. SAM, the only methyl group donor, is generated primarily through the folate pathway in normal cells but in case of malignant cells where glycolysis is increased multifold, SAM is also generated through the one-carbon metabolism pathway as an offshoot to the glycolysis pathway.

We showed that the levels of SMAR1 can be altered by changing the nutrient supply to the cell i.e, by changing the cellular metabolism which in turn causes the change in the methylation status of the SMAR1 promoter. We found that MeCP2 interacts with both HDAC1 and HDAC2 in untreated cells. We also observed that in untreated cells there is methylation of H3K9 and H3K27 and these methylation marks are lost upon glucose deprivation. Apart from these findings we also observed an overall decrease in the levels of HDAC1 and HDAC2 upon glucose deprivation. We can speculate that since the overall levels of the HDACs go down and there is a loss of methylation, together it causes the transcriptional activation of the tumor suppressor gene *SMAR1*. To validate this further we treated the cells with Trichostatin A which is a general HDAC inhibitor and this treatment allowed transcription of SMAR1 gene.

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

SMAR1 triggers cell cycle arrest and apoptosis through transcriptional regulation of specific target genes. SMAR1-dependent regulation of the up-regulated protein calnexin was further studied. To delineate the mechanism of

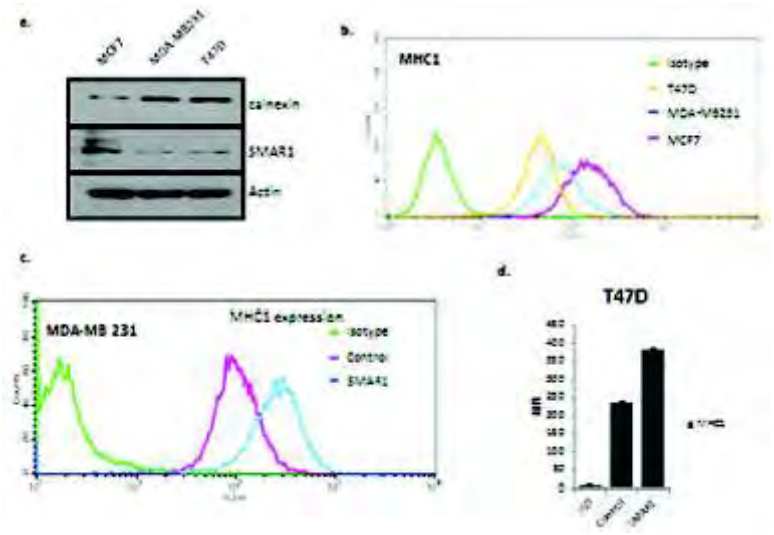

Fig. 3: SMAR1 regulates MHC1 expression in different grade of breast cancer cells

how SMAR1 regulates calnexin gene expression, a bioinformatics analysis of calnexin promoter was performed. Interestingly SMAR1 and GATA2 binding sites were observed proximal to each other in calnexin promoter. Chromatin immunoprecipitation confirms the binding of SMAR1 and GATA2 on calnexin promoter. SMAR1 forms triple complex with GATA2 and HDAC1. Recruitment of HDAC1 results in deacetylation of GATA2, under deacetylated condition GATA2 acts as repressor resulting in downregulation of calnexin gene. This study mechanistically highlights the co-ordinated regulation of calnexin gene by SMAR1 and GATA2. SMAR1 controls the expression of these proteins suggesting direct role of SMAR1 in ER homeostasis.

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

Mechanism of action of sulforaphane and ITC derivatives on SMAR1 mediated alternative splicing

A multitude of natural compounds which are components of our day to day diet have been identified to possess anticancer activity. Isothiocyanates (ITCs) are the most widely studied class of compounds exhibiting anticancer activity. One of the profoundly studied ITC is sulforaphane (SFN). SFN is found in vegetables of *cruciferae* family and exhibits several different modes of action to culminate cancer cell proliferation and survival.

Here we report a novel target of SFN and chemically synthesised derivatives of ITCs. SFN treatment leads to the increased expression of a nuclear matrix binding protein, SMAR1. In the absence of LOH, SMAR1 leads to variable exon incorporation in CD44 gene and thus leads to CD44 isoforms, which confer metastatic potential in several types of cancers. Thus SMAR1 negatively regulates the alternative splicing of CD44. Enhanced expression of SMAR1 was observed when LOH containing cell lines such as MDAMB231 were treated with small compounds like SFN. Various derivatives of ITCs were then synthesized and SMAR1 expression was checked. Of these derivatives, SCS-OCL-381 leads to maximum expression of SMAR1 at much lower concentration as compared to the parent compound. Also, SCS-OCL-381 treatment leads to decreased CD44 variable exon inclusion.

No change was observed at the transcript level upon treatment with SFN/SCS-OCL-381. Therefore, we speculated that SMAR1 must be getting stabilized. MG132 treatment shows SMAR1 stabilization. Thus, SFN/SCS-OCL-381 might be preventing the ubiquitination of SMAR1 and thus inhibiting the incorporation of CD44 variable exons. Further, various SMAR1 D-Box mutants were prepared and SMAR1 expression upon transfection of these mutants was checked. MDA-MB 231 cells were then treated with SFN/SCS-OCL-381. Further experiments suggest that mutation at the N-terminal D-Box (mutant M1) might be preventing the binding of SFN/SCS-OCL-381 to SMAR1 protein and thus decreasing its stabilization owing to no change in CD44 variable incorporation.

It was thus concluded that SFN/SCS-OCL-381 treatment leads to the stabilization of SMAR1 by blocking the ubiquitination machinery to recognize the protein and the elevated levels of SMAR1 in turn lead to the decrease in the incorporation of CD44 alternative exons thus inhibiting metastasis. Further studies are being carried out to decipher the structural aspects of SMAR1 ubiquitination prohibition.

Future Research Plans

- Maintenance of cellular homeostasis through transcriptional regulation of MAR binding protein SMAR1 and its role in embryonic development.
- Regulation of catalytic subunit of telomerase by SMAR1
- Role of SMAR1 in regulation of PKM alternative splicing

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Structural and functional studies on components of the nuclear pore complex

Background

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

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

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Aims and Objectives

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

Work Achieved

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

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

Native isolation of sub-complexes of the human NPC, and structural analysis by cryo-EM methods

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

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

We are currently reconstituting Nup93●Nup205 and Nup93●Nup62●Nup54●

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

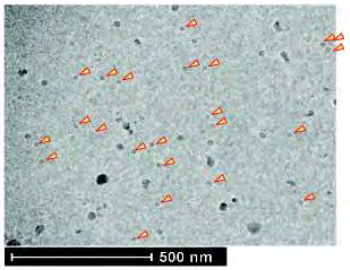

Figure 1

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

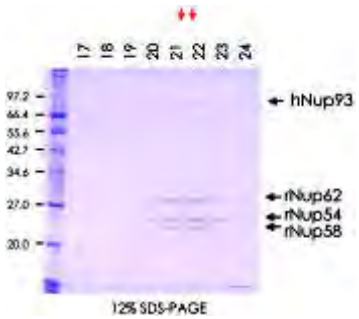

Figure 2

Fig. 2: Size exclusion chromatography profile of the Nup93●Nup62●Nup54●Nup58 complex and SDS-PAGE scan to show the composition of the * marked peak elution.

Based on immunoprecipation assay, we could reconstitute Nup93●Nup62●Nup54●Nup58 quaternary complex (See figure 2) in E. coli system. The complex is purified in large amount and its crystallization work is in progress.

Future Research Plans

- Crystallization of Nup93●Nup62●Nup54●Nup58 quaternary complex.
- Native isolation of the Nup93Nup205 and Nup62 subcomplex from stably expressed HEK cell lines followed by low resolution and high resolution cryo-electron microscopy studies.
- Characterization of various nucleoporin complexes by size exclusion chromatography coupled to multi angle light scattering.

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Regulation of aPKC activity by Nup358 dependent SUMO modification

Background

The aPKC sub-family proteins have been well known for their role in generation and maintenance of polarity by their asymmetric localization within the cell. Broadly, there are three well characterized polarity complexes, namely, the Par3-Par6-aPKC complex, Scribble-Dlg-Lgl and the Crumbs-PALS complex. aPKC as a component of the Par polarity complex is central to its functionality. Downstream to Par complex, aPKC is known to phosphorylate and regulate a range of polarity proteins, namely, Lgl, Par3, Crumbs, GSK3β, MARCKS, LGN and Lin5/NuMA. Phosphorylation can affect the target protein in various ways; for example, phosphorylation of Par3 by aPKC causes it to dissociate from the Par3-Par6-aPKC complex, while phosphorylation of Lgl prevents it from localizing to the apical membrane. Two activation modes of aPKC involving phosphorylation are known; one by phosphoinositide-dependent kinase-1 (PDK1)-mediated phosphorylation of specific threonine residue (T410 in PKCζ and T408 in PKCλ) in the activation loop (A-loop) and the other by mTORC2-directed phosphorylation of T560 in the turn motif (TM) of PKCζ. Additionally, aPKC can be activated in a spatio-temporal manner by binding with the Cdc42~GTP-Par6 complex through its PB1 domain. Interestingly, it is found that during epithelial polarization and asymmetric division of neural stem cells, aPKC could make mutually exclusive complex with Par3 and Lgl. This appears to be important for defining the membrane domains required for generation and maintenance of cell polarity. aPKC members are also involved in other signaling pathways.

Like phosphorylation, SUMOylation, another post-translational modification involving covalent conjugation of SUMO with the target protein, affects the

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behavior of modified proteins in many ways. SUMO proteins are highly conserved across different species and have been shown to be involved in diverse cellular processes. Four paralogs exist in mammals, SUMO1, SUMO2, SUMO3 and SUMO4. SUMO1 shares ~50 % homology with SUMO2/3. SUMO2 however shows ~95 % homology with SUMO3. Involvement of SUMO4 in protein conjugation is less studied. The consequence of SUMOylation could be different based on the SUMO paralog involved in the modification. Poly-SUMOylation usually occurs with SUMO2/3, but not by SUMO1. SUMO conjugation requires three sets of enzymes; E1, the SUMO-activating enzyme (consists of SAE1 and SAE2 subunits), E2, the SUMO-conjugating enzyme (UBC9) and a group of SUMO E3 ligases. SUMO E3 ligases include members of protein inhibitor of activated STAT (PIAS) family, Pc2 and the nucleoporin Nup358. Few of the proteins known to be SUMOylated in vivo by Nup358 include topoisomerase II, borealin and Ran. SUMO specific proteases (SENPs) perform maturation of immature SUMO and deconjugation of the SUMO moiety from SUMOylated substrate proteins.

We recently showed that Nup358 interacts with and acts upstream of aPKC in regulating cell polarity during neuronal differentiation. Nup358 at its C-terminal region harbors two internal repeats (IRs), which have been shown to possess SUMO E3 ligase activity. Nup358 also interacts with other components of Par polarity complex, Par3 and Par6. Preliminary results suggested that aPKC is SUMOylated. Based on this observation, we were interested in understanding the molecular details and functional relevance of the process.

Aims and Objectives

- To study and characterize SUMO modification of aPKC
- To investigate the functional significance of aPKC SUMoylation
- To examine the role of Nup358 as a possible SUMO E3 ligase for aPKC

Work Achieved

Nup358, a well characterized SUMO E3 ligase, was shown to interact with the Par polarity complex and thereby regulate polarity in differentiating neurons. To check whether any of the members of this complex is post-translationally modified by SUMO conjugation, we overexpressed GFP-tagged version of conjugable (SUMO1GG) or non-conjugable (SUMO1G) form of SUMO1 along with HA-PKCζ in HEK293T cells and monitored for the presence of a modified band in the GFP immunoprecipitates. We could detect high molecular weight bands for HA-PKCζ (Fig. 1A) in cells co-transfected with SUMO1GG, but not with SUMO1G. The results suggested that PKCζ was specifically modified with SUMO1 (Fig. 1A).

To test the endogenous SUMOylation, immunoprecipitation of aPKC was performed in HEK293T lysates using specific antibodies and analyzed the immunoprecipitates for the presence of SUMO1 or SUMO 2/3. The results clearly indicated that endogenous aPKC is modified by SUMO1 (Fig. 1B). However, no specific SUMO2/3 positive bands were detected with aPKC immunoprecipitate, suggesting that although PKCζ gets modified by SUMO2/3 in overexpressed conditions (Fig. 1B), endogenous aPKC is preferably modified by SUMO1. SUMOylation of PKCζ was also confirmed using an in vitro SUMO conjugation assay (Fig. 1C). Consistent with the ability to get SUMOylated, aPKC also interacted with the SUMO E2 enzyme Ubc9 (Fig. 1D).

Covalent attachment of SUMO to the target protein occurs at specific lysine (K) residues, mostly within the consensus sequence ψKxE/D (ψ, a bulky aliphatic residue; K, lysine; x, any amino acid; E, glutamic acid; D, aspartic acid), by formation of an isopeptide bond between the target lysine and the carboxyl-terminal glycine residue of SUMO peptide. To identify the lysine residue in PKCζ involved in SUMO conjugation, we searched for the presence of consensus

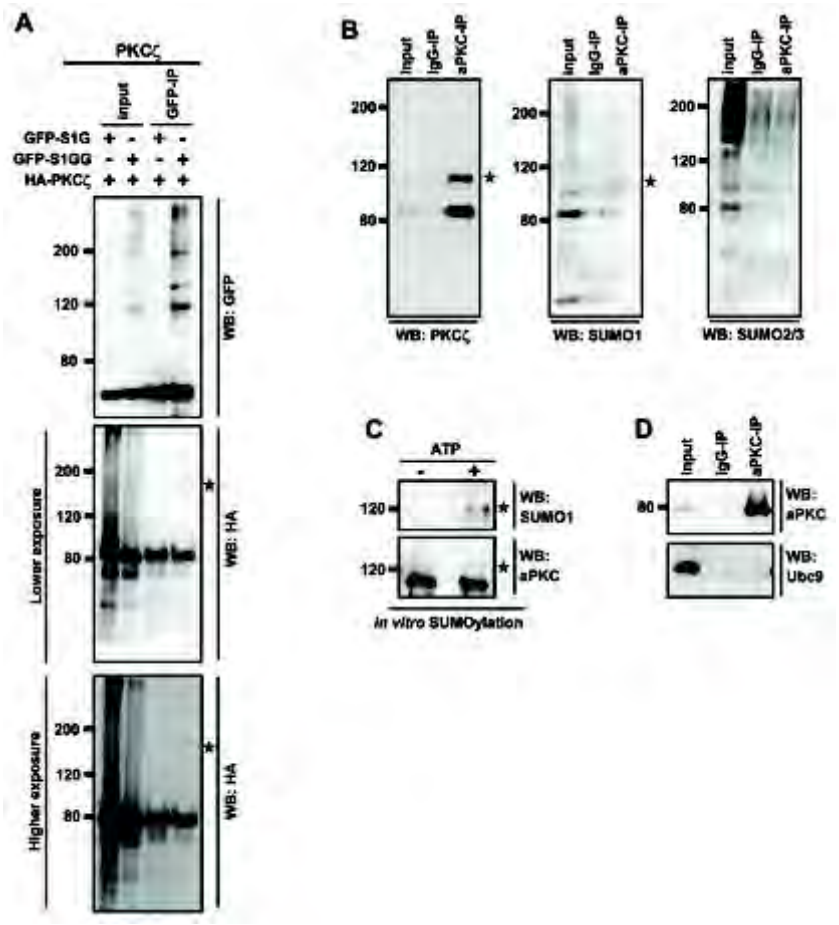

Fig. 1: SUMOylation of aPKC. (A) Lysates prepared from HEK293T cells co-expressing HA-PKCζ, with GFP-SUMO1G or GFP-SUMO1GG were subjected to immunoprecipitation (IP) using GFP-specific antibodies and the immunoprecipitates were analyzed for the presence of specific proteins by western blotting (WB) using indicated antibodies. * indicates SUMO modified bands. (B) HEK293T cells were lysed and IP was performed with control mouse IgG (IgG-IP) or mouse anti-aPKC (aPKC-IP) antibodies and the immunoprecipitates were subjected to western analysis using SUMO1 or SUMO2/3 antibodies. (C) SUMOylation of recombinant PKCζ was performed using in vitro SUMOylation kit as per manufacturer's instruction. The products were analyzed by western blotting using indicated antibodies (D) HEK293T cells were lysed and subjected to IP using mouse IgG (IgG-IP) or mouse anti-aPKC antibodies (aPKC-IP) and western analysis was performed to monitor the presence of Ubc9.

SUMOylation sites in PKC using the SUMOplot™ software (Abgent-http://www.abgent.com/sumoplot). The analysis predicted three potential K residues in PKCζ (K225, K284 and K378) with relatively higher score (Fig. 2A). All the three K residues were mutated to arginine (R) individually and in combination. Immunoprecipitation assays suggested that when all the three lysine residues were mutated to arginine, SUMOylation of PKCζ was significantly reduced. All the single and double mutants were significantly SUMOylated as compared to control [PKCζ-wild type (wt) co-transfected with GFP-SUMO1G] (Fig. 2B). As the triple mutant (K225/284/378R) showed almost complete loss of SUMOylation, we refer to this as PKCζ-SUMOylation-defective mutant (PKCζ-Smut).

Next, we wished to analyze the consequence of SUMOylation on the activity of aPKC. As a critical player in many cellular processes, aPKC exerts its effect through phosphorylation of key proteins. To test whether SUMOylation affects aPKC's kinase activity, we monitored the phosphorylation status of one of its targets, Lgl. We co-expressed FLAG-tagged Lgl1 (one of the Lgl isoforms) with HA-PKCζ-wt, HA-PKCζ K to R single mutants (K225R, K284R, K378R), double mutants (K225/284R, K284/378R, K378/225R) or triple mutant (K225/284/378R, also called PKCζ-Smut), and analyzed the extent of Lgl1 phosphorylation using a phospho (p)-Lgl specific antibody. PKCζ-wt expression significantly enhanced Lgl1 phosphorylation, as compared to control vector transfected cells. PKCζ mutants K225R, 284R, and K225/284R also showed kinase activity comparable to that of the wild-type PKCζ. However, although PKC-K378R, K284/378R, K378/225R mutants had reduced activity as compared to that of PKC-wt, these mutants were still capable of phosphorylating Lgl to a significant level as compared to HA-vector control. Importantly, the PKCζ-Smut did not show any increased activity as compared to that of HA-control (data not shown), indicating that SUMOylation enhances the aPKC activity in vivo. Furthermore, co-immunoprecipitation experiments suggested that PKCζ-Smut, as compared to PKCζ-wt, exhibited better interaction with Lgl1 (data not shown). This is consistent with the previous finding that phosphorylation of Lgl compromises with its ability to bind to aPKC. These experiments suggested that SUMOylation could increase the kinase activity of aPKC.

To further test the activity of SUMOylated aPKC, recombinant GST-PKCζ was SUMOylated using in vitro SUMO1 conjugation system in the presence and absence of ATP and was then used for in vitro kinase assays. As expected, PKCζ was modified with SUMO only in the presence of ATP and resulted in its

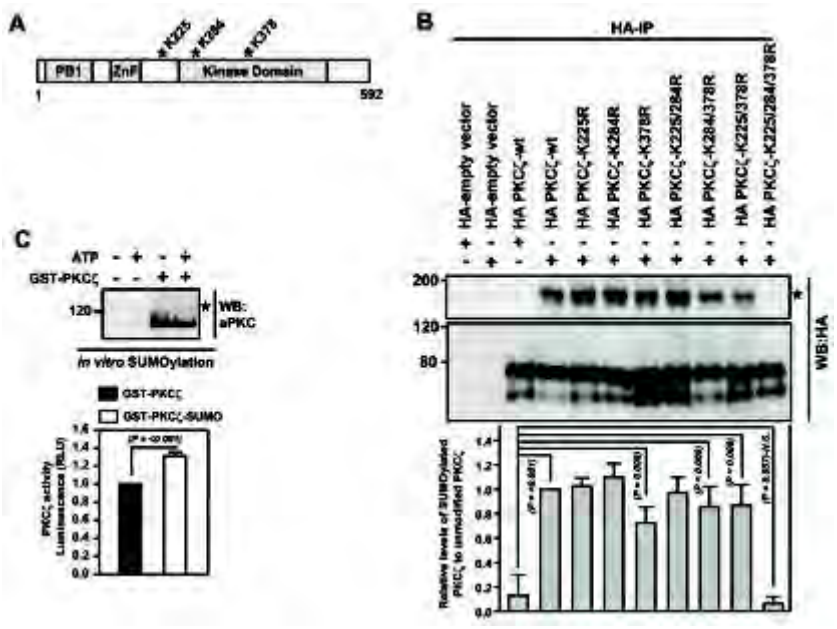

Fig. 2: Identification of SUMOylation sites in PKCζ. (A) Schematic diagram of PKCζ depicting the domain architecture: PB1 (Phox and Bem 1), ZnF (zinc-finger) and kinase domains. Potential lysine (K) residues that were predicted by SUMOplot software as SUMOylation sites are marked with asterisks. (B) HEK293T cells were transfected with GFP-SUMO1G or GFP-SUMO1GG along with HA-empty vector control, HA-PKCζ-wt, HA-PKCζ lysine (K) to arginine (R) single mutants (K225R, K284R, K378R), double mutants (K225/284R, K284/378R, K378/225R) or triple mutant (K225/284/378R). Cells were lysed 36 h post-transfection and co-IP was performed using anti-HA antibodies, followed by western analysis with indicated antibodies. The graph represents quantitative data depicting levels of SUMOylated aPKC relative to unmodified PKCζ in the corresponding immunoprecipitates. Error bars indicate standard deviations, *n = 3*, P values calculated by Student's *t* test. (C) In vitro kinase assay was performed using in vitro SUMOylated PKCζ. GST-PKCζ was subjected to in vitro SUMOylation reaction using recombinant proteins. The reactions were performed in the presence (+) or absence (-) of ATP. The reactions were subjected to immunoblotting using anti-aPKC antibodies. * indicates SUMOylated PKCζ. The PKCζ from in vitro SUMOylation reactions was further assayed for the activity using a commercial kit as per manufacturer's instruction. The graph represents quantitative data showing relative activity of PKC derived from indicated reactions, expressed as relative luciferase units (RLU). Error bars indicate standard deviations, *n = 3*, P values calculated by Student's t test.

enhanced activity (Fig. 2C). The results indicated that SUMOylation enhanced the activity of PKCζ. Only a small fraction of the total PKCζ was SUMOylated in vitro (~ 3 %), which accounted to an increase of ~ 30 % kinase activity. The in vitro studies thus indicated that SUMOylation of PKCζ increased its activity approximately by 10-fold.

SUMO E3 ligases mediate the last step in SUMO conjugation, by transferring SUMO from E2 to the substrate. Role of these ligases become extremely crucial when the target modification needs to be regulated in a spatio-temporal manner. Earlier reports showed that Nup358, a well characterized SUMO E3 ligase, interacts with aPKC. We wished to investigate if Nup358 functions as the E3 ligase for aPKC SUMOylation. For this, we analyzed the extent of aPKC SUMOylation in HEK293T cells treated with Nup358 specific short interfering RNA (siRNA). Our results suggested that the extent of aPKC modification was significantly reduced in Nup358 depleted cells as compared to control siRNA treated cells (Fig. 3A).

Further, we monitored the activity of aPKC by assessing the phosphorylation status of its substrate, Lgl1, in Nup358 deficient cells. Our results showed that there was about 80 % reduction in the p-Lgl1 levels in Nup358 knockdown cells as compared to control cells (Fig 3B). Interestingly, ectopic expression of HA-PKCζ in Nup358 knockdown cells rescued the phosphorylation of Lgl1 to levels comparable to the control siRNA treated cells. These results indicated that Nup358 is important for aPKC activation.

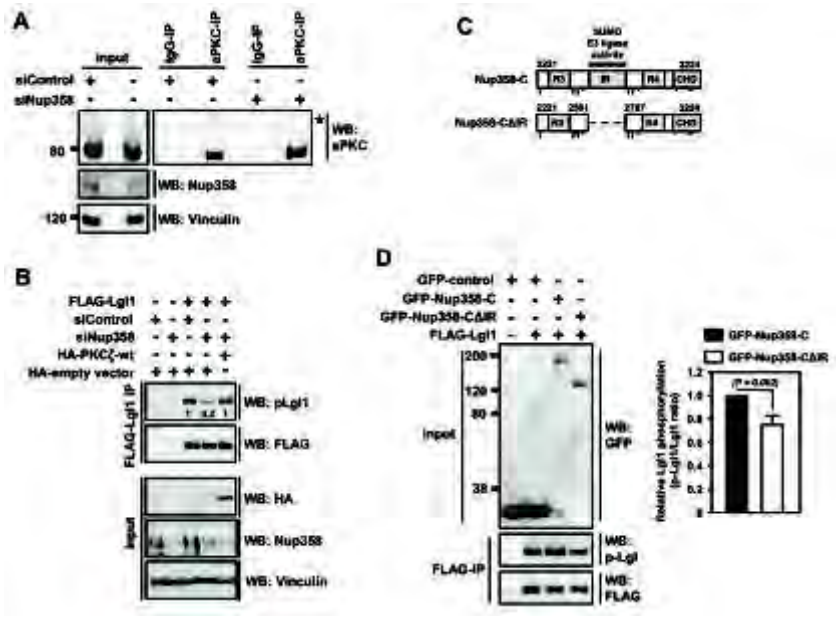

Fig. 3: Nup358 acts as E3 ligase for aPKC SUMOylation. (A) HEK293T cells were transfected with control (siControl) or Nup358 specific (siNup358) siRNA and were lysed and analyzed for the levels of indicated proteins using specific antibodies by western blotting (WB). * indicates SUMO-modified band. Extent of Nup358 depletion was monitored with Nup358 specific antibodies. Vinculin was used as loading control. (B) Cells were initially transfected with siControl or siNup358 and were retransfected with FLAG-Lgl1 with (+) or without (-) HA-PKCζ. Cell lysates were analyzed by WB using indicated antibodies. (C) Schematic depiction of C-terminal region of human Nup358 (Nup358-C) with amino acids marked in number. Nup358-C contains two RanGTP binding domians (RB3 and RB4), internal repeat (IR) that acts as SUMO E3 ligase and cyclophilin homology domain (CHD). Dashed line shows the deleted region in Nup358-C mutant (Nup358-CΔIR). (D) Cells were co-transfected with indicated constructs and lysates were immunoblotted for indicated proteins. Graph represents quantitative data depicting the relative p-Lgl1 levels compared to total Lgl1 levels. Error bars indicate standard deviations, *n = 3*, P values calculated by Student's *t* test.

We wished to investigate if the activation of aPKC required Nup358's E3 ligase function. Ectopic expression Nup358-C (Fig. 3C), a fragment of Nup358 having the E3 ligase activity, considerably increased the extent of endogenous aPKC SUMOylation in HEK293T cells (data not shown). Moreover, compared to Nup358-C, expression of an E3 liagse-defective mutant of Nup358-C (Nup358-CΔIR), activated endogenous aPKC to much lesser extent and almost comparable to GFP-control, as monitored by the phosphorylation status of Lgl (Fig 3C, D). Collectively, these results support the conclusion that Nup358 stimulates the aPKC activity by functioning as its SUMO E3 ligase.

In brief, our studies reveal a new mode of activation of aPKC through Nup358 dependent SUMOylation. Recently, it was shown that PKCα gets SUMOylated, which inhibits its activity. Additionally, SUMOylation has been shown to regulate the function of PKCθ in T cell synapse organization and activation. Although Nup358 has been shown to be an important player in cell polarity in diverse cellular contexts, the molecular mechanism by which it functions in this process is still unclear. Further investigations will look into the contribution of Nup358 in cell polarity by functioning as a SUMO E3 ligase for aPKC.

Future Research Plans

- Understand the functional relevance of Nup358 mediated aPKC SUMOylation in cell polarity.

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AKT signaling prevailing in mesenchymal stromal cells modulates the functionality of hematopoietic stem cells via intercellular communication

Background

Cells comprising the micro-environment of hematopoietic stem cells (HSCs) modulate their fate via cell-cell or cell-ECM interactions, and also thorough secretion of various cytokines. AKT is one of the important signaling pathways that integrate numerous upstream signals from cytokine receptors to drive cell proliferation, growth and survival. Conversely, a constitutive activation of AKT in the HSCs depletes their pool, and also becomes a cause of their neoplastic transformation. Long-term engraftment defects have been observed in HSCs from AKT1/AKT2 double knockout mice. Phosphatidylinositol-3 kinase (PI3K) /AKT signaling negatively regulates HSC quiescence.

Although such cell-autonomous effects of AKT signaling have been studied widely, very few reports are available about the non-cell-autonomous influence of AKT signaling in the stromal cells on HSC fate. Activation of AKT signaling in endothelial cells has been shown to support self-renewal and expansion of HSCs via secretion of angiogenic factors; however, the effect of activated AKT signaling in the mesenchymal stromal cells (MSCs) has not been studied. Since MSCs form a special niche for the HSCs, the signaling mechanisms prevailing in them could be expected to have direct implications in the HSC fate.
In the present study, we examined the effect of modulation of AKT1 in the MSCs on the fate of the HSCs co-cultured with them.

Aims and Objectives

- To study the effect of AKT signaling prevailing in the mesenchymal stromal cells on the functionality of HSCs
- To elucidate the mechanism involved in the process

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Work Achieved

Mesenchymal stromal cells modified with a constitutively active AKT1 expand phenotypically defined HSCs, but affect their fucntionality.

To examine the effect of a constitutively active AKT1 in the stromal cells on the HSCs interacting with them, HSCs were co-cultured with M210B4 cells (M2) and M2 cells stably expressing a constitutively active AKT1 (M2-AKT) for 10 days and the ouput cells were analysed on a flow cytometer. We found that the total number of hematopoietic cells (Fig. 1A) and absolute numbers of Lin-Sca-1$^+$c-Kit$^+$ (LSK), LSK-CD34$^-$ HSCs (Long-term HSCs, LT-HSCs) as well as LSK$^-$CD34$^+$ HSCs (Short-term HSCs, ST-HSCs) (Fig. 1B) were significantly higher in the M2-AKT co-cultures, as compared to the M2 co-cultures. These data show that

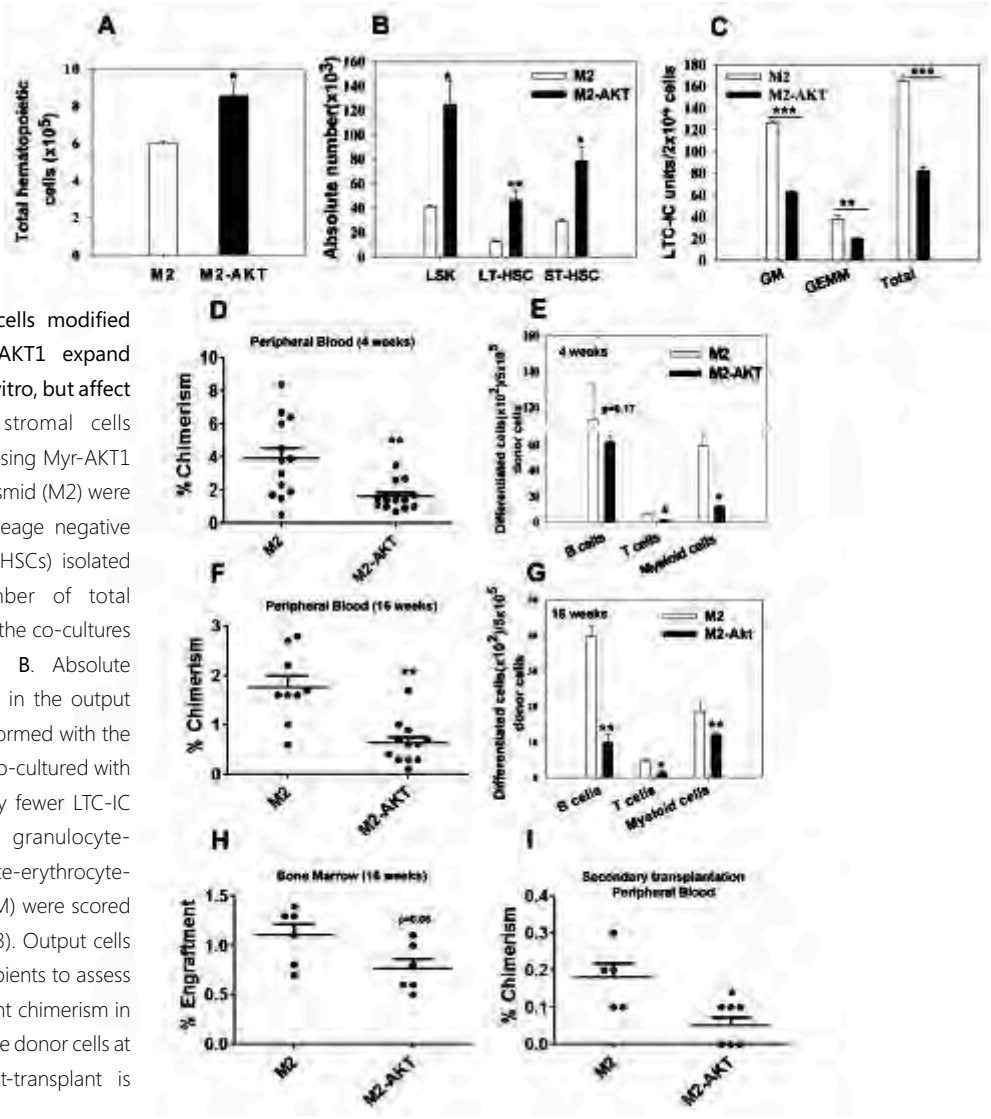

Fig. 1: **Mesenchymal stromal cells modified with constitutively activated AKT1 expand phenotypically defined HSCs in vitro, but affect their functionality.** M210B4 stromal cells transfected with a plasmid expressing Myr-AKT1 (M2-AKT) or with a backbone plasmid (M2) were co-cultured for 10 days with lineage negative fraction (HSC-enriched fraction; HSCs) isolated from bone marrow. **A.** Number of total hematopoietic cells generated in the co-cultures is graphically illustrated (n=3). **B.** Absolute numbers of various HSC subsets in the output populations. **C.** LTC-IC assay performed with the output cells shows that the cells co-cultured with M2-AKT cells contain significantly fewer LTC-IC units. Colonies belonging to granulocyte-monocyte (GM) and granulocyte-erythrocyte-monocyte-megakaryocyte (GEMM) were scored using morphological criteria. (n=3). Output cells were infused into irradiated receipients to assess their engraftment capacity. Percent chimerism in receipients' peripheral blood by the donor cells at 4 **(D)** and 16 weeks **(F)** post-transplant is

lillustrated. 10-12 mice were kept in each group. Number of donor-derived B, T and myeloid cells in the peripheral blood of the recipients at 4 **(E)** and 16 weeks **(G)** post-transplant are graphically represented. **H.** Percent engraftment of donor cells in the bone marrow of the recipients at 16 weeks post-transplant. **I.** Percent chimerism established by the HSCs sorted from the bone marrow of primary recipients in the peripheral blood of the secondary recipients at 4 weeks post-secondary transplant. 5-6 mice were kept in each group. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

constitutive activation of AKT1 in the stromal cells expands phenotypically defined HSCs.

The output cells were subjected to in vitro long-term culture initiating cells (LTC-IC) assay and in vivo transplantation assay to assess their functionality. The output cells from M2-AKT co-cultures contained significantly fewer LTC-IC units (Fig.1C), showing that the expanded cells had compromised functionality.

The output cells (CD45.1) were infused into irradiated recipients (CD45.2), and the engraftment levels were monitored by analyzing peripheral blood (PB) after 4 and 16 weeks post-transplant. The output cells from M2-AKT co-cultures showed significantly reduced chimerism in the PB of the recipients at both time points (Fig.1D and 1F). The significantly fewer T, B and myeloid cells observed in the PB of the recipients transplanted with cells from M2-AKT co-cultures (Fig.1E and 1G) showed that these cells could not form blood cells as efficiently as their control counterparts. Surprisingly, the BM analysis done at 16 weeks post-transplant revealed that though the level of engraftment in M2-AKT set showed a clear downward trend, the difference was statistically non-significant (Fig. 1H). When the engrafted cells from the BM of the primary recipients were sorted and infused in equal numbers into irradiated secondary recipients, the output cells from M2-AKT co-cultures failed to engraft in the secondary hosts (Fig.1I). These data showed that the HSCs co-cultured with M2-AKT stromal cells were dysfunctional.

Silencing of AKT1 in the stromal cells restricts HSC expansion, but boosts their functionality

The detrimental effect of constitutive activation of AKT1 in the stromal cells on functionality of co-cultured HSCs led us to hypothesize that silencing of AKT1 may have the reverse effect. To test this, output cells from HSCs co-cultured with stromal cells stably expressing AKT1-specific shRNA (M2-shAKT) or scrambled sequences (M2) were compared with those co-cultured with M2-AKT cells. The output of hematopoietic cells was significantly reduced in M2-shAKT co-cultures, as compared to M2 and M2-AKT co-cultures (Fig.2A). M2-shAKT co-cultures produced significantly lower absolute numbers of LSK and LT-HSCs (Fig 2B); suggesting that silencing of AKT1 in the stromal cells restricts the HSC pool.

LTC-IC assays were then performed with the output cells. Contrary to the phenotypic data, the cells grown on M2-shAKT showed a significantly higher

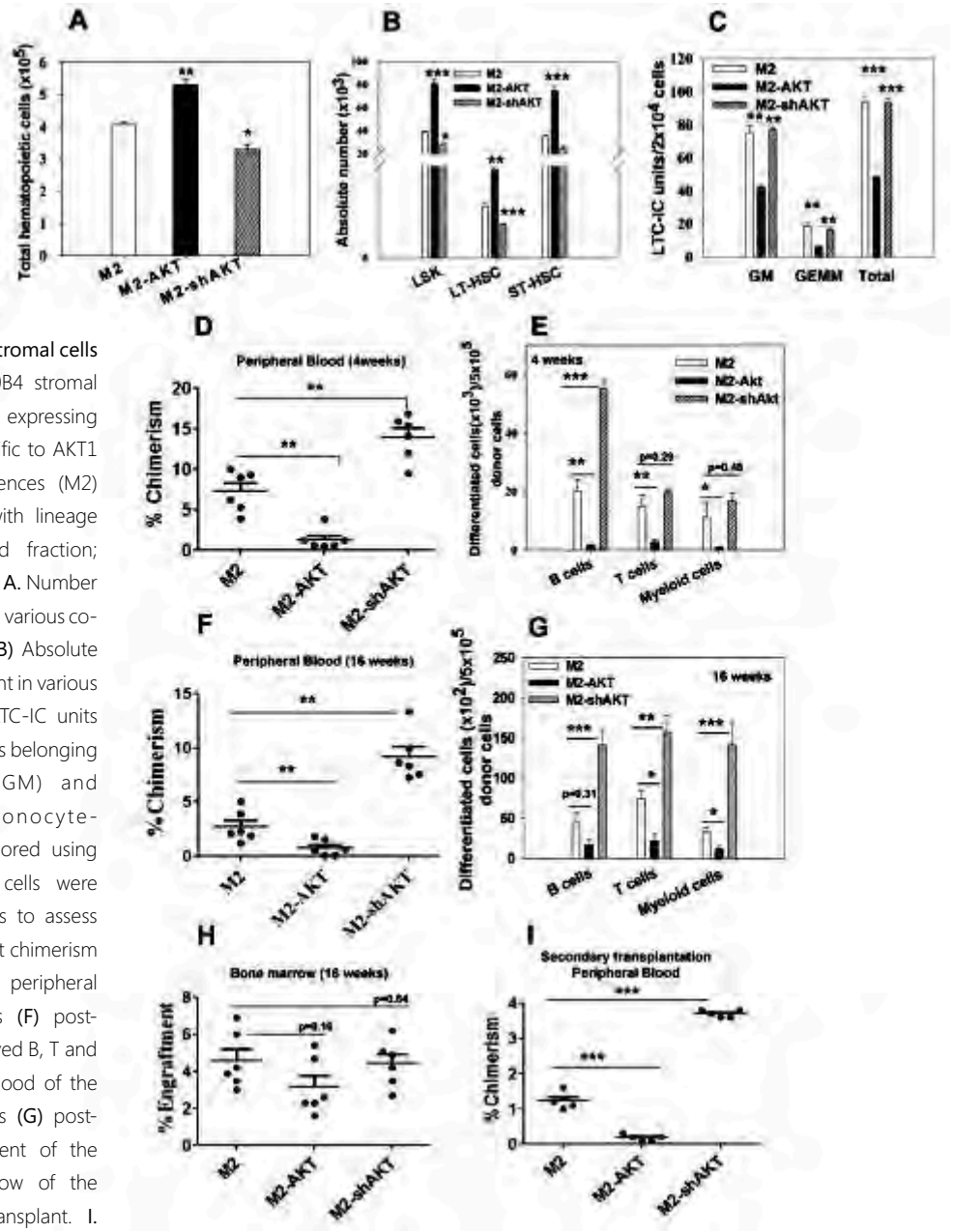

Fig. 2. : Silencing of AKT1 in the stromal cells boosts HSC functionality: M210B4 stromal cells transfected with a plasmid expressing Myr-AKT1 (M2-AKT), shRNA specific to AKT1 (M2-shAKT) or scrambled sequences (M2) were co-cultured for 10 days with lineage negative fraction (HSC-enriched fraction; HSCs) isolated from bone marrow. A. Number of hematopoietic cells generated in various co-cultures is graphically depicted. (B) Absolute numbers of the HSC subsets present in various co-cultures. C. Enumeration of LTC-IC units present in the output cells. Colonies belonging to granulocyte-monocyte (GM) and granulocyte-erythrocyte-monocyte-megakaryocyte (GEMM) were scored using morphological criteria. Output cells were infused into irradiated receipients to assess their engraftment capacity. Percent chimerism of donor cells in the recipients' peripheral blood at 4 (D) and 16 weeks (F) post-transplant. Number of donor-derived B, T and myeloid cells in the peripheral blood of the recipients at 4 (E) and 16 weeks (G) post-transplant. H. Percent engraftment of the donor cells in the bone marrow of the recipients at 16 weeks post-transplant. I. Percent chimerism established by the engrafted HSCs sorted from the bone marrow of primary recipients in the peripheral blood of the secondary recipients at 4 weeks post-secondary transplant. 5-6 mice were kept per set. Data represent two independent experiments. *p≤0.05, **p≤0.01, ***p≤ 0.001

number of LTC-IC units, as compared to those cultured on M2-AKT cells (Fig.2C). The numbers of LTC-IC units in the output cells from M2-shAKT and M2 co-cultures were comparable, indicating that silencing of AKT1 in the stromal cells did not affect LTC-IC output, while its activation had a detrimental effect.

The co-cultured cells were subjected to in vivo transplantation assays. Flow cytometry analyses of PB of the recipients done at 4 and 16 weeks post-

transplant showed that the output cells from M2-shAKT co-cultures established a significantly higher level of chimerism in the PB of the recipients, as compared to those from M2-AKT and control M2 co-cultures (Fig.2D and 2F). The absolute numbers of T, B and myeloid cells were also significantly higher in the PB of mice transplanted with cells from M2-shAKT co-cultures than in the mice that received cells from M2-AKTco-cultures (Fig.2E and 2G). These data revealed that the HSCs from M2-shAKT co-cultures were efficient in forming blood cells, whereas those from M2-AKT co-cultures showed a compromised ability to do so.

The differences in the numbers of HSCs in the BM of mice in all three sets at 16 weeks post-transplant were not statistically different (Fig.2H). But when the engrafted cells from primary recipients' marrow were sorted and infused into secondary recipients in equal numbers, the cells from M2-shAKT set engrafted with higher efficiency, as compared to the control set, whereas those from the M2-AKT set failed to engraft in the secondary recipients (Fig.2I).

Collectively, these data demonstrate that silencing of AKT1 in the stromal cells restricts the expansion of HSCs, but boosts their functionality; in sharp contrast, activation of AKT1 in the stromal cells expands HSCs, but affects their functionality.

Stromal AKT1 down-regulates Cxcr4 expression in the HSCs

Since SDF1α-CXCR4 axis plays a crucial role in the functionality of HSCs, we examined the expression of Cxcr4-specific mRNA in the sorted LSK HSCs from the co-cultures.The expression of Cxcr4 was drastically reduced in HSCs co-cultured with M2-AKT and was significantly up-regulated in HSCs co-cultured with M2-shAKT (Fig. 3A), suggesting that down-regulation of Cxcr4 could be one of mechanisms involved in the M2-AKT-mediated dysfunction of HSCs.

Differential intercellular trafficking pathways adopted by stromal cells expressing activated AKT vis-à-vis shAKT.

Since activation or silencing of AKT was done in the stromal cells, but the effect was seen in the HSCs co-cultured with them, we speculated that perhpas some intercellular communication mechanisms are operative in these stromal cell-mediated effects on the HSCs. To this end, we added GAP27, which is one of the peptide inhibitors of GAP junctions, in the co-cultures and examined whether it affects Cxcr4 gene expression in the HSCs co-cultured with M2-AKT and M2-

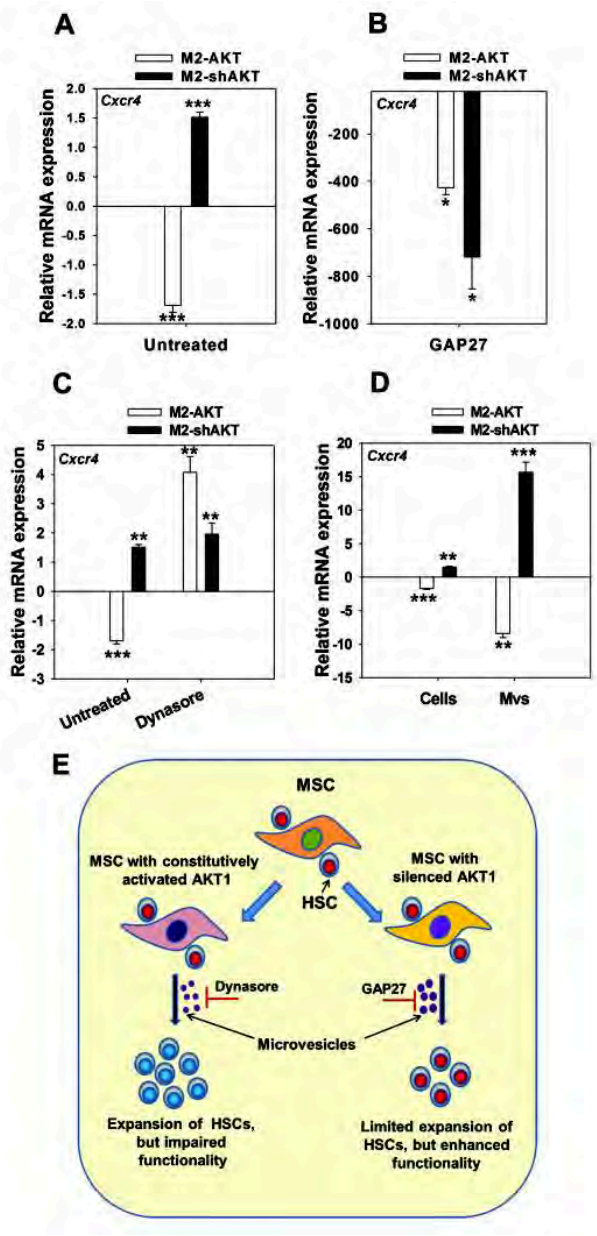

Fig. 3: Differential intercellular trafficking pathways adopted by stromal cells expressing activated AKT vis-à-vis shAKT. **A.** Sort-purified LSK-HSCs co-cultured with stromal cells modified with constitutively active AKT1 (M2-AKT) show a significant down-regulation of *Cxcr4* mRNA, as compared to those co-cultured with stromal cells having silenced AKT1 (M2-shAKT) as assessed by q-RT-PCR. Beta actin was used as an internal control and expression in the LSK HSCs co-cultured with control stromal cells (M2) was used as calibrator. (n=3). Effect of addition of GAP27 **(B)** or Dynasore **(C)** on *Cxcr4* mRNA expression in the LSK-HSCs co-cultured for 48 hours with stromal cells having a constitutively activated AKT (M2-AKT) or silenced AKT (M2-shAKT). **D.** Effect of stromal cells and microvesicles (Mvs) isolated from their conditioned media on Cxcr4 expression in LSK-HSCs. Data were normalized with beta actin and expression in the HSCs co-cultured with M2 cells was used as a calibrator. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. **E. Graphical summary.** AKT1 signaling prevailing in the stromal cells regulates HSC functionality via intercellular communication pathways. Stromal cells with a constitutively active AKT1 secrete micro-vesicles (Mvs) which are taken up by the HSCs via dynamin-dependent endocytosis (sensitive to Dynasore); whereas the stromal cells with silenced AKT1 produce Mvs that are transferred through GAP junctions (sensitive to GAP27).

shAKT. Surprisingly, we found that incorporation of GAP27 in the co-culture further aggravated the M2-AKT-mediated loss of Cxcr4 expression in the co-cultured HSCs (Fig.3B, white bar), suggesting that GAP27 perhaps blocked transfer of a positive regulator of Cxcr4 from M2-AKT to the HSCs. However, GAP 27 strikingly reversed the salutary effect of M2-shAKT on Cxcr4 expression in the co-cultured HSCs (Fig.3B, black bar); indicating that GAP junction-mediated communication plays an important role in the salutary effects of M2-shAKT cells.

Endocytosis is yet another important pathway involved in intercellular communication. When we added Dynasore, a noncompetitive cell-permeable inhibitor of dynamin that blocks dynamin-dependent endocytosis in cells, in the co-cultures, we found that inhibition of endocytosis significantly rescued the down-regulation of *Cxcr4* in the HSCs co-cultured with M2-AKT (Fig.3C, white bars), suggesting that intercellular transfer mechanism between stromal cells with activated AKT and HSCs involves dynamin-dependent endocytosis. In contrast, addition of Dynasore in the co-cultures established with M2-shAKT did not affect its salutary effect on *Cxcr4* (Fig. 3C, black bars), showing that perhaps dynamin-dependent endocytosis does not play a major role in the intercellular interaction between stromal cells lacking AKT1.

Consistent with the data obtained with Dynasore, the micro-vesicles (Mvs) secreted by M2-AKT strongly affected the *Cxcr4* expression in the HSCs and this effect was significantly higher than that of the cells themselves (Fig.3D; right white bar vs. left white bar). Interestingly, though the effect of M2-shAKT cells on *Cxcr4* was not sensitive to Dynasore, the Mvs secreted by them exerted a strong salutary effect on the *Cxcr4* expression in the HSCs, which was significantly higher than the cells themselves (Fig.3D; right black bar vs. left black bar), suggesting that perhaps the Mvs of M2-shAKT cells are taken up by the HSCs via dynamin-independent pathway.

In summary, we have demonstrated that activation of AKT1 signalling in mesenchymal stromal cells affects the HSC functionality via endocytosis-mediated mechanisms; whereas silencing of AKT1 in them controls the expansion of HSCs, but boosts their functionality via GAP junction-mediated intercellular communications (Fig.3E).

This is perhaps the first report demonstrating that AKT1 signaling prevailing in the MSCs regulates HSC functionality through various intercellular communication mechanisms. These findings could have important implications in the use of MSCs in regenerative medicine.

Future Research Plans

- To identify the molecular composition of the microvesicles secreted by M2-AKT and M2-shAKT stromal cells
- To examine whether the stromal cells of patients suffering from bone marrow failure have a constitutively active AKT

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Protection from adverse lung and cerebral pathology induced by *Plasmodium berghei* ANKA: A possible role for malaria specific Immunoglobulin A

Background

Plasmodium falciparum, which causes fatal and life-threatening complications like cerebral and placental malaria, utilizes several redundant ligands to invade the erythrocyte membrane to evade immune surveillance. Among the ligands, EBA-175, the ligand used for invasion by merozoites, comprises of several regions viz. regions I-VI. It is also secreted by microneme onto the surface of merozoites and is shed at or around the point of invasion. While the regions I and II have the Duffy Binding Domains, regions III-V have variable functions and the C-terminal Cysteine-rich region VI is followed by a transmembrane domain (TMD) with a cytoplasmic tail as shown in Fig. 1A. It is reported that region VI of EBA-175 of *P. falciparum* (PfrVI) participates in the formation of a tight junction complex with the host membrane, dimerization of the EBA-175 and also in trafficking to microneme. Moreover, the PfrVI has sequences that are conserved across the DBL family proteins. For example, it contains eight highly conserved Cys residues (Fig. 1B, marked in bold) and a ROM4 cleavage site needed for shedding of EBA-175 form the junctional complex for completion of the invasion cycle. Shedding of this junctional complex occurs irrespective of whether EBA-175 is used as a primary ligand or not. However, the nature of immune response elicited by this domain and its in vivo role has not been investigated in detail.

Antibody response against region II, EBA-peptide 44 (42 aa within the region V) can block the binding of native EBA-175 to human erythrocytes and also inhibits merozoite invasion *in vitro*. It appears that antibodies against EBLs and RBLs are elicited in a kinetic manner i.e. initially to EBA175, EBA181, EBA140 (involved in sialic acid-dependent pathway), and later to PfRh2 and PfRh4

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(involved in sialic acid-independent pathway). The success of passive immunization also highlights the role of Immunoglobulin (Ig)- based therapies since anti-EBA-175 IgG has been able to block the invasion by ~90%. Further, animal models based on passive immunization with mono specific antibodies (raised against synthetic peptides) or adoptive transfer of B-cells (specific to malarial antigens) have also highlighted the role of adaptive immune response against malaria infection. In addition, susceptibility of B-cell-deficient hosts has, once again, underlined the role of antibodies in fighting infection.

Hence, it is curious whether or not antibody response, exclusively directed towards the membrane proximal region of PfrVI, can have any role during the erythrocyte invasion step. It appears that the rVI of EBA-175 can play an important role during the erythrocyte invasion step. In this regard, we have observed that the rVI- derived peptide sequences, i.e. Mpep3 and Mpep4, elicit predominant IgA isotype in Balb/C background. In view of these observations, we have attempted to understand the utility of this IgA in the context of experimental animal infection. Since, the Mpep4 sequence has good homology with the rVI domain of *Plasmodium berghei* ANKA (PbA), we have attempted to understand the role of IgA, if any, during the onset of infection. Our results show that the IgA can substantially improve the cerebral pathology of C57BL/6 mice. This can pave the way for understanding the role of IgA in malarial infections.

Aims and Objectives

- To investigate the nature and utility of the immune responses generated by region VI of EBA-175.

Work Achieved

IgA response is centered on the N-terminal side of the Mpep3 and Mpep4.

The antisera of C57BL/6 mice have exhibited IgA specific to Mpep3 (Fig. 1C), Mpep4 (Fig. 1D) and PfrVI (Fig. 1E). It should be noted that the recombinant PfrVI is useful for verification of the immune response since it contains both Mpep3 and Mpep4 sequences and hence, acts as independent antigen. We have also ascertained that the goat anti-mouse IgA-HRP, used in our studies did not bind to mouse-IgG. The panels Fig.1C-1E indicate that both Mpep3, Mpep4 elicit IgA but not IgG response in about 30 independent immunizations performed over 3 years with different antigen preparations. However, the PfrVI elicits IgG in addition to the IgA (Fig.1E). Although, not dramatic, the IgA response in Balb/C has been found to be always stronger than that of C57BL/6 mice. Moreover, there is also no cross-reactivity by the observed IgA i.e. the IgA

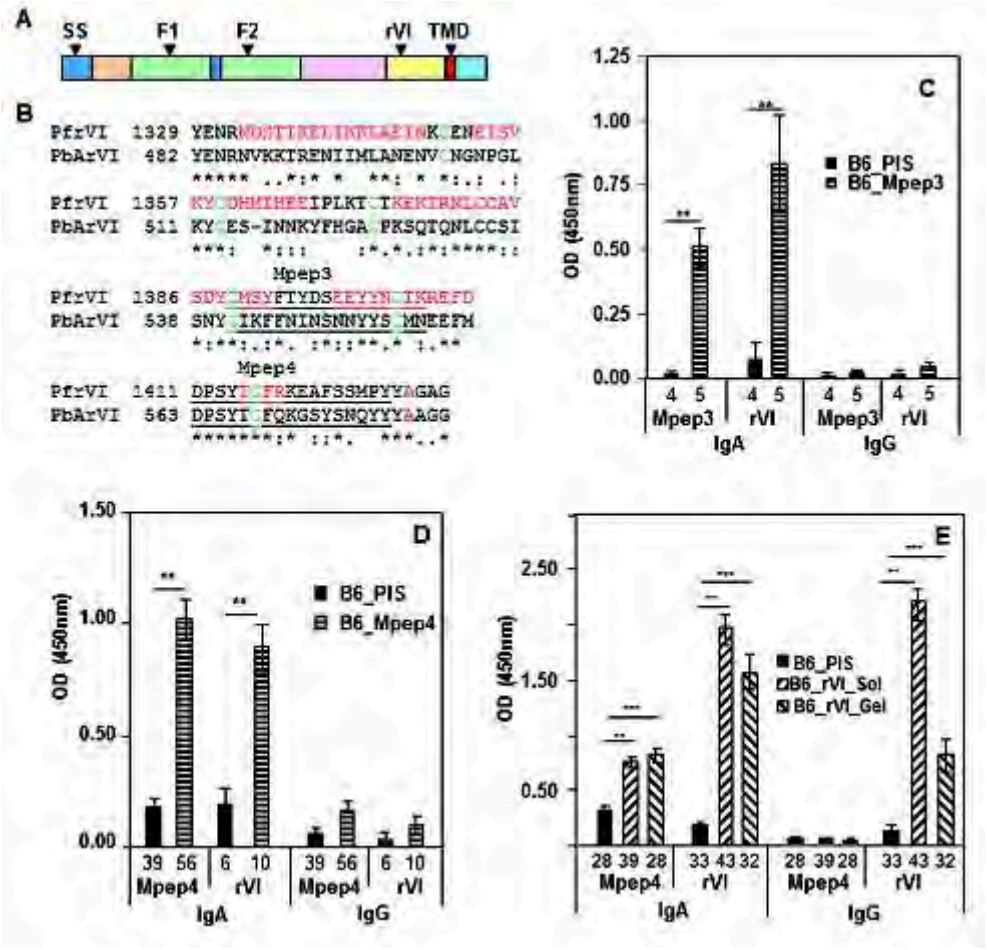


Fig. 1: Immune responses of Mpep3, Mpep4 and PfrVI in mice and human endemic population: A: Diagrammatic presentation of domains of EBA-175 of *Plasmodium falciparum*. The markings SS, F1, F2, rVI, TMD respectively represent signal sequence, duffy binding domains 1 and 2, region VI and transmembrane domains. **(B) Homology between PfrVI and PbArVI:** The PfrVI and PbArVI have 44% homology between them and the PfrVI contains a cleavage site (shaded region) of PfROM4 protease. The peptides sequences, Mpep3 (17mer) and Mpep4 (18mer), used in the study are underlined. **(C-E) Immunoglobulin profile elicited by the antigens Mpep3 (C), Mpep4 (D) and PfrVI(E) in C57BL/6 mice:** The immunoglobulin isotype was determined by ELISA. The antigen coated on the plate is shown below the group of bars and the isotype detected is shown below for each group. A sample with an OD value which were at least two standard deviations (SD) higher from that of specific to Mpep3 does not cross react with Mpep4 and vice versa. Panel E also illustrates that the IgA response elicited by PfrVI (both in-solution and in-gel form of antigens) is certainly centred on the Mpep4 sequence since Mpep4-coated wells showed stronger IgA than IgG. Similar IgA response against Mpep3 is also seen upon PfrVI immunization. Thus, both Mpep3 and Mpep4 elicited only IgA response, while PfrVI elicited both IgA and IgG upon immunization.

Mpep3 and Mpep4 antisera inhibit erythrocyte invasion *in vitro*:

We next examined whether or not the observed IgA can interfere in *in vitro* invasion of *Plasmodium falciparum* 3D7 (Pf) merozoites. The *in vitro* invasion assay, in the presence of mice serum, was done as per established procedures. As seen in Fig. 2A, even with 50 fold diluted peptide anti-sera or anti-PfrVI anti-sera, about 50% inhibition of invasion was observed. We consistently observed best inhibition with anti-Mpep4 anti-serum in all our *in vitro* inhibition assays. This suggests that the IgA, specific to PfrVI and Mpep4 can inhibit *in vitro* invasion of Pf merozoites.

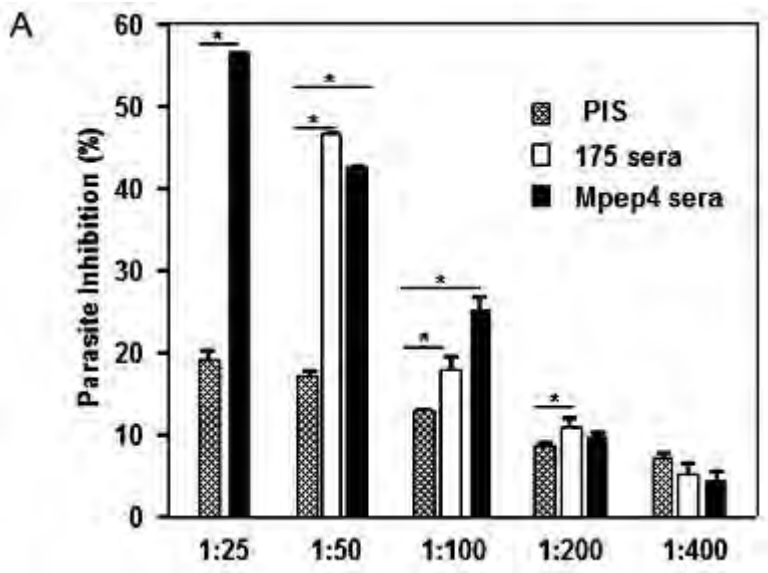
the negative controls (described in methods sections) were considered positive. **(C)** Represents the average data of one of the five independent Mpep3 immunizations of C57BL/6 mice. **(D)** Represents the average data of seven independent immunizations of Mpep4 of C57BL/6 mice. **(E)** Represents the average data of five independent PfrVI immunizations of C57BL/6 mice. The 'sol' and 'gel' form respectively represent the antigen, PfrVI, in solution (purified through Ni-NTA column) and isolated from SDS-PAGE gel specific to the PfrVI band respectively. The number of mice used for obtaining the average for each bar is given below.

Fig. 2: In vitro invasion Assay: Inhibition of Erythrocyte Invasion by immune sera: Inhibition of erythrocyte invasion at different dilutions of the indicated immune sera. The data shown are an average of 3 independent experiments.

Mpep4 specific IgA improves survival and overall cerebral pathology:

We next examined the role of IgA in mice during infection. The homology between PfrVI and PbArVI (Fig.1A) gave us the opportunity to probe the protective role, if any, of the IgA. Two parasite strains were used for this study viz. PbA-1, MRA-671 (MR4, USA). The characteristics of PbA-1 are similar to MRA-311, which usually exhibited average parasitemia (30-50%). The average life span of infected mice was 8-13 days post infection (dpi) and the parasitemia and cerebral pathology were similar to several published reports. On the other hand, the MRA-671 always exhibited typical parasitemia in the range 12-18% and an average life span of 8-13 days dpi of control mice.

Immunization of mice with three different doses 5µg, 10µg and 20µg of Mpep4 elicited strong IgA immune response and the titres of the individual subjects were similar among the group of mice studied. In mice immunized with 5µg of Mpep4, the progression of PbA-1 was slower initially but reached ~ 40% in 11days dpi in comparison to the control mice for which the parasitemia reached over 55% by day 8. Similarly, for the MRA-671 strain, in mice immunized with 5µg of Mpep4, the progression of parasitemia was slower and reached parasitemia upto 60 % and survived at least 15 days longer, whereas the unimmunized control mice died with 12-15 % parasitemia. For all the mice that survived, the brain sections were examined by histopathology. We specifically looked for sequestration of infected RBC (iRBC) in the brain tissue, perivascular hemorrhage and damage to endothelial lining in the H&E stained sections of the brains. Histopathology of brain sections of mice immunized with Mpep4 (for various immunization doses) for both strains (PbA-1 and MRA-671) clearly showed several fold lesser sequestration of iRBC, little or no damage to microvasculature and no sign of perivascular hemorrhage (Fig. 3, panels B1, B2



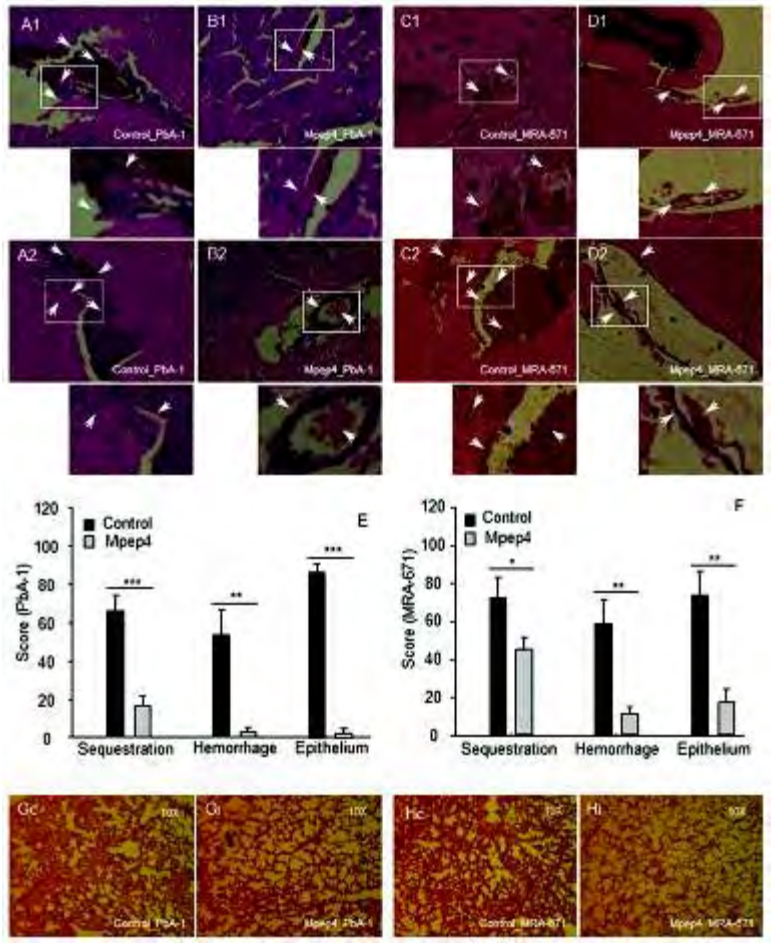


Fig. 3: IgA improves cerebral pathology of mice challenged with PbA: (A-H): Histology of brain sections of the infected Mpep4 immunized C57 BL/6 mice: The controls and immunized mice were sacrificed on the last day of the curve (Fig. 3A and 3C) for quantitative histopathological examination of the brain sections as described in methods. The panels A1, A2 (PbA-1) and C1 and C2 (MRA-671) are representative of brain sections of unimmunized, infected controls and the B1, B2 (PbA-1) D1, D2 (MRA-671) were of Mpep4 immunized mice. The panels E (PbA-1) and F(MRA-671) represent the quantitation of sequestration, hemorrhages and damage to epithelial lining observed in various sections of controls (black bars) and Mpep4 immunized mice (grey bars). The Mpep4 immunized mice consistently showed far less sequestration and no damage to the microvasculature or sign of perivascular hemorrhages as compare to that of unimmunized mice while the control mice showed higher iRBC sequestration, damaged epithelial lining of microvasculature and perivascular hemorrhages despite of similar peripheral parasitemia scored. The panels shown were a representative of three repetitions. The panels labeled with Gc, Hc and Gi and Hi are representative lung sections of mice infected with PbA-1 and MRA-671 infected mice. This data shown is a representative of three independent experiments which showed similar results.

and D1 and D2) as compared to the brain sections of unimmunized C57 mice, which showed higher sequestration of iRBC, damaged epithelial lining of microvasculature and frequent perivascular hemorrhages (Fig. 3, panels A1, A2, C1, and C2). In >75% tissue sections examined, we noted that the small blood vessels had remained intact and the damage to endothelial lining was observed only in the case of large blood vessels as well as hemorrhages (Fig. 3E and 3F). A similar, markedly improved pathology was also seen in lungs of PbA-infected mice upon immunization. The panels, Fig. 3Gc and Fig.3Hc, show extreme congestion of the alveolar space, while the immunized mice show a normal appearance (Fig. 3Gi and 3Hi) despite the high parasitemia. These results suggest that the IgA can offer protection against parasite-mediated sequestration and damage of endothelial lining. Overall, the immunized mice did not exhibit any symptoms of experimental cerebral malaria described in the literature.

Our study, for the first time, demonstrates the utility of anti-malarial IgA. Based on the literature, it is clear that malaria induces high levels of IgG and the

resultant response, in principle, can compete with the FcR occupancy. In this regard, the IgA described here can have an obvious advantage. In addition to this, antibody-based therapies involving both IgG_1 and IgG_3 subtype may be disadvantageous since they can bind FcγRs present on cells (platelets, B-cells, endothelial cells and placental tissue) that do not eliminate *P. falciparum* and may lead to triggering of inhibitory FcγRIIb, as observed among endemic patients. It is apparent form the literature that the immunoglobulin subtypes IgM, IgG and IgE were all implicated in various pathologies i.e. the pathology associated with rosette formation, placental malaria and severe malaria. Interestingly, the IgA isotype has not been shown to be responsible for any undesired pathology in malarial infection. This possibility opens-up the debate on IgA- based anti-malarial therapy. In the study involving western Kenyan children samples, it was observed that the IgA- associated immune complex has a protective effect against cerebral pathology, unlike IgG1, IgG3 and IgE-associated immune complexes. In another study, the retinoic acid, a major oxidative metabolite of Vitamin A, induces IgA isotype switching mainly through RARα in human B cells and Vitamin A deficiency increases the mortality in African children, with malaria being an all-important cause of death of children of this age. This is because human FcαR is constitutively expressed on monocytes, neutrophils, eosinophils, Kupffer cells and dendritic cells, which can capture and process the IgA- opsonized parasite. It is relevant to note that the receptor for IgA in mice (human CD89 equivalent) has not been confirmed. Moreover, IgA is the second most abundant antibody (~ 1-4% of total serum protein) in humans and mice. In addition the malaria, associated IgA, i.e. positive serum of endemic patients, also appears to inhibit the in vitro invasion of *P. falciparum* merozoites. Notably, the IgA detected among the endemic Indian population was significant only among individuals who had multiple clinical attacks of *Plasmodium* infection. It was also reported that the IgA titers were found to increase with age among the seropositive individuals.

The summarize, our study will serve as an important starting point for the development of IgA- based therapy against malaria infection. Monoclonal antibodies against the Mpep4 sequence may serve as a potent antibody-mediated therapy to provide an alternative approach to combat malaria successfully.

Future Research Plans

- Determination of how IgA specific memory is maintained in the system & the characteristics of the cells will be the course of future investigations.

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Molecular mechanisms for regulation of ionotropic glutamate receptors by their auxiliary subunits

Background

Ionotropic glutamate receptors (iGluRs) form the cornerstones of fast excitatory neurotransmission in the mammalian central nervous system. They are essential for many basic nervous system functions, including learning and development, and are involved in a remarkable range of neuronal diseases. Despite their physiological importance, our understanding of these receptors is hampered by a lack of insight into their complex structures and working mechanisms. iGluRs are regulated by a family of transmembrane auxiliary proteins that exist as macromolecular complexes at synapse. Till date several of these transmembrane auxiliary subunits have been discovered that not only regulate the gating properties of iGluRs but also their distribution and trafficking. However, till-date the molecular mechanisms and structural insights into these processes is unknown due to lack of atomic structures of auxiliary subunits or their complexes with cognate iGluRs. We have proposed to undertake the structure-function analysis of iGluR auxiliary subunits and their complexes.

Aims and Objectives

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

Work Achieved

Our goals for the first year included cloning of all the known iGluR-auxiliary proteins, construct screening and optimization for their expression. We have

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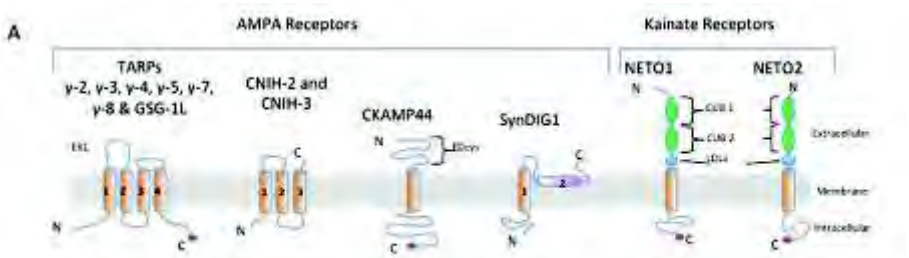

B

i) Auxiliary subunit	Expression detection via Western analysis	ii) Soluble interacting proteins	Expression detection via Western analysis	iii) iGluR receptor clones	Expression detection via Western analysis
TARP γ-2		Neuritin		GluA1	
TARP γ-5		Noelin1	Yes	GluA2	Yes
TARP γ-8		Brorin	Yes	GluA3	Yes
CNIH-2	Yes	KRIP6	Yes	GluA4	
CNIH-3	Yes			GluK1	
CKAMP44	Yes			GluK2	Yes
SynDIG1				GluK3	Yes
NETO1	Yes			GluK4	
NETO2	Yes			GluK5	

Fig. 1: (A) Shows schematic diagrams of all the auxiliary proteins of iGluRs identified till date. (B) Table summarizing all the full-length clones of auxiliary subunits, soluble interacting proteins and iGluRs now available with us. The clones for which expression was detected by Western analysis are indicated.

now successfully cloned/obtained 9 out of 13 identified auxiliary proteins except the TARP Y-3, Y-4, Y-7 and GSG-1L (Figure 1). We have expression clones for all the AMPA receptor subunits (GluA1- GluA4) and Kainate receptor subunits (GluK1-GluK5). Clones of soluble interacting proteins of iGluRs e.g. Neuritin, Noelin1, Brorin and KRIP6 that have been shown to modulate iGluR receptor functions and neurogenesis have also been obtained. Our Western analysis showed expression of 5 out of 9 cloned auxiliary subunits namely: CNIH-2, CNIH-3, CKAMP44 (AMPA auxiliary subunits) and NETO1, NETO2 (Kainate auxiliary subunits). iGluR receptors GluA2, GluA3 (AMPA subtype), GluK2 and GluK3 (kainate subtype) also express. Our soluble interacting partners of iGluRs; Noelin1, Brorin and KRIP6 also show positive expression (Figure 1). Further construct optimization and screening of non-expressing clones is ongoing.

We went on to make several deletion constructs of NETO1 and NETO2 proteins as proposed. Suitable overexpressing clones for the ectodomain as well as TM domain containing protein have been identified. We were able to overexpress and purify both rat and human NETO1 ectodomain from transiently transfected mammalian cells to homogeneity. Size exclusion profile of the purified protein revealed monodispersed homogenous protein preparation suitable for crystallization trials (Figure 2). We have identified potential conditions that may be optimized to yield crystals suitable for X-ray diffraction and data collection. Some of the crystals for rat NETO1 ectodomain diffracted weakly at the synchrotron to about ~ 6Å. Similarly, promising crystallization hits have also

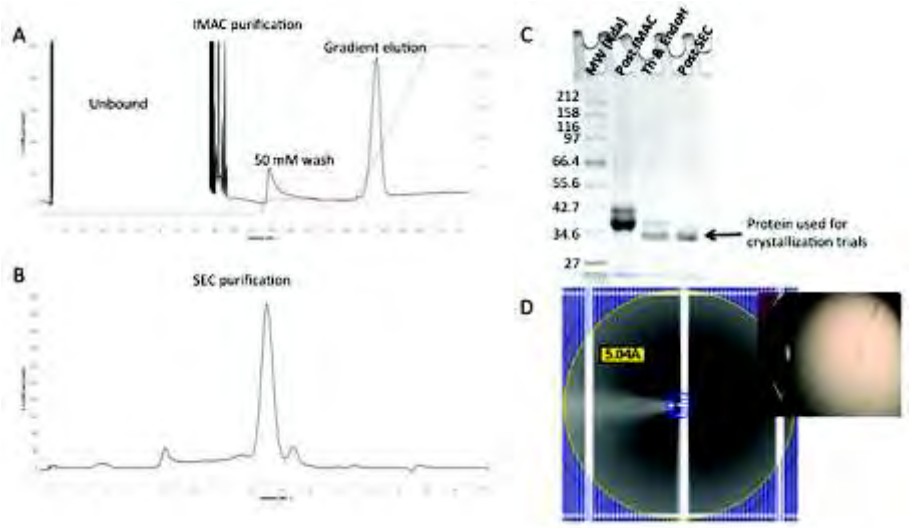

Fig. 2: Expression and purification of the rat NETO1 extracellular domain. (A) IMAC (Ni-NTA) elution profile (B) Size exclusion profile of the IMAC purified and thrombin + Endo H digested protein. Samples were run on 10/300 Superdex 200 column equilibrated in 20mM Tris-HCl pH 8.0, 150 mM NaCl (C) SDS PAGE for NETO1 extracellular domain expressed in HEK293 GnTI- cells; Lane 1: molecular weight markers (kDa); Lane 2: Post IMAC indicates eluate from a Ni^{2}+ affinity column; Th indicates cleavage of the C-terminal His tag by thrombin & Endo H indicates glycosidic cleavage; PostSEC indicates size exclusion chromatography purified protein. (D) Crystals of rate NETO1ECD that diffracted to ~5Å.

been obtained for the human NETO1 ectodomain as well (Figure 3). Further optimization of crystallization conditions to yield better diffracting crystals is ongoing. Interaction analysis and stabilization of NETO1 ectodomain complexes with GluK2 (kainate) receptors is also being optimized.

Expression and purification of full length iGluR receptors GluA2, GluK2 and GluK3 was also carried out successfully to perform the above mentioned interaction studies and complex formation with auxiliary proteins.

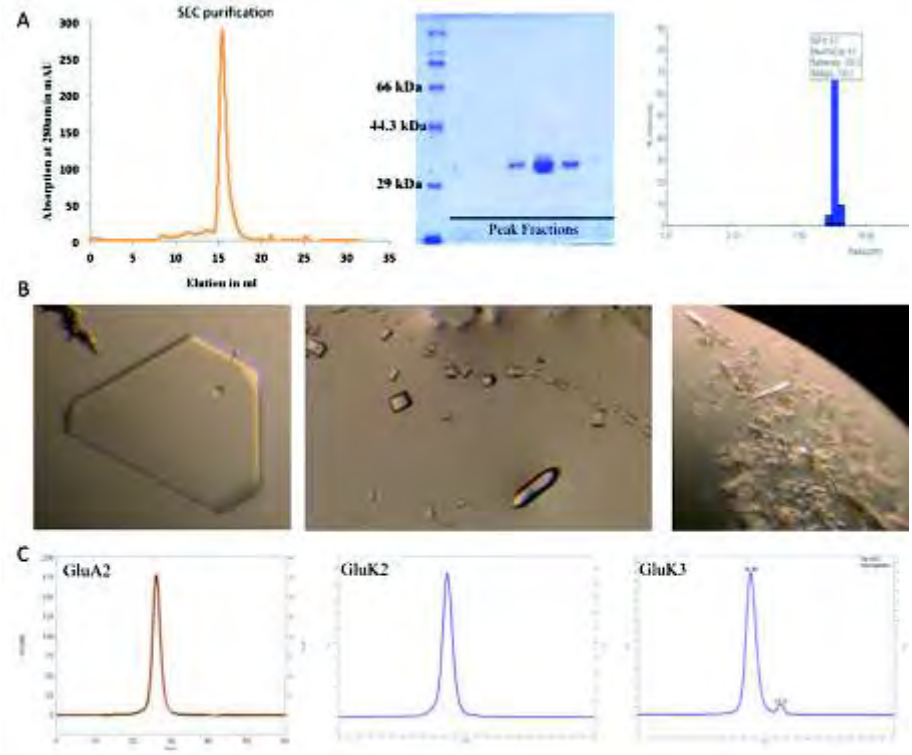

Fig. 3: (A) Size exclusion profile of the IMAC purified and thrombin + Endo H digested human NETO1 ecto domain. Samples were run on 10/300 Superdex 200 column equilibrated in 20mM Tris-HCl pH 8.0, 150 mM NaCl (B) Crystallization hits obtained for the human NETO1 ectodomain. (C) Size exclusion profiles of the purified rat GluA2 (AMPA), GluK2 and GluK3 (Kainate) receptors are shown. Intrinsic tryptophan fluorescence was used to monitor eluting protein loaded onto a superose 6 10/300 (GE) column pre-equilibrated with 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM DDM.

Future Research Plans

- Purification of intact GluK2 receptors and complex formation with NETO1 and NETO2 proteins.
- Structure determination of GluK2-NETO complex via X-ray crystallography or single particle cryo-EM.
- Functional assays via electrophysiological recordings.

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Epoxyazadiradione, a potent therapeutic agent attenuates breast tumor growth and angiogenesis through targeting PI3K/AKT pathway

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Background

Breast cancer is one of the most aggressive endocrine related cancer which has been considered as common malignancy affecting female worldwide. In spite of numerous therapeutic agents available to treat breast cancer, development of chemoresistance and recurrence of disease is frequently observed day by day. Although several potent cytotoxic, hormonal and estrogen receptor (ER) targeted agents have been developed for treatment of breast cancer, the disease free survival of the patients remains unsatisfactory. Moreover, several breast cancer-targeted agents are available which are extremely effective in treating ER+ breast cancer. However, treatment of triple-negative breast cancer (TNBC) patients lacking estrogen receptor (ER), progesteron receptor (PR) and human epidermal growth factor receptor 2 (HER2) has been challenging due to heterogeneity and devoid of well defined molecular targets. Nearly about 20% of breast cancer patients are TNBC and commonly observed in younger patients. Thus, identification of novel effective and selective agents against TNBC is essential for the management of this subtype of breast cancer.

Neem plant is well-known for its diverse applications in traditional medicine in Indian subcontinent for many years. Various parts of this tree are being used over the years as the home made remedies for several pathological conditions including hyperglycaemia, ulcer, malaria, cancer and dermatological complications. Structural diversity in the secondary metabolites of neem plant and more importantly their insecticidal efficacy and pharmacological activities has been explored in last five decades. Over 150 triterpenoids have been isolated and structurally characterized from neem plant, majority of which belongs to tetranortriterpenoids (limonoids). On the basis of structural

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diversity, neem limonoids can be classified broadly into two groups; (i) basic/ring-intact limonoids possessing 4,4,8-trimethyl-17-furanylsteroidal skeleton (e.g. azadirone, azadiradione, gedunin etc.) and (ii) C-seco limonoids with rearranged framework generated through C-ring opening (e.g. salannin, nimbin, azadirachtin A etc). Various neem limonoids including nimbolide, azadirachtin A, gedunin, azadirone and several other ring-intact limonoids have been tested for their cytotoxic potency against various cancer cell lines in vitro. However, mode of action and anti-carcinogenic activity of these compounds under *in vivo* conditions are not well explored. Our continuous effort to search for potent anti-carcinogenic plant derived metabolites has prompted us to screen the neem limonoids against breast cancer cell lines and further investigate the molecular mechanism underlying this process. Previous studies have shown that neem derived epoxyazadiradione limonoid exhibit anti-feedant properties. Further, it has been shown that epoxyazadiradione acts as anti-inflammatory agent by attenuating macrophage migration inhibitory factor (MIF) mediated macrophage migration. However, anti-cancer activity of epoxyazadiradione, a neem limonoid is not studied yet. We report that epoxyazadiradione acts as an anti-cancer agent in both TNBC and ER+ breast cancer models.

In this context, we report the probable anti-cancer activities of neem derived epoxyazadiradione limonoid under *in vitro* and *in vivo* conditions. It is noteworthy that out of ten major limonoids, epoxyazadiradione is highly potent cytotoxic agent. It induces apoptosis in both TNBC and ER+ breast cancer cells through mitochondrial dependent Caspase 3 and 9 activation. We have also shown that epoxyazadiradione induces apoptosis which is ROS and AIF independent. Our findings suggest that it significantly attenuates breast cancer cell viability, migration and vasculogenesis. It inhibits PI3K/Akt mediated AP-1 activation and suppresses the expression of MMP-9, Cox2, OPN and VEGF leading to attenuation of breast tumor progression, angiogenesis and metastasis. Taken together, our study demonstrates that epoxyazadiradione may act as a potential therapeutic agent for control of TNBC and ER+ breast cancer.

Aims and Objectives

- To indentify potent neem derived limonoids as anti-cancer agent in TNBC and ER+ breast cancer models.
- To study whether epoxyazadiradione (limonoid) derived from neem extracts attenuates PI3K/Akt pathway in these models.

- To investigate the anti-tumorigenic potential of epoxyazadiradione in breast cancer under *in vivo* conditions.

Work Achieved

A number of studies demonstrate that there is several anti-cancer therapies are available which can induce cell shrinkage and lead to apoptosis. However, they are relatively ineffective against the triple negative breast cancer (TNBC) cells lacking estrogen, progesterone and Her/neu receptors. To target these cells, we have extracted and purified 10 major limonoids (1: Epoxyazadiradione; 2:

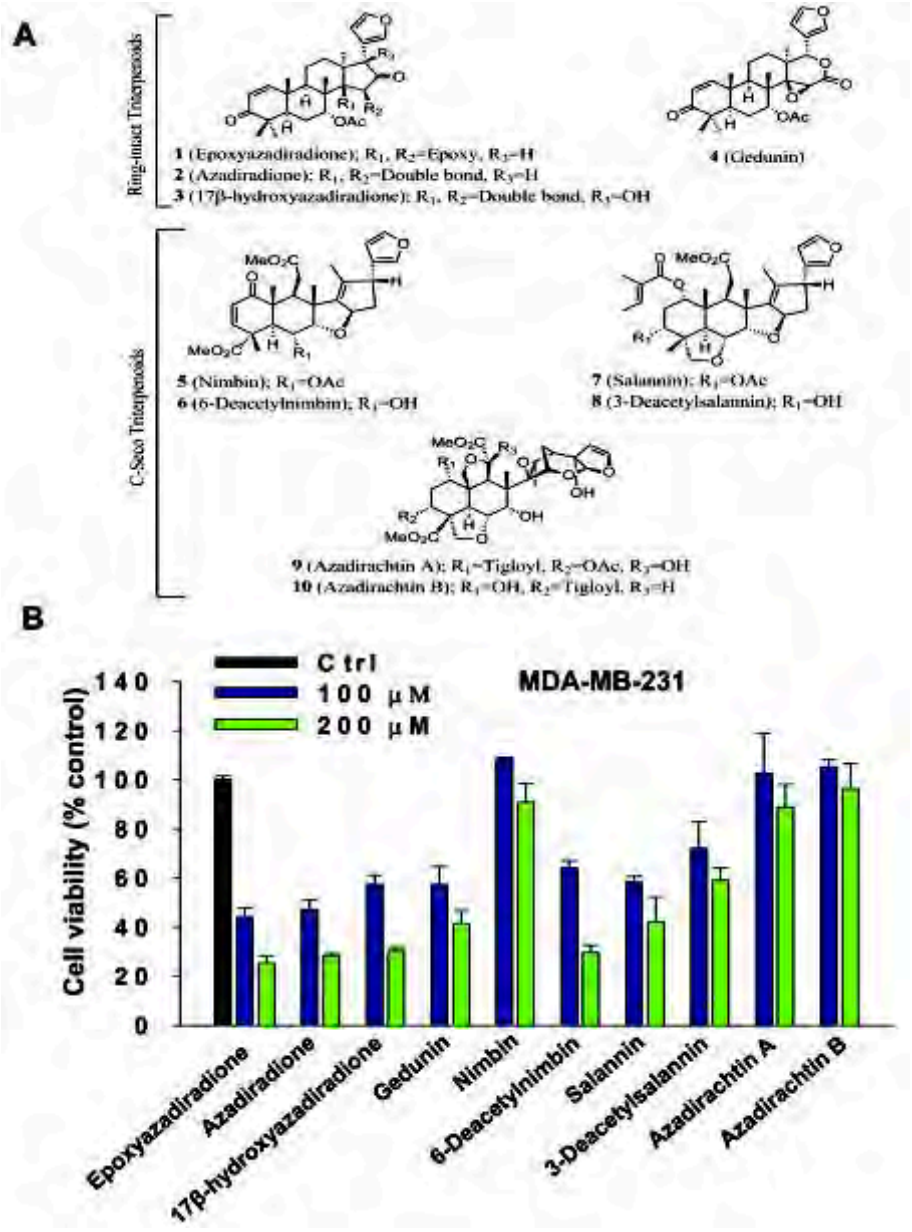

Fig. 1: Epoxyazadiradione a neem limonoid exhibits potent cytotoxic effect in breast cancer cells. (A) Structure of ten major limonoids isolated from neem. (B) MDA-MB-231 cells were seeded into 96-well and treated with ten major neem limonoids (1: Epoxyazadiradione; 2: Azadiradione; 3:17β-hydroxyazadiradione; 4: Gedunin; 5: Nimbin 6: 6-Deacetylnimbin; 7: Salanin; 8: 3-Deacetylsalannin; 9: Azadirachtin A; 10: Azadirachtin B) for 24 h at 100 and 200 μM and cell death was analyzed using MTT assay. The inhibition of the percentage of cell viability is represented into bar graph. Values are represented in mean ± SEM of three independent experiments.

Azadiradione; 3: 17β-hydroxyazadiradione; 4: Gedunin; 5: Nembin 6: 6-Deacetylnimbin; 7: Salanin; 8: 3-Deacetylsalanin; 9: Azadirachtin A; 10:

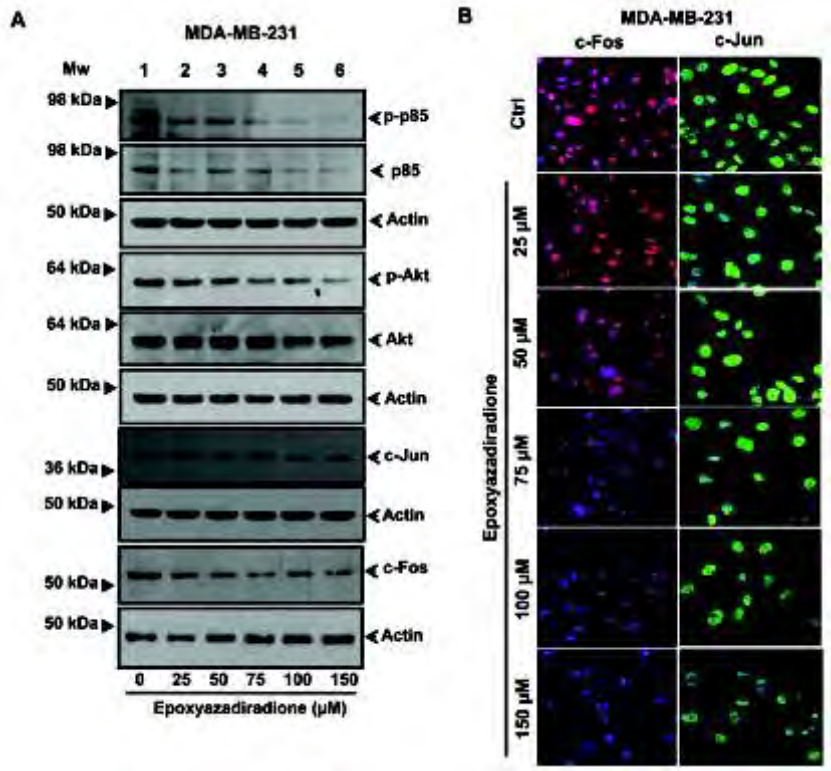

Fig. 2: Epoxyazadiradione attenuates PI3K/Akt phosphorylation and expression of c-Fos and c-Jun. **(A)** MDA-MB-231 cells were treated with increasing concentrations of epoxyazadiradione (0-150 μM) for 24 h. The level of phosphorylation of p85 and Akt and c-Jun and c-Fos were analyzed by immunoblot. Actin was served as loading control. **(B)** The expressions of c-Fos and c-Jun were detected by confocal microscopy in epoxyazadiradione (0-150 μM) treated MDA-MB-231 cells. Nuclei were stained with DAPI. Micron bar represent 20 μm.

epoxyazadiradione downregulates phosphorylations of p85 and Akt drastically in a dose dependent manner in these cells (Fig. 2A). We have then evaluated the expression of c-Jun and c-Fos in epoxyazadiradione treated cells by western blot and immunofluorescence. The results showed that the expressions of c-Jun and c-Fos were blocked by epoxyazadiradione in these cells (Fig. 2A and 2B). Overall, these results demonstrate that epoxyazadiradione downregulates PI3K/Akt-mediated AP-1 activation in breast cancer cells.

To investigate the effect of epoxyazadiradione on *in vivo* breast tumor growth in orthotopic mice model, we have injected MDA-MB-231-Luc cells into mammary fat pad of NOD/SCID mice. After 10 days, tumor bearing mice were randomly divided into three groups (5 mice each). Vehicle or two doses of epoxyazadiradione (25 mg/Kg body wt and 100 mg/Kg body wt) were injected intraperitoneally (i.p.) twice a week for 6 weeks. Tumor volumes were measured twice a week (Fig. 3A). Further, these tumor growths were monitored in a real time manner using In Vivo Imaging System (IVIS) (Fig. 3B). At the end of experiments, mice were sacrificed, tumors were dissected, photographed and weighed (Fig. 3C and 3D). Interestingly, our data showed that epoxyazadiradione significantly reduced tumor volume and weight as compared to vehicle treated mice (Fig. 3A-3D). Taken together, our *in vivo* data demonstrate that epoxyazadiradione attenuates breast tumor growth.

Future Research Plan

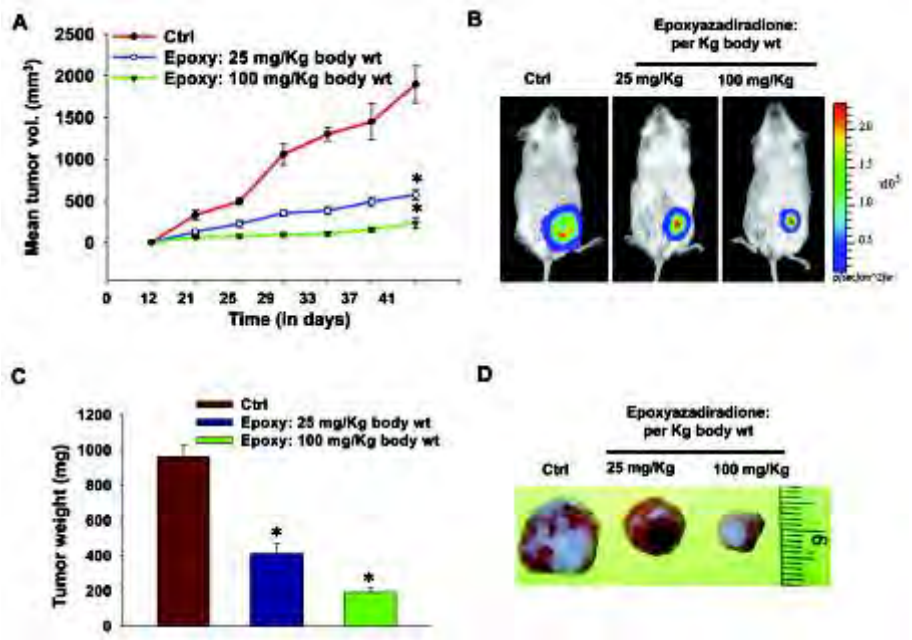

Fig. 3: Epoxyazadiradione suppresses breast cancer growth and angiogenesis under *in vivo* conditions. **(A)** MDA-MB-231-Luc (2×10^6) cells were injected orthotopically into NOD/SCID mice and then 25 and 100 mg/Kg body wt of epoxyazadiradione was injected intraperitoneally (i.p.) twice a week for 6 weeks. Tumor volumes were measured twice a week, analyzed statistically and represented graphically (mean ± SEM, n=5; *, $p < 0.02$ compared to untreated control tumor). **(B)** Photographs of bioluminescence imaging of representative tumor bearing NOD/SCID mice were analyzed using IVIS as indicated conditions. (**C** and **D**) Tumors were excised, photographed, weighed and analyzed statistically. Bar graph represents mean tumors weight (Mean ± SD, n=5; *, $p < 0.005$).

- To study the role of CCL5 in regulation of stroma-tumor interaction leading to tumor growth and metastasis in breast cancer and melanoma models.

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

Background

Inflammation is a complex set of reactions involving a set of cytokines, chemokines and adhesion molecules. There are several secreted chemokines, cytokines and their specific ligands that are expressed in the inflamed microenvironment, and a joint venture of pro- and anti-inflammatory functions are initiated together by infiltrating leukocytes, tissue fibroblasts, epithelial and endothelial cells. Chemokine receptors and cell adhesion molecules present on the cell surface are known to be involved in the migration of immune cells into the inflamed tissue. These chemokines and cell adhesion molecules are well studied in the migration of cells. However, intrinsic signaling from these receptors that can perturb 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 heterotrimers consisting of α-, β-, and γ-subunits, which transduce signals from surface receptors to intracellular effectors. Upon receptor activation, the G-protein complex dissociates into α and βγ-subunits, which in turn recruits 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.

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CCR6 is a GPCR, which is expressed on various immune cells and interacts with its specific chemokine, CCL20. CCR6 plays an important role in various diseases such as experimental autoimmune encephalitis (EAE), inflammatory bowel disease, psoriasis, chronic hepatitis, rheumatoid arthritis, chronic pulmonary sarcoidosis, cancer metastasis and graft-versus-host disease. How CCR6 signaling affects differentiation and function of CD4 T cells is not known.

Aims and Objectives

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

Work Achieved

We induced colitis in C57BL/6 mice by giving dextran sodium sulfate (DSS) in drinking water or by adoptive transfer of naïve CD4 T cells ($CD4^+Foxp3rfp^-CD44^-CD45RB^{hi}$ cells) into RAG1-/- mice. $CCR6^+CD4^+$ T cells showed significantly increased expression of RORγτ and IL-17 during colitis, as compared to controls, and CCR6-/- mice were protected from colitis. To test the contribution of CCR6 on RORγτ expression, we injected $CCR6^{-/-}$ CD4 T cells ($CD45.2^+$) in the $CD45.1^+$ congenic mice, and mice were given DSS. Compared to the $CD45.1^+(CCR6^{+/+})$ CD4 T cells, $CD45.2^+(CCR6^{-/-})$ cells did not increase RORγτ expression. To faithfully monitor the effect of CCR6 on RORγτ expression in $Foxp3^+$ Tregs, we adoptively transferred naïve CD4 T cells from $CCR6gfp^{+/-}::Foxp3rfp^{+/+}$ mice to $RAG1^{-/-}$ mice. During colitis, $CCR6gfp^+Foxp3rfp^+$ Tregs showed significant increased RORγτ expression compared to the $CCR6gfp^-$ Tregs. Addition of CCL20 (CCR6 ligand) in Treg and Th17 lineages

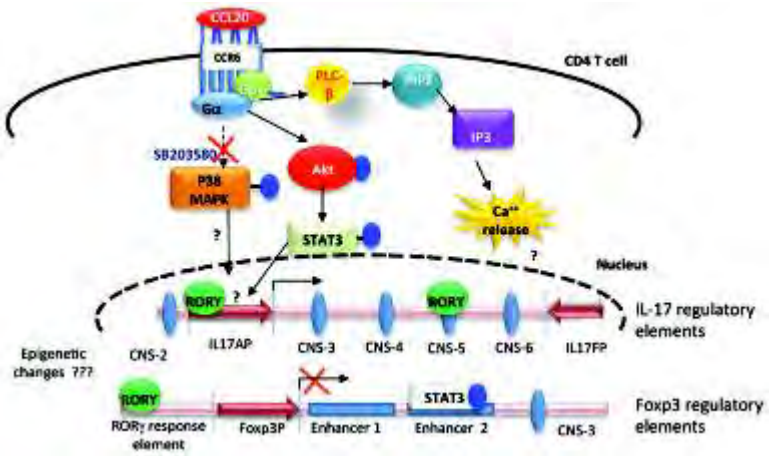

Fig. 1: Schematic representation of CCL20-CCR6 signaling in CD4 T cells.

differentiation culture conditions significantly increased RORγτ and IL-17 expression. Binding of CCL20 with CCR6 induced phosphorylation of Akt, mTOR and STAT3, which in turn promoted the binding of RORγτ specifically on IL-17A promoter and CNS5, and increased IL-17 reporter activity. CCL20-CCR6 signaling promotes differentiation of Th17 cells, and designing strategies to block CCR6 signaling would help in controlling gut inflammation and autoimmunity.

Future Research Plans

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

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Signaling cues guiding mesoderm induction and its derivatives

Background

Embryogenesis involves complex and fine-tuned interactions among various signalling cues with their temporo-spatial influence attributing to the precision in development. The gatrulating embryo in vertebrate consists of three germ layers that execute the well orchestrated body plan culminating in the development of an organism. Mesoderm, the middle of the three embryonic germ layers, gives rise to cells of various vital systems including haematopoietic, cardiovascular, reproductive, excretory, urogenital etc. during early development. Several signaling pathways have been investigated by various investigators including ours, to assess their roles during mesoderm specification and further differentiation to various derivatives. Using murine embryonic stem cells (ESCs) as a model system we have demonstrated earlier the Wnt-BMP synergy to underlie mesoderm induction (Verma and Lenka, 2010). The canonical Wnt activation at early stage of ESCs differentiation was shown to induce mesoderm through upregulation of BMP4 and Brachyury, whereas subsequent cardiomyogenesis was attenuated that was associated with down-regulation of BMP4 and Wnt5a and thus elucidating a fine tuned temporal Wnt-BMP4 crosstalk during cardiac differentiation. Indeed, there exist conflicting reports on the role of Wnt/β-catenin signaling during cardiac differentiation and haematopoiesis. Naito et al. (2006) have shown the influence of stage specific activation of Wnt/β-catenin pathway during ESCs differentiation. While Wnt activation at early stage promoted cardiac differentiation and suppressed the differentiation into hematopoietic and vascular cell lineages, the reverse was true with the late stage activation of Wnt/β-catenin signalling. Kattman et al. (2006) have reported that, mouse ESCs

Participants

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could give rise to two waves of progenitors marked by expression of the VEGF receptor Flk-1. While the first wave of Flk-1^{+} cells functioned like hemangioblasts giving rise to hematopoietic cells and endothelium, the second wave gave rise to cardiomyocyte, endothelium, and smooth muscle cells. Similarly, contradictory findings exist regarding the role of BMP signalling during cardiovascular development. Yuasa et al (2005) have shown transient inhibition of BMP signalling by Noggin inducing cardiomyogenesis from mouse ESCs, while contradicting to this report, combinations of activin A and BMP2 have been shown to induce cardiovascular development in human ESCs (Kim et al., 2011). Thus a concrete blueprint of mesoderm cell fate and its derivatives modulated by various cell intrinsic and extrinsic cues during early development is still debated. We have tried to explore the cell fate decision machinery addressing how different signalling mechanisms integrate and crosstalk in a spatio-temporal manner during mesoderm induction and its subsequent differentiation into cardiac mesoderm and other derivatives.

Aims and Objectives

- Generation of Stable transgenic Bry-LIG/PIG and Mesp1-Venus clones to demarcate the mesodermal and cardiogenic mesodermal cells respectively during ESCs differentiation and quantify them.
- Exploring the temporo-spatial influence of Wnt-BMP during cardiac mesoderm specification and other mesodermal derivatives.

Work Achieved

To discern the role of canonical Wnt signalling in specifying mesoderm, Bry-LIG clones (described in previous report) were differentiated in presence of BIO, the GSK-3β inhibitor that prevents the degradation of β-catenin and facilitates the latter's entry into the nucleus. Flow cytometry quantification of d5 EBs revealed that, Wnt activation at d0-2 of differentiation increased the Brachyury driven EGFP expression by 3 fold when compared with vehicle control thereby demonstrating the positive influence of canonical Wnt at early stage of ESCs differentiation in promoting mesoderm induction. This also confirmed our previous report (Verma and Lenka, 2010) where brachyury transcripts were seen upregulated upon Wnt activation. Since the stated study also demonstrated cardiomyogenesis to be attenuated following canonical Wnt activation, it hinted at two possibilities, (1) generation of other mesodermal derivatives at the expense of cardiac ones following Wnt activation mediated mesoderm induction, (2) specification to all mesodermal derivatives with equal efficiency, however with impediment of cardiac mesodermal cells in their

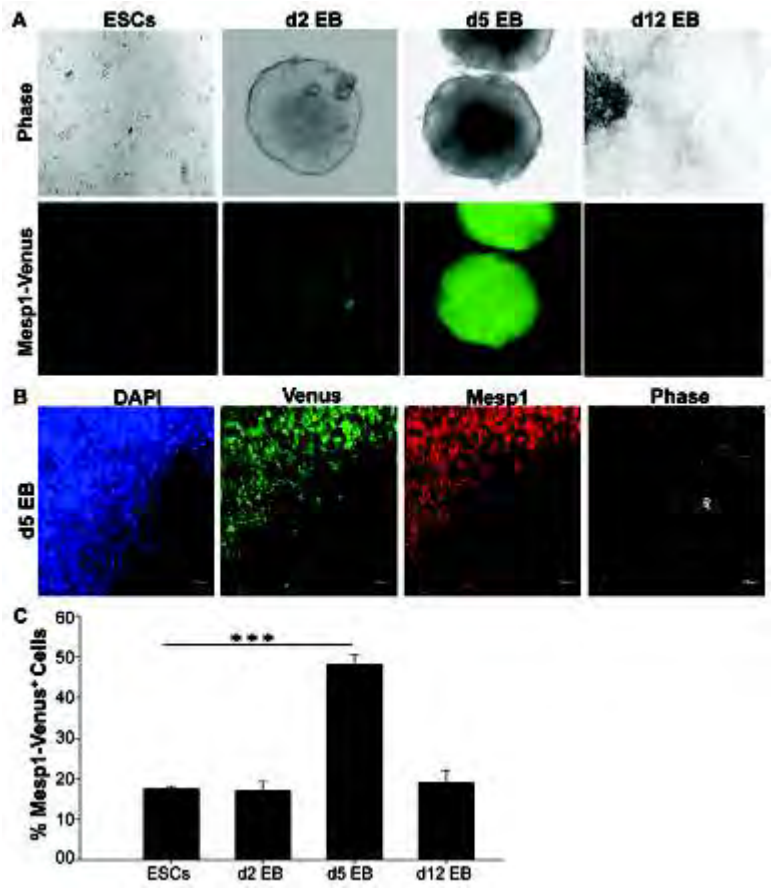

Fig. 1: A. Mesp1 promoter driven Venus expression profile during differentiation of Mesp1-Venus ESC clones. B. Colocalization of Mesp1 driven Venus expression with endogenous Mesp1 authenticates the specificity of Venus expression in Mesp1^{+} cardiomyogenic progenitor cells during differentiation of ESCs. C. Flow cytometry quantification of Mesp1-Venus^{+} cells during various stages of ESCs differentiation reveals d5 to be the optimum stage for maximum cardiomyogenic progenitor generation.

further differentiation into cardiomyocytes. Accordingly, to discern Wnt influence on cardiogenic mesodermal cells and demarcate "live' the cardiac mesoderm induction from ESCs *in vitro*, a number of stable transgenic Mesp1-Venus ESC clones were established, since Mesp1 is considered as the earliest marker of cardiovascular development in vivo. Mesp1 is expressed transiently during early mesoderm specification in the primitive streak, the latter migrating anterolaterally along with the cardiac mesoderm. As could be seen in Fig. 1A, the Mesp1 driven Venus expression was not observed to an appreciable level in ESCs during maintenance; however, its expression peaked at d5 during differentiation that diminished subsequently. The authenticity of cardiac mesoderm specific Venus expression was assessed by monitoring the co-localization of Venus and endogenous Mesp-1 in these clones (Fig. 1B). Further quantification of Mesp1-Venus cells by flow cytometry revealed maximum generation of Mesp^{+} cells at d5 of differentiation when compared with ESCs, d2 EB and d12 EB respectively (Fig. 1C). To investigate the role of Wnt signalling in the generation of Mesp1^{+} multipotent cardiac progenitors, ESCs were differentiated in presence of BIO. Flow cytometry quantification revealed that

Wnt activation decreased the expression of $Mesp1^+$ cells at d2 and d5 of ESCs differentiation when compared with DMSO control. Interestingly there was an increase in the $Mesp1^+$ cells at d12 of ESCs differentiation upon Wnt activation when compared with DMSO control. Together it suggests Wnt signalling not only inhibits the generation of cardiac mesodermal cells at early stage but also impedes their further differentiation to cardiomyocytes by retaining $Mesp1^+$ progenitors at latter stages too. Hence, the possibility of Wnt activation favoring the generation of other mesodermal derivatives rather than cardiac becomes apparent. Accordingly, flow cytometry quantification for Flk1, a haematopoietic progenitor marker, was carried out. While its expression in d2 EB during differentiation was undetectable, the same upon Wnt activation showed significant increase when compared with DMSO control. Further, cKIT, a marker for haematopoietic stem cells (HSCs) that arise from the Flk^+ haematopoietic mesoderm was quantified at various stages of ESCs differentiation. It was observed that, Wnt activation not only promoted the generation of $cKIT^+$ cells (4-8 fold induction), but also expedited the same with maximum induction at d2, when compared with vehicle control at the respetive time points. This suggested Wnt promoting haematopoietic differentiation from mesoderm at the expense of cardiac ones.

Since our earlier report suggested Wnt activation modulating BMP4 expression in a temporal fashion, we further investigated the role of BMP signaling and the synergy, if any, with Wnt in modulating the generation of $Mesp1^+$ multipotent cardiac progenitors. ESCs were differentiated either in presence or absence of BMP and/or Wnt activators and inhibitors and flow cytometry was carried out to quantify the $Mesp1^+$ cells. Intriguingly, BMP4 activation also led to decrease in generation of $Mesp1^+$ cells similar to that seen with Wnt. However, BMP inhibition with Chordin did not have appreciable influence. Furthermore, simultaneous activation of Wnt and BMP4 signalling at early stage of ESCs differentiation showed drastic reduction in $Mesp1^+$ cells when compared to either Wnt or BMP4 activation alone. However, the reciprocal inhibition of either could reverse the inhibitory effect of the activators thereby suggesting the probable synergy between the two signalling pathways operational during cardiogenic mesoderm specification.

Further, to discern the role of Wnt and BMP signalling during cardiac differentiation, ESCs were exposed to the respective activators or inhibitors during differentiation. BIO treatment during differentiation initiation stage (d0–2) resulted in attenuation in cardiac differentiation with percentage EBs

beating (54.7±8.6 %) and number of beating clusters/EB (1.62±0.6%) decreasing at d10 of differentiation compared to DMSO control (% Beating EBs: 100%; beating cluster/EB: 3.8429±0.76). Conversely, inhibiting Wnt signalling by Dkk1 at early stage of ESCs differentiation had opposite effect with marginal increase in beating clusters/EB (5.6±0.5393), when compared to control (4.3±0.60). Similar effect was noted with BMP activation/inhibition as well. Attenuation in cardiac differentiation was further intensified with simultaneous activation of Wnt and BMP4 signalling at early stage of ESCs differentiation with significant decrease in percentage of EBs beating and in the number of beating clusters/EB when monitored at d10 of differentiation. Efforts are ongoing to ascertain their temporal influence, if any, and also to determine whether or not both act synergistically or independent of each other in specifying, and differentiating into various mesodermal derivatives.

Future Research Plans

We would further like to investigate the temporo-spatial influence of Wnt-BMP-Notch and the possible cross-talk among those during hemato-endothelial and cardiomyogenic differentiation. Determining various genetic and epigenetic modulators during the same would also shed light on these critical developmental events.

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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 seven different ongoing projects from the lab., the project on "Effect of feeding of nutraceuticals on haematopoiesis and megakaryopoiesis of mice" is described in detail below.

Dietary nutraceuticals belonging to the class of Polyunsaturated fatty acids (n-3 and n-6 PUFAs) have effects on diverse physiological processes impacting normal health and chronic diseases, such as the regulation of plasma lipid levels, cardiovascular and immune function, insulin action and neuronal development and visual function. Ingestion of PUFAs will lead to their distribution to virtually every cell in the body with effects on membrane composition and function, eicosanoids synthesis, cellular signaling and regulation of gene expression. Cell - specific lipid metabolism, as well as the expression of fatty acid-regulated transcription factors, likely plays an important role in determining how cells respond to changes in PUFAs composition.

Available literature as well as our initial studies suggests that n-3 and n-6 PUFAs influence megakaryopoiesis and thrombopoiesis in in vivo and in vitro models. Therefore in the present study, we orally administered N3 or N6 PUFAs individually as well as in combination and studied their effect on hematopoiesis and thrombopoiesis in mice.

Aims and Objectives

Based on the above background, the specific aims of the project were as follows:

- To Study effect of oral administration of mice with PUFAs on the hematopoietic stem/progenitor (HSPC) pool of the mice.
- To study the effect of oral administration of mice with PUFAs on megakaryopoiesis and thrombopoiesis of mice.
- To check metabolism of the PUFAs by determining the fatty acid composition of PUFAs in the bone marrow cells.

Work Achieved

We fed C57BL6 mice with PBS/ALA/DHA/LA/AA daily once for 10 days by oral administration and then examined their bone marrow and peripheral blood cells for stimulation of hematopoiesis and megakaryopoiesis. It was observed that there was a significant increase in Total nucleated cells (TNC Fig.1a) in all PUFA fed sets as compared to the control which were fed with PBS. The stimulation of HSCs was evident by an enhancement in LSK subset (Fig.1b) and side populationand (Fig.1c) In order to test the functionality of marrow cells from fed and unfed mice we performed CFU and LTC assays and found that indeed the marrow cells from fed mice harbored significantly higher

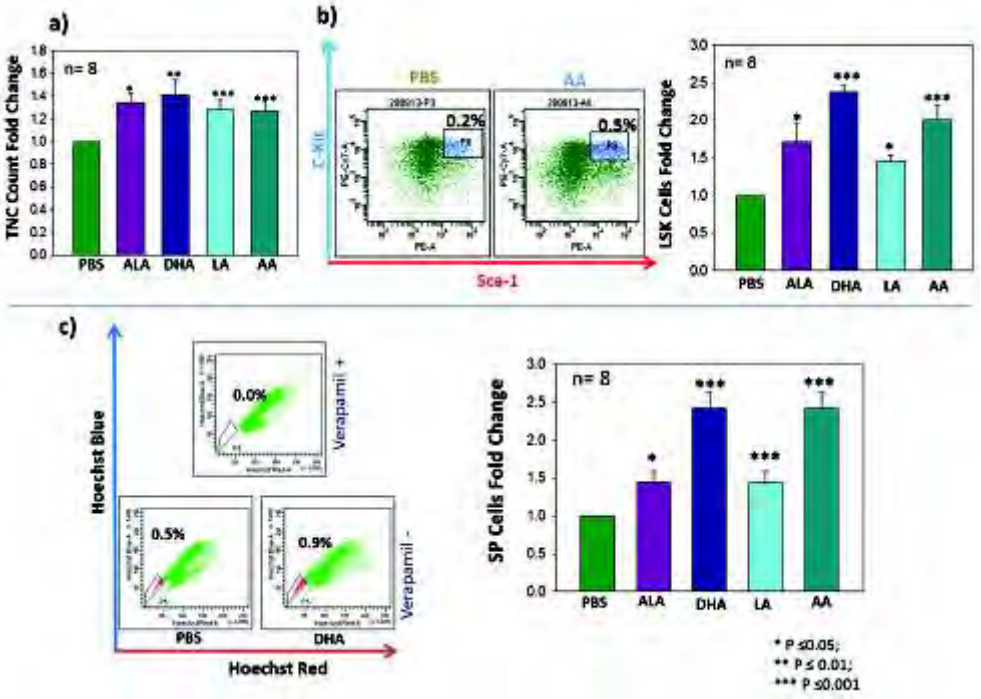

Fig. 1: Intake of PUFAs in mice significantly enhances a) TNC count; b) LSK population - Left panel shows flow cyometry profile of one sample, right panel shows cumulative data of 8samples c) SP cells - Left panel shows flow cyometry profile of one sample, right panel shows cumulative data of 8samples.

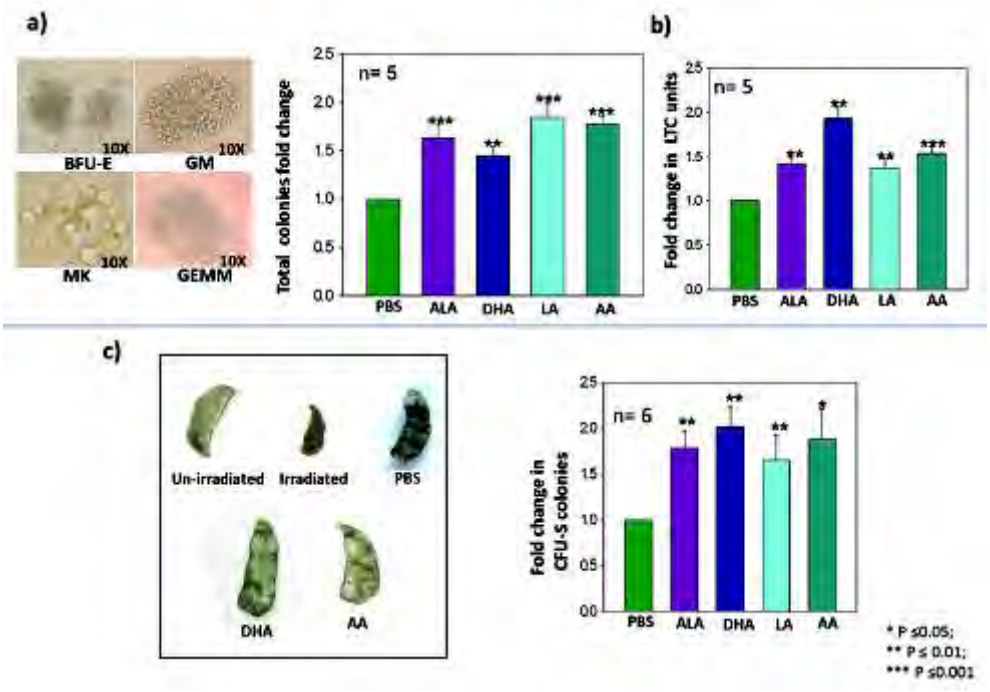

Fig. 2: Feeding of PUFAs in mice stimulates **a)** progenitor pool in mice -CFU - left panel shows images of various progenitor colonies formed in CFU assay and right panel shows total colonies formd in different sets in 5 samples **b)** Long term repopulation in BM-MNCs –LTC –cumulative data of 5 samples; **c)** Promotes in vivo progenitor colonies formation-CFU-S-left panel shows increased number of CFU-S colonies from BM-MNCsand right panel data from 6 samples.

progenitors (CFU-Fig.2a) and primitive cells (LTC-Fig.2b).The in vitro data could be extrapolated to in vivo condition. Bone marrow from fed and unfed mice were infused in irradiated recipients to check CFU-S as well as engraftment. The colony forming unit spleen assay also clearly indicated that there was

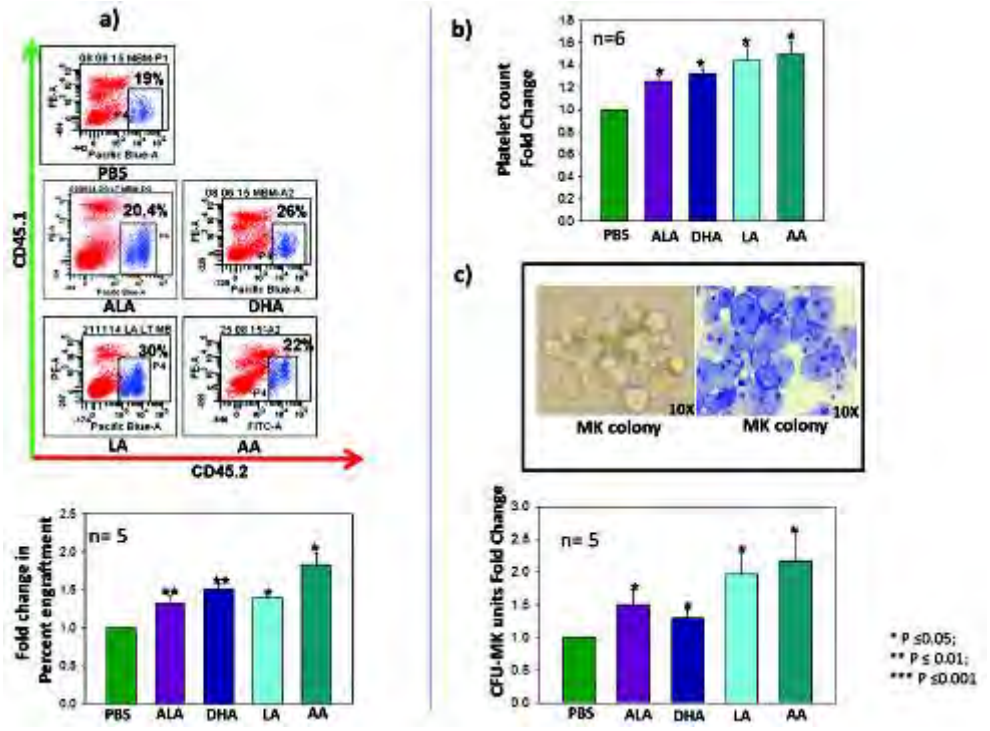

Fig. 3: Oral administration of PUFAs in mice increases **a)**long term engraftment potential - flow profile of one representative sample in the top panel and cumulative data of 5 samples in the bottom panel **b)** platelet production- cumulative data of 6 samples **c)** megakaryopoiesis - top panel – images of MK colony, bottom panel- cumulative data of 5 samples

enhancement in progenitor cells in the fed mice. (Fig2c). We further addressed the issue whether fed mice marrow have improved engraftment potential. We

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Understanding the molecular mechanism of persistence of memory

Background

We are interested in understanding how long term memory persists over time for years, even though the proteins maintaining it in the brain have a much shorter half life. In the learning memory field, while there are many genes identified which regulates short term and long term memory, the persistence of memory phase is not understood very well. During my previous work I found the protein synthesis regulator Orb2 is an important player in this process. Orb2 behaves like a prion-like protein, undergoes regulated oligomerization in a neuronal activity dependent manner. If the regulated prion-like oligomerization is interfered, long term memory forms but does not persist over time. Taking Orb2 as our entry point towards understanding the molecular mechanism of persistence of memory.

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Aims and Objectives

- What regulates the activity dependent prion-like oligomerization of Orb2.
- What Orb2 translates downstream to maintain memory.
- In neurodegenerative diseases is there any crosstalk between Orb2 pathway and the toxic amyloids.

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Structural and functional studies on mycobacterial proteins

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Background

Our group has been interested in understanding the structural and functional aspects of the mycobacterial stress proteins. *Mycobacterium tuberculosis*, the pathogen causing tuberculosis, has a well defined stress response and our laboratory has been involved in the characterization of its heat shock proteins. Among the heat shock proteins, GroEL1, a chaperonin homologue that deviates substantially from the canonical chaperonin properties, is one such protein. Mycobacteria lacking GroEL1 have been shown failure in forming biofilms and granulomas, although general growth is unaffected. Therefore, GroEL1 has been linked to the pathogenicity. We, therefore explored how depletion of GroEL1 will affect the growth of mycobacteria through different growth conditions.

Morevoer, biochemically, *M. tuberculosis* GroEL1 deviates significantly from the canonical homolog, *E. coli* GroEL. GroEL1 fails to function as a chaperone owing to its impaired oligomerization. The difference extends to the functionally important C-termini; while the canonical chaperonins harbor a Gly-Met rich C-terminus, GroEL1 bears His-rich C-terminus. We have studied how the differential C-terminus and oligomerization affect GroEL1 activity. Since, phosphorylation has been demonstrated to modulate oligomerization of GroEL1, we have used phosphomimetic mutants of GroEL1 to study its activity.

Chaperonins have been demonstrated to enhance the folding of co-expressed heterologous proteins and also their fitness by masking their mutations. However, the precise effect of chaperone overproduction on the cellular

proteome and growth parameters has not been investigated. We therefore have investigated the effect of overexpression of chaperonins on the fitness of the organism and its metabolism. For this, a simple assay system was developed using *E. coli* conditional mutant strain that lacks chaperonins and chaperonin expressing plasmids, as explained in the following section.

Aims and Objectives

- Exploring the function of GroEL1, using Δ*groEL1 M. tuberculosis*.
- Genetic analysis of the GroEL1 mutants in depletion strain of E. coli.
- Exploring the structural aspects of GroEL1 in its functional complexes.

Work Achieved

Growth of *M. tuberculosis* lacking GroEL1 was monitored under different culturing conditions. Compared to the wildtype, these strains exhibited growth defects during hypoxic (low aeration) growth, suggesting a link between GroEL1 and hypoxia, a condition which mycobacteria encounters during infection (Fig. 1).

Studies with the phosphomimetic GroEL1 variants have demonstrated that, indeed oligomerization is a pre-requisite for the chaperonin function (Fig. 2). Moreover, structural and phylogenetic analysis proposed a canonical quaternary structure and conservation pattern for GroEL1, suggesting a conserved mechanism of action for GroEL1. Based on these observations and the prediction that mycobacteria hosts higher number of metalloproteins, we have proposed a phosphorylation mediated chaperonin activity for GroEL1 (Fig. 2).

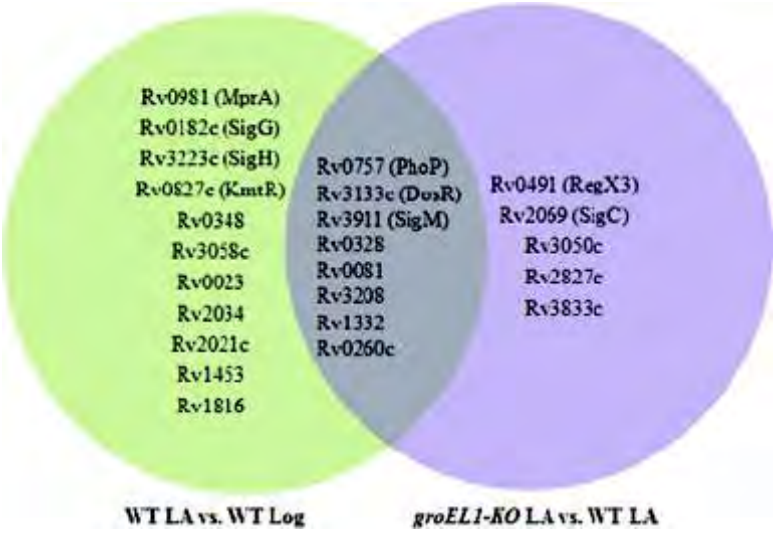

Fig. 1: Venn diagram representing the regulons affected on subjecting WT and *groEL1-KO* to low aeration stress. The green circle represents the genes differentially expressed on subjecting WT to low aeration stress in comparison to WT log phase. The purple circle represents the genes differentially affected in *groEL1-KO* vs. WT under low aeration. The intersection represents the genes affected in *groEL1-KO* and WT in low aeration stress.

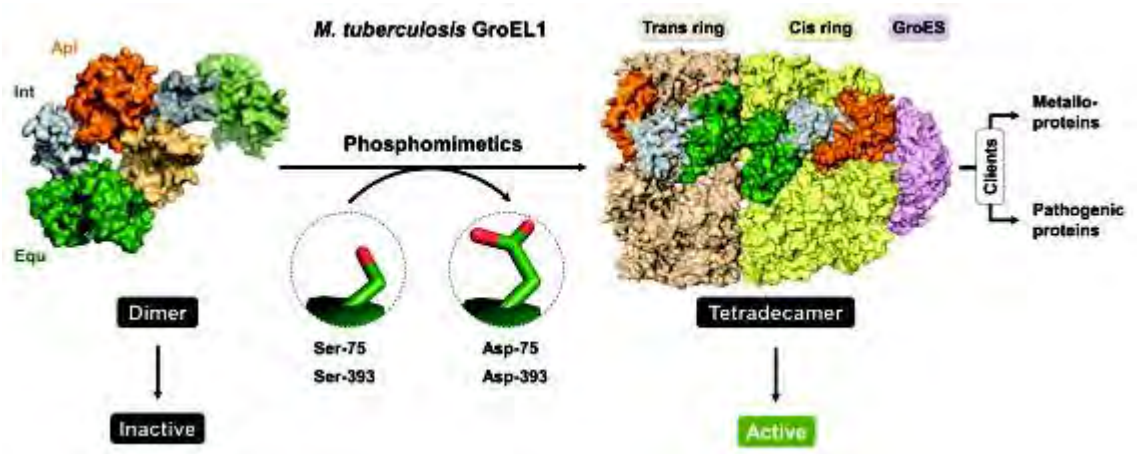

Fig. 2: Functional significance of Phosphorylation in GroEL1. Schematic representation of the activation of GroEL1. GroEL1 that generally exists as a dimer and thus inactive as a chaperone, gets activated upon phosphorylation on two indicated Serine residues by assembling into the tetradecamer. It is supposed that, as an active chaperone, GroEL1 might be involved in folding the pathogenesis related proteins or metalloproteins. Api, Int and Equ indicate the different domains of GroEL1, the apical, intermediate and equatorial, respectively.

In the chaperonin-fitness project, we have obtained interesting correlations between chaperonin overexpression and fitness. Overexproduction of chaperonins in *E. coli* resulted in enhaced growth and competitive advantage over the low chaperonin producing control strain. Moreover, proteomic analysis followed by Flux Content Analysis (FCA) revealed that the chaperonin overproduction enhanced carbon and enrgy metabolism via the preferential folding of certain metabolic enzymes (Table 1).

Future Research Plans

- Exploring the rationale for the growth defects of GroEL1 mutant *M. tuberculosis* under low aeration.
- Exploring phosphorylation stata of GroEL1 *M. tuberculosis* and identifying its kinase.
- Exploring the pleotrophic effects of chaperonin overexpression in correlation with the cellular metabolism under different growth conditions.

Table 1: Proteins enriched upon chaperonin overproduction.

GroEL-High*	GroEL-Low*
Enolase (EC:4.2.1.11)	Trigger factor (EC:5.2.1.8)
6-phosphogluconate dehydrogenase, decarboxylating (EC:1.1.1.44)	DNA-directed RNA polymerase subunit alpha (EC:2.7.7.6)
Malate dehydrogenase (EC:1.1.1.37)	Phosphoglycerate kinase (EC:2.7.2.3)
Isocitrate lyase (EC:4.1.3.1)	Outer membrane protein C
Thymidine phosphorylase (EC:2.4.2.4)	Outer membrane protein F
Isocitrate dehydrogenase [NADP] (EC:1.1.1.42)	Fructose-bisphosphate aldolase class II (EC 4.1.2.13)
Dihydrolipoyl dehydrogenase (EC:1.8.1.4)	UDP-galactopyranose mutase (EC:5.4.99.9)
DNA polymerase III beta subunit protein (EC:2.7.7.7)	Succinyl-CoA ligase [ADP-forming] subunit beta (EC:6.2.1.5)
Transaldolase B (EC:2.2.1.2)	Maltose-binding periplasmic protein
Spermidine/putrescine-binding periplasmic protein	Trehalose-6-phosphate hydrolase (EC:3.2.1.93)
Pyrimidine-specific ribonucleoside hydrolase (EC:3.2.2.-)	Mannose-6-phosphate isomerase (EC:5.3.1.8)
Chaperonin 60, GroEL	Aspartate aminotransferase (EC:2.6.1.1)
Beta-galactosidase (EC:3.2.1.23)	Lactose operon repressor (LacI)

* GroEL-High and GroEL-Low indicates high and low chaperone producing *E. coli* strains.

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

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

Background

One of the antiviral defense mechanisms whose understanding has gained momentum in the recent decade is the small non-coding RNA mediated interference (RNAi). RNAi is a mechanism of eukaryotic posttranscriptional gene silencing that relies on ~22 nucleotides long non-coding RNAs binding to their complementary sites on target mRNAs. In insects, RNA silencing pathways are very well distinguished and characterized in the Dipteran insect *D. melanogaster*. Also, the studies on role of small non-coding RNAs in intricate host-viral interactions are limited to *Drosophila melanogaster*. However, such information is scanty in many other economically important insect species such as *Bombyx mori*, a genetic model system for the largest and most diverse insect order, Lepidoptera, which includes many devastating agricultural pests. Most of the components of RNA silencing machinery still need to be identified and characterized in the *B. mori*. The domesticated silkworm, *B. mori* is an insect of high economic importance in the countries like India and China. *Bombyx mori* nucleopolyhedrosis virus (BmNPV) that infects *B. mori*, is a natural pathogen of great economic significance in sericulture inflicting more than 60% crop loss annually. BmNPV is a baculovirus and belongs to a large and diverse group of double-stranded DNA viruses, which include many devastating pathogens infectious to several economically important arthropods, particularly insects of the order, Lepidoptera.

My lab group is interested in understanding the role of small non-coding RNA mediated defense and counter-defense in the antiviral immune system of the domesticated silkworm, *B. mori* against its viral pathogen, BmNPV.

Participants

Uddhav Ambi, *Project Assistant*
Ria Anand, *Project Trainee*
Suhas Kharat, *Research associate*

Collaborators

Dr Arun Kumar KP, *CDFD, Hyderabad*

Aims and Objectives

- Identification of important components of RNAi machinery in *B. mori* by taking *D. melanogaster* as a model.
- Prediction and characterization of small non-coding RNAs and their mRNA targets encoded by the host, *B. mori* as well as its viral pathogen, BmNPV.
- Analysis of miRNA(s) role in resistance/susceptibility of *B. mori* against BmNPV infection.

Work Achieved

Key players of small RNA biogenesis: All the well-known key components of miRNA, siRNA and piRNAs biogenesis pathways were found to be expressed in *B. mori*. Besides, expression of all these genes was upregulated upon BmNPV infection. Our double-stranded RNA based knockdown of these components have suggested their role in miRNA/siRNA/piRNA biogenesis pathways. Intriguingly, we found 6 different isoforms of Loquacious transcript.

Isoforms of Loquacious: Our computational analysis of *B. mori* ESTs and RNA-Seq data resulted in identification of 6 alternative spliced transcripts of Loqs. Interestingly, these isoforms differ by only few residues at exon 2 and 6 as shown in Figure 1A. All these 6 isoforms were cloned and sequenced. Transcripts, which were lacking bases for "VMR" motif on exon 2 (X3, X5 and X6) were found to be upregulated upon BmNPV infection (Figure 1B).

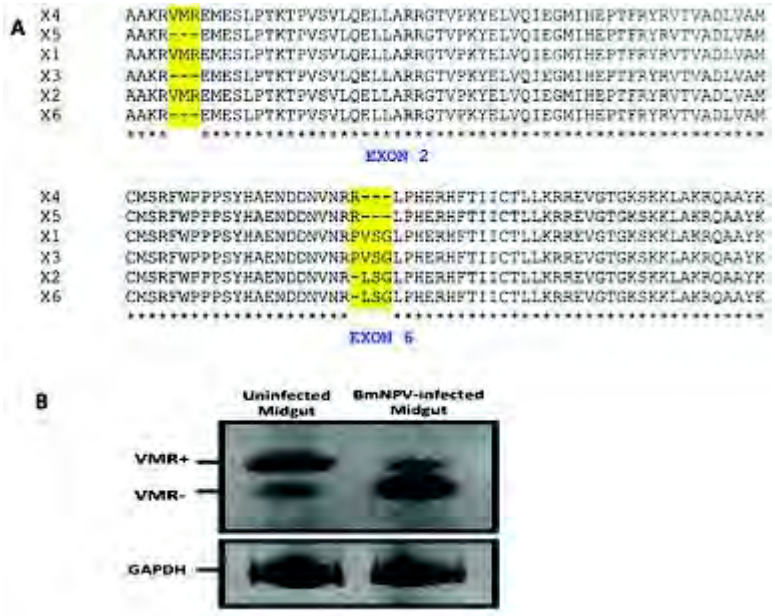

Fig. 1: A) Multiple Sequence Alignment of 6 isoforms of Loqs. Variable residues are highlighted in yellow. B) RT-PCR for VMR region in BmNPV infected and uninfected midgut tissues of *B.mori*

Identification of differentially expressed miRNAs:

To understand the role of miRNAs in resistance/susceptibility of B. mori against BmNPV infection, the differentially expressed miRNAs in resistant (SBNP-1) and susceptible strains (CSR2) of *B. mori*, known miRNAs of *B. mori* were downloaded from miRBase database, and BLAST against the small RNA sequences generated by RNA-Seq of midgut and fat body tissues of these two strains. The susceptible and resistant samples were then compared to fetch out the differentially expressed miRNAs. As a result, we found 37 miRNAs, which were differentially expressed among resistant and susceptible strains of *B. mori*. Out of these 37 miRNAs, 19 were found to expressed in fat body tissues, whereas 11 miRNAs showed expression in midgut tissues. Rest of the 7 miRNAs were found to be expressed in both the tissues.

Expression validation of differentially expressed miRNAs:

Real time PCR was done to validate the expression of 37 differentially expressed miRNAs identified by comparing the RNA-Seq data of resistant and susceptible varieties of *B. mori* against BmNPV.

Future Research Plans

- To understand the role and functional specificity for each of the different isoforms of Loqs protein towards a particular small non-coding RNA biogenesis pathway, we will analyze the effect of overexpression and knockdown of each of the Loqs isoforms on small non-coding RNAs expression.
- Prediction of mRNA targets of some of the very highly differentially expressed miRNAs in the host, *B. mori*, and the virus, BmNPV.

Publications

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

Conferences attended

Participated in the RNA Biology Meet 2016 at CCMB, Hyderabad, 8-10 January 2016.

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

Biological systems are complex adaptive systems endowed with properties such as complexity, robustness and evolvability. Despite active research, the relationship between complexity, robustness and evolvability still is 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

- Creation and characterization of complete point mutation space (~ 3000 mutants) to capture fitness and binding landscape of survivin.
- Developing the mechanistic insight of the effect of these mutations in a structural framework.

Participants

Collaborators

Dr. Chandra Shekar Prabhakar, *CCMB, Hyderabad*
Dr. Csaba Pal, *BRC, Szeged, Hungary*

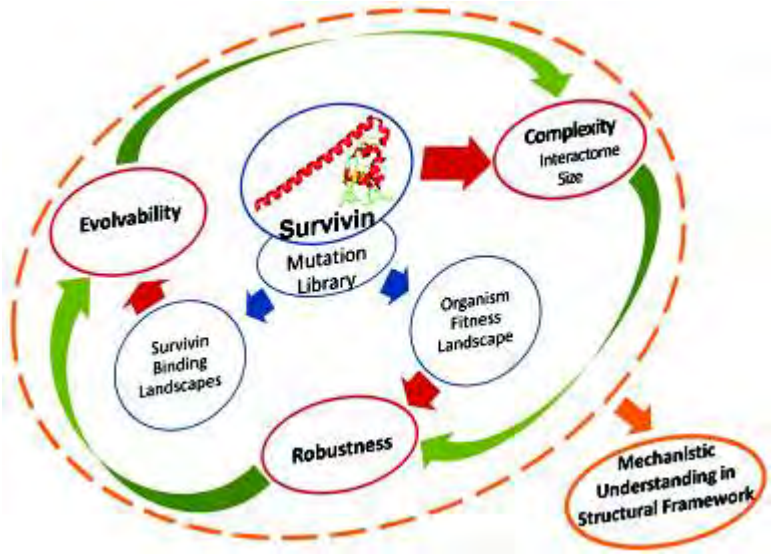

Fig. 1: The scheme depicts the primary goals and approaches of the work. The green arrows indicate the accepted relationship between complexity, robustness and evolvability of biological systems.

Work Achieved

Survivin interacts with ~ 55 proteins. Through computational methods and literature survey, I have downsized this number to 11 direct inter-actors and only 3 interfaces of survivin.

I have also finished molecular cloning of survivin and its direct inter-actors for their use in phage-display experiments.

Future Research Plans

- Phage-display technique will be established.
- 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.

AWARDS / HONOURS / MEMBERSHIPS

Wellcome Trust/DBT IA Early Career Fellowship

EXTRAMURAL FUNDING

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

CONFERENCES / WORKSHOPS

Attended (Md. Zahid Kamal), 7th Peptide Engineering Meeting (PEM7-2015), 5-7, December, 2015, Pune, India

Talk on "Origin and evolution of various interaction of survivin, a hub in protein-protein interaction network" (Md. Zahid Kamal), 2015 NNMBC Meeting, 27- 30, December, 2015, Pune, India

Attended (Md. Zahid Kamal), 2nd Bangalore School on Population Genetics and Evolution, 25 January- 6 February, 2016, Bengaluru, India

Attended (Md. Zahid Kamal), Metabolic Modeling Workshop – MetaMod 2016, 14 - 16 March, 2016, Pune, India

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

Participants

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Collaborators

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Dr. Manas Kumar Santra, *NCCS, Pune*

Background

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

Aims and Objectives

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

Work Achieved

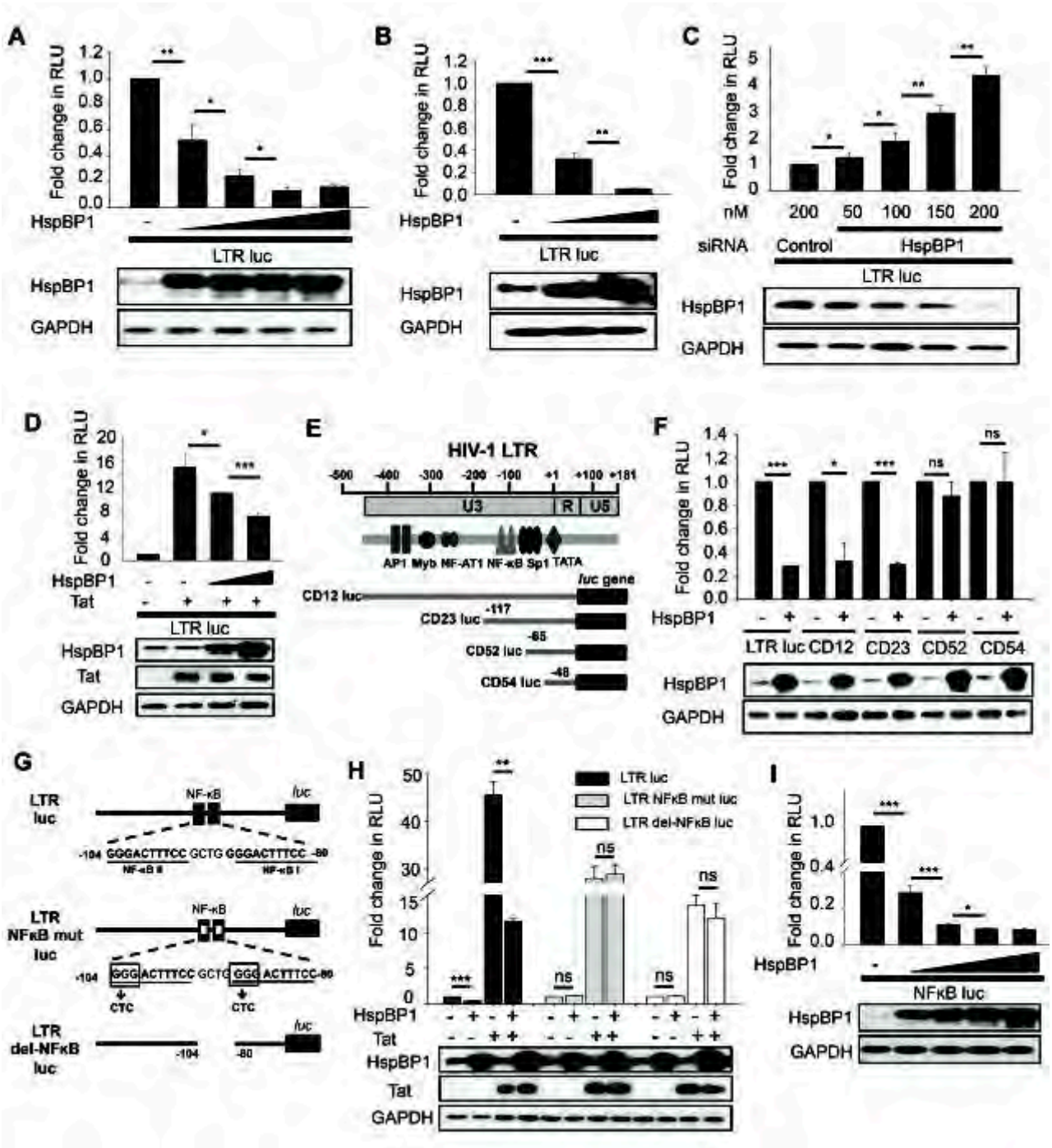
Role of viral regulatory proteins Tat and Nef in HIV pathogenesis

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

Fig. 1: HspBP1 inhibits HIV-1 promoter activity through NF-κB enhancer region.

(A) HspBP1 inhibits LTR-driven gene-expression in dose-dependent manner in HEK293T cells. Cells were co-transfected with increasing amounts of HspBP1 construct and the HIV-1 LTR luciferase reporter

complex to the LTR promoter. The pTEFb complex then hyper-phosphorylates the C-terminal domain of RNA polymerase II increasing the processivity of polymerase, which leads to elongation of transcription. There are convincing evidences that Tat also functions independently of TAR element to activate the LTR promoter. A previous report from our lab has shown Tat binding to the NF-



construct followed by luciferase assay after 36h of transfection. (B) HspBP1 over-expression inhibits LTR promoter activity in dose dependent manner in Jurkat cells. Jurkat cells were co-transfected with 0.5 and 1 µg of HspBP1-construct along with LTR-luc. Luciferase assays were performed 36h post transfection. (C) HspBP1 silencing enhances HIV-1 LTR driven gene expression in dose-dependent manner. Control siRNA and siRNA against HspBP1 were transfected in HEK293T cells. LTR-luc was also transfected 24h after siRNA-transfection. Luciferase activities were determined 24h post second transfection. (D) HspBP1 inhibits LTR-driven gene-expression in presence of Tat. HEK293T cells were co-transfected with indicated plasmids and luciferase assays were performed 36h post transfection. (E) Schematic representation of HIV-1 LTR and LTR-luciferase mutant constructs. (F) Luciferase reporter assays depicting inhibition of LTR promoter activity by HspBP1 through NF-κB and SP1 binding sites. HEK293T cells were co-transfected with various LTR luc constructs and HspBP1. Luciferase assays was performed 36h post-transfection. (G) Schematic representation of NFκB mutants of HIV-1 LTR promoter. (H) Effect of HspBP1 on HIV-1 LTR promoter is specifically through NF-κB binding sites. HEK293T cells were co-transfected with indicated plasmids and reporter assays were performed 36h post transfection. (I) HspBP1 inhibits NF-κB driven gene expression in dose-dependent manner. HEK-293T cells were co-transfected with increasing concentrations of HspBP1 vector and luciferase reporter construct under the control of five NF-κB binding sites. Luciferase activities were determined 36h post-transfection. Error bars represent the mean ± SD values and significance is defined as * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

κB enhancer sequences on the LTR promoter as one of the mechanisms underlying TAR independent transactivation. Thus, DNA binding activity of Tat could be also one of the potential mechanisms of TAR independent Tat mediated regulation of cellular gene expression. In this context, we have recently 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 involving the 68-72 amino acid motif of Tat protein. Down regulation of an NFκB family transcription factor C-Rel by Tat could also be a viral strategy to induce persistent infection in T cells.

The Human Immunodeficiency Virus Type 1 encodes a 27 KDa protein, Nef, which has come a long way from being termed as a negative factor to being one of the most important proteins of HIV-1. However, its role in HIV-1 replication and gene expression remains to be clearly understood. In a recent study, we have compared the gene expression profile of wild type and Nef deleted HIV-1 infected T-cells. Our preliminary analysis of the expression profile indicates that Nef may be responsible for deregulation of lipid metabolism in infected cells. We have also shown earlier that HSP40 and HSF-1 interacts with Nef protein and positively regulate HIV-1 replication. Although involvement of different heat shock protein family members in viral pathogenesis has been reported earlier, a clear understanding of their role in viral replication and infectivity remains to be elucidated. We have now initiated a comprehensive study of all the HSP protein family members during HIV infection. Each family is represented by different HSP members and their isoforms, encoded by different genes. Our expression profiling results targeting HSP family members indicate that a significant number of genes belonging to HSP40 and HSP70 family are differentially expressed during infection. We have now cloned many of these isoforms and further characterization of the individual role of these isoforms in HIV-1 infection is currently in progress. We are also looking at the role of different HSPs in viral replication and infectivity by knocking down individual HSPs and analyzing viral production and infectivity. Our initial results suggest that HSPs play an important role in viral replication and infectivity.

We have also been studying the role of HSP70 binding protein; HspBP1, a co-chaperone molecule of HSP70. HspBP1 negatively affects the binding of substrate to HSP70 by accelerating nucleotide exchange of ATP domain. It has also been reported that HspBP1 levels increase in the serum of HIV-1 infected individuals. HSP70 is associated with various phases of HIV-1 life cycle and

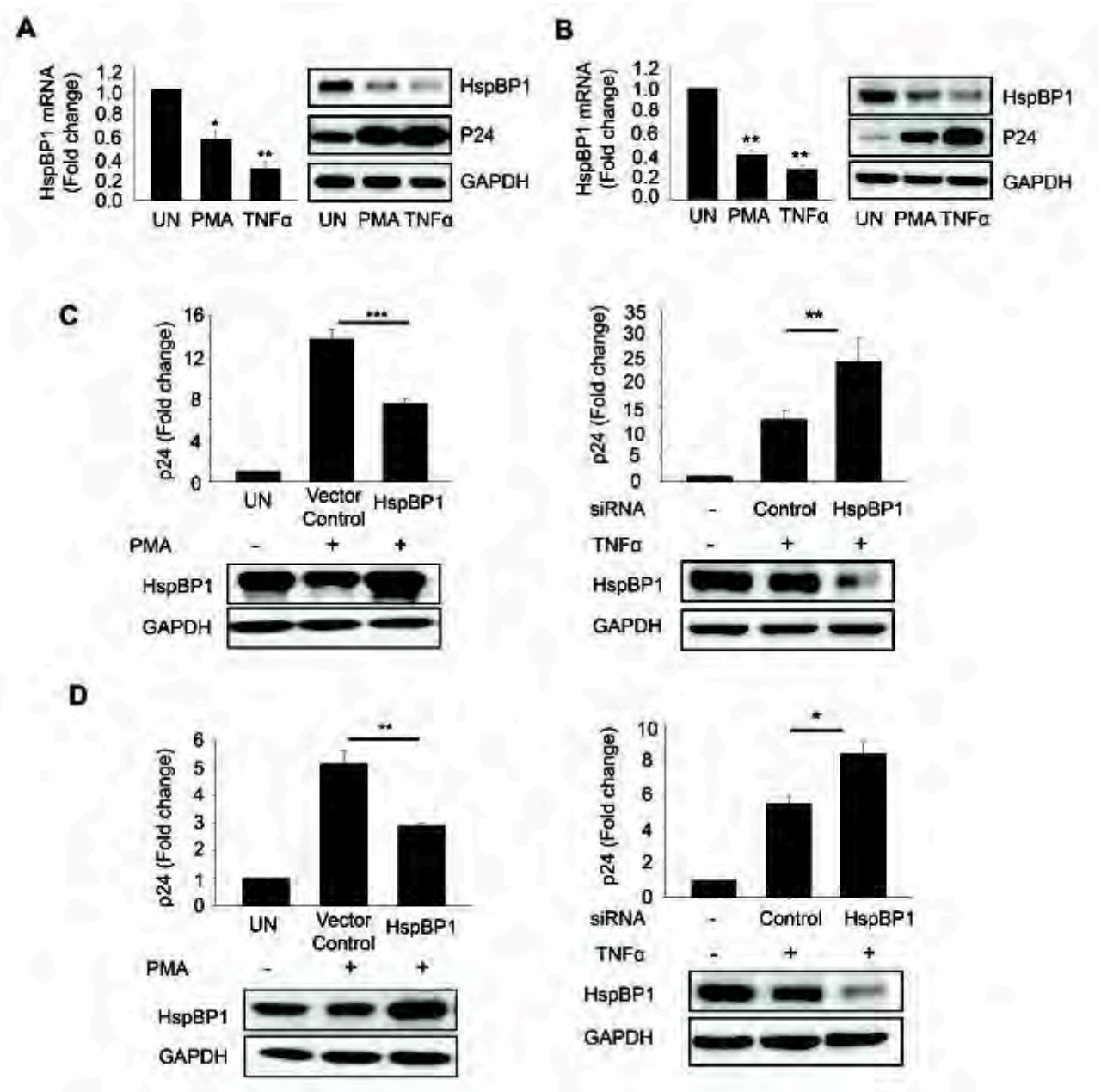


Fig. 2: HspBP1 inhibits activation of latently infected cells.

Activation of latently infected cells (A) ACH2 and (B) U1 leads to depletion in HspBP1 levels. ACH2 and U1 were stimulated with PMA (50ng/ml) and TNFα (10ng/ml). 36h post treatment, cells were harvested for RNA and protein lysate preparation. Expression of HspBP1 was analysed by qRT-PCR and immunoblotting. (C) HspBP1 over-expression inhibits virus production whereas HspBP1 can regulate various HSP70 activities; so it is worth studying the role of HspBP1 during HIV-1 infection, if any. We have observed earlier that expression of HspBP1 was down-modulated during HIV-1 infection in T-cells. We have also shown that silencing of HspBP1 seems to increase HIV-1 gene expression whereas its over-expression leads to inhibition of HIV-1 replication. Our results also indicated that over-expression of HspBP1 significantly reduces LTR-driven gene expression while silencing enhances it. Our recent studies clearly show that HspBP1 inhibits HIV-1 LTR mediated gene expression and viral replication

knockdown causes an increase in virus production in ACH2 cells. Nucleofection was performed to over-express or knockdown HspBP1 in these cell lines. 24h post nucleofection, cells were stimulated with PMA (50 ng/ml) or TNFα (10ng/ml). 24h post activation, p24 ELISA was performed with the supernatant to determine the amount of virus produced. HspBP1 over-expression and knockdown was confirmed by immunoblotting. (D) HspBP1 over-expression inhibits virus production whereas knockdown causes an increase in virus production in U1 cells. Error bars represent the mean ± SD values and significance is defined as ** $p \leq 0.01$ and *** $p \leq 0.001$.

by interacting with NF-κB enhancer sequences in the LTR promoter (Fig-1). We have also shown that HspBP1 competes with p65 of NF-κB heterodimer for recruitment on the κB enhancer site. Furthermore, we have shown that HspBP1 levels are increased in latently infected cells and upon activation, the levels of HspBP1 goes down. Recent results obtained with over-expression and silencing experiments show that HspBP1 inhibits activation of latently infected cells (Fig-2). Taken together, our study suggests that HspBP1 inhibits HIV-1 gene expression and replication by restricting p65 from binding to NF-κB enhancer sequence on the viral promoter (Fig-3)

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

Studies done till date to elucidate the pathways involved in HIV-1 induced T cell depletion has revealed that apoptosis underlie the etiology; however, a clear molecular understanding of HIV-1 induced apoptosis has remained elusive. Furthermore, HIV-1 infection is known to be associated with the hijacking of a number of cellular factors including the cell cycle associated molecules. The subversion of the host cell cycle during HIV-1 infection progression includes arresting of the normal cell cycle at mitosis, specifically at the boundary of the G2-M phase. We have initiated studies intending to look into the role of cell-cycle associated proteins in HIV-1 pathogenesis. In this context, we have performed differential gene expression analysis using PCR array specific for cell cycle associated genes. Our results show differential gene expression of a significant number of cell cycle associated genes. One such identified gene, Cyclin-F, is being currently studied to identify its role in HIV infection.

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

The current therapeutic strategy involving the use of reverse transcriptase and protease inhibitors in combination (HAART) has proven to be useful in controlling the virus but is not sufficient to eradicate the virus from the patients. Extensive work is being done throughout the globe to identify new ant-HIV therapeutic strategies. We have been also involved in identification of novel anti-HIV molecules and study of their potential use as microbicides. We have screened a library of pharmacologically active bio-molecules which are known to target cellular pathways for identification of novel anti-HIV molecules, with ultimate objective to identify novel cellular targets for inhibition of HIV-1. We

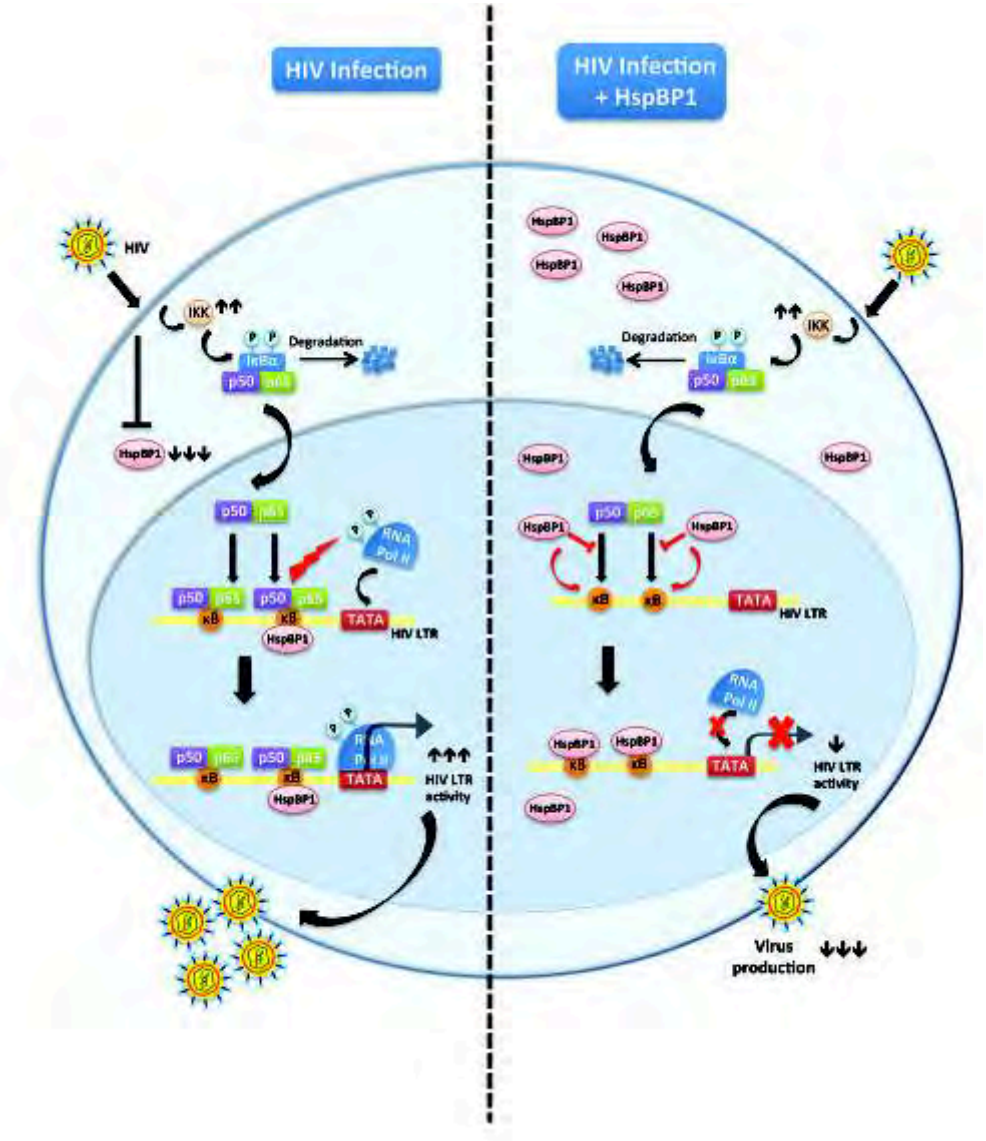


Fig. 3: Restriction of HIV-1 by HspBP1: Proposed mechanistic model.

Left, HIV-1 infection causes down-modulation of HspBP1 expression. Therefore, HspBP1 cannot inhibit HIV-1 production, consequently leading to efficient virus production. Right, when HspBP1 is over-expressed in an infected cell, HspBP1 interacts with NF-κB enhancer region (κB sites) on the HIV-1 LTR promoter and inhibits LTR driven gene-expression. The binding of HspBP1 to κB site obliterates the binding of NF-κB hetero-dimer (p50/p65) to the same region, leading to repression in HIV-1 transcription.

have been successful in identifying several promising bioactive molecules from the screening of this library and further characterization of few of these molecules are currently in progress. Identification of such novel targets may lead us to a novel therapeutic strategy to inhibit the virus.

Future Research Plans

Our results till date indicate that heat shock proteins play an important role during HIV-1 infection. We now intend to elucidate the role of individual heat shock protein isoforms in HIV-1 replication and pathogenesis, with specific reference to HSP70 and HSP40 isoforms. We are continuing characterization of few novel Nef interacting host cell proteins identified previously, for their

functional relevance in HIV life cycle. Identification of differentially expressed genes and their relevance to HIV induced cell death will be continued, with a focus on cell cycle associated proteins and autophagy. 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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A proteomic map of the unsequenced *Leishmania* vector *Phlebotomus* papatasi using cell line

Background

At least 20 species of the parasite of the genus *Leishmania* are known pathogens and they cause leishmaniasis in animals and humans. Leishmaniasis is a vector-borne disease transmitted by blood-sucking sand flies from infected patients or animals to healthy individuals. The parasite leads a digenetic life cycle as a motile extracellular promastigote within the gut of the sand fly and as an intracellular amastigote within infected host macrophages. Over 500 species of sand flies are documented till today and are assigned to three genera: *Phlebotomus*, *Sergentomyia* and Lutzomyia. The characteristic of vectorial capacity for leishmaniasis is mainly attributed to the genus *Phlebotomus* in the Old World and genus *Lutzomyia* in the New World. Development of resistance and toxicity to drugs to control leishmaniasis is a newer problem and the best way to limit leishmaniasis is to avoid sand fly bite and therefore the greater interest lies in the control of sand flies. To understand more the *Leishmania*-sand fly interactions, genomic and proteomic studies are being reported from different laboratories. We have carried out proteomic profiling of *P. papatasi* whose genome sequence is not yet reported.

Aims and Objectives

In the present study, we want to map the global proteome of the *P. papatasi* using a cell line as a model, utilizing a comparative proteogenomic-based approach.

Work Achieved

Continuous cell line PP-9 (cell line established from embryonic stage of *P. papatasi*) developed by Prof. Robert B. Tesh (presently at the University of Texas

Participants

S. Khobragade, *SRF*
M. Jhamdade, *SRF*
M. Mandania, *JRF*
M S Jadhav, *Technical Officer*
A N Atre, *Technical Officer*

Medical Branch) was used in this study. The cell lysate from PP-9 cells was resolved on SDS-PAGE and in-gel trypsin digestion (1:20 trypsin) of different protein bands was carried out. This was followed by extraction of peptides and peptide fractions were dried using vacuum drying process. In addition, cell lysate was also subjected to in-solution trypsin digestion. The peptides solution was fractionated by strong cation exchange chromatography. These fractions were completely dried, reconstituted and desalted and subjected to mass spectrometry analysis.

In total 16 LC-MS/MS runs were carried out and the mass spectrometry data generated in this study was searched against a three frame translated *P. papatasi* transcriptome database and a combined protein database of four related dipterans using 'Mascot' and 'Sequest' search algorithms. In this study 41,460 MS/MS spectra were acquired, which resulted in identification of 12,802 peptide spectrum matches. In total 4,052 unique peptides that passed the 1% false discovery rate threshold were identified from three frame translated transcript database and related dipteran protein database searches. This in turn resulted in identification of 1,313 unique proteins in *P. papatasi*.

Four dipteran species, namely *Ae. aegypti*, *An. gambiae*, *C. quinquefasciatus and D. melanogaster* have their genomes completely sequenced and these insects are closely related to sand flies. A combined protein database was generated from the genomes of these four insects and was used for searching the *P. papatasi* mass spectrometry data. This has resulted in the identification of 1,381 unique peptides that mapped to 583 proteins from the four related dipterans. Majority of the identified 569 peptides mapped to 192 proteins in Ae. aegypti followed by 520 peptides mapping to 166 proteins in *D. melanogaster* and 423 peptides mapped to 113 proteins from *An. gambiae*. The least number of peptides (310) mapped to 112 proteins in *C. quinquefasciatus*.

The genome sequence of sand flies including P. papatasi is unavailable as of today. In this study, to overcome the non-availability of genome data for proteomic analysis, the published transcriptome data was used for the proteogenomic analysis. We generated a three frame translated database from published transcriptome data of *P. papatasi* in order to identify protein coding genes. *P. papatasi* mass spectrometry data was searched against the three frame translated transcript database and has resulted in identification of 3,380 unique peptides. This in turn resulted in the identification of 927 translated protein entries in the three frame database.

These proteins when classified based on their functionality, were found to contain all the sets that are essential for cellular organization, viability and proliferation. Because the PP-9 cells were established from embryonic tissue, proteomic profile obtained from mass spectrometry analysis showed stark absence of proteins that are associated with terminally differentiated cells or organs. For example salivary gland enzymes and proteins which were previously reported in several proteomic studies of sand flies were not identified in this study. However, because PP-9 cell line is of embryonic origin, few proteins such as Asrij [required for maintenanceof the Stem Cell Niche], Endophilin b [regulation of membrane dynamics in developing egg chambers], Midline fasciclin [mediates cell adhesion and signaling, plays multiple developmental role] and Rings lost [required for ring canal growth in germline cysts] were detected. The present proteogenomic analysis of *P. papatasi* illustrates the value of high-resolution MS-derived peptide data in mapping the proteome of sand flies. Our study reports the largest catalog of *P. papatasi* proteome till date and many proteins identified in this study were not previously reported previously in sand flies.

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Neuro-immuno-pathogenesis in murine experimental cerebral malaria

Background

Plasmodium falciparum inflicts malaria that can turn into potentially fatal cerebral malaria, which is characterized by breached blood-brain barrier, cerebral sequestration of parasitized RBCs and lymphocytes infiltration. These pathological features are expressed in experimental cerebral malaria (ECM) in *Plasmodium berghei* ANKA (PBA)-infected C57BL/6 mice. Studies with this murine malaria model have shown the role of cytokines and chemokines in ECM development. But the expression of these molecules at different stages of disease progression was not known. Therefore, we characterized the neuro-immunopathological changes and the expression of cytokines, chemokines and inhibitory ancillary molecules during different phases / time points after parasite infection in mice that developed ECM. Moreover, infectious pathogens have evolved a range of strategies that can positively or negatively modulate immune activation, thereby altering the host's immune function to their own benefit. The outcome of microbial infections is dependent on the balance between pro-inflammatory and regulatory immune responses. Priming of naive T cells and their differentiation into effector cells needs to be balanced by switching off these cells at an appropriate stage of infection, in order to prevent tissue damage (immune pathology). Further, the balance between pro-inflammatory and regulatory immune responses in determining optimal T cell activation is vital for the successful resolution of microbial infections and it has been reported that negative stimulatory molecules suppress the effector responses during chronic infections. Although the interactions between CTLA-4 or CD28 and B7-1/B7-2 are the archetypal receptor ligands in T cell-APC activation, a number of additional functional receptors and B7 family members

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have been identified recently. These include programmed death ligand 1 (PD-L1), also known as B7-H1, and PD-L2 (B7-DC), that interact with programmed death 1 (PD-1) on T cells, resulting in regulation of T cells. *P. falciparum* infection in humans is associated with higher expression of the inhibitory receptor PD-1 and T cell dysfunction. The brains of C57 mice developing ECM are immunologically active, as reported earlier, due to infiltration of lymphocytes in the tissue and its involvement in behavioral alterations and immunopathology of the disease. The blockade of PD-L1 and LAG-3 resulted in rapid clearance of parasites in murine malaria. The role of inhibitory molecules seems to independently regulate host resistance to *Plasmodium*-induced acute immune pathology in murine ECM. However, the role of the expression of these molecules in the brain during the progression of disease in ECM mice has not been clearly reported. We have studied expressions of these molecules along with other molecules at different time points starting from parasite infection in C57 to ECM stage, in different areas of brain.

Aims and Objectives

To study:

- Host-parasite interactions.
- Studies on murine malaria pathogenesis/.
- Parasite-macrophage interactions.

Work Achieved

C57BL/6 mice were infected with PBA parasites. Animals were classified on the basis of neurological features induced by the parasites and ECM, including paralysis and coma. Progression of the disease was correlated with parasitemia, cytokines and chemokines. Expression of the genes for cytokines, chemokines and ancillary inhibitory molecules and histopathological changes in the different areas of brain were also examined. A significant increase in the levels of cytokines and chemokines were detected in sera and a selective up-regulation in mRNA expression of genes for cytokines, chemokines and inhibitory molecules was observed in different areas of the brain in 5 dpi animals, as compared to 3 dpi animals. During the disease progression, the animals did not show any lesion in the cerebellum, cerebrum, hippocampus, midbrain and olfactory bulb of the brain in 3dpi animals, whereas the tissues from 5dpi animals showed parasite accumulation with edema, while the tissue from ECM mice showed hemorrhages. The mRNA levels of the genes for receptors of TNF and IFN-γ in the brain were up-regulated significantly only in mice that had developed ECM. These studies show complex expression profiles of cytokines,

chemokines and ancillary inhibitory molecules in mice developing ECM from PBA parasite infection, coinciding with parasitemia, histopathological changes and neuro behavioral features. These observations provide a kinetic view of ECM development for the first time. These results show drastic transformation of ECM developing C57BL/6 mice, from PBA parasite infection through different stages to ECM. Increased alteration in histology were observed during disease progression in different parts of the brain, lung, spleen and liver tissues, as well as up-regulation of sera cytokines and chemokines, and enhanced expression of different genes of cytokines, chemokines and inhibitory molecules in the brain tissue. This could be due to pRBC accumulation, activation of resident brain cells and infiltration of immune cells in the brain. These studies also revealed the fluctuations and selective expression of these genes in different areas of the brain at different time points during ECM development.

The parasite induces up-regulation of both, pro-inflammatory molecules as well as inhibitory molecules simultaneously in the same areas of brain tissue. It appears that the collective immune response including both pro-inflammatory and inhibitory molecules might have a role in the disease progression in ECM developing mice. Further, the significantly increased expression of cytokines and chemokines and tissue alteration particularly in the brain clearly coincided with the appearance of neurological characters in the infected mice. These studies show that some molecules are expressed progressively though the advancement of the disease after infection, from the beginning, whereas expression of other genes is correlated with appearance of clinical features, and some specific molecules are up- regulated only after onset of ECM.

Future Research Plans

Studies on the interactions of parasitized RBCs with macrophages.

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

Background

Breast cancer incidences appertains to greater than 1.3 million women each year throughout the world with a mortality rate of about 14% of the total cancer-related death. Presently, breast cancer incidences are observing an age shift pattern in India as the average age of developing breast cancer has shifted from 50 - 70 years to 30 - 50 years age group. The primary reason for the high mortality of breast cancer is the lack of awareness about the disease in women, diagnosis in later stages, unavailability of suitable sensitive and specific markers and lack of proper screening protocols at the healthcare facilities. Breast cancer is characterized as heterogeneous in nature and has different tumor subtypes such as luminal (luminal A and luminal B), human epidermal growth factor receptor 2 (HER2) enriched, basal-like and normal breast-like subtype. In the current cancer treatment practice, tumor histological factors and subtypes are mainly used to determine the therapy for the patient. The foremost screening test for breast cancer is mammography but it very often gives false negative results in different age groups, not able to detect breast cancer and most importantly fails to detect the tumor in young women as dense breast tissue decreases mammographic sensitivity. Hence, there is an urgent need to come up with potential biomarkers which can support the clinicians in early diagnosis as well as differentiating and identifying the breast cancer subtypes.

Quantitative proteomic and metabolomic profiling of body fluids, tissues, or other biological samples to identify differentially expressed proteins / metabolites represents a very promising approach for discovering novel potential biomarkers. Proteins and metabolites associated with breast cancer

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identified through proteomic and metabolomic profiling technologies could be useful as biomarkers for the early diagnosis, assessment of prognosis, prediction of therapeutic effect and treatment monitoring. In this work, we plan to identify non-invasive potential biomarkers for breast cancer using high throughput mass spectrometry based proteomic, lipidomic and volatomic approaches in Indian scenario.

Aims and Objectives

- Identification of urinary proteomic biomarkers in HER2 enriched breast cancer using multipronged quantitative proteomic approaches.
- Investigation of serum phospholipid alterations in breast cancer using mass spectrometry.
- Identification of urinary volatile metabolites involving breast cancer towards early diagnosis.

Work Achieved

Identification of urinary proteomic biomarkers in HER2 enriched breast cancer using multipronged quantitative proteomic approaches.

Although biomarker discoveries through various proteomic approaches of tissue and serum samples have been studied in breast cancer, urinary proteome alterations in breast cancer is least studied. Urine, being a non-invasive biofluid and a significant source of proteins has potential in early diagnosis of breast cancer. The breast cancer heterogeneity at the molecular level (subtypes) is well defined and it is a very important aspect to decide the line of treatment according to the subtype of breast cancer. Breast cancer related to the HER2 subtype has molecular markers but the diagnostic tests for confirming these markers are expensive, time consuming and utilize invasive sampling. The present study used complementary quantitative gel based and gel free proteomic approaches to find a panel of urinary protein markers that could discriminate HER2 enriched (HE) subtype breast cancer from the healthy controls. Gel based DIGE analysis identified 30 non-redundant differentially expressed proteins in which 11 up-regulated and 19 were down-regulated. Representative 2-D DIGE image of control vs. HE urine including expression levels of selected proteins are shown in Fig. 1A-1C. Gel free iTRAQ analysis yielded 78 differentially expressed proteins with minimum two unique peptides out of which 26 proteins showed up-regulation and 52 proteins showed down-regulation. Label free SWATH analysis detected 132 non-redundant proteins being differentially expressed out of them 49 proteins were up-regulated and the rest showed down-regulation. A total of 183 differentially expressed

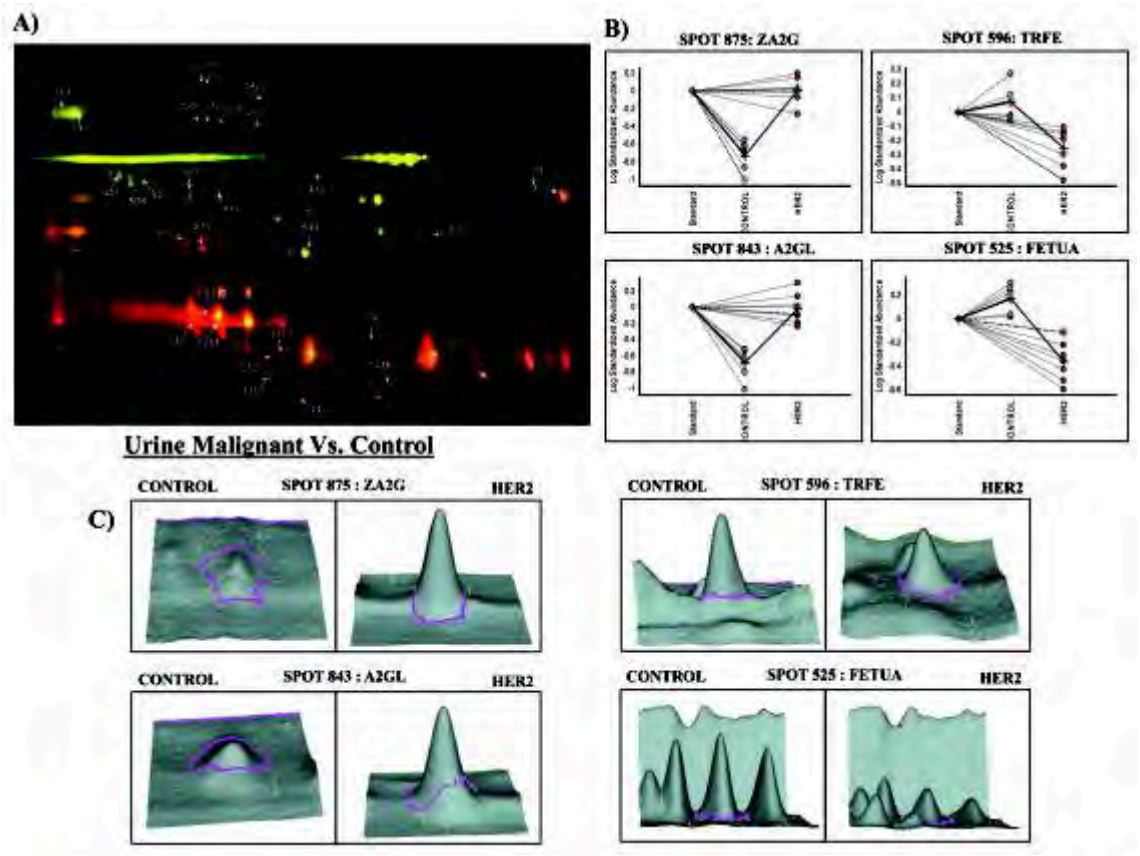

Fig. 1: The representative DIGE image for A) Urine Malignant Vs. Control, B) Expression profile of identified proteins obtained through BVA module of DeCyder software and C) 3-D View of identified proteins obtained through BVA module of DeCyder software.

proteins were identified using three complementary approaches viz. 2D-DIGE, iTRAQ and SWATH. The differentially expressed proteins were subjected to various bioinformatics analyses for deciphering the biological context of these proteins using PANTHER, DAVID and STRING. The bioinformatics analysis revealed that differentially expressed proteins were involved majorly in binding, receptor activity, enzyme regulator activity and transporter activity. Many physiological pathways were found to be altered due to these differentially expressed proteins, some of which are interleukin signaling pathway, VEGF and FAS signaling pathway, Wnt signaling pathway, p38 MAPK pathway, integrin signaling pathways, inflammation mediated by chemokine and cytokine signaling pathway, EGF receptor signaling pathway. Multivariate statistical analysis was undertaken to identify the set of most significant proteins, which could discriminate HE breast cancer from healthy controls. Immunoblotting and MRM based validation in a separate cohort testified a panel of 21 proteins including ZA2G, A2GL, RET4, ANXA1, SAP3, SRC8, GELS, KNG1, CO9, CLUS, CERU and A1AT could be a panel of candidate markers that could discriminate HE breast cancer from healthy controls.

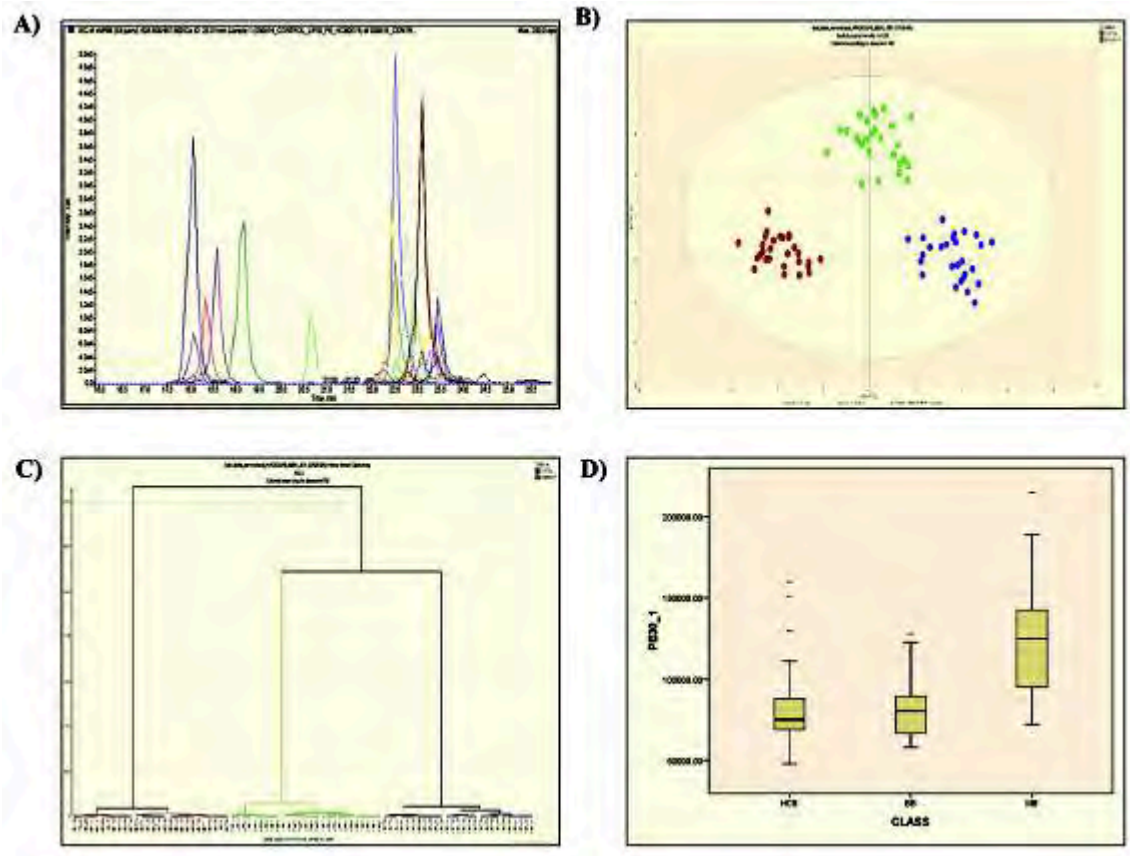

Fig. 2. Serum phospholipid analysis A) Representative MRM chromatogram of a PC obtained from serum of malignant patient, B) OPLS-DA score plot discriminating malignant, benign and controls groups, C) Hierarchical clustering analysis showing clustering of malignant, benign and healthy controls. D) Box-and-whisker plots illustrating the significant differences in phospholipids concentration among malignant patients, benign patients and healthy controls.

Investigation of serum phospholipid alterations in breast cancer using mass spectrometry

Phospholipids, major constituents of cell membranes are involved in various physiological processes including cell signaling, migration and invasion. Cancer development is complex mechanism, which depends on the interplay between these intricate processes. As the newly proliferating tumor cells would demand more basic components including phospholipids that are used in physiological process. Hence, in this study we investigated serum phospholipid alteration in breast cancer which will give more insights on breast cancer progression. For the analysis of phospholipids, a lipidomic approach using LC-MS/MS with the help of multiple reaction monitoring (MRM) was adopted (Fig. 2A). MRM transitions were developed by using head group specific losses of each phospholipid class such as phospatidylcholine (PC), phophatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS). We have analysed phospholipids alterations in 28 serum samples from each group of benign, malignant and controls by multivariate statistical analysis. Orthogonal-partial least squares discriminant (OPLS-DA) classification model was clearly able to classify malignant patients, benign patients and healthy controls (Fig. 2B).

Hierarchical clustering analysis resulted in distinct clusters of malignant, benign and healthy controls shown in Fig. 2C. Out of 210 targeted phospholipids, 31 statistically significant phospholipids were identified. Amongst these, 11 phospholipids show specific alterations in malignant breast cancer than benign and controls with higher VIP score (>1.4), P value (<0.01) and AUC value (>0.70). LPC 16:4, PE 38:7, PE 27:3, PE 30:1, PS 42:11, PC 38:2 showed progressive increase towards malignant from control and benign (Fig. 2D) whereas SM 34.2, PE 42:2, PC 36:0 and PE 44:4 progressively decreased in malignant patients. In our study we majorly observed the alteration of PC, SM, and PE group of phospho lipids. High levels of PC and SM have been reported to be related with cell migration and metastasis. PE helps in maintaining fluidity of membrane and regulates Ca^{+2} transportation which in turn activate intracellular signaling and help in cell differentiation. These findings are not only helpful to distinguish breast cancer from healthy controls and benign but also useful to understand the biochemical pathways involved in breast cancer.

Identification of urinary volatile metabolites involving breast cancer towards early diagnosis

The diagnostic tests available for breast cancer at present are expensive and involve invasive procedures which makes unsuitable for screening of large population. It is a well-known fact that with the diagnosis at early stages of disease, the 5-year-survival rate is increased by 3-4 folds. Various omics based biomarker discovery for diagnostic and theranostic aspects of breast cancer are currently in practice. Our group aims towards a novel approach of analysis of volatile organic metabolites (VOMs) consumed or released by cancerous cells using non-invasive body fluids. It is highly sensitive, does not require invasive procedures and intervention of specialized medical staff. Therefore, this diagnostic approach can be easily disseminated through countries where the economic resources and advanced infrastructure are not available. This study aims towards establishment of the urinary metabolomic biosignatures of breast cancer patients and healthy individuals (control group) and to explore the VOMs as potential biomarkers in breast cancer diagnosis at early stage. Solid-phase micro extraction (SPME) as pre-concentration technique was used with gas chromatography–mass spectrometry (GC-MS) was utilized to identify the metabolomic patterns of 69 breast cancer patients and 70 healthy individuals (controls) represented in Fig 3A. 98 VOMs belonging to distinct chemical classes like organic acids, ketones, sulphur derivatives, ethers, benzene derivatives etc. were detected and identified in breast cancer and control

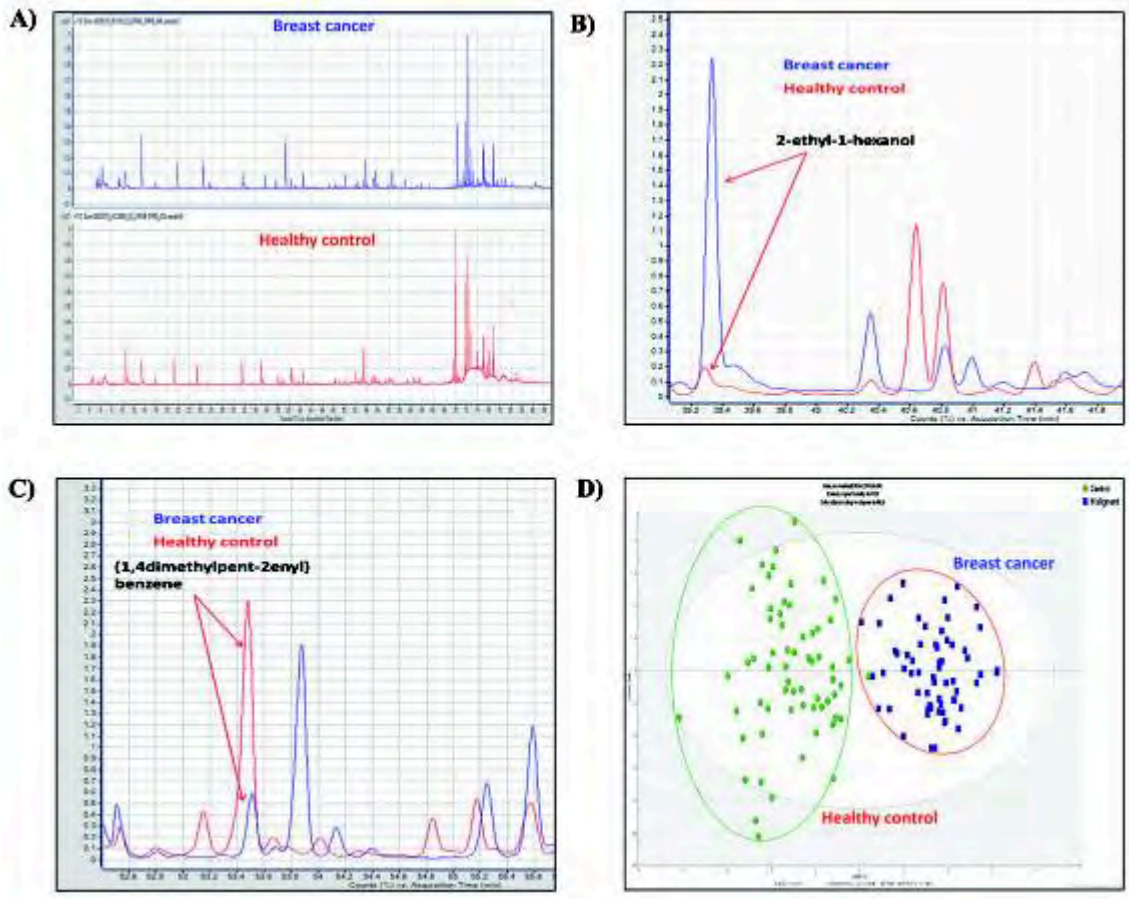

Fig. 3: The representative images for A) Breast Cancer Vs. Healthy Control GC chromatogram, B) Up-regulated expression profile of 2-ethyl-1-hexanol VOM, C) Down-regulated expression profile of 1,4 dimethylpent-2-enyl benzene VOM and D) Multivariate Statistical analysis through OPLS-DA based classification model of Breast Cancer and Healthy Controls.

groups. The univariate fold change analysis (threshold of 1.5) revealed 17 VOMs to be having elevated levels (Fig. 3B) while 8 VOMs had decreased levels (Fig. 3C). The data obtained was further subjected to multivariate statistical analysis to visualize clustering patterns of disease and to detect the VOMs that are able to differentiate cancer patients from healthy individuals. Very clear discrimination within breast cancer and control groups was achieved using different modelling approaches like PLS-DA and OPLS-DA (Fig. 3D).

Future Research Plans

- Investigation of subtype level serum proteome alterations in breast cancer using multipronged quantitative approaches.
- Investigation of subtype level serum metabolite alterations in breast cancer using untargeted and targeted approaches.
- Identification of salivary volatile metabolites involving breast cancer towards early diagnosis.

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

Background

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

Aims and Objectives

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

Work Achieved

As stated in the future plans of the previous year, the role of Ras isoforms in anti-leishmanial immunotherapy is being examined in detail.

Future Research Plans

We will continue examining how Ras isoforms play a role antileishmanial immunotherapy and immunoprophylaxis.

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

Background

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

Aims and Objectives

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

Collaborators

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Work Achieved

We are presently identifying the intermediates recruited to the CD40 receptor at different time points on stimulation with three different doses of CD40 ligand using LCQ-Orbitrap system and then validating the intermediates identified by western blotting.

Future Research Plans

- To complete of identification of intermediates and their validation.
- Identification of role of different signalling intermediates by analyzing their expression profile as a function of dose and time.

Publications

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- Arkyajyoti Mukherjee, Sayoni Roy, Bhaskar Saha, Debasri Mukherjee. Spatio-Temporal regulation of PKC isoforms imparts signaling specificity. Frontiers in Immunology. 2016. 7 (45): 1-7. [First paper as corresponding author]

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Role of complement in pandemic influenza A (H1N1) 2009 virus infection

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Background

The pandemic influenza A (H1N1) 2009 virus caused significant morbidity and mortality worldwide. Moreover, it is now circulating seasonally and causing a significant disease burden. Hence, it is important to delineate the immune factors that influence its control. Earlier, the complement system has been shown to provide protection during seasonal influenza infection however, the role of individual complement pathways was not yet clear. In the present study we therefore have asked what role intact complement and its individual complement pathways play in controlling the pandemic influenza virus infection, and whether the pandemic influenza virus is susceptible to neutralization by various complement pathways. Our data show that deficiency of intact complement results in heightened vulnerability to the pandemic influenza virus infection in mice leading to complete mortality, and synergy between the classical and alternative pathways is necessary for efficient protection.

Aims and Objectives

- To dissect the role of individual complement pathways in pandemic influenza A(H1N1) 2009 virus neutralization.
- To dissect the role of individual complement pathways in controlling pandemic influenza A(H1N1) 2009 virus infection.

Work Achieved

Neutralization of pandemic influenza A(H1N1) 2009 virus by complement

We first sought to determine whether the pandemic influenza virus is susceptible to neutralization by all the three pathways. We thus measured the

complement-mediated neutralization of the virus by treating it with mouse complement under pathway specific conditions. The results showed that the virus is susceptible to the classical pathway (CP)-mediated neutralization, but resistant to the alternative pathway (AP) and lectin pathway (LP) mediated neutralization. Earlier studies have shown that antibodies can also act as C3 acceptor surface hence we tested the possibility whether the pandemic virus becomes susceptible to AP-mediated neutralization when coated by antibodies. The data revealed that the virus indeed becomes susceptible to neutralization by AP when coated by antibodies. Together these results suggest that the virus becomes susceptible to complement either by CP or by AP only when coated by antibodies.

Next, we addressed whether CP and AP work synergistically to neutralize the pandemic virus. We thus tested if the CP-mediated deposition of C3b onto the viral surface leads to activation of the AP loop and thereby augment neutralization. Examination of the CP-mediated neutralization of the virus using $FB^{-/-}$ mouse plasma showed significant reduction in the extent of neutralization compared to the normal mouse plasma suggesting that synergy does exist between the CP and AP during the pandemic virus neutralization. Further, C5-deficient plasma was able to neutralize the virus as efficiently as the C5-sufficient plasma. It is thus evident that MAC-mediated lysis is not necessary for the neutralization of the pandemic influenza virus.

Since influenza A(H1N1) pdm09 virus is a human pathogen, we also tested if the virus is susceptible to human CP-, AP- and LP-mediated neutralization. The results were essentially similar. Intrigued by the finding that the pandemic influenza virus is resistant to AP-mediated neutralization, we next asked whether seasonal influenza A virus, A/Perth/16/2009(H3N2), is also resistant to AP-mediated neutralization. Our results showed that unlike the pandemic influenza virus, seasonal influenza virus is susceptible to AP-mediated neutralization. These results intrigued us to investigate whether the difference in AP-mediated neutralization of the above two viruses is due to the difference in the ability of their surfaces to allow C3b deposition. Activation of C3 near their surfaces using purified complement components confirmed our presumption: C3b deposition was efficient on the H3N2 viral surface, but not on the pandemic influenza virus unless coated by the antibodies. Thus, there exists distinct difference in the susceptibility of these two viruses towards AP-mediated neutralization.

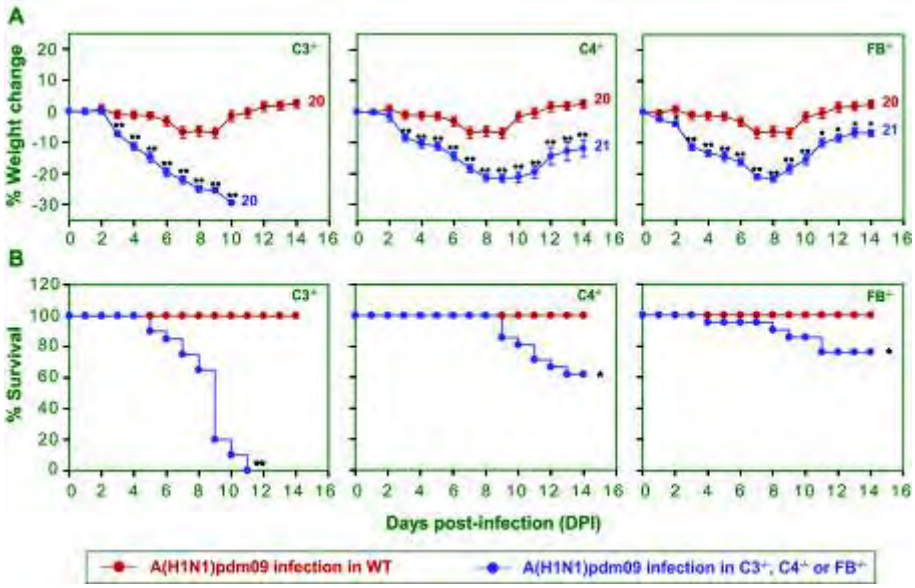

Fig. 1: Complement deficient mice are highly susceptible to A(H1N1)pdm09 virus infection. A) Percentage of body weight loss during the infection in $C3^{-/-}$, $C4^{-/-}$ and $FB^{-/-}$ mice in comparison to wild-type (WT) mice. Body weight was normalized to their initial body weight. The numbers at the end of the lines indicate the total number of animals utilized in each group. Statistical comparisons were performed between the WT mice and the complement deficient mice ($C3^{-/-}$, $C4^{-/-}$ and $FB^{-/-}$). *p < 0.01 and **p < 0.001. B) Percentage of survival during the infection in $C3^{-/-}$, $C4^{-/-}$ and $FB^{-/-}$ mice in comparison to WT mice. *p < 0.02; **p < 0.001.

Role of individual complement pathways in controlling pandemic influenza A(H1N1) 2009 virus infection in mice

Because the pandemic virus is susceptible to complement-mediated neutralization, we next asked whether presence of intact complement is necessary for controlling its infection in mice. Thus, we performed infection experiment in $C3^{-/-}$ mice which are devoid of complement effector function. These mice showed severe illness with significant weight loss leading to 100% mortality by day 11 post-infection (p.i.) (Fig. 1A & 1B). In contrast, WT mice showed only 10% weight loss at the peak of infection, and all mice fully recovered at day 12 p.i (Fig. 1A & 1B), strongly establishing that complement plays a protective role during the pandemic influenza virus infection. Next, to determine the contribution of the individual pathways, we infected $C4^{-/-}$ (deficient in CP and LP) and $FB^{-/-}$ (deficient in AP) mice. Both the knockout strains showed significant weight loss compared to the WT mice (Fig. 1A) with 32% mortality in $C4^{-/-}$ and 24% mortality in $FB^{-/-}$ mice (Fig. 1B). These data support the in vitro virus neutralization data that cooperativity between the pathways is needed for providing protection against the pandemic influenza virus infection.

To look into the possible cause of lethality, we examined the effect of the deficiencies on the histopathological changes and viral clearance in the lung. We observed an overall higher degree of inflammation in $C3^{-/-}$ and $FB^{-/-}$ mice compared to the WT mice, but not in $C4^{-/-}$ mice i.e., the lethality in mice did not correlate well with exacerbated inflammatory changes. The viral clearance in the lung however showed a better correlation with lethality: WT mice effectively cleared the virus from the lung by day 7 p.i., but the same was not observed in

$C3^{-/-}$ mice. The viral load also remained high in $C4^{-/-}$ and $FB^{-/-}$ mice lungs, but was lower compared to that observed in $C3^{-/-}$ mice. Intriguingly, initial virus load did not differ between the WT and complement deficient mice suggesting that complement-mediated protection seen in WT mice was a late phenomenon which occurred after day 5 p.i.

Interestingly, the lethality in mice correlated with decreased antibody response against influenza hemagglutinin. To establish the causal relationship between reduction in response to HA and higher lethality in $C3^{-/-}$ mice, we performed rescue experiment with immune sera from the virus infected WT and $C3^{-/-}$ mice collected 7 days p.i., a time point at which significant mortality was observed. Injection of WT immune sera to $C3^{-/-}$ mice infected with the virus provided significant protection, while injection of $C3^{-/-}$ immune sera failed to provide any protection. Earlier C3a and C5a have been shown to shape the acquired immunity hence we sought to characterize the role of C3a and C5a during the infection. Blocking C3aR/C5aR signaling using receptor antagonists resulted in high degree of lethality in the WT mice infected with the pandemic influenza virus suggesting that these peptides are critically involved in boosting the influenza-specific immune responses.

In summary, our data reveal the importance of cross-talk between the CP and AP that provides sufficient trigger (C3b deposition and C3a/C5a production) required for efficient protection against the pandemic influenza virus infection. Based on our data and earlier studies, we propose the following model for complement-mediated protection during the pandemic influenza H1N1 2009 virus infection: i) recognition of the pandemic influenza virus by antibodies triggers the activation of both CP as well as AP leading to C3b deposition and

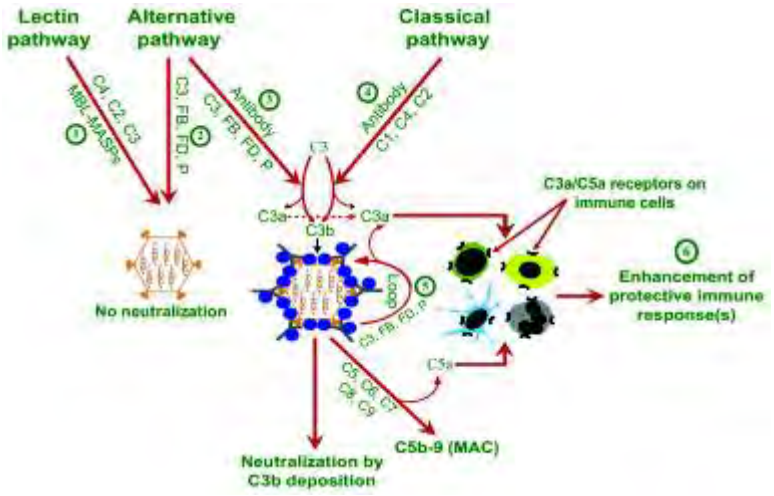

Fig. 2: Model for the interplay between CP and AP during A(H1N1)pdm09 infection. Synergy between CP and AP is critical to contain influenza A(H1N1)pdm09 virus infection. 1) The virus is resistant to LP-mediated neutralization. 2) It is also resistant to AP-mediated neutralization. 3) It becomes susceptible to AP-mediated neutralization when coated with virus-specific antibodies. 4) It is susceptible to CP-mediated neutralization. 5) Augmentation of CP initiated complement activation through activation of AP-loop results in enhanced CP-mediated neutralization. 6) Complement activation products, C3a and C5a help in the enhancement of protective immune responses owing to activation of immune cells.

direct neutralization of the virus to a certain extent, ii) the complement activation fragments C3a and C5a generated as a result of complement activation enhance the effector T cell as well as B cell responses, and iii) the effector CD8+ T cells and antibodies then efficiently contain the virus infection (Fig. 2)

Future Research Plans

- To investigate the role of locally produced C3a, C4a and C5a during viral infections.
- To define the immune responses generated during viral infections in the presence of locally produced C3a, C4a & C5a.
- To delineated the immune mechanisms responsible for locally-produced C3a, C4a & C5a-mediated protection against viral infections.

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FBXO31 degrades MDM2 to facilitate p53-mediated growth arrest following genotoxic stress

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Background

Cancer is one of the foremost causes of death worldwide. It manifests due to uncontrolled proliferation of genomically unstable abnormal cells. Such abnormal cells are generated due to the the transformation of normal cells either due to the activation of proto-oncogenes and/or the inactivation of tumor suppressor genes. One of the most fundamental and extensively studied anticancer mechanisms is the large increase in the levels of the tumor suppressor p53, which occurs following DNA damage. The increased p53 then mediates growth arrest and/or apoptosis. The mutational or functional inactivation of the p53 gene observed in more than 50% of human cancers shows the importance of its anticancer mechanism.

p53 is maintained at a low level in unstressed cells. The major negative regulator of p53 is MDM2, an E3 ubiquitin ligase that interacts directly with p53 and promotes its polyubiquitination-mediated proteasomal degradation through 26S proteasome. Following DNA damage, p53 is stabilized due to rapid degradation of MDM2. Originally it was proposed that MDM2 degradation was caused by auto-ubiquitination; however, subsequent experiments showed that the E3 ubiquitin ligase activity of MDM2 is not required for its own degradation.

Previous studies showed that F-box protein FBXO31 functions as a tumor suppressor. It arrests the cells at the G1 phase of the cell cycle through proteasomal degradation of cyclin D1. Further, it acts as a dedicated DNA damage checkpoint protein. Both p53 and FBXO31 can induce growth arrest,

and we have found that after DNA damage there is a posttranslational increase in FBXO31 levels, as there is for p53. These considerations prompted us to ask whether there is a functional relationship between FBXO31 and p53.

Aims and Objectives

- Does FBXO31 has any role in the stability of p53 under genotoxic stresses?
- What is the molecular mechanism of regulation?

Work Achieved

Does FBXO31 have any role in the stability of p53 under genotoxic stresses?

First, we asked whether the ability of FBXO31 to induce growth arrest results, at least in part, from the regulation of p53 levels. Toward this end, we have generated MCF7 (p53-positive) stable knockdown cells expressing either control nonsilencing (NS) shRNA or an FBXO31 shRNA (FBXO31-KD). NS and FBXO31-KD cells were treated with the DNA-damaging agent camptothecin or γ-irradiation, and the levels of p53 and MDM2 were analyzed by immunoblotting. Since post-translation levels of MDM2 decrease rapidly following genotoxic stress, we therefore monitored the levels of p53 and other proteins at early times after the induction of DNA damage. Levels of FBXO31 increased significantly in MCF7 NS cells within 45 minutes of treatment with either camptothecin (Fig. 1A) or γ-irradiation (Fig. 1B), as was consistent with previous results. This increase in FBXO31 levels was accompanied by decreased MDM2 levels and increased p53 levels in MCF7 NS cells (Fig. 1A and 1B). Following camptothecin or γ-irradiation treatment of FBXO31-KD cells, there was a marked reduction in FBXO31 levels, as expected, but notably, MDM2 and p53 levels remained constant (Fig. 1A and 1B). Similar results were obtained in p53-positive IMR90 cells (data not shown).

A previous study suggested that following DNA damage the apparent decrease in MDM2 levels as monitored by immunoblotting is caused in actuality not by MDM2 degradation but rather by a conformational change in MDM2 that results in the masking of epitopes recognized by monoclonal anti-MDM2 antibodies. Later, this conclusion has been challenged by other studies. We performed two additional experiments to confirm that MDM2 is truly degraded following DNA damage in NS cells but not in FBXO31 KD cells. First, in the experiments described above, MDM2 levels also were monitored using a polyclonal anti-MDM2 antibody (N20). Similar to the results with the AB1 monoclonal anti-MDM2 antibody, we found that after DNA damage MDM2

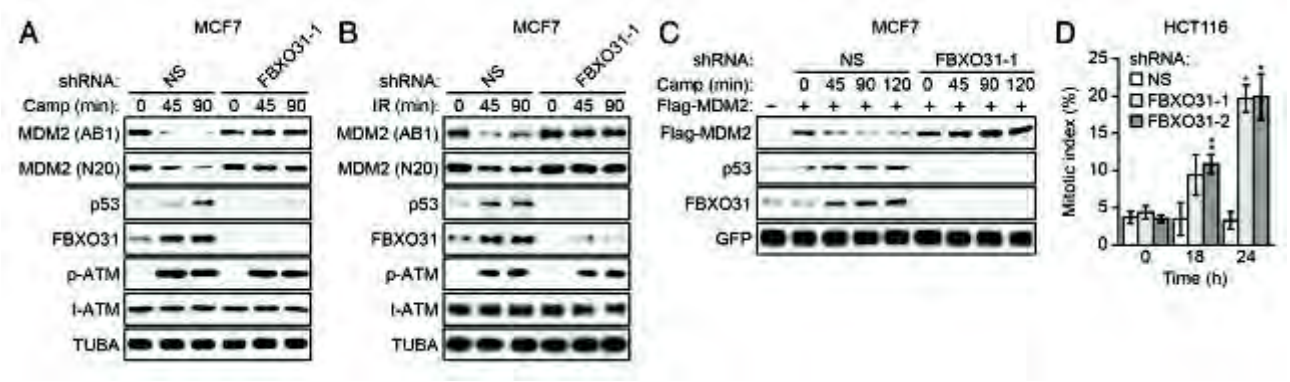

Fig. 1: FBXO31 is required for decreased MDM2 and increased p53 levels following DNA damage. (A and B) Immunoblot monitoring MDM2 [using a monoclonal (AB1) or polyclonal (N20) antibody], p53, FBXO31, phosphorylated ATM [p-ATM(1981)], and total ATM (t-ATM) in MCF7 cells expressing NS or FBXO31 shRNA and treated in the presence (45 or 90 min) or absence (0 min) of camptothecin (A) or γ-irradiation (IR) (B). α-tubulin (TUBA) was monitored as loading control. (C) Immunoblot monitoring Flag-MDM2, p53, and FBXO31 in MCF7 cells expressing Flag-MDM2 and NS or FBXO31 shRNA and treated in the presence or absence of camptothecin. GFP, expressed from a cotransfected plasmid, was used as a transfection and loading control. (D) Mitotic index analysis of HCT116 cells expressing NS or FBXO31 shRNA. Error bars indicate SD. *P≤ 0.05, **P≤0.01.

levels decreased in cells expressing the NS shRNA, but not in FBXO31 KD cells (Fig. 1 A and 1B). Second, we ectopically expressed an N-terminal Flag-tagged MDM2 in control or FBXO31 KD MCF7 cells and, after DNA damage, monitored MDM2 levels using an anti-Flag antibody. The immunoblot results in figure 1C show that after camptothecin treatment in MCF7-NS cells, the levels of ectopically expressed Flag-MDM2 decreased, and this decrease was accompanied by increased levels of endogenous p53. In contrast, after camptothecin treatment in FBXO31-KD cells, the levels of ectopically expressed Flag-MDM2 and endogenous p53 were unaffected (Fig. 1C).

The finding that in FBXO31-KD cells p53 levels failed to increase following DNA damage suggested that growth arrest would not occur efficiently. To test this prediction, we measured the mitotic index of NS and FBXO31-KD cells in the presence of nocodazole to trap cells in mitosis. After DNA damage, cells harboring p53 will arrest in G2 and G1, whereas cells lacking p53 will progress through the cell cycle and enter mitosis.

These experiments were performed in p53-positive HCT116 cells, which previously have been shown to undergo p53-dependent growth arrest in a mitotic index assay. Similar to the other p53-positive cell lines analyzed above, in FBXO31 KD HCT116 cells, MDM2 levels did not decrease and p53 levels did not increase after DNA damage. The results demonstrate that at 18 and 24 h following γ-irradiation, the mitotic index of FBXO31 KD HCT116 cells was markedly higher than that of control HCT116 cells expressing an NS shRNA (Fig. 1D). Notably, the difference in the mitotic index between control and FBXO31 KD HCT116 cells correlated with levels of p53 and the p53 target p21, which plays a critical role in p53-mediated growth arrest. Collectively the results suggest that FBXO31 regulates p53 activation under getoxic stresses.

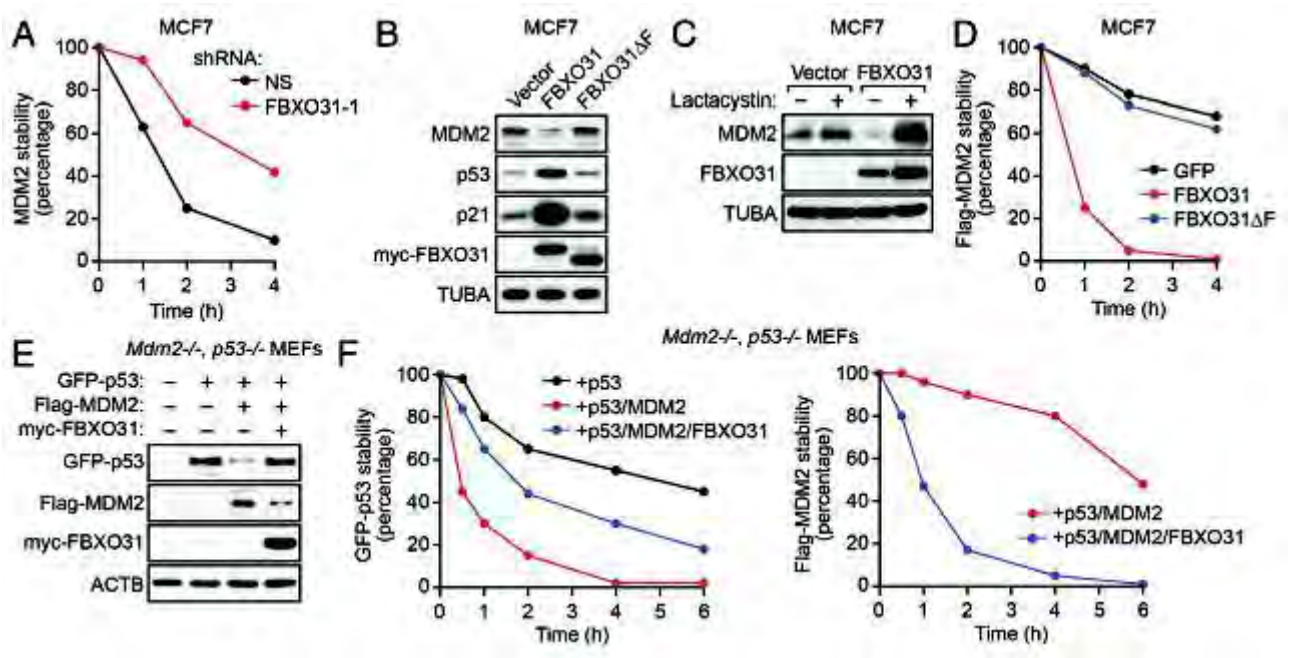

Fig. 2: FBXO31promotes the proteasomal degradation of MDM2. (A) Quantification of a cycloheximide-chase/immunoblot assay monitoring MDM2 stability in MCF7 cells expressing NS or FBXO31 shRNA following treatment with cycloheximide. The graph shows the ratio of the relative levels of MDM2 and PCNA (control) at each time point; time 0 was set to 100%. (B) Immunoblot monitoring MDM2, p53, and p21 in MCF7 cells expressing empty vector, FBXO31, or FBXO31? F. (C) Immunoblot monitoring MDM2 in MCF7 cells expressing vector or FBXO31 and treated in the presence or absence of lactacystin. (D) Quantification of a cycloheximide-chase/immunoblot assay monitoring Flag-MDM2 stability in cycloheximide-treated MCF7 cells expressing GFP (control), FBXO31, or FBXO31? F. (E) Immunoblot monitoring GFP-p53, Flag-MDM2, and myc-FBXO31 in *Mdm2*$^{-/-}$, *p53*$^{-/-}$ MEFs coexpressing combinations of p53, MDM2, and FBXO31. β-Actin (ACTB) was monitored as a loading control. (F) Quantification of a cycloheximide-chase/immunoblot assay monitoring GFP-p53 and Flag-MDM2 stability in cycloheximide-treated *Mdm2*$^{-/-}$, *p53*$^{-/-}$ MEFs coexpressing combinations of p53, MDM2, and FBXO31.

FBXO31 promotes proteasomal degradation of MDM2

The results described above suggested that FBXO31 may directly mediate the degradation of MDM2, therefore we performed a series of experiments to confirm this possibility. First, we measured the half-life of endogenous MDM2 using a cycloheximide-chase/immunoblot assay. The results show that the half-life of MDM2 was substantially longer in FBXO31 KD MCF7 cells than in control cells (Fig. 2A).

We next asked whether ectopic expression of FBXO31 would result in the degradation of endogenous MDM2. The immunoblot of figure 2B shows that ectopic expression of FBXO31 resulted in decreased levels of MDM2, which, as expected, were accompanied by increased levels of p53 and p21. In contrast to wild-type FBXO31, ectopic expression of an FBXO31 derivative in which the F-box had been deleted (FBXO31? F) failed to result in decreased levels of MDM2 or increased levels of p53 and p21.

Consistent with our finding that FBXO31 affected MDM2 stability, the addition of the proteasome inhibitor lactacystin blocked the ability of ectopically expressed FBXO31 to decrease MDM2 levels (Fig. 2C). In addition, we found that mRNA levels of MDM2 were unaffected by ectopic FBXO31 expression or after FBXO31 knockdown by quantitative RT-PCR (qRT-PCR). Moreover, ectopic expression of FBXO31, but not FBXO31? F, substantially reduced the half-life of MDM2 in MCF7 cells (Fig. 2D).

Finally, to confirm the antagonistic roles of MDM2 and FBXO31 on p53 levels, we performed reconstitution experiments in homozygous knockout mouse embryo fibroblasts (MEFs) lacking MDM2 and p53 ($MDM2^{-/-}$, $p53^{-/-}$ MEFs). We ectopically expressed GFP-p53 alone, GFP-p53 and Flag-MDM2, or GFP-p53, Flag-MDM2, and myc-FBXO31 and measured p53 protein levels by immunoblotting. As expected p53 levels were significantly down regulated in the presence of MDM2 (Fig. 2E). Notably, expression of FBXO31 led to decreased levels of MDM2 and a restoration of p53 levels. To confirm that these effects resulted from alterations of protein stability, the half-lives of p53 and MDM2 were measured by a cycloheximide-chase/immunoblot assay. We found that the half-life of p53 was markedly reduced in the presence of MDM2 and that expression of FBXO31 led to a large decrease in the half-life of MDM2 and a concomitant increase in the half-life of p53 (Fig. 2F).

FBXO31 Interacts Directly with MDM2.

F-box proteins direct substrate specificity to the SCF ubiquitin ligase complex by interacting directly with their target proteins. To test whether FBXO31 and MDM2 interact, we performed a series of co-immunoprecipitation experiments. First, MCF7 cells were stably transduced with a retrovirus expressing myc-tagged FBXO31, and FBXO31 was immunoprecipitated using an anti-myc antibody. We found the presence of MDM2 in the FBXO31 immunoprecipitate. Similarly, we found the presence of myc-FBXO31 in the MDM2 immunoprecipitate in reciprocal co-immunoprecipitation experiments.

Protein kinases such as ATM, AKT, cyclin-dependent kinases (CDKs), casein kinase 1 (CK1), and mammalian the target of rapamycin/S6K1 (mTOR) phosphorylate MDM2. We asked whether any kinase- mediated phosphorylation is essential for FBXO31- mediated MDM2 degradation. We analyzed the effect of chemical inhibitors of these kinases on MDM2 levels in MCF7 cells after ectopic expression of FBXO31. We found that FBXO31 fails to downregulate MDM2 upon treatment of cells with the two different ATM inhibitors, KU-55933 and caffeine.

FBXO31 promotes polyubiquitylation of MDM2

Mostly, F-box proteins direct the polyubiquitylation of their substrates, resulting in proteasome-mediated degradation. We performed a series of experiments to determine whether FBXO31 can direct polyubiquitination of MDM2. In these experiments, cells were treated with the proteasome inhibitor MG132 to

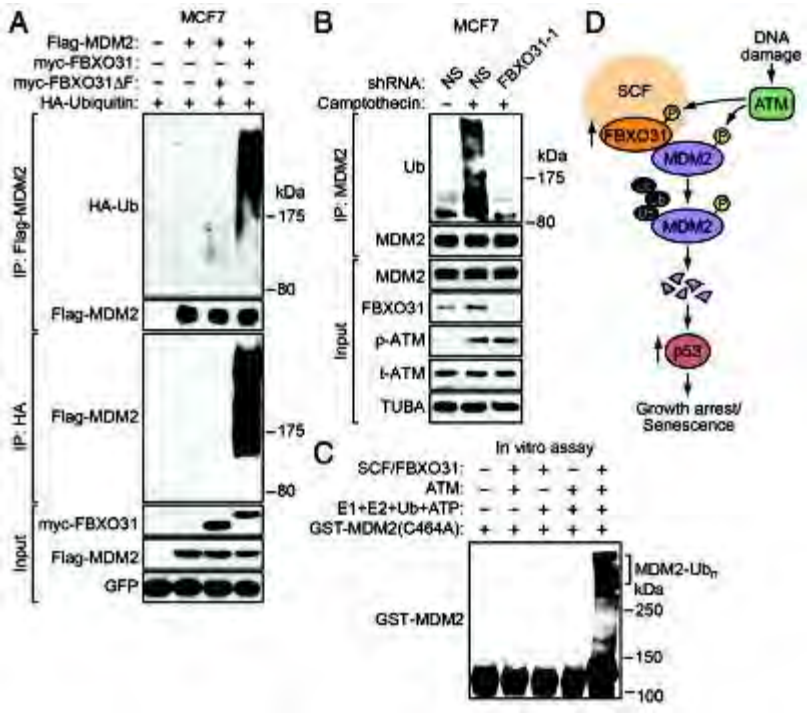

Fig. 3: FBXO31 directs polyubiquitination of MDM2. **(A)** In vivo assay monitoring ubiquitination of Flag-MDM2 in MCF7 cells expressing Flag-MDM2, HA-ubiquitin, and either myc-FBXO31 or myc-FBXO31ΔF. GFP expressed from a cotransfected plasmid was used as transfection/loading control. **(B)** In vivo assay monitoring the ubiquitination of endogenous MDM2 in MCF7 cells expressing NS or FBXO31 shRNA and treated in the presence or absence of camptothecin. **(C)** In vitro ubiquitination assay monitoring the ability of a purified FBXO31/CUL1/SKP1/ROC1 complex to ubiquitinate GST-MDM2(C464A). **(D)** Model. Ub, ubiquitin.

minimize degradation of polyubiquitinated proteins. In the first experiment, MCF7 cells were cotransfected with plasmids expressing Flag-MDM2, HA-tagged ubiquitin, and either myc-FBXO31 or myc-FBXO31ΔF. Polyubiquitination of MDM2 was assessed by immunoprecipitation of Flag-MDM2 followed by immunoblotting for HA-ubiquitin. The results in figure 3A show that ectopic expression of FBXO31, but not FBXO31ΔF, resulted in polyubiquitination of MDM2. Similar results were obtained in a reciprocal coimmunoprecipitation experiment. Notably, FBXO31-directed polyubiquitination of MDM2 was lost after treatment of cells with the ATM inhibitor KU-55933.

To confirm these results, we performed another in vivo ubiquitination experiment involving cotransfection of plasmids expressing Flag-MDM2, His-tagged ubiquitin, and either myc-FBXO31 or myc-FBXO31ΔF. His-ubiquitin–conjugated proteins were purified under stringent, denaturing conditions, followed by immunoblotting for Flag-MDM2. The results confirm that ectopic expression of FBXO31, but not FBXO31ΔF, resulted in polyubiquitination of MDM2.

We also used this His-ubiquitin pull-down assay to confirm the antagonistic relationship of MDM2 and FBXO31 on polyubiquitination of p53. In p53-negative H1299 cells we ectopically expressed His-ubiquitin with GFP-p53

alone, GFP-p53 and Flag-MDM2, or GFP-p53, Flag-MDM2, and myc-FBXO31. Polyubiquitinated p53 was detected by purifying His-ubiquitin–conjugated proteins followed by immunoblotting for GFP-p53. The results show that ectopic expression of MDM2 resulted in a substantial increase in polyubiquitination of p53, which was counteracted by the coexpression of FBXO31.

The ubiquitination assays described above were performed with ectopically expressed proteins. We next performed an additional in vivo ubiquitination experiment to confirm that endogenous FBXO31 could polyubiquitinate endogenous MDM2. Extracts from untreated or camptothecin-treated MCF7 cells expressing either an NS or FBXO31 shRNA were immunoprecipitated with an anti-MDM2 antibody, and the immunoprecipitate was analyzed by immunoblotting with an anti-ubiquitin antibody. As expected, camptothecin treatment led to a large increase in polyubiquitylated MDM2 in NS cells (Fig. 3B). Notably, knockdown of FBXO31 substantially reduced the amount of polyubiquitinated MDM2 in camptothecin-treated cells. These results indicate that FBXO31 is required for polyubiquitination of MDM2 following DNA damage.

Finally, we performed an in vitro ubiquitination assay. Previous studies have shown that MDM2 has auto-ubiquitinating activity in vitro in the presence of an E1 ubiquitin-activating enzyme and E2 ubiquitin-conjugating enzyme. Therefore, we used a previously described catalytically inactive MDM2 mutant, MDM2 (C464A). In addition to MDM2(C464A), the reaction mixtures contained or lacked the known cofactors (E1, E2, ubiquitin, and ATP), ATM, and the SCF/myc-FBXO31 complex purified from transfected 293T cells. The results show that in vitro polyubiquitination of MDM2(C464A) was dependent on the addition of the known cofactors, ATM, and the myc- SCF/FBXO31 complex (Fig. 3C).

The results collectively demonstrated an essential role of FBXO31 in MDM2 degradation following genotoxic stress, which is summarized in the schematic model in Fig. 3D.

Summary of the work

The tumor suppressor p53 plays a critical role in maintaining genomic stability. In response to genotoxic stress, p53 levels increase and induce cell-cycle arrest, senescence, or apoptosis, thereby preventing replication of damaged DNA. In

unstressed cells, p53 is maintained at a low level. The major negative regulator of p53 is MDM2, an E3 ubiquitin ligase that directly interacts with p53 and promotes its polyubiquitination, leading to the subsequent destruction of p53 by the 26S proteasome. Following DNA damage, MDM2 is degraded rapidly, resulting in increased p53 stability. Because of the important role of MDM2 in modulating p53 function, it is critical to understand how MDM2 levels are regulated. In this report, we show, for the first time, that the F-box protein FBXO31 is responsible for promoting MDM2 degradation. Following genotoxic stress, FBXO31 is phosphorylated by the DNA damage serine/threonine kinase ATM, resulting in increased levels of FBXO31. FBXO31 then interacts with and directs the degradation of MDM2, which is dependent on phosphorylation of MDM2 by ATM. FBXO31-mediated loss of MDM2 leads to elevated levels of p53, resulting in growth arrest. In cells depleted of FBXO31, MDM2 is not degraded and p53 levels do not increase following genotoxic stress. Thus, FBXO31 is essential for the classic robust increase in p53 levels following DNA damage.

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 pathways associated with cancer.

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Interplay of insulin and glucose in mediating insulin responsive Glut4 translocation

Background

Insulin maintains glucose homeostasis by stimulating glucose uptake from extracellular environment to adipose and muscle tissue through glucose transporter (GLUT4). Insulin resistance plays a significant role in pathologies associated with type2 diabetes. It has been previously shown that hyperinsulinemia can lead to insulin resistance. In these studies very high levels of insulin was used to achieve insulin resistance. We hypothesized that one of the causes of type 2 diabetes could be insulin synthesis in the absence of glucose stimulation. We used CHO cell line, stably expressing Myc-GLUT4-GFP along with human insulin receptor to study the effect of hyper insulinemia in the presence of low glucose (6.5 mM) or high glucose (20 mM). The insulin responsiveness of these cells was assessed by FRAP, FACS and subcellular fractionation. The results suggest that exposure of cells to insulin in low glucose conditions made these cells insulin resistant within 10 passages, while the same level of insulin in the presence of high glucose did not result in insulin resistance. These results clearly suggest that hyper insulinemia combined with hypoglycaemia may lead to insulin resistance and may be one of the causes for the type2 diabetes.

Aims and Objectives

- Study the effect of insulin in presence of high and low levels of glucose on Glut4 translocation

Participants

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Work Achieved

Myc-GLUT4-GFP translocates to plasma membrane upon insulin stimulation in CHO cells

Translocation of GLUT4 transporter from intracellular compartment to the cell surface in response to insulin is well characterized in adipocytes and muscle cells. To study the effect of prolonged exposure of insulin on GLUT4 translocation we used Myc-GLUT4-GFP construct, stably expressing in CHO cells and responsive to insulin [24;25]. The Myc tag fused is at the N-terminus end of GLUT4 in such a way that it is exposed to extracellular side of cell surface which can be detected by fluorescent labelled myc antibodies whereas GFP is fused at C-terminus of GLUT4, exposing it to the cytoplasmic side of the plasma membrane. Insulin responsive GLUT4 trafficking to cell membrane was assessed by FRAP (Fluorescence Recovery after Photobleaching) and subcellular fractionation. Glut4-CHO cells were seeded on cover slips and a small region of plasma membrane was selected and photo bleached and time for GFP fluorescence recovery at photo bleached area was assessed. GFP fluorescence recovery was assessed in the presence or absence of insulin stimulation in order to measure the insulin responsive GLUT4 translocation. Time taken to recover 50% fluorescence intensity after Photobleaching is rapid after addition of insulin compared to basal level, suggesting GLUT4 exocytosis rate is increased upon insulin stimulation (Fig. 1), suggesting a robust insulin responsive Glut4 translocation in these cells. In addition, subcellular fractionation of cytoplasm and plasma membrane was performed using sequential ultracentrifugation after stimulation by insulin followed by immunoblotting with GLUT4 antibody further confirming that GLUT4 translocation to plasma membrane from cytoplasmic vesicles is increased in response to insulin. Both the experiment suggests that CHO cells expressing GLUT4 receptor are highly insulin responsive and can be monitored by GLUT4 trafficking in vivo and in-vitro.

Prolonged exposure of insulin to insulin sensitive cells in low glucose cause resistance

Insulin stimulates glucose uptake in adipocytes and skeletal muscle cells primarily by stimulating the transport of glucose transporter type 4 (GLUT4) to plasma membrane from specialized GLUT4 storage vesicles (GSV). We tested the effect of hyper insulinemia and or hyperglycemia on the insulin responsiveness of these cells. The Glut4 expressing CHO cells were maintained in medium containing different levels of insulin and glucose. The schematic of the experimental procedure is shown in Fig. 2A. After 10 passages the cells were

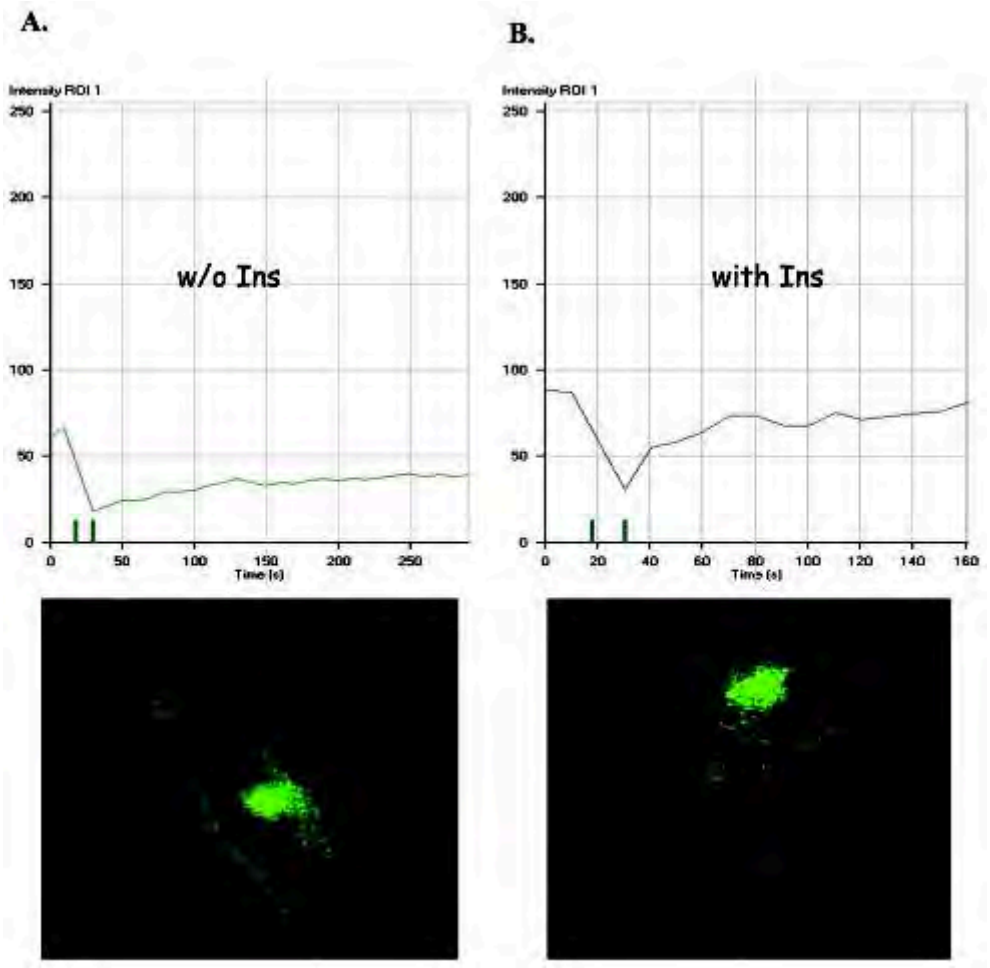


Fig. 1: CHO cells expressing Myc-GLUT4-GFP is insulin responsive. Insulin responsiveness of Glut4-CHO cells was assessed by FRAP analysis. A small region of the cell membrane was photobleached using full intensity of argon laser and GFP fluorescence recovery time to the photobleached area (before and after adding the insulin) was analysed in the absence **(A)** and presence **(B)** of insulin. The graph was plotted between GFP fluorescence intensity in the photo-bleached area and time (sec).

tested for insulin responsiveness by FRAP (Fluorescence recovery after Photobleaching), subcellular fractionation of GLUT4 containing vesicles and FACS).

For the FRAP experiment a small area in the plasma membrane was photobleached and fluorescence recovery in presence and absence of insulin stimulation was recorded. Graph was plotted for the time taken to recover 50% GFP fluorescence and a reduce time indicates a faster translocation and a good insulin response. As expected the cells maintained in medium without insulin showed a robust insulin responsive Glut4 translocation in both high and low glucose growth conditions (Fig. 2B and 2C the first two bars), whereas cells maintained in insulin and low glucose medium showed a complete loss of insulin responsive Glut 4 translocation (Fig. 2B bars 3 and 4), however the cells maintained in insulin and High glucose conditions still retained insulin responsive Glut4 translocation (Fig. 2C bars 3 and 4). Further, low glucose

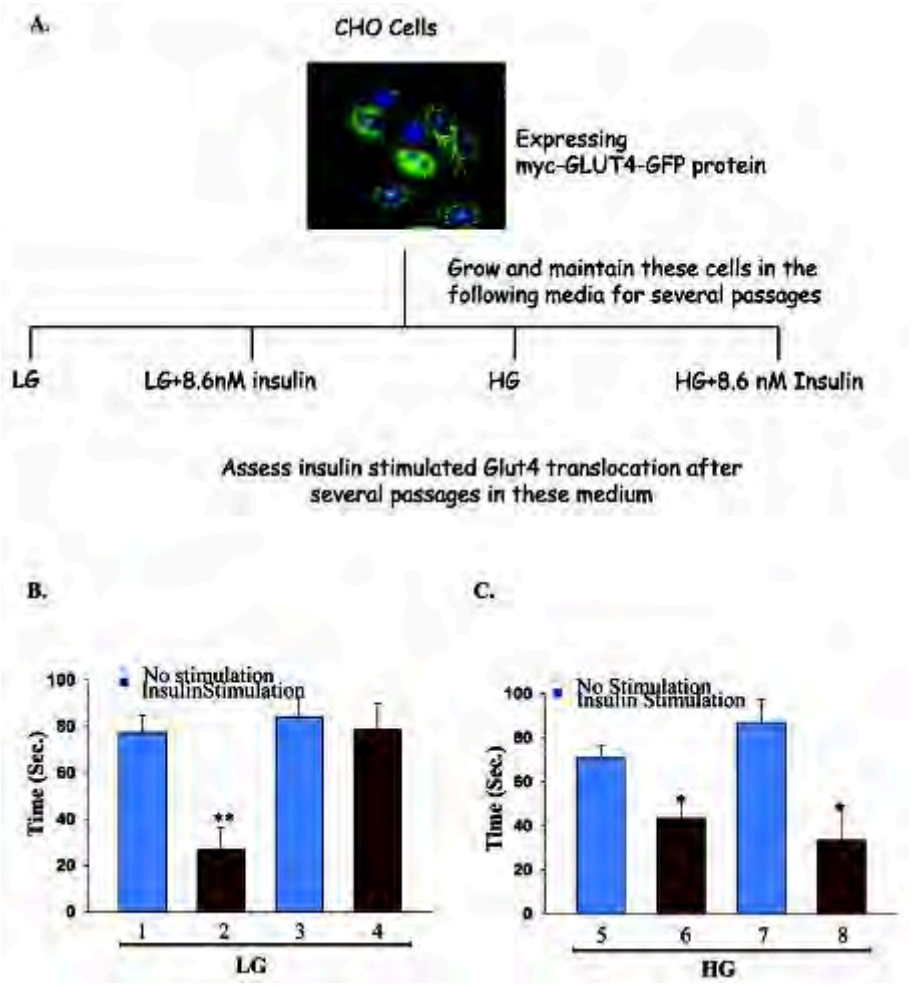


Fig. 2: Insulin responsiveness of CHO cells is reduced upon prolonged exposure to insulin in low glucose containing medium. Schematic representation of experimental design to assess the insulin responsiveness of the Glut4-CHO cells exposed to insulin (A). CHO cells expressing Myc-GLUT4-GFP was maintained in a media with or without insulin in low or high glucose for several passages. FRAP analysis of Myc-GLUT4-GFP expressing CHO cells after 10 generation of insulin exposure in low glucose or high glucose medium was performed. The GFP fluorescence recovery time for each was recorded before (Blue bars) or after (Brown bars) insulin (8.6 nM) stimulation and graph was plotted for the time (sec) to recover 50% of GFP fluorescence and represents an average of three biological repeat experiments. The recovery time for the cells maintained in low glucose medium without insulin (bars 1 and 2) and with insulin (Bars 3, 4) is shown in the top panel (B), while The recovery time for the cells maintained in high glucose medium without insulin (bars 5 and 6) and with insulin (Bars 7, 8) is shown in the lower panel (C). Statistical analysis was done by t-test where * indicates $P<0.05$ and ** indicates $P<0.01$.

condition alone in the absence of insulin did not affect the insulin responsiveness of CHO cells. This data suggest that long time insulin exposure in low glucose conditions leads to insulin resistance however cells exposed to similar levels of insulin but with higher levels of glucose are still insulin responsive.

In order to further confirm this phenomenon, we performed subcellular fractionation of GLUT4 expressing CHO cells. The cells were lysed in hypotonic buffer and sequential ultra-centrifugation was done as described in materials and method. The fractions were resolved on SDS-PAGE, transferred to PVDF membrane and probed for GFP (Glut4), Transferrin receptor (endosomal) and N-Cadherin (Plasma membrane) antibody. We find an increased GLUT4 translocation to the membrane in response to insulin in cells which were maintained in medium lacking insulin (Fig. 3A and B). On the other hand, cells which were maintained in presence of insulin and low glucose for 10 passages,

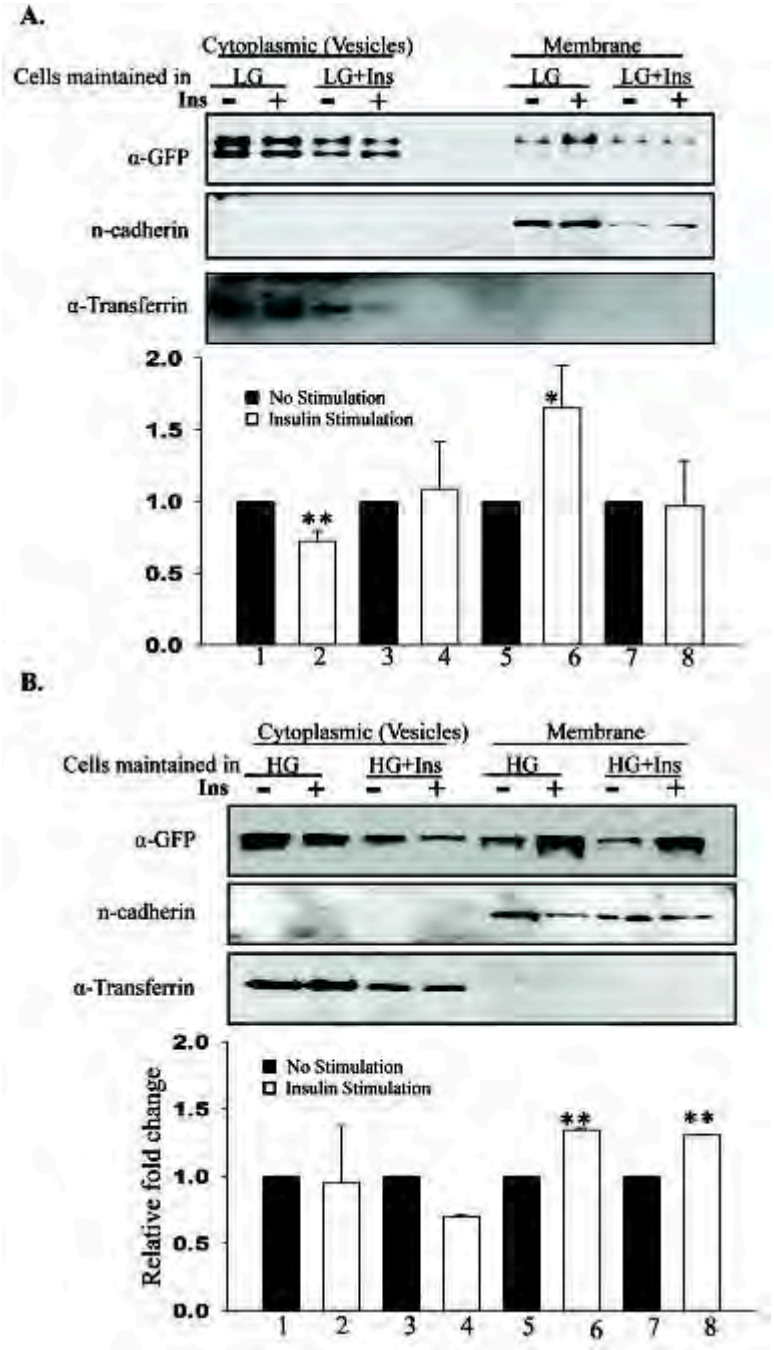


Fig 3: Glut4 translocation to the plasma membrane is reduced in cells maintained in medium containing insulin and low glucose. Glut4-CHO cells were lysed and subcellular fractionation was performed through sequential ultracentrifugation. Vesicle and membrane fractions were analyzed for GLUT4 by western blot using GFP antibody. The blot was stripped and probed with N-Cadherin and Transferrin receptor antibody as fraction markers. The normalized band intensity was plotted for each sample and is represented as bar graph below the western blots. Normalized Glut4 levels in cytoplasmic vesicle fraction (1-4) and membrane fractions (5-8) for cells maintained in low glucose media is indicated **(A)** Similarly for the Glut4-CHO cells maintained in high glucose medium the results are indicated **(B)** The relative band intensity was calculated from three independent experiments after normalization with N-cadherin and transferrin receptor for each fraction. Statistical significance was calculated by t-test using systat 3.5 where $P<0.05$ (*), $P<0.01$ (**).

showed no significant change in GLUT4 levels on membrane to cytoplasmic vesicles in response to insulin (Fig. 3A). In contrast, cells which were maintained in insulin and high glucose medium throughout 10 passages, showed increase insulin stimulated membrane associated GLUT4 (Fig. 3B). The transferrin receptor (endosomal protein) did not show any change in cytoplasmic to membrane translocation in response to insulin and is mainly located in intracellular compartment (Fig. 3A and B).

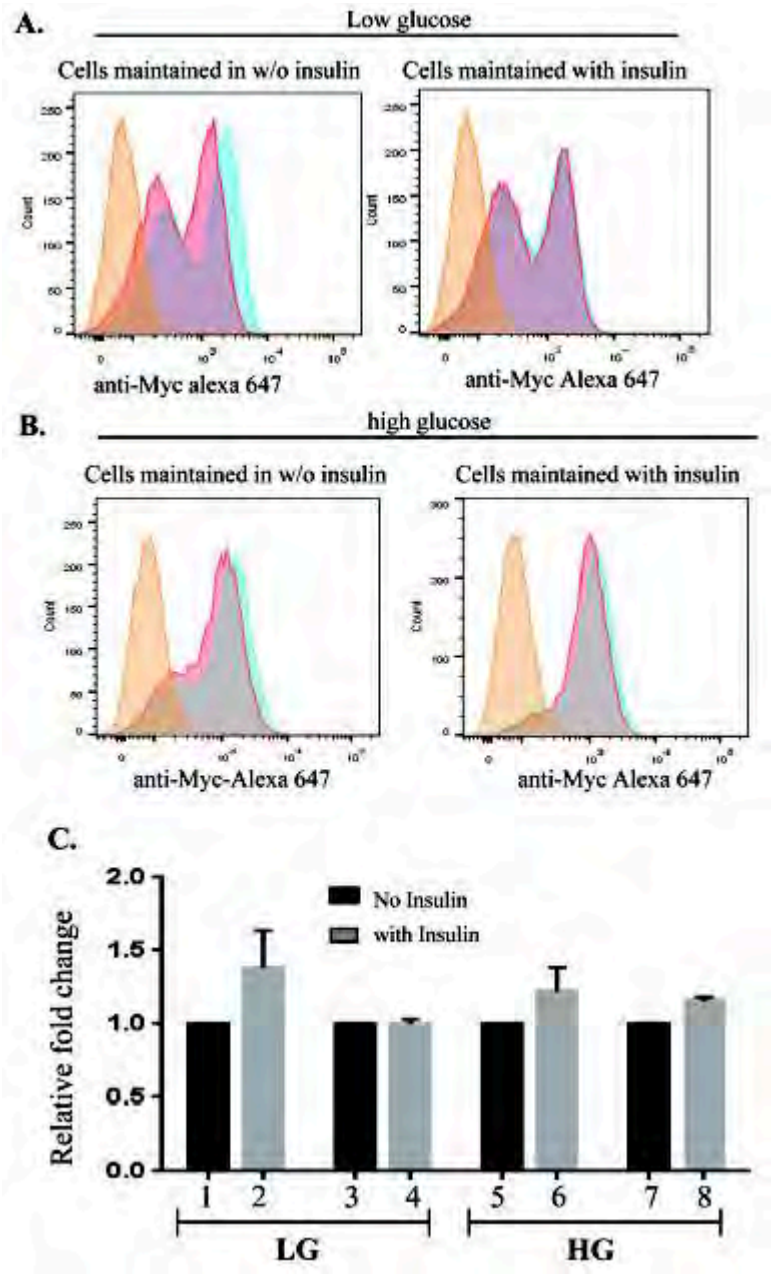


Fig 4: FACS analysis of insulin responsive GLUT4 translocation to plasma membrane. The Glut4-CHO cells were fixed and stained with anti-myc mouse monoclonal antibody without permeabilization to monitor the membrane expression of Glut4. The Fluorescence intensity for the population of cells maintained in low glucose medium (A) and high glucose medium (B) was measured. Mean fluorescence intensity (MFI) was calculated using Flowjo software (V10) and Relative MFI was calculated by normalizing with no insulin MFI and graph was plotted between relative fold changes upon insulin stimulation for cell populations that were grown in indicated medium (C). Statistical analysis was done using Sigma plot 12.0 (Systat software Inc., CA, USA)

Similar results were also observed by FACS analysis, where Glut4-CHO cells was stimulated by insulin and stained with anti-Myc antibody (sc-40, santacruz) without permeabilizing the cells. The total plasma membrane associated GLUT4 was estimated by FACS and mean fluorescence intensity (MFI) was calculated and plotted for cells grown in different medium. An increased MFI indicates an increased Plasma membrane associated Glut4 suggesting a good insulin responsiveness. The Relative Mean fluorescence intensity (MFI) of cells

which was maintained in low glucose and no insulin media were increased significantly in response to insulin (Fig. 4A left panel) compared to those cells which were maintained in insulin and low glucose (Fig. 4A right panel). However, cells which were maintained in high glucose and in presence or absence of insulin didn't show insulin resistance (Fig. 4B). The relative increases in MFI from multiple experiments are averaged and depicted in Fig. 4C. In addition, we assessed whether prolonged exposure to insulin and glucose affects the total levels of GLUT4 protein. We prepared protein lysates from cells, after 5 and 10 passages of insulin exposure in presence and absence of insulin and assessed Glut4 expression levels by western blot using GFP. We find no significant change in GLUT4 protein levels, suggesting GLUT4 protein biosynthesis is not affected by long term insulin exposure but only its translocation to the membrane. Altogether, these results strongly suggest that increased level of insulin exposure to insulin responsive cells (Adipocytes and skeleton muscles) in low glucose condition leads to insulin resistance.

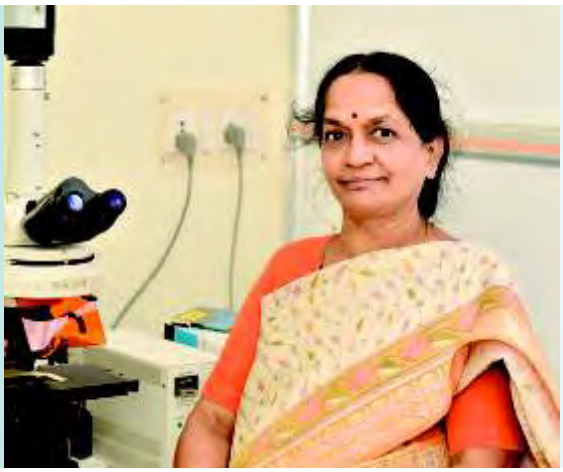
Insulin is the main regulatory hormone which maintains glucose homeostasis in the blood by acting on insulin sensitive cells (adipocytes and skeleton muscle cells) to internalize glucose by GLUT4 transporters from the extracellular space. Insulin is preferentially produced and secreted by β cells of the pancreas in response to glucose elevation in the blood. At short period of glucose stimulation, insulin is mainly regulated at the translation level. Previously, we have identified PDI as an insulin 5'UTR trans-acting protein that increases insulin production.

PDI expression is up-regulated in various cellular stress conditions including ER stress or metabolic disorders. This increased level of PDI may result in high insulin production even in absence of glucose. Moreover, during metabolic disorders and obesity insulin secretion pathways are affected which leads to increased insulin levels without corresponding increase of plasma glucose. So we believe that prolonged higher levels of insulin exposure of the responder cells (adipocytes and skeleton muscle cells) in the absence of increased glucose may result in insulin resistance. We used CHO cell line which was stably expressing human insulin receptor and GLUT4 glucose transporter. We maintained the cells till 10 passages in presence of insulin (8.6 nM) and low (6.5 mM) or high (20 mM) glucose. The cells were then again tested for insulin responsiveness through GLUT4 translocation by FRAP, FACS and subcellular fractionation. The results suggest that prolonged exposure of insulin in low glucose reduces the sensitivity for insulin compared to control cells which were

maintained in absence of insulin. In contrast prolonged exposure of insulin in high glucose did not show significant reduction of insulin responsiveness. However, the total GLUT4 protein expression is not affected in response to insulin. So it is very likely that insulin signalling pathway might be playing critical role in development of insulin resistance. Thus individuals with hyper insulinemia may develop insulin resistance if they are also exposed to hypoglycaemia, however the insulin resistance can be averted if sufficient levels of glucose is provided to them at intervals.

Future Research Plans

We plan to identify the molecular basis of this insulin resistance using the Glut4-CHO cell system as these provide a very controlled system to understand the complex pathways involved in development of insulin resistance which is one of the major causes of Type II diabetes.



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Role of mammalian Target of Rapamycin (mTOR) signalling pathway in survival, proliferation and invasion of human gliomas

Background

Glioblastoma (GBM) is the highly predominant form of life threatening primary malignant gliomas and astrocytomas. GBM has extremely poor prognosis with median survival period of <1 year of patients post diagnosis. The mammalian Target of Rapamycin (mTOR) signalling network downstream to EGFR/PI3K/AKT pathway regulates cell growth, proliferation and survival in normal and cancer cells. The central component of the pathway, the mTOR protein kinase, nucleates two distinct multi-protein complexes mTORC1 and mTORC2 to affect the biological activities of the pathways. The mTOR pathway is frequently activated in GBMs and is therefore an attractive target for intervention.

The microenvironment of solid tumors comprising of hyper-reactive stroma and Tumor Associated Macrophages (TAMs) link between inflammation and tumor progression. The other factors contributing to invasion include extracellular matrix (ECM) components that trigger extrinsic signalling by binding to cell adhesion receptors to manipulate cell cytoskeletal structure. The signalling cascades activated influence cell migration and invasive potential of tumor cells. The levels/proportions of ECM components- fibronectin, hyaluronic acid, collagen and laminin are imbalanced during cancer invasion process. The altered adhesive capacity of tumors contributes to migration of tumor cells from primary site to surrounding normal tissues that leads to tumor recurrence.

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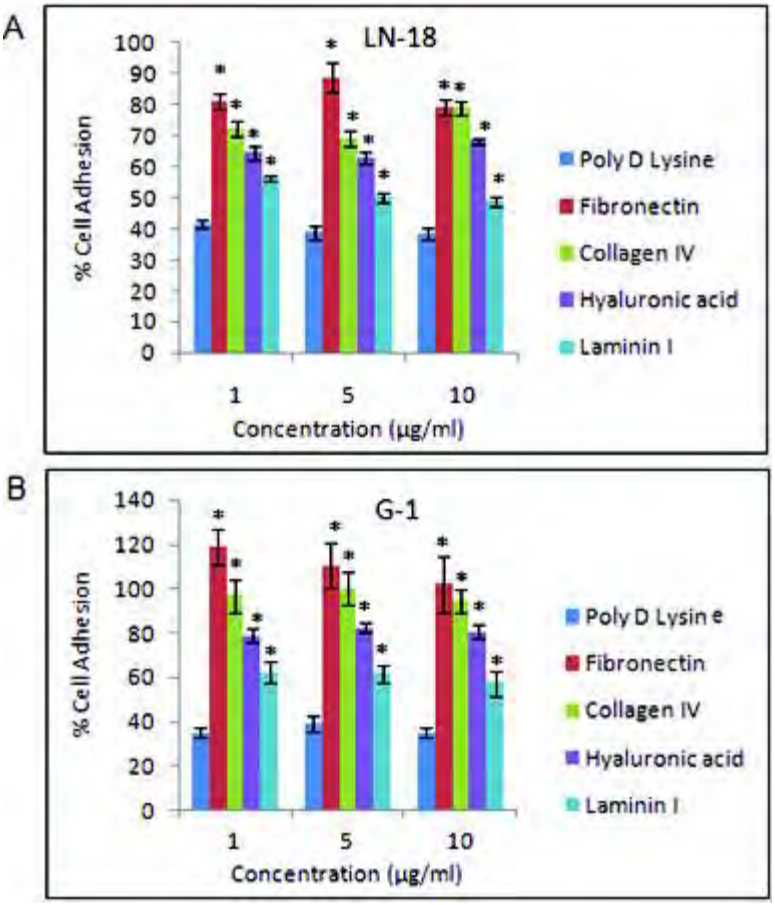

Fig. 1: Adherence of GBM cells to extracellular matrix components (ECMC): Cell adhesion assay was performed with LN-18 cells (A) and primary culture -G-1 cells (B). Cells were seeded onto surface coated with serial concentrations (1, 5, 10 µg/ml) of ECM components- collagen-IV, fibronectin, hyaluronic acid and laminin-1. Poly D lysine was used as negative control. The graphs represent percentage of cell adhesion +/- SEM of three similar experiments performed in triplicates. *p–value <0.05 Poly D lysine vs ECM components.

Aims and Objectives

- Since multiple pathways and a number of transcription factors that are activated downstream of mTOR pathway play an important role in tumor progression and invasion, the study aimed-
- To explore the potential of mTOR inhibitors to control invasion in GBM cells with focus on adhesion and extracellular components and study the molecular mechanisms involved in the process.

Work Achieved

Experiments performed with human GBM cells using LN-18 cell line and primary cultures –G1 revealed that adhesion to extra-cellular matrix components was in the order of fibronectin> collagenIV> hyaluronic acid> laminin-1(Fig.1).In G1 cells, the enhanced adhesive potential was significantly decreased by mTOR inhibitors- rapamycin(RAP), temisirolus (TEM),torin (TOR) and PP242 (Fig.2). Flow cytometric analysis demonstrated that G1 cells abundantly expressed integrin heterodimers- α3β1> α2β1 > α6β1 and CD44 receptors compared to α5β1 and ICAM1 receptors. The mTOR inhibitors, TEM and TOR did not affect the expression of the integrin subunits and receptors.

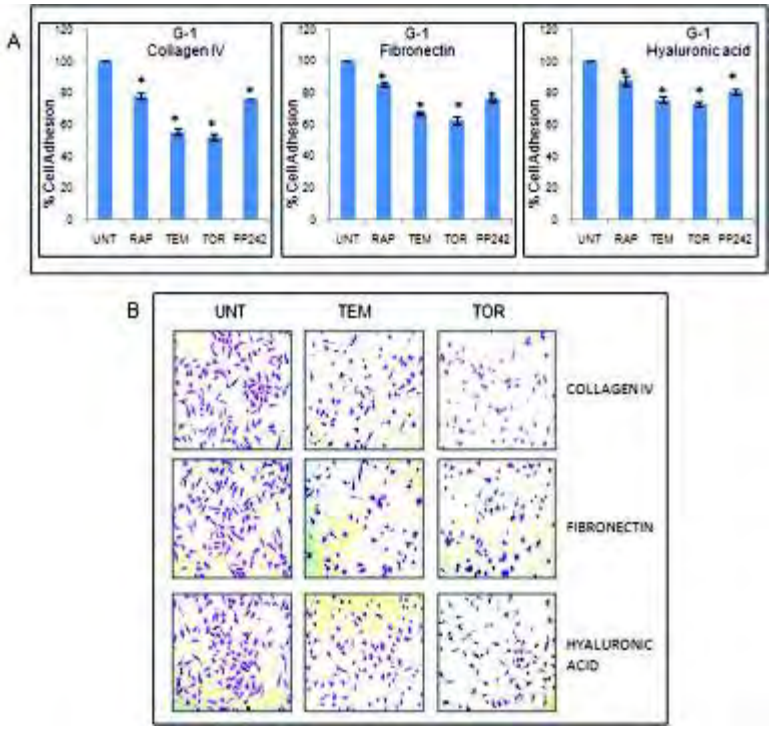

Fig. 2: Effect of mTOR inhibitors on adhesion of GBM cells on ECM components in GBM. G-1 cells were pretreated with mTOR inhibitors-Rapamycin-RAP (10μM), Temisirolimus-TEM (5μM), Torin-TOR (100nM) or PP-242 (100nM) for 12h and seeded on ECM components-collagen-IV, fibronectin and hyaluronic acid in the presence of inhibitors. After 6 hr the cells were stained with crystal violet and absorbance of the eluted dye was measured at 595nm. A. The graphs represent percentage of cell adhesion +/- SEM of three similar experiments performed in triplicates with G-1 cells treated with inhibitors. *p–value <0.05 Untreated vs. treatment with inhibitors. B. Representative images of adhesion of G-1 cells pretreated with TEM and TOR.

Integrin-mediated signalling results in activation of various signalling pathways leading to activation of transcription factor, c-Jun. Activation of c-Jun is mediated by the JNK pathway that regulates cell proliferation, apoptosis, invasion and inflammatory response. c-Jun/JNK pathway aberrations enhance self-renewal and the tumor-initiating capacity of human glioma stem cells. In this study, we found that the expression of phosphorylated FAK, - JNK and -ERK 1/2 and c-Jun enhanced in response to collagen IV and hyaluronic acid as assessed by western blotting analysis. The enhanced expression of these proteins was reduced by TEM and TOR (Fig.3). Collectively, the findings suggested that the mTOR inhibitors inhibited FAK/JNK/ERK mediated pathway that was activated on binding of cells to ECM components.

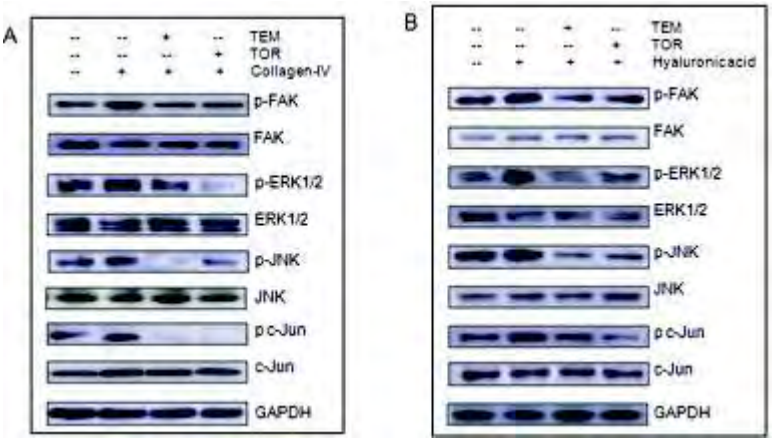

Fig. 3: Effect of mTOR inhibitors on phospho FAK, ERK1/2, JNK and c-Jun induced by ECM components. Levels of phospho and total -FAK, ERK1/2, JNK and c-Jun proteins were assessed by immunoblotting. G-1 cells were pretreated with temisirolimus-TEM (5μM) and Torin-TOR (100nM)for 12h and seeded on- A. collagen-IV and B. hyaluronic acid in the presence of inhibitors . After 6 hr, total cell lysates were prepared and probed for phosho and total FAK, ERK1/2, JNK and c-Jun. GAPDH was used as loading control. Images are representative blots of two independent experiments.

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Understanding the mechanism of transformation elicited by a novel long non-coding RNA - Ginir

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Background

The recent surge of information regarding evolutionary conservation, functionality and annotation of sequences from human and mouse genome have led to a stunning revelation that nearly 99% of the genome does not encode proteins. This raises a pertinent question as to whether the major non-coding bulk of DNA has essential regulatory roles. Importantly, while the coding content is functionally conserved across evolution, non-coding sequences perform short lived lineage-specific roles. With huge number of long noncoding RNAs getting continuously mined from deep sequencing of eukaryotic genome, their involvement in a variety of roles in both gene expression and re-modelling of the eukaryotic genome is constantly being unfathomed. Here, we report functional studies with pair of long intergenic non-coding (Linc) RNA pair identified in our lab - Ginir and Giniras which has a critical role in maintenance of cellular homeostasis. This RNA pair termed as Genomic Instability Inducing RNA functions as a pair of sense (Ginir) and anti-sense (Giniras) transcripts and is localized to Chromosome X in mouse.

Aims and Objectives

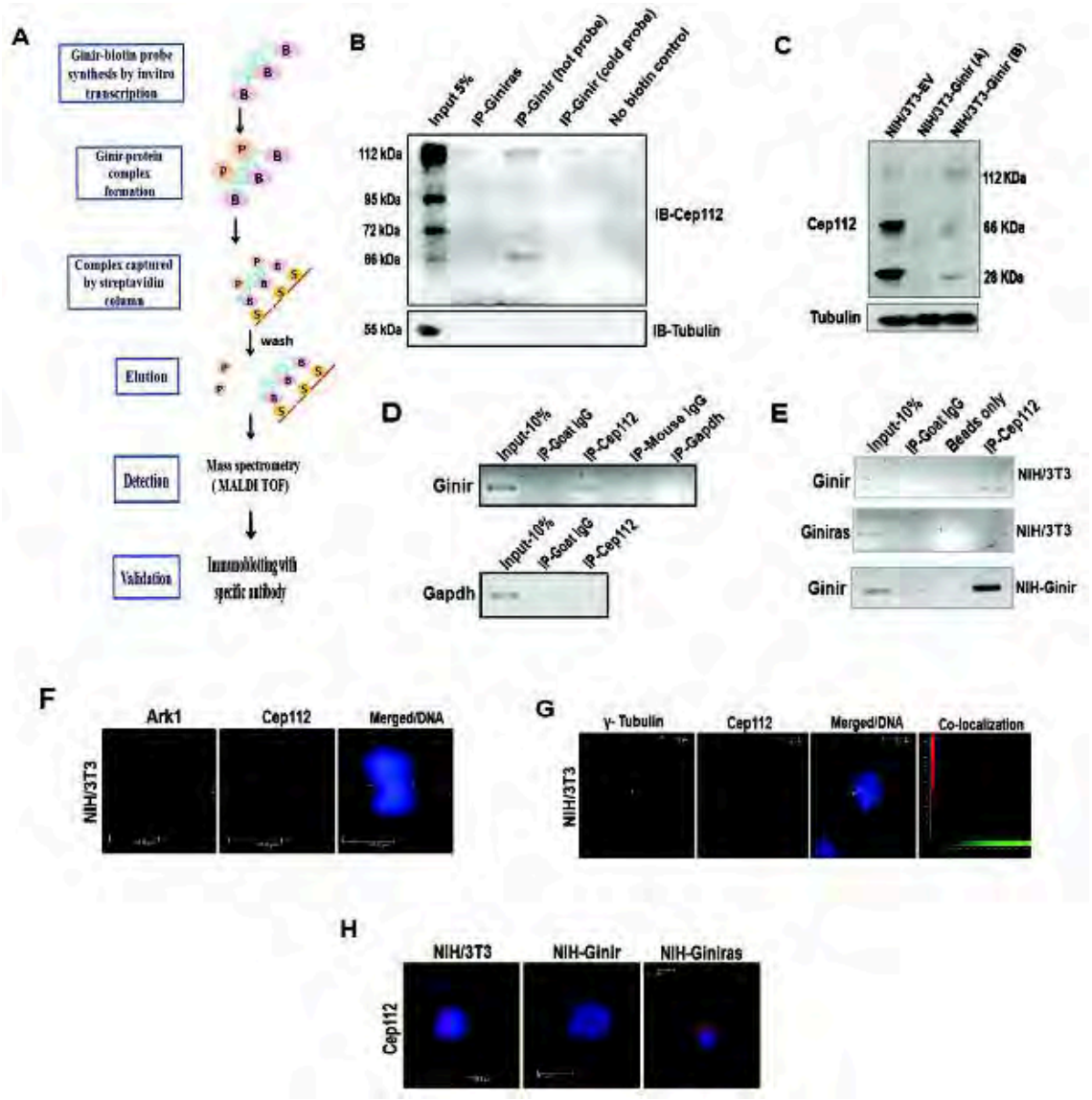
- Delineate mechanisms through which non-coding RNA –Ginir is involved in cell growth regulation.
- Identification of protein targets through which Ginir RNA possibly mediates cellular functions and evokes transformation.
- Deciphering pathways through which non-coding RNA Ginir and its cellular targets interact and mediate cellular homeostasis.

Fig. 1: Ginir physically interacts with centrosomal protein Cep112 in mouse cells

A) Schematic representation of RNA pull down assay using biotinylated RNA probe. B) Western blotting of Ginir bound proteins obtained with RNA pull down using biotinylated Ginir and Giniras probes in NIH/3T3 cells. Blot was probed with anti-Cep112 antibody. Both hot (biotin probe) and cold probes for Ginir were used for pull down. Un-biotinylated probe served as control. Blot was stripped and probed for tubulin as control for non-specific binding. C) Western

Work Achieved

LincRNAs are known to mediate their functions via varied mechanisms like either interaction with proteins, direct interaction with DNA or chromatin or through interaction with miRNAs. Our earlier data has elucidated functional role of non-coding RNA-Ginir in mediating cellular transformation and tumorigenicity in immune-compromised mice. Main focus of present study was to understand the mechanisms through which Ginir may be involved in mediating these effects on cell growth de-regulation. Our initial experiments suggested that Ginir may be mediating its biological effects through interaction with proteins. Hence, we began by identifying interacting protein partners for Ginir by performing biotin affinity RNA pull down assay and followed it up with



blot analysis of Cep112 protein expression in Ginir overexpressing cells in comparison to control NIH/3T3 cells. Tubulin was used as control for equal loading of protein lysates. RNA immunoprecipitation (RIP) with NIH-Ginir (D) and NIH cells (E) with Cep112 antibody followed by RT-PCR with Ginir and Giniras specific primers. (D) Gapdh was used as control for non-specific amplification F). Co-localization experiments for Cep112 and centrosomal marker Ark1 in NIH/3T3 cells using immune-fluorescence. Cells were counterstained with the nuclear probe DAPI (blue). Scale bar, 10 μm. G). Co-localization of Cep112 with centrosomal marker γ-tubulin validating the centrosomal localization of Cep112 by immune-fluorescence staining. DAPI was used for counterstaining. Scale bars, 10μm. H). confocal microscopy with Cep112 antibody in NIH/3T3, NIHGinir and NIHGiniras cells showing change in subcellular localization of Cep112 protein in NIHGinir cells. Scale bars, 10μm.

mass spectrometry analyses. The strategy used for target protein identification is outlined in Fig. 1A. Proteins interacting with Ginir that were commonly detected from NIH, NIH-Ginir cells and mouse brain lysates that showed higher confidence index were analyzed on the basis of their binding propensity to Ginir using prediction tools like cat RAPID and RPISeq. Based on MALDI-TOF MS confidence index and the interaction scores we chose two novel proteins Cep112 and Kif20b as eligible protein partners for Ginir RNA. Cep112 (centrosomal protein of 112 KDa) or Ccdc46 or Macoco is a centrosomal protein with ATPase domain. Ensembl Genome browser shows Mus musculus Cep112 to have 18 possible splice variants & 12 predicted protein isoforms, out of which protein isoforms of 112 KDa & 28 KDa are the predominant ones. Our immunoblotting data indicated that NIH3T3 cells expressed three isoforms-isoform-1 (112 KDa), isoform-2 (66 KDa) & isoform-3 (28 KDa) (Fig.1C). Interestingly, NIH-Ginir stable transfectant cells (A and B) showed significant down-regulation of Cep112 expression (Fig. 1C). To determine interaction of Cep112 with Ginir, we performed biotin RNA pull down assay Here, a specific interaction of Ginir with both 66 and 112 kDa isoforms of Cep112 was detected (Fig 1B). Next, we confirmed this interaction of Cep112 with Ginir by in vivo RNA immunoprecipitation with Cep112 antibody followed by PCR using Ginir specific primers. GAPDH was used as a control for non-specific binding (Fig. 1D). In NIH/3T3 cells we obtained amplification of Ginir in Cep112 bound elute although the binding was lower due to less expression of Ginir in NIH/3T3 cells. Interestingly, Giniras lacked interaction with Cep112 in the same cells suggesting that Cep112 interaction significantly occurred only with Ginir RNA (Fig.1E). To investigate whether lower level of Cep112 expression in NIH cell could be due to lower presence of Cep transcripts, we performed RT-PCR using Cep112 specific primers. The data indicated that Ginir affected Cep112 only at protein level but not at RNA level. Interestingly the subcellular localization of Cep112 also was affected upon ectopic expression of Ginir. Cep112 has normally a cytoplasmic or centrosomal localization as evident from its co-localization with ARK-1 and γ tubulin but in Ginir overexpressing cells Cep112 was found partitioned in the nucleus as well (Fig.1E,F,G). We have independently demonstrated interaction of Brca1 with Cep112 protein.

Kif20b is an interacting protein partner for Ginir

Next, our mass spectrometry analyses showed that Kif20B was another putative interactor for Ginir. Bioinformatics tools like RPISeq and cat RAPID used to estimate RNA-protein binding scores showed high propensities of binding of Ginir to Kif20b (Fig 2A,B). Kinesin superfamily proteins (KIFs) are microtubule-

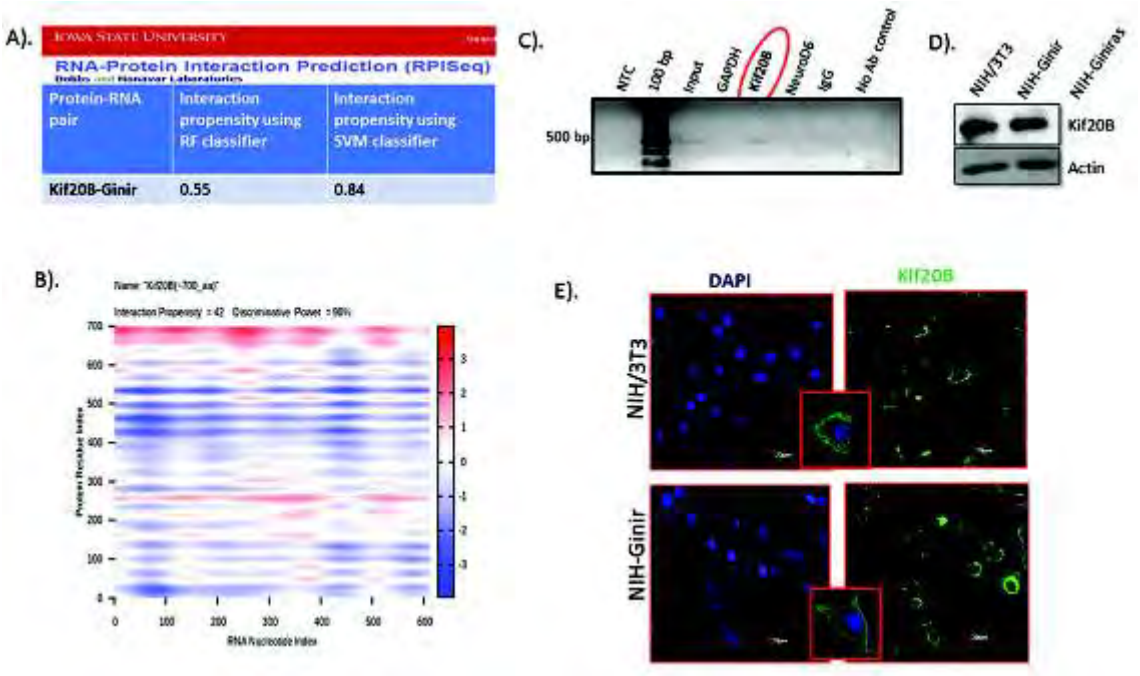


Fig. 2: Kif20B, a member of Kinesin superfamily 6, interacts with Ginir.

A,B) Estimation of interaction propensities for Ginir and Kif20B through sequence based bioinformatics tools RPISeq (A) and cat RAPID (B). (C). RNA Immunoprecipitation with Kif20B in NIH-Ginir cells showing enrichment of Ginir in PCR analysis. (D). Expression of Kif20B in NIH/3T3 and NIH-Ginir cells through western blotting. Actin was used as loading control. (E). Immunofluorescence showing localization of Kif20B in NIH/3T3 and NIH-Ginir cells.

dependent molecular motors important in cell division and play prominent roles as microtubule stabilizers and these activities are fundamental to cellular morphogenesis and mammalian development. Our RNA immunoprecipitation data demonstrated that Kif20b was interacting with Ginir RNA (Fig. 2C). Interestingly, a change in localization of Kif20b to the membrane was detected in NIH-Ginir cells instead of a cytoplasmic presence in NIH cells (Fig.2D). Strikingly, the levels of Kif20b protein did not alter significantly in NIH-Ginir cells as compared to NIH-3T3 cells (Fig.2E).

Ginir causes mitotic defects and impaired cytokinesis

Interestingly, NIH/3T3-Ginir cells were enriched with a pre-ponderant population of multinucleated giant cells as seen in phase contrast micrographs (Fig.3A) and by DAPI staining (Fig. 3B). This led us to determine whether ectopic presence of Ginir was responsible for this effect. Our data indicated that Ginir caused defects in cytokinesis leading to multiple cycles of karyokinesis followed by mitotic dys-regulation and occurrence of giant cells and these giant cells showed nuclear positivity of Ginir as assayed by RNA-FISH (Fig. 3C) and were proliferative as indicated by Ki67 positivity (Fig.3D). Various reports suggest that a cell population enriched with giant cells contribute to generation of cancer stem-like cells and these are responsible for tumor growth, heterogeneity, and chemo-resistance. Centrosomes play an important role in mitotic regulation and thus centrosomal defects may lead to aberrant spindle formation and defective cytokinesis. These defects were visualized in terms of staining with

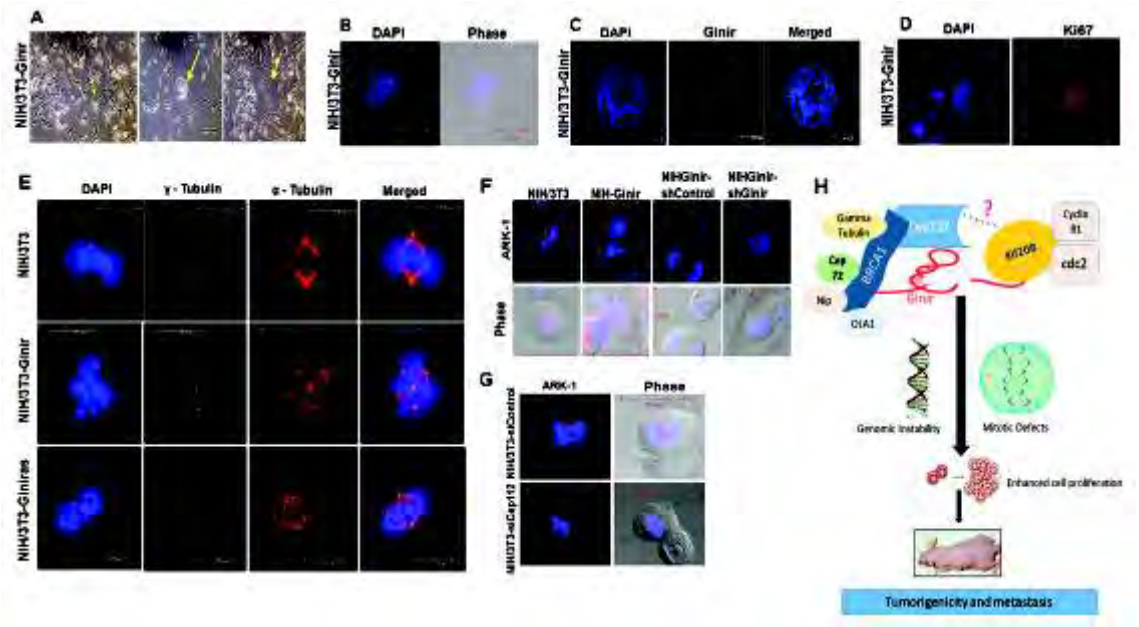


Fig. 3: Ginir causes mitotic defects and impaired cytokinesis leading to tumorigenic phenotype

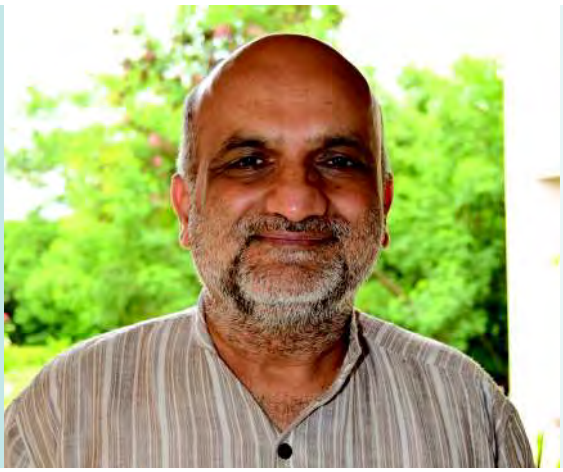
(A) Phase contrast micrographs showing multinucleated giant cells in cells stably overexpressing Ginir; 10X magnification. Arrows and arrowheads point to the multinucleated giant cells amongst the fast proliferating NIH-Ginir cells. (B). DAPI staining of NIH-Ginir cells showing presence of multiple nuclei within a giant cell. Scale bars- 20µm. (C). RNA FISH using Ginir specific LNA probe (green) in NIH-Ginir cells showing nuclear localization of Ginir in giant cells. Blue color indicates nuclear staining with DAPI. Scale bars- 10µm. (D). Confocal image of Ki67 staining in NIHGinir cells showing intense staining in a multinucleated cell. Scale bars- 20µm. (E). Co-staining of alpha and gamma tubulin in cell lines. Chromosomes and nuclear material was stained with DAPI. Scale bars- 10 µm. (F) Immunofluorescence for Ark1 /Aurora A (centrosomal marker) in cells. DAPI was used for nuclear staining. Scale bars- 20µm. (G) Confocal images showing Ark1 staining in NIH/3T3-siCep112 and NIH/3T3-siControl cells. DAPI (blue) was used to stain nuclei. Scale bars- 20µm.(H). Model depicting mechanism of action of Ginir non-coding RNA in genomic instability and tumorigenesis mediated through its protein partners Cep112, and Kif20b.

Ark-1 and co-staining of gamma and alpha-tubulin in Ginir overexpressing cells (Fig. 3E, F). Our speculation that Ginir induces mitotic defects by downregulating the levels of Cep112 was validated in an experiment wherein Cep112 protein was knocked down using siRNA followed by immunostaining for ARK-1. Interestingly, knock down of Cep112 in NIH/3T3 cells resulted in similar mitotic defects as observed in case of Ginir overexpression in the same cells thus indicating role of Cep112 in mitotic regulation (Fig.3G). Besides defects in mitotic spindle segregation, Ginir also induced defects in DNA damage responses, together culminating in high levels of genomic instability. Also, our preliminary data indicated interaction of Cep112 with Kif family proteins and this could be a major contributing factor for improper cytokinesis.

Our study provides experimental evidence for interaction of Ginir with Cep112 and Kif20B. We postulate that Ginir may be responsible for inducing genomic instability by forming a ternary complex with Cep112 and Kif20b (Fig 3H) thereby causing defective cytokinesis and propelling cells towards oncogenesis.

Future Research Plans

- Generate mechanistic insight into non-coding RNA- Ginir action in human cells.
- Understand protein and RNA target network through which Ginir may be mediating genomic instability and effecting cytokinesis in mouse cells.



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Human microbiome - Indian perspective

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Background

The human gastrointestinal tract harbors a complex microbial ecosystem and plays an important role in human physiology, metabolism, immune homeostasis and overall health. Dysbiosis of microbiota in turn, induces chronic inflammation in the mucosa and is shown to be related with many gastrointestinal disorders. Moreover, disruption in normal microbiota in lung, oral and skin has also been associated with many disorders. Several factors are known to influence microbiota including diet, age, geographic location, socio-economic condition and ethnicity. Although India has large cultural, dietary, geographical and environmental variation systematic studies on Indian microbiome are lacking.

Aims and Objectives

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

Work Achieved

Pune Microbiome study – molecular analysis of human microbiome

The study was aimed to explore the microbial communities associated at different body locations in healthy human beings and understanding the changes in abundance and diversity of the microbial flora as a function of age. Human oral, skin and stool samples from 54 healthy participants belonging to three generations from rural and semi-urban population of Vadu HDSS area Pune district, India were collected. Members from families are grouped

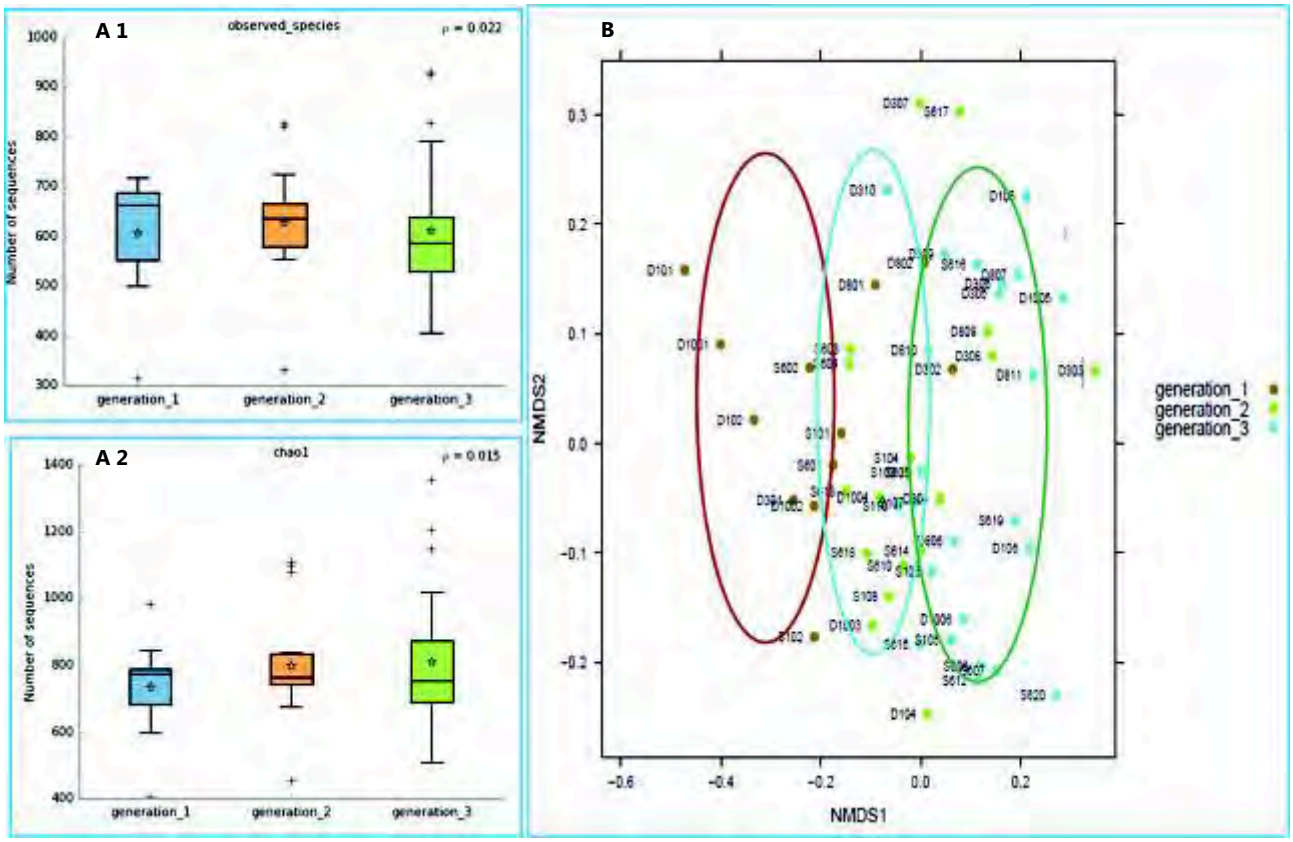

Fig. 1: Statistically significant differences were found in the first, second and third generation members in (A1) Observed species and (A2) Chao1 alpha diversity indices. (B) Bray Curtis NMDS plot showing gradient structure of microbiome composition in first, second and third generation members.

according to generations. First generation members had age 50 years or more, Second generation members had age between 25 to 40 years and third generation members had age between 3 to 15 years. Stool, oral washings and skin swabs were collected. The microbiome composition of oral and stool was studied using illumina Miseq sequencing technology with more than 1,00,000 sequences per sample. Phylum *Proteobacteria* was found to be most abundant followed by *Bacteroidetes* and *Firmicutes* in oral and Firmicutes were found to be most dominant in stool followed by *Bacteroidetes* and *Proteobacteria* (Fig.1). Microbial communities were found to display complex population dynamics, both in frequency and absolute density. Based on alpha and beta diversity, statistically significant differences were found in the first, second and third generation members. Further studies on beta diversity to check the distinctive community structure within the generations, we used two dimensional non-metric multidimensional scaling (NMDS) plots based on bray-curtis metric. This revealed a gradient structure of taxonomic composition in which second generation members occupy intermediate position between first and third generation members (Fig.1). The findings also show a significant impact of cooking fuel on oral and gut microbiome and necessitate further investigation

into the mechanisms and their clinical consequences. Collectively, this data represents future characterization of the ecology and translational applications of the human microbiome in diverse Indian population.

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

The object of this study was to decipher the gut microbial community structure of 13-14 years children from India and Finland. Additionally, the project also focused on understanding the influence of factors like birth mode and secretor status of FUT2 (*Fucosyl Transferase*) gene on the gut microbial flora in these two geographically distinct settings. The study included 99 subjects (Finnish, n=52 and Indian, n=47). Fecal samples were used for microbial community studies, while blood samples were used for FUT2 SNP (Single Nucleotide Polymorphism) rs601338 analysis. The observations suggest that there are significant differences in the gut bacterial community structure between Indian and Finnish children (as illustrated in Fig. 1A.) Further analysis to find out the key contributors for these differences revealed that genera like *Blautia*, *Bifidobacteria*, *Megasphaera* and *Prevotella* are differentially abundant between these populations (Figure. 1B). Furthermore, our findings suggest that the factors like birth mode and FUT2 secretor status have significant influence on abundance of specific bacterial groups like Dialister, Actinomyces (Birth mode: Vaginal and C-section) and *Akkermansia*, *Dialister* and *Coprococcus* (FUT2 Secretor status).

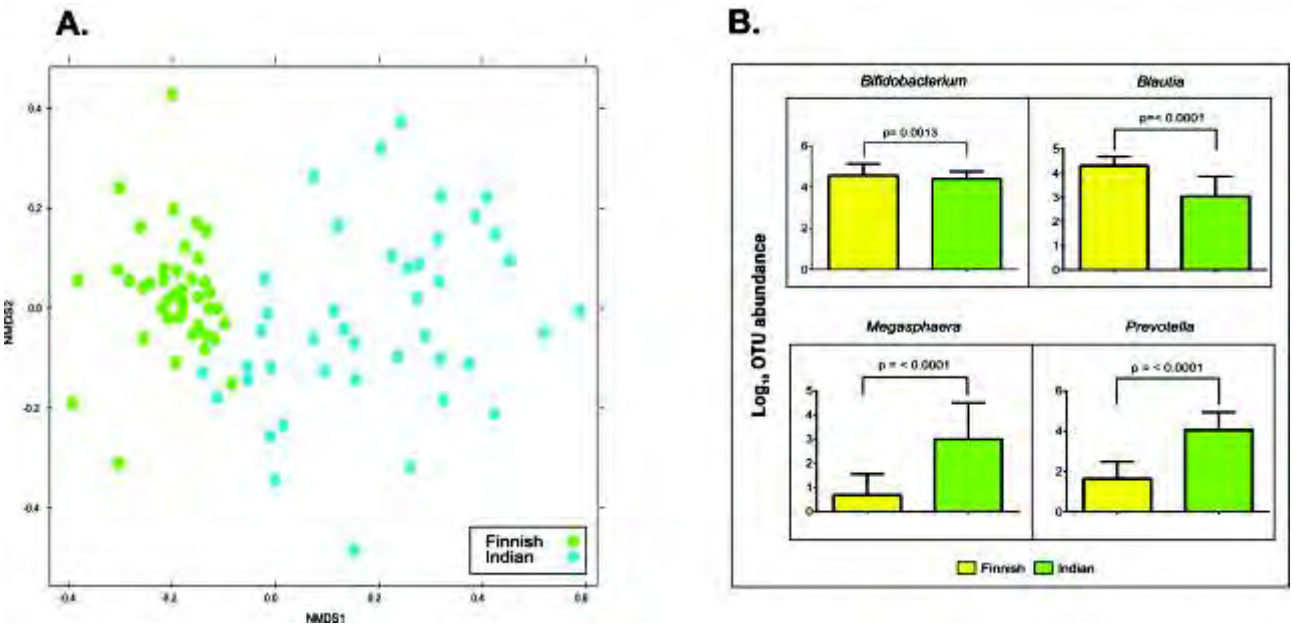

Fig. 2: Bacterial community analysis (A) Nonmetric Multi-Dimensional Scaling (NMDS) of Bray-Curtis distances based on the prevalence of phylotypes (B) Bar graph (Mean with SD) as determined from the abundance of specific genera within Indian (n=42) and Finnish (n=41) subjects; p-values (Mann Whitney test) indicate significant differences in abundances of bacterial groups between two cohorts.

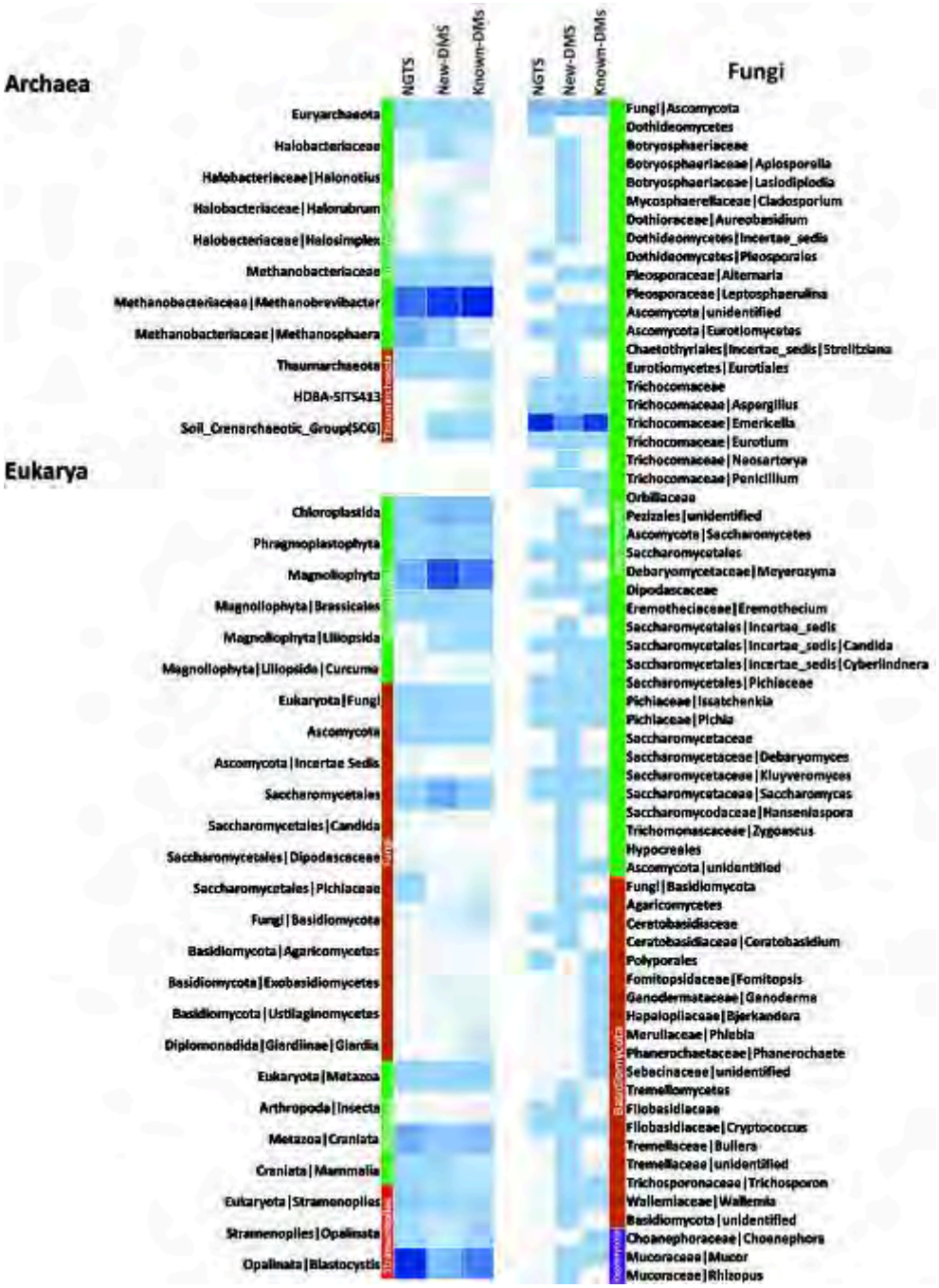

Fig. 3: Heatmap showing abundance of different members of archaeal, eukarya and fungal components of NGTs, New-DMs and Known-DMs subjects

Archaeal, Eukaryotic and fungal Dysbiosis in diabetic subjects

Gut archaeal, fungal and eukaryal studies are lagging behind Eubacteria; few studies concerning archaea fungi and eukaryote present in human gut are clear indications that these microbes together with eubacteria forms very complex ecosystem in the gut and their functional role in human health and diseases needs to be evaluated thoroughly. Amplicon studies using archaeal 16S rRNA

gene from three pools of samples (NGTs, New-DMs and Known-DMs); indicated that gut archaea are dominated by *Euryarchaeota* and *Thaumarchaeota* phyla. The former being the most dominated phylum occupying more than 99% reads of all three groups. The gradual increase in *Methanobrevibacter* (which was also the most abundant taxa in all groups) and associated decrease in *Methanosphaera* abundance from NGTs to New-DMs to Known-DMs subjects was observed (Fig.3). Eukaryotic 18S amplicon sequencing revealed that there were four phyla: Chloroplastida, Metazoa, Stramenopiles, and Metamonada found predominating in gut environment. Members of Stramenophile especially members of genus *Blastocystis* were found abundant in all groups (Fig.3). Fungi, particularly members belonging to Saccharomycetales were abundant in New-DMs compared to NGTs and Known-DMs. From the fungal ITS data, it was obseved that OTUs belonging to phyla Ascomycota were most dominant followed by *Basidiomycota* and *Zygomycota* to be least dominant. From the Ascomycota group; *Aspergillus* and *Emericella*, the two alternative forms of same fungus predominated most of the sequences (Fig.3).

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

Background

Leishmaniasis is an infectious disease that is endemic in 88 countries across the world, affecting nearly 12 million people. Leishmaniasis is caused by the parasite 'Leishmania'. There are three important forms of Leishmaniasis, one among them being the cutaneous leishmaniasis (CL). CL is characterized by skin lesions and scars which may eventually progress to form ulcers. CL is widely prevalent in the tropical and sub-tropical regions of the world and the current treatment of this disease relies on the administration of antimony based compounds. These compounds have their associated toxicity and moreover in parts of India, there is a growing incidence of resistance against these antimony based drugs. Second line treatment of leishmaniasis involves the administration of parenteral formulations such as pentamidine, Paromomycin etc while the only oral agent available to treat leishmaniasis is miltefosine. The current chemotherapy for leishmaniasis therefore faces problems related to difficulty in administration of compounds, associated toxicity and side effects as well as the emergence of drug resistance. Thus, there is a need to identify compounds with characteristic anti-leishmanial properties.

Aims and Objectives

The aim of the current work is to carry out the *in silico* design and screening of coumarin derivatives thereby identifying compounds exhibiting anti-leishmanial properties.

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Work Achieved

In-silico design and screening of compounds:

Compounds like coumarins have known to possess potent anti-fungal properties. Recent studies based on drug repurposing have now identified that coumarins like auraptene have anti-protozoan activities as well. To further exploit the possibility of using coumarins as anti-leishmanial agents, we have designed a set of coumarin derivatives *in silico*. During the screening, a combination of shape matching methods as well as QSAR techniques was used. The top five compounds were further selected for screening against *Leishmania major* promastigotes. Of the five compounds that we have tested *in vitro*, only two of the compounds displayed efficacy over the promastigotes and their IC50 values were determined. (Figure 1a) We further screened these two compounds in Balb/c mice infected with *Leishmania major*. Mice were infected in the left hindlimb footpad. Progression of infection was monitored in the form of thickness of the footpad every week. Compound was administered orally daily once at a dose of 5mg/kg/bwt. Of the two compounds tested, treatment of only one compound resulted in significant reduction of the footpad swelling. (Figure 1b) Parasite load in the draining lymph nodes was quantified and

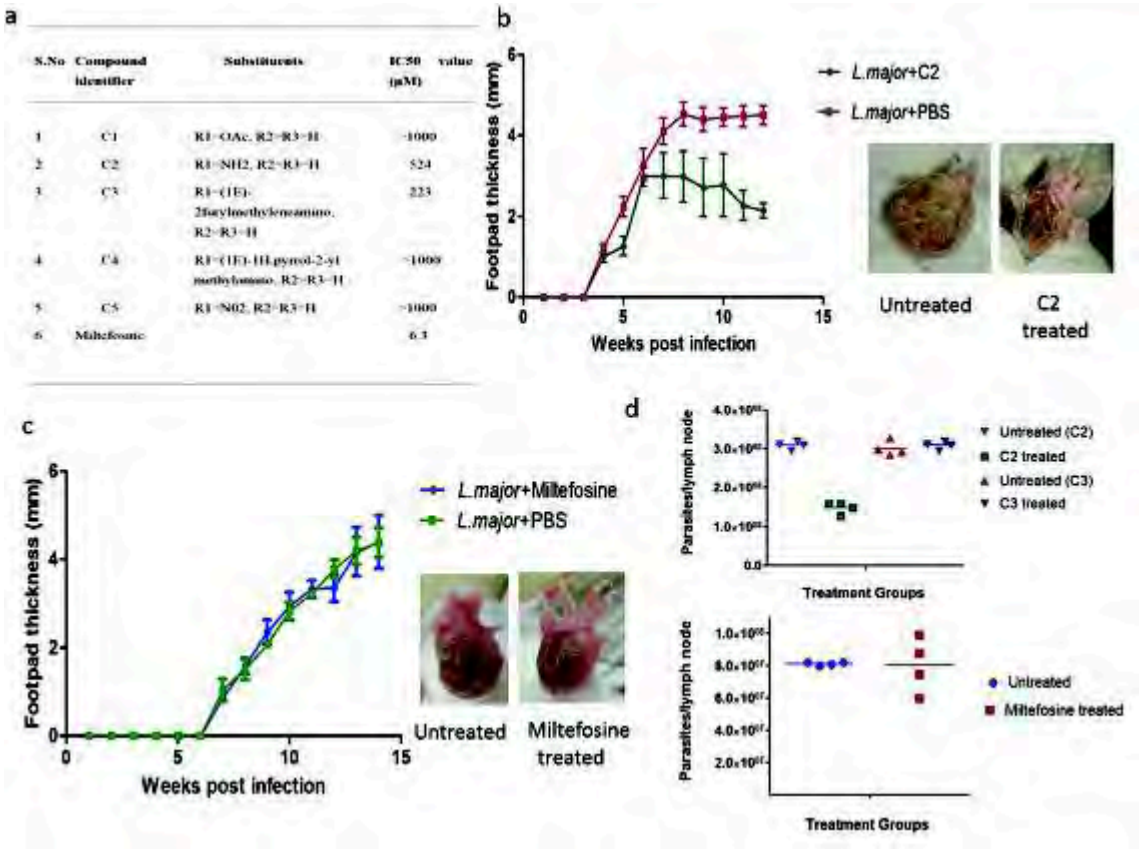

Fig. 1: a) IC50 values of compounds and miltefosine post 48h of treatment. Compounds giving less than 50% inhibition have been reported to have IC50 >1000 μM. b) Footpad swelling of the treated mice (n=4). Data summarizes the (Mean±SEM) with *p <0.05,

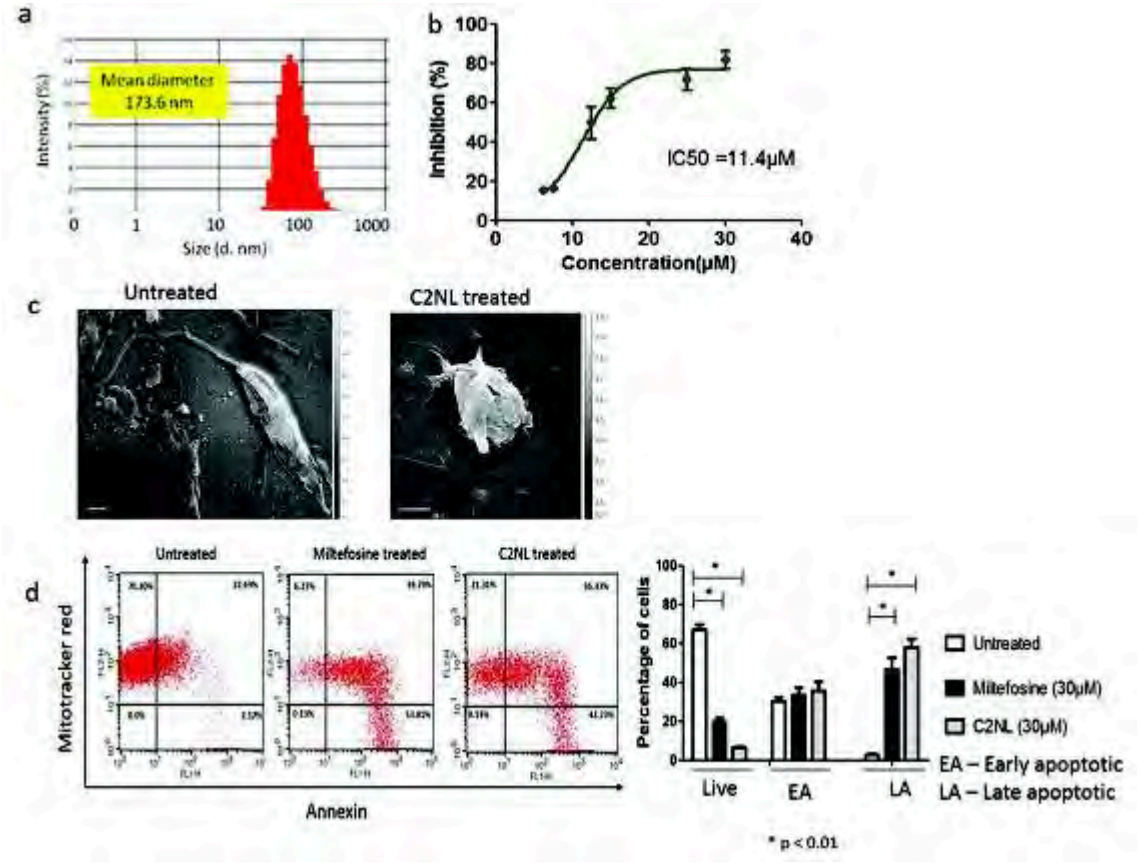

Fig. 2: a) DLS indicating the hydrodynamic diameter of the nanoliposomes. IC50 graph showing the efficacy of nanoliposomes over *Leishmania major* promastigotes. b) SEM images of the untreated and the nanoliposomes treated promastigotes. White bar corresponds to 1 μm. c) Plots indicate the reduction of mitochondrial membrane potential and the induction of apoptosis in the miltefosine and the C2NL treated *L.major* promastigotes.

compared with that of the control (infected but untreated) mice. (Figure 1d) On similar lines, we also compared the effect of miltefosine (only known oral drug for the treatment of CL). At a similar dose (5mg/kg/bwt), there was no reduction in the footpad swelling in the mice being treated with miltefosine. (Figure 1c) This was in line with the previous studies which report the standard dose of miltefosine for CL is >25mg/kg/bwt. This could be attributed to the low bioavailability of miltefosine at the site of infection. Thus the efficacy of our compound with the standard drug was compared.

Development of nanoliposomes and their anti-leishmanial properties:

To further enhance the solubility of the compound, to improve its efficacy and to study the mode of action of the compound, a nanoliposomal formulation was prepared. Encapsulation of the drug in the form of nanoliposomes further enhanced the efficacy of the compound. (Figure 2a) *L.major* promastigotes treated with the nanoliposomes showed morphological changes such as size reduction as well as loss of motility. To futher confirm our hypothesis; we have performed SEM analysis of the treated promastigotes. *L.major* promastigotes

were treated with the nanoliposomes at a concentration of 30μM for a time period of 48h. The treated promastigotes exhibit size reduction and loss of flagella. (Figure 2b) To further characterize the anti-leishmanial properties of the compound, *L.major* promastigotes were treated with 30μM of the nanoliposomes for a time period of 48h. Dual staining with annexin and mitotracker red resulted in the identification of cells that exhibit apoptosis as well as reduction in mitochondrial membrane potential. The efficacy of the nanoliposomes was also compared with that of the standard drug miltefosine (Figure 2c). Our study, at present indicated that the coumarin derivative identified exhibits anti-leishmanial property and its efficacy and solubility can further be enhanced by encapsulating it in the form of nanoliposomes.

Future Research Plans

To further characterize efficacy of the nanoliposomes and study their anti-leishmanial properties

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Role of peroxiredoxin-3 (Prx-3) in redox regulation of hyperglycemia-induced contractile dysfunction in diabetic cardiomyopathy

Background

Mitochondria are principle source of ROS under hyperglycemic conditions. Most of the superoxide ($O_2^{\cdot-}$), produced at electron transport chain (ETC), is dismutated under physiological conditions by mitochondrial Manganese superoxide dismutase (MnSOD) to form hydrogen peroxide (H_2O_2). Even though, MnSOD relieves mitochondrial oxidative stress caused by $O_2^{\cdot-}$ it further enhances a different type of oxidative stress by dismutating $O_2^{\cdot-}$ into H_2O_2 that can damage cellular macromolecules such as proteins, lipids, and nucleic acids, especially after its conversion to hydroxyl radical (OH.) by Haber-Weiss reaction. Recently, our laboratory has demonstrated that Monoamine Oxidase-A (MAO-A), present in the outer mitochondrial membrane is also an important source of H_2O_2 and involved in development of DCM. Based on these results, mitochondrial antioxidants are projected to be the first line-of-defense mechanism against ROS generation in the mitochondria and thus, may improve the myocardial performance in diabetes mellitus. Mitochondrial H_2O_2 can be decomposed by Glutathione peroxidases (GPxs) 1, 4 and Peroxiredoxins (Prxs) 3, 5. However, an absence of catalase in mitochondria of myocytes and reduced scavenging of H_2O_2 by GPx1 highlights the importance of Prxs in removal of mitochondrial H_2O_2.

Peroxiredoxins are thiol-dependent antioxidants which reduce H_2O_2 at its cysteine residues containing active sites. Prxs are present in six isoforms Prx-1 to -6. Of these, Prx-3 contains mitochondrial localization sequence and exclusively found in mitochondria. Prx-5 is also linked with mitochondria in addition to nucleus and peroxisomes. The ability of Prx-3 and Prx-5 as active

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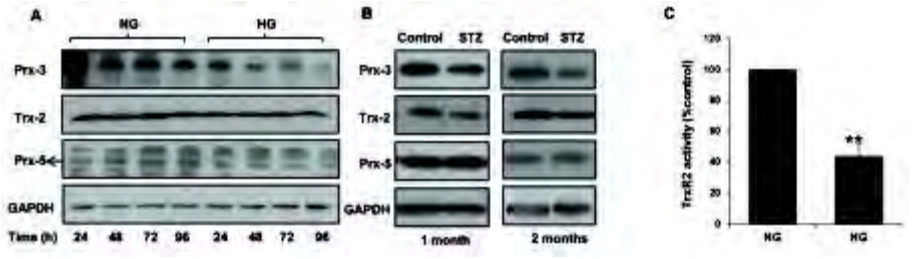


Fig. 1: Hyperglycemia reduces mitochondrial antioxidant protein expression Prx-3 and its electron donors Trx-2 and TrxR activity both *in vitro* and *in vivo*.

antioxidants depends on their recycling by the mitochondrial electron donor Thioredoxin-2 (Trx-2) complex. The reduced form of Trx-2 is then regenerated by Thioredoxin reductase-2 (TrxR2) at the expense of NADPH. Trx-2 and TrxR2 reside in the mitochondrial matrix and operate independently from the cytosolic Trx network. According to kinetic studies, Prx-3 has emerged as a principle scavenger of H_2O_2 in mitochondria. The greater efficiency of mitochondrial Prx-3 and -5 together with Trx-2, TrxR2 and NADPH, may attribute to protection against high glucose induced oxidative stress. Infact, overexpression of Prx-3 has been reported to prevent the left ventricular remodeling after myocardial infarction in transgenic mice, and Prx-3 also has crucial role in contractile function of skeletal muscle by regulating mitochondrial homeostasis. Prx-5 overexpression protects mitochondrial DNA damage induced by H_2O_2 and also human tendon cells against apoptosis and loss of cellular function during oxidative stress. However, the role of mitochondrial Prx in DCM prevention has not been explored to its full potential.

Aims and Objectives

To examine the effect of Prx-3 induction on oxidative stress induced myocardial damage in diabetic condition.

Work Achieved

Our previous work provided strong evidence that mitochondrial MAO-A is an important source of oxidative stress in the heart and that MAO-A-derived reactive oxygen species (ROS) contribute to DCM. Further we sought to understand the role of the mitochondrial antioxidant protein Peroxiredoxin-3

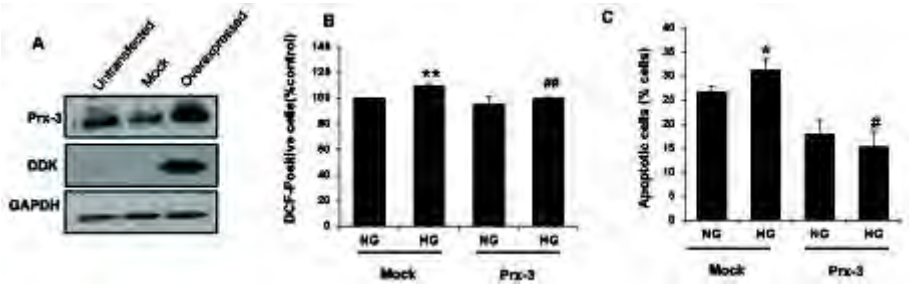
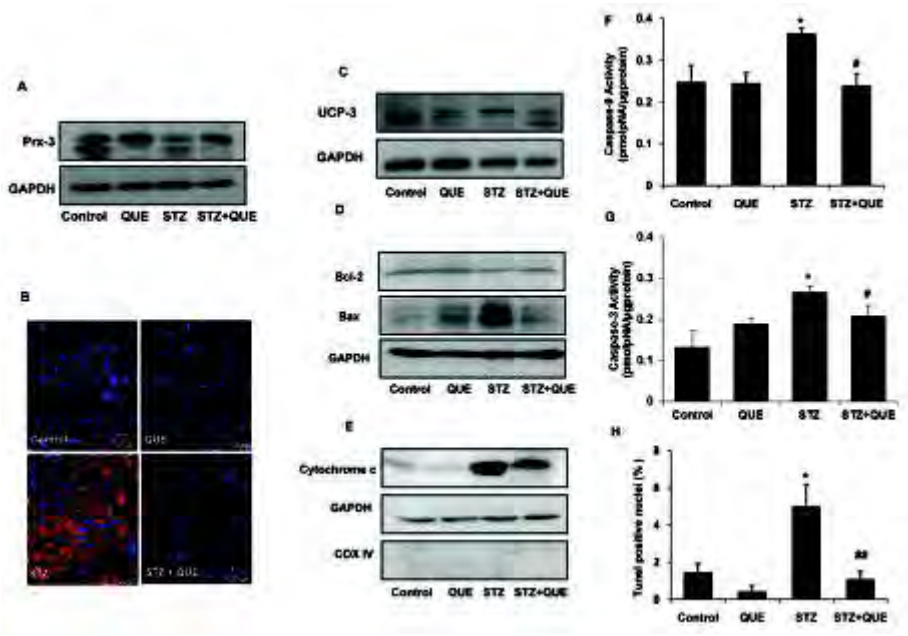


Fig. 2: Prx-3 overexpression reduces oxidative stress, prevents apoptosis under high glucose conditions

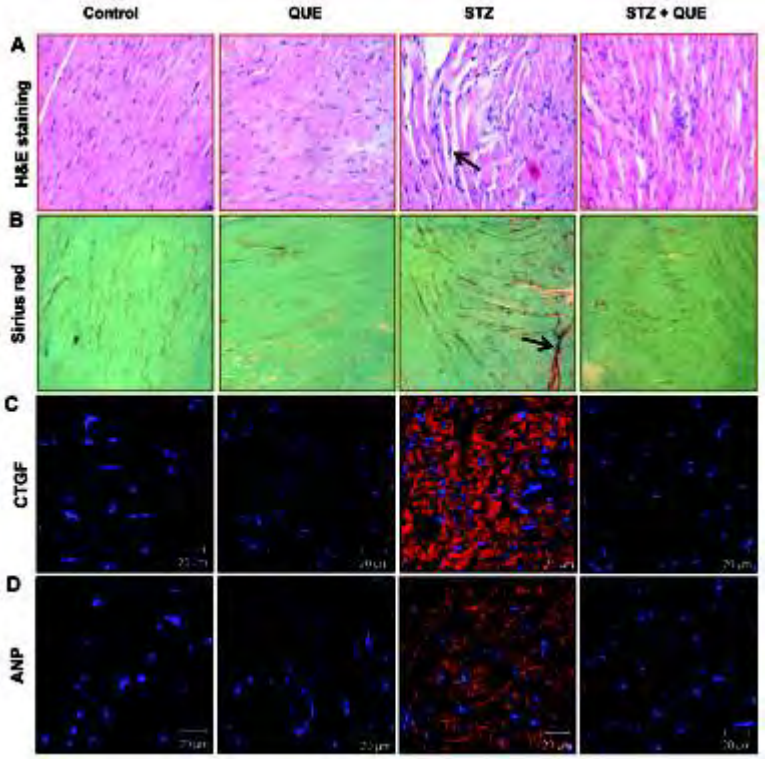
Fig. 3: Prx-3 induction by quercetin reduces oxidative stress and myocardial apoptosis in STZ induced diabetic rats



(Prx-3) in redox regulation of hyperglycemia-induced contractile dysfunction in diabetic cardiomyopathy

We observed a decrease in the expression of Prx-3 in the hearts of streptozotocin (STZ) induced diabetic rats, and also high glucose treated H9c2

Fig. 4: Prx-3 induction by quercetin attenuates diabetes-induced changes in myocardial histology, cardiac fibrosis and hypertrophy



cardiac cells, which may augment oxidative stress mediated damage. Hence we hypothesized that overexpression of Prx-3 could prevent the cardiac damage associated with diabetes. In this study we used quercetin (QUE) to achieve Prx-3 induction in vivo, while a Prx-3 overexpressing H9c2 cell line was employed for carrying out in vitro studies. Diabetes was induced in Wistar rats by a single intraperitoneal injection of STZ. Quercetin (50mg/kg body weight) was delivered orally to hyperglycemic and age matched control rats for 2 months. Quercetin treatment induced the myocardial expression of Prx-3 both in control and STZ rats. Prx-3 induction by quercetin prevented diabetes induced oxidative stressas confirmed by decrease in expression of markers such as 4-HNE and mitochondrial uncoupling protein, UCP-3. It was also successful in reducing cardiac cell apoptosis, hypertrophy and fibrosis leading to amelioration of cardiac contractility defects. Overexpression of Prx-3 in cultured H9c2 cardiac cells could significantly diminish high glucose inflicted mitochondrial oxidative damage and apoptosis, thus strengthening our hypothesis. In conclusion, these results suggest that diabetes induced cardiomyopathy can be prevented by elevating Prx-3 levels thereby providing extensive protection to the diabetic heart.

Future Research Plans

Further, efforts are under way to investigate the role of the redox-sensitive transcription factor nuclear factor E2-related factor 2 (Nrf2)/ Kelch-like ECH-associated protein 1 (Keap1), pathway which is the master regulator of cytoprotective response including antioxidants in diabetic cardiomyopathy.

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

Background

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

Aims and Objectives

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

Work Achieved

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

In order to determine whether genes involved in endocytosis and vesicular transport indeed regulate the pluripotency of stem cells, we used commercially available siRNAs against 112 genes implicated in the process of endocytosis. siRNAs were introduced individually into each well of a 96 well plate in which mESCs were plated. In order to prime mESCs to differentiate, LIF was withdrawn

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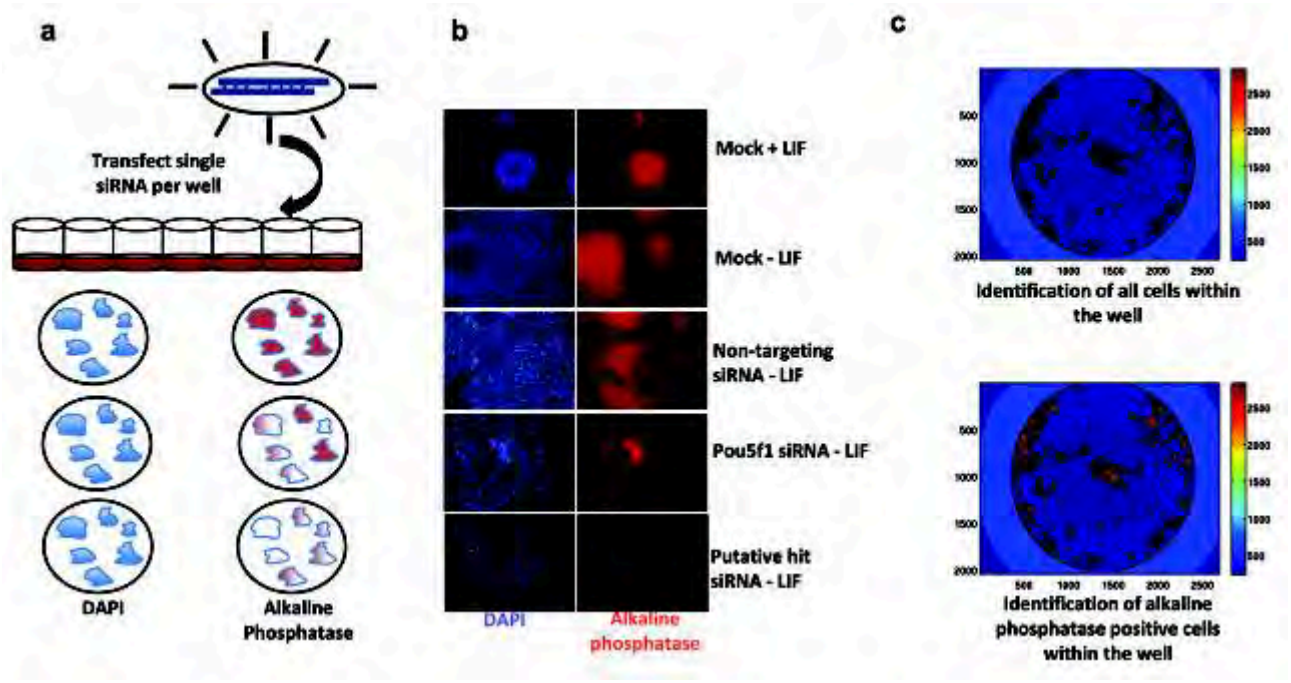

Fig. 1: Schematic of siRNA screen to knockdown genes involved in endocytosis in mouse embryonic stem cells: (a) Schematic of siRNA screen. mESCs were plated in 96 well plates. 24 hours post plating, individual siRNAs were introduced into each well at a final concentration of 50nM. At the time of transfection,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

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

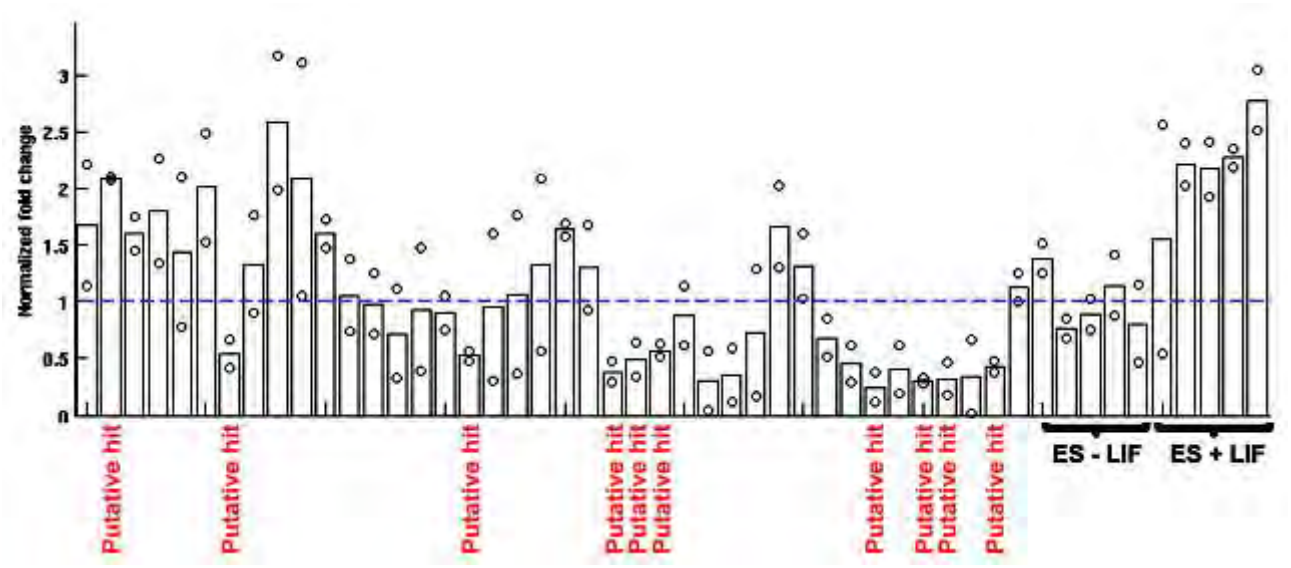

Fig. 2: Knockdown of genes involved in endocytosis and vesicular trafficking impact pluripotency of mouse embryonic stem cells: Graphs showing normalized fold change in Alkaline phosphatase staining compared to staining observed in mESCs grown under ES-LIF conditions. Each screen was repeated twice and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

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

Establishing tools and techniques to generate genetically modified mice using CRISPR-Cas9 technology:

The CRISPR-Cas9 technology of gene editing allows modification of genetic loci and the generation of genetically-modified mice with relative ease. Using this technology we are trying to build the following:

1) Knockout mice lacking expression of specific genes involved in trafficking.
2) Knock-in mice carrying fluorescent reporters at the endogenous locus of genes involved in trafficking.

These tools will permit us to understand and track the role of specific endocytic genes at various stages of early development. Towards this goal, we are initially establishing embryo isolation (Fig. 3a), culture and transfer into pseudopregnant female mice (Fig. 3b), followed by monitoring of successful implantation and development (Fig. 3c, 3d). We have successfully established these in our setting. We are currently developing reagents based on CRISPR-Cas9 technology to enable us to modify specific genetic loci and are about to start injecting these into mouse embryos prior to transfer into pseudopregnant females.

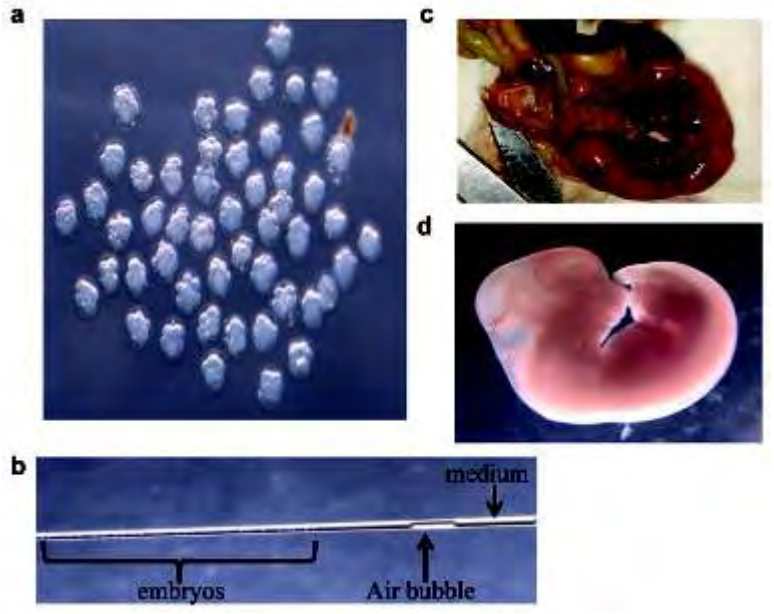

Fig. 3: Isolation of mouse embryos and their transfer into pseudopregnant female mice: (a) Representative image showing e2.5 mouse embryos isolated by flushing the oviduct and uterine horn. (b) Image showing a capillary loaded with embryos prior to transfer into a pseudopregnant female mouse. (c) Representative image showing successfully implanted and developed embryos in the uterine horn after successful transfer. Asterisks show sites of implantation. (d) Image showing an embryo isolated at e13.5 post transfer.

Future Research Plans

- Validation of targets from the siRNA screen is under progress.
- Isolation of endosomes from embryonic stem cells and differentiated cells and the determination of contents of these endosomes by mass spectrometry is underway.
- Development of knockout mice using CRISPR-Cas9 technology in order to understand the role of endocytic genes during early mouse embryonic development is under progress.

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

Background

In eukaryotes, cells integrate cues from their surrounding environment and interpret these signals in order to retain their capacity to transition between a cycling state and a resting state, termed quiescence. The ability of cells to retain viability during quiescence and their entry or exit into cell cycle when needed, is necessary for complex multicellular processes. This transition is associated with changes in gene expression accompanied by alterations in chromatin modifications. Dysregulation of this balance can lead to a wide range of hypo and hyper-proliferative 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*.

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

Participants

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Aims and Objectives

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

Work Achieved

To understand the genomic program of quiescence-proliferation axis and the entry into and exit from quiescence, we have utilized an inducible classic model of human diploid fibroblasts in which quiescence and proliferation can be induced by serum deprivation and stimulation. Transcriptome analysis of the cells during this process revealed a large number of differentially expressed lncRNAs during entry into and exit from cell cycle indicating their crucial roles during this process. We have further categorized lncRNAs expressed at different stages of the quiescence and proliferation program. Currently we are focusing on the candidates induced immediately upon serum stimulation. Functional characterization of the candidates is in progress.

Future Research Plans

- Functional characterization of lncRNAs induced at early stages of cellular quiescence.
- Functional validation of the candidates in other quiescence models.
- Mechanism of action of selected candidates in driving cellular proliferation.
- Understand the temporal regulation of lncRNAs during the quiescence-proliferation axis.

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Role of IL-3 in prevention of cartilage and subchondral bone damage in osteoarthritis

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Background

Osteoarthritis (OA) is a chronic disease of articular joints that leads to degeneration of both cartilage and subchondral bone. Chondrocytes in articular cartilage are highly sensitive to pathological changes and any injury that leads to irreversible physical damage to cartilage induces inflammatory microenvironment in the joints. The proinflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are the key players in the pathophysiology of OA. These cytokines enhances the degenerative processes of cartilage by downregulating the expression of genes involved in maintenance of cartilage matrix, and promote hypertrophy and apoptosis of chondrocytes. Importantly, these cytokines induce expression of matrix metalloproteinases (MMPs), which degrade the cartilage matrix. The degenerative changes in articular cartilage gradually percolate into the subchondral bone region, as both are interdependent. Inflammation attracts immune cells and osteoclast precursors, thereby increasing osteoclast formation and bone resorption in subchondral bone region.

Interleukin-3 (IL-3), a cytokine secreted by activated T lymphocytes is a broadly acting hematopoietic-regulatory protein. Previously, we have documented that IL-3 is a strong inhibitor of osteoclast formation and pathological bone resorption. Also, IL-3 has anti-inflammatory activity in vivo and indirectly protects cartilage and bone damage in murine models of inflammatory and rheumatoid arthritis. However, the role of IL-3 on chondrocyte biology and pathophysiology of OA is not yet delineated. Recently, we demonstrated that IL-3 increases the expression of the chondrocyte specific genes Sox9 and Col2a

in mouse chondrocytes which were downregulated by IL-1β, and it also decreases IL-1β and TNF-α-induced expression of MMP-3 and MMP-13 in both mouse and human chondrocytes. Importantly, IL-3 decreases cartilage degeneration in vivo in mouse model of human OA. In further studies we investigated the role of IL-3 on cartilage matrix degradation in vitro in human mesenchymal stem cells (MSCs) and subchondral bone damage in mice OA.

Aims and Objectives

- To investigate the role of IL-3 in chondrocyte differentiation from human MSCs.
- To evaluate the role of IL-3 on degeneration of subchondral bone in mice OA.

Work Achieved

IL-3 reduces IL-1β-induced matrix degradation in human MSCs

To assess the translational implications of protective effect of IL-3 on cartilage damage in vivo in mice, we determined the effect of IL-3 on cartilage degeneration induced by IL-1β in micromass pellet cultures of human MSCs. We first examined the preventative effect of IL-3 on matrix degradation by treating pellet cultures with IL-1β and IL-3 from day 1 to 21. Histological evaluation showed that the matrix in IL-3 treated chondrogenic pellets was less degraded compared to IL-1β. Quantitation of percent matrix deposition and percent matrix degradation confirmed that IL-3 significantly reduces the matrix degradation induced by IL-1β.

Next, we determined whether IL-3 could help in repairing IL-1β-induced matrix degradation. For this, the cell pellets were primed with IL-1β for first 10 days and IL-3 was added from day 10 to 21. Histological analysis showed that IL-3 reduced matrix degradation induced by IL-1β, and increased matrix deposition. These results suggest that IL-3 shows a protective effect when the process of matrix degradation was initiated by IL-1β. Further, we examined the therapeutic effect of IL-3 on IL-1β induced matrix degradation by incubating cell pellets with IL-1β from day 1 to 21; and IL-3 was added from day 10 to 21. Interestingly, we observed that IL-3 showed therapeutic effect and decreases degradation of matrix under severe inflammatory conditions. Thus, IL-3 showed the preventive and therapeutic effects on cartilage degeneration induced by IL-1β in human MSCs. These observations support that the decreased matrix degradation by IL-3 is a result of transcriptional downregulation of MMPs.

IL-3 decreases degeneration of subchondral bone in OA mice

We have earlier shown that IL-3 decreases cartilage degeneration in vivo in mouse model of human OA. Articular cartilage and subchondral bone constitute the important components of joint articulation. In OA, the

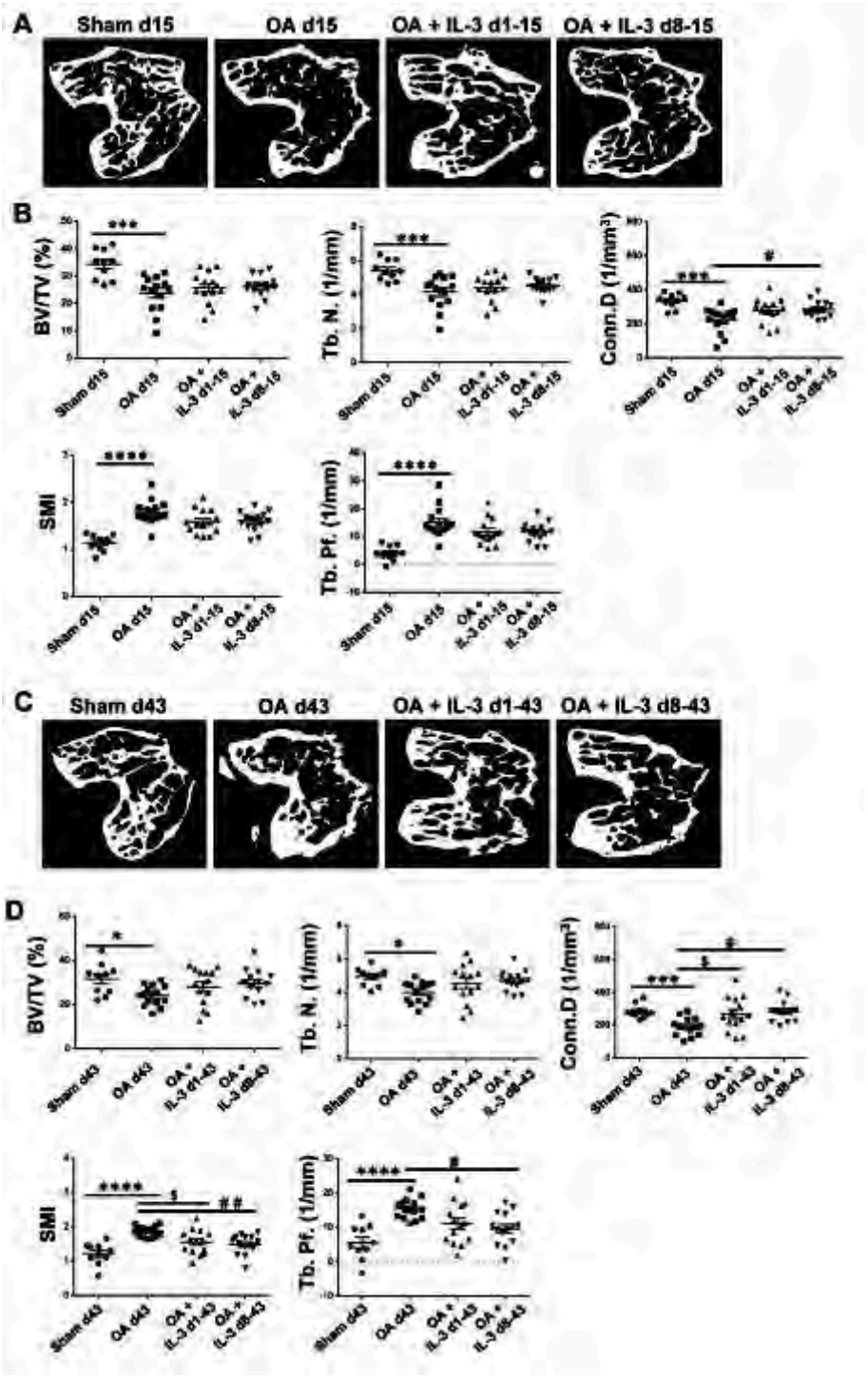
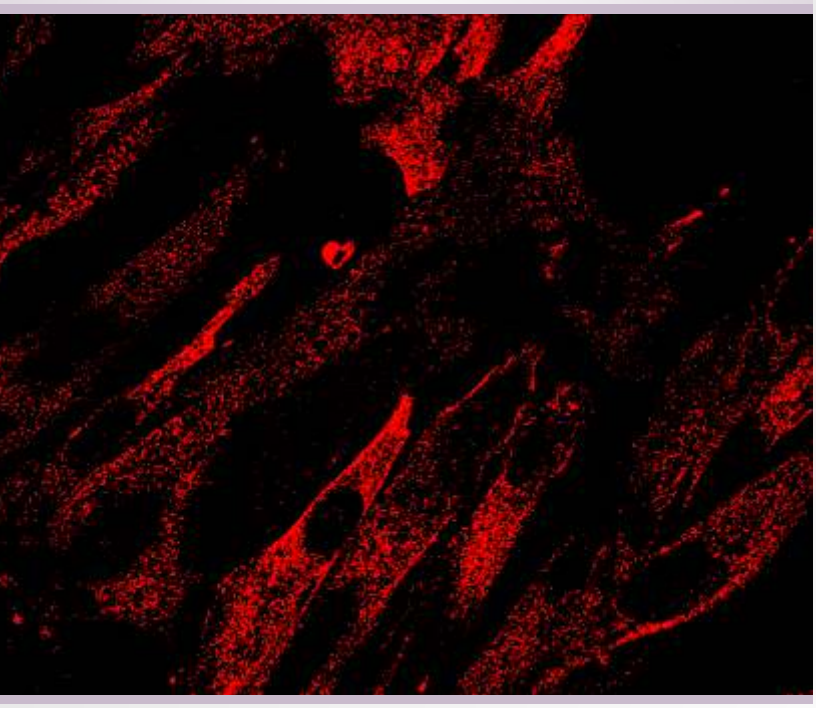


Fig. 1: IL-3 reduces degradation of subchondral bone in OA mice. ACL-transected mice were injected intra-articularly with IL-3 using four different treatment regimens, which were categorized into two different time points (day 15 and day 43 post-surgery) and two different therapies (preventive and therapeutic). Whole knee joints were subjected to µ-CT for evaluation of subchondral bone. Representative binary radiographs show the trabecular bone structure of various experimental groups at day 15 (A) and 43 (C). Various trabecular bone indices reflecting the quality of subchondral bone and topological parameters including BV/TV, Tb. N., Conn.D, SMI and Tb. Pf. (B & D) were quantified at day 15 (B) and 43 (D) using µ-CT reconstructions. Data is presented as mean ± SEM of 10–14 mice per group. Significance was calculated by a one-way ANOVA with a post hoc Tukey's multiple comparisons test between 1) OA versus sham; 2) OA + IL-3d1 groups versus OA and 3) OA + IL-3d8 groups versus OA. *p<0.05 or ***p< 0.001 or ****p < 0.0001 for OA versus sham; $p < 0.05 for OA + IL-3d1 versus OA; #p < 0.05 or ##p < 0.01 for OA + IL-3d8 versus OA

degenerative changes in articular cartilage gradually percolate into subchondral bone region as both are interdependent. To evaluate the role of IL-3 on subchondral bone damage, we first confirmed the erosion of trabecular bone in the femoral subchondral region in mouse model of human OA. Next, mice were injected intra-articularly with IL-3 using four different treatment regimens, which were categorized into two different time points (day 15 and day 43 post-surgery) and two different therapies (preventive and therapeutic). Whole knee joints were subjected to microcomputed tomography (μ-CT) for evaluation of subchondral bone damage. The representative images of trabecular microarchitecture of femoral subchondral bone showed betterment upon IL-3 treatment in both preventive and therapeutic regimens at day 15 (Fig. 1A). The trabecular microarchitecture in IL-3 treated OA mice showed a significant increase in connectivity density (Conn.D) in therapeutic treatment regimen and a trend towards improvement in structure model index (SMI) and trabecular pattern factor (Tb. Pf.) in both preventive and therapeutic treatment regimens. However, no effect on trabecular bone volume fraction (BV/TV) and trabecular number (Tb. N.) was seen in IL-3 treated mice (Fig. 1B). At day 43, the representative images of trabecular microarchitecture of femoral subchondral bone also showed betterment upon IL-3 treatment in both preventive and therapeutic treatment regimens (Fig. 1C). IL-3 showed significant preservation of SMI and Conn.D in both the treatment regimens while Tb.Pf was significantly protected in therapeutic treatment regimen. A trend of improvement in BV/TV and Tb. N. was observed in both the treatment regimens (Fig. 1D). These results suggest that in addition to preventing the cartilage damage, IL-3 also plays an important role in preserving femoral subchondral bone microarchitecture in OA. Thus, we provide the evidence that IL-3 has therapeutic potential in amelioration of degeneration of both articular cartilage and subchondral bone microarchitecture associated with OA.

Future Research Plans

Our in vitro studies using mouse and human chondrocytes, and also in vivo studies in mice strongly suggest that IL-3 has a multifaceted role in amelioration of cartilage and subchondral bone damage in addition to its role in modulating the inflammatory responses associated with OA. We plan to do further studies in large animal model using clinical cases of osteoarthritis in dogs.



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 core 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 barrier-maintained facility for the breeding, maintenance and supply of small laboratory animals viz. inbred and mutant mice, rats, rabbits etc. for the ongoing research projects of the Institute. The following is the list of various laboratory animals maintained at the facility:

MICE:

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

Genetically engineered mutant mice (knock-out, transgenic and mutant mice - 39 lines)

RATS:

- WISTAR

RABBITS:

NEWZEALAND WHITE

The Team

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

* BALB/c with cataract mutation
# Outbred

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

The breeding programs for laboratory animals are designed to meet the requirements of the investigators with minimum wait periods for majority of the strains maintained in the facility. Complete scientific support and advice is extended as per demand to the Scientists and their group members for the conduct of experiments under IAEC approved projects.

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

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

Proteomics Facility

Dr. Srikanth Rapole
(Facility In-Charge)

The Team

Dr. Varsha Shepal, *Technical officer*

Mr. Venkatesh Naik, *Technician*

4800 MALDI-TOF/TOF

4000 Q-Trap LC-MS/MS

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

4800 LC-MALDI TOF/TOF system (AB Sciex) is a tandem time-of-flight MS/MS system that is used for high-throughput proteomics research. The system identifies proteins by determining accurate masses of peptides formed by enzymatic digestion. Additionally, the system can more definitely identify and characterize proteins by isolating and fragmenting a molecular ion of interest and measuring the fragment ion masses. The number of samples analyzed is approximately 684 samples including 262 external samples from April-2015 to March-2016.

4000 Q-Trap LC-MS/MS system (AB Sciex) is a hybrid triple quadrupole/linear ion trap mass spectrometer. The system is ideal for proteomic applications including post-translationally modified proteins discovery, protein identification, and biomarker validation. The number of samples analyzed is approximately 135 samples including 12 external samples from April-2015 to March-2016.

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

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

EKSIGENT NANO-LC and SPOTTER

EKSIGENT MICRO-LC

AGILENT GC-MS

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

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

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

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

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

Bioinformatics and High Performance Computing Facility

Dr. Shailza Singh
(Facility In-Charge)

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

Hardware Infrastructure

SGI Altix XE 1300 Cluster

Head Node:

SGI Altix XE 270 Serve.

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

Compute Nodes:

SGI Altix 340 Servers

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

SGI Cluster Software Stack:

SLES Ver 11

SGI ProPack 7

SGI Foundation Software Ver 2.0

Interconnect:

24-Ports Gigabit Ethernet Switch

GPU Computing HP Proliant SL6500

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

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

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

Integrated Graphics ATI RN50/ES1000 with 64 MB memory

2x NIVIDIA Tesla 2090 6 GB GPU computing module

Specialized Workstations:

HP Elite 8200 CMT PC

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

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 (DISCOVERY STUDIO 3.0), Protein Families (DISCOVERY STUDIO 3.0), Protein Health (DISCOVERY STUDIO 3.0), Protein Refine (DISCOVERY STUDIO 3.0), Profiles-3D (DISCOVERY STUDIO 3.0)

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

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

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

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

Molecular Dynamics: CHARMM, GROMACS, NAMD, MOIL

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

ab initio modeling: GAUSSIAN

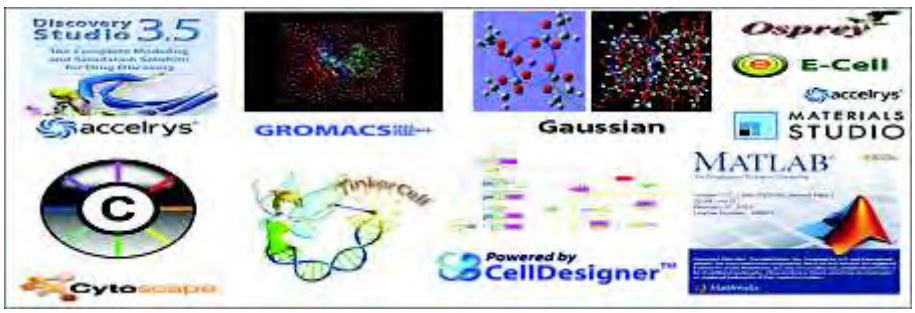
Systems Biology Tools: Virtual Cell, M-cell, Cell Designer, GEPASI, Cytoscape, Osprey, E-Cell, SimBiology

Artificial Intelligence: SVMlight and SNNS

Material Modeling and Simulation: Material Studio 5.5

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

Statistical packages: MATLAB and R



Library

The NCCS library has a collection in frontier areas of biotechnology having relevance to NCCS research activities. The library holds approximately fourteen thousand bound journals, three thousand books and two hundred thirteen NCCS Ph.D. theses. It subscribes to nineteen scientific journals and twenty eight other periodicals in print form.

In the development of its collections, the library's priority is to support NCCS research activities. The library collection is expanded in consultation with the scientists at NCCS. The library's print collections are growing by approximately 400 volumes per year. The library is equipped with Linux-based SLIM21 with RFID Interfaced library software for library house keeping operations and Web-OPAC for online searching of library documents. The library has also installed the barcode technology for circulation (issue & return) of library documents. The library also maintains its information (in Hindi & English) on its webpage, which includes free Online Medical database links, NCCS research publications list, library forms, 'NCCS in News', a collection of the Ph.D. theses of NCCS research scholars and a list of NCCS alumni and other Scientific Grants\Funds and fellowship-related links. During the period under review, the library has created a Digital Archive of the Ph.D. theses of NCCS research scholars, NCCS Publications, NCCS Annual Reports and Book Chapter archives, which are accessible through the NCCS Intranet.

Additional documentation facilities include local area network for library activities and PubMed database access, a number of CD-ROMS for books, journals & Ph.D. theses. The library is listed in the Union Catalogue of Biomedical Serials in India created by the National Informatics Centre, New Delhi and continues to be a member of the Medical Library Association of India.

The Library provides access to 734 online Journals of various Publishers, such as Springer, John Wiley, Nature Publishing group, Mary & Libert, Oxford, Elsevier Science Direct (including one online book series, Methods in Enzymology), and through the 'DeLCON' DBT Online journal consortia. In addition, it also subscribes to nine extra online journals related to the NCCS research activities.

In addition to the above, the library provides services for scanning documents for plagiarism using the iThenticate Anti-Plagiarism Software. We also use the Turnitin Anti-Plagiarism Software for scanning of Ph.D. theses provided by the Savitribai Phule Pune University. The library has also set up an open access repository for the research publications of the scientists of NCCS, which is available at http://nccs.sciencecentral.in/

The Team

Mr. Krupasindhu Behera, *Technical Officer*

Mr. Rameshwar Nema, *Technical Officer*

Computer Section

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

Expansion of LAN

A secured wireless LAN has been installed and configured in the additional locations of the NCCS Campus.

New NCCS website

A new HP server was configured having Linux CentOS 7.0 operating system for hosting new NCCS website conforming to Digital India initiative. This new website is anticipated to go live on the Foundation Day of NCCS. An additional new server having CentOS 7.0 OS was configured for hosting Dr. Shekhar Mande's (TNF-Core) application server.

ERP / Project Management and Accounting Software

The software requirement specification document for Integrated Project Management and Finance & Accounting software was prepared. We are collaborating with C-DAC, Pune for customized development and implementation of this software for NCCS.

The computer section constantly upgrades its systems and as part of this exercise, the section has upgraded its connectivity lines from 6 Mbps(1:1) ILL to 10 Mbps (1:1) bandwidth.

Regular maintenance

Regular maintenance and up-dating of the NCCS website and intranet website is done by the computer section. A new facility was created on the intranet, to host all circulars/ notices/ memorandums, etc., issued by the administration from time-to-time. Several operating systems and common application software were installed/updated on user computers at NCCS. These include MS Office 2010, Adobe Suite X, Sigma Plot Suite 12.0 and Reference Manager 12.0. The Paypack software takes care of staff salary process that includes TDS and EPF deductions.

The Team

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

The following systems are maintained in the virtualized environment:

a) WEB server, DNS server, SMTP Mail server.

b) File server and Print server

c) Setting up four Linux Proxy servers for Internet access.

d) Windows DHCP / DNS and Antivirus server

General Assistance

The computer section has made provision for cable and Wi-Fi internet connectivity in the newly inaugurated auditorium, to ensure smooth functioning during the presentations made by distinguished speakers. The computer section provides assistance in preparations of presentations /slides and also provides full technical support throughout the duration of seminars, symposiums, conferences.

Other Facilities

1) FACS Core Facility

The Team

- **Dr. L. S. Limaye** (Facility In-charge)
- Hemangini Shikhare
- Pratibha Khot
- Amit Salunkhe
- Ashwini Kore
- Dnyaneshwar Waghmare
- Atul Khirwale, (Operator provided by BD and posted in NCCS under BD-NCCS STEM CELL COE from Dec.2011)
- Pooja Shinde, Consultant (From June 2014-June 2015)

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

The usage of the seven equipments 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	7955	2533	---	---	10488
FACS Canto II (Old)	10542	---	---	---	10542
FACS Canto II (New)	12683	---	---	---	12683

STERILE SORTING:

EQUIPMENT	SORTING	ACQUISITION **
FACS Aria II SORP	542	1589
FACS Aria III SORP	179	638
FACS Aria III Standard	200	1618

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

BD Pathway 855:

Imaging of samples can be done for live cell staining (96 well plates) as well as for fixed cells in the form of sections on slides. Images are captured using mercury arc lamp and transmitted light in montage form (e.g. 8X8, 10X10). Macros are done as per the user's request.

Samples analyzed for users from outside NCCS:

As workload of outsider samples was increased, so since June 2012 NCCS had made a policy to charge the people from outside NCCS for their samples. For academic and research institutes, the charges are nominal and for private institutes/ companies the charges are higher. Institutes like AFMC, ARI, IISER, Biochemistry and Zoology departments of the S. P. Pune University have utilized our facility from April 2015- March 2016. We had received 289 samples for services like surface/ intracellular staining and DNA cell cycle analysis.

Activities under BD-NCCS COE programme:

1. National Workshop on Basic Flowcytometry and cell sorting

We organized a "National hands-on workshop on Basic Flowcytometry and cell sorting" under the BD-NCCS Centre of Excellence in 'Stem Cell Research'. It was conducted at NCCS, Pune from June 22-24, 2015. Four students (1 from NCCS & 3 from outside NCCS) were short listed for the program. The program was conducted by a BD Application specialist with help from our operators.

2. Basic Course on Cytometry (BD FACSCanto II)

We organized a 3 days basic hands-on course for those students with no/minimal experience in flow cytometry under BD-NCCS Centre of Excellence in 'Stem Cell Research.' It was conducted at NCCS, Pune from April 22-24, 2015. Five students (3 from NCCS & 2 from outside) were short listed for the program. The program was conducted by a BD Application specialist with help from our operators.

3. Canto-II training and examination:

We organized training on Canto-II during this period. 16 students from NCCS had received training in different batches from Sept 2015- Oct 2015. We also conducted an examination for the trained students on 2-3 November 2015. 16 Students appeared for the exam, of which 15 students successfully qualified it and started using the instrument independently.

Science Day Activities:

On the occasion of the National Science Day on 28 February 2016, facility operator Ashwini Kore and Dnyaneshwar Waghmare presented a poster entitled "NCCS Flow Cytometry Core Facility", which was open to all.

2) Imaging facility

The Team

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

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

1. Zeiss LSM510 META

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

2. Leica SP5 II

This is a high-end Broadband Confocal Laser Scanning Microscope with 4 cooled spectral PMTs and unique AOBS, technology equipped with CO2 incubator, fully motorized, automated and computer controlled Microscope Leica DMI 6000. The Lasers are Blue Diode Laser 405nm, Ar Laser with 458nm, 488nm, 476nm, 496nm, 514 nm lines, DPSS 561nm, HeNe 594nm and HeNe 633nm with a scanning stage and incubation chamber for live cell experiments. The software for Confocal imaging 3D imaging and reconstruction, Dye Finder, Time lapse, colocalization, FRET (SE & AB), FRAP are also available.

3. Olympus Fluoview 10

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

The numbers of samples imaged during this year were approximately 4000 in-house samples, plus 200 received from various other institutes.

3) DNA sequencing facility

The Team

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

The central sequencing facility is located at the Microbial Culture Collection (MCC) and houses two instruments (3730 and 3730xl) from Applied Biosystems along with all the software required for sequence and data analysis. The facility offers services related to the identification of microorganisms; sequencing of plasmids, PCR products and cloned inserts; primer walking; and genotyping and fragment analysis to researchers from NCCS and other organizations. The number of samples run on the sequencers in the central sequencing facility was 2215, during 2015-2016. In addition, the facility serves as the back-bone of culture authentication and identification for MCC's preservation activities and has run a total of 29333 sequencing reactions during this period.

4) IVIS Imaging System

The Team

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

The In-Vivo Imaging System facility is a common central facility of NCCS. This instrument can be used for bioluminescent and fluorescent imaging of cells or whole small animals in vitro or in vivo as well as ex vivo condition. Currently, the IVIS imaging system is being used by more than 25-30 researchers from various laboratories in NCCS and by collaborators from other institutes. All scholars are using bioluminescence as well as fluorescence imaging in different tissue culture plates (96 well, 12 well, 24 well plate and 1.5 ml tube etc) as well as in different strains of mice (NOD/SCID, NUDE, C57, Balb/C mice etc).

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

IVIS Imaging System

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

Standard filter sets for IVIS Imaging System

Fluorescence Filters

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

Spectral Imaging Filters

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

Features:

- High-sensitivity in vivo imaging of fluorescence and bioluminescence.
- High throughput (6 mice) with 26 cm field of view.
- High resolution (upto 60 microns) with 3.9 cm field of view.
- Dual 12-position emission filter wheels (24-position total) and 12-position excitation wheel.
- A set of four filter pairs for fluorescent imaging come standard with the instrument, in addition to a set of four background filters for subtraction of tissue autofluorescence.
- 25 x 25 cm alignment grid on the imaging platform ensures consistent accurate placement of animals for imaging.

- Spectral imaging filters that acquire images at different wavelengths (ranging from 560 nm to 660 nm) facilitate 3D diffuse tomographic reconstruction and determination of the depth and location of a bioluminescent reporter.
- Heated animal shelf (up to 40° C).
- NIST traceable absolute calibrations.
- Class I Laser Product.

5) Central Sterilization Facility

The Team

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

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

Microbial Culture Collection

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

Background

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

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

Aims and Objectives

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

Work Achieved

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

a. Normal' Cultures: All the cultures which are isolated by each institute.
b. Three star' Cultures: All normal cultures are then screened by Piramal Life Science Limited (PLSL), Mumbai for four different activities. Three star cultures are the ones which show one or more of these activities

c. Re-fermented' Cultures: All the three star cultures undergo second level of screening (fermentation) to check whether they retain the activity. Re-fermented cultures are those three star cultures which retain their activity after second fermentation.
d. Scale-up' Cultures: Re-fermented cultures that have shown potentially novel molecules/compounds during screening at PLSL and have been selected for large scale fermentation by PLSL are designated as 'Scale-up' cultures. These cultures are being sent by PLSL to MCC.

Culture Preservation Status

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

Cultures passage status

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

Cultures dispatched to Academia/Industry

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

Identification of cultures

So far, a total of 7938 pure 'Three Star' cultures have been received at MCC and all of them are now identified. The DNA sequencing was done using three/ four different universal 16S rRNA gene specific primers. Preliminary classification of these three star sequences was done using Ribosomal Database Project (RDP) using Bayesian naïve classifier. Those that could not be identified by sequencing even after multiple trails, were identified using Matrix-assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry. Since September 2013, all normal category cultures are also being processed for 16S rRNA gene sequence based identification and MALDI based identification. From that 6710 have been sequenced and identified and 7803 have been identified by MALDI

Fatty acid methyl ester (FAME) analysis

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

Services

Supply of Cultures

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

General Deposit

MCC is continually receiving cultures for general deposit from several institutions across India and overseas. Authentication of the taxonomic identity of the cultures is done by rRNA gene sequencing (>1200 bp). Upon authentication and preservation, an aliquot of the Deposit is sent to the depositor for confirmation and accessioned in MCC collection. So far, MCC has authenticated 1477 cultures (1210 bacterial and 267 fungal) in its collection. While all of these have been preserved by two different methods: two cultures stocks in 20% glycerol are stored at -80°C and two vials in Liquid nitrogen (-196° C); preservation by lyophilization is in process under which 168 lyophilized ampoules are stored at 4° C.

IDA/Patent deposits

In addition to the general deposits, MCC is also receiving deposits for IDA from Indian and overseas institutes. So far, MCC has accessioned 75 bacterial cultures under IDA and 2 safe deposits. On priority, all IDA deposits have been preserved by lyophilization in addition to -80°C and LN storage. For each culture, 14 lyophilized vials are prepared and two of these are sent to the depositor and the remaining vials are stored at MCC at 4°C.

Identification services

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

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

In addition to the sequencing of deposit cultures for authentication, a total of 16904 cultures for bacteria and fungi together (including 964 for paid service) were sequenced at MCC. For bacterial identification 16S rRNA gene sequence was used whereas for fungi ITS region sequence was used.

Phylogenetic analysis

MCC has received 35 requests for bacterial identification and phylogenetic analysis. Currently MCC is providing phylogenetic analysis based on two methods; Neighbor joining and Maximum parsimony. For phylogenetic analysis, only type strain sequences from databases like RDP and EZTaxon are used. The alignment is done in ClustaX software. Phylogenetic trees are constructed in MEGA software.

MALDI-TOF typing

Matrix-assisted Laser Desorption/Ionization (MALDI-TOF) mass spectrometers are frequently used for the rapid and sensitive analysis of biomolecules. One of the main uses of MALDI-TOF-MS is in the identification of proteins, by peptide mass fingerprinting (PMF).Since its installation in April 2013; the methods for sample preparation and analysis have been standardized for the Bruker MALDI-TOF MS. So far, only internal MCC cultures have been run on the instrument with very high congruence to rRNA gene sequence identification. MCC has provided MALDI analysis as a part of bacterial identification paid service for 14798 cultures. Apart from this 28345 cultures from various research and MCC-BRC project have been identified using MALDI. In addition to identification of cultures by MALDI-TOF, customers also require creation of a MALDI database for which the number of replicates required is large and needs additional consumables along with technical expertise. A revised fee structure for MALDI-TOF typing of single cultures as well as database creation is submitted to the SAC meeting and is approved.

FAME Analysis

MCC has start providing FAME analysis as a service in February 2013. Since then, a total of 279 (including 79 for paid service) bacterial cultures have been analyzed on the MIDI system.

DNA-DNA hybridization and GC (mol %)

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

Cyanobacterial Culture Collection

The cyanobacterial culture collection at MCC has started functioning and is working actively in the identification and preservation of cyanobacteria belonging to all the groups.

ISO Certification

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

Future Research Plans

Additional Services to be offered

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

Anaerobic microbes

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

Hazard group 3 microbes

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

Supply of cultures to Industry

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

Awards / Honours / Memberships (MCC Scientists)

Dr. Prashant Singh

- Selected in the Indian National Science Academy (INSA) Bilateral Exchange Programme of Indian Scientists for the year 2015 for visiting Czech Republic.
- Awarded the Young Investigator Grant, 2015 of the Department of Science and Technology (DST), India for working on the taxonomy of Indian cyanobacteria

Dr. Om Prakash Sharma

- Selected as Visiting Fellow of Indian National Science Academy (INSA) in Environmental Microbiology to develop the Indo-Israel bilateral program in wastewater treatment for the year 2016 in collaboration with Volcani research Centre ARO, Israel.
- Selected for ICMR, Human Resource Development (HRD) Long term Fellowship (2016), to conduct research in the area of human gut microbiology in collaboration with School of Biomedical Sciences, Florida State University, USA

Dr. Praveen Rahi

- Awarded start up research Grant (Young Scientist), of 24.36 Lakh (2015), funded by SCIENCE & ENGINEERING RESEARCH BOARD, DST, GOI.
- Awarded the Young Associate (Life Science 2015), Maharashtra Academy of Science (MACS), Pune, India.
- Received International Travel Grant (October 2015), Department of Biotechnology, India

Dr. Amit Yadav

- Member of Indian Phytopahological Society (IPS)

Dr. Venkata Ramana

- Received a postdoctoral fellowship to work in University of Stuttgart, Germany.

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

Conferences / Workshops (attended by MCC Scientists)

Dr. Amit Yadav

Challenges in Detection and Taxonomy Studies of Phytoplasmas Associated with Plants and Insect Vectors in India. Oral Presentation in Technical Session 17: Plant Mollicutes, Challenges in Research. At the 6th International Conference on Plant, Pathogens and People; Challenges in Plant Pathology to Benefit Humankind. February 23-27, 2016, New Delhi, India

Conferences / Events Organized by MCC

- Organized a 'One day hands-on training workshop in gene annotation' for M. Sc. Part II Students in eight batches of 40 students, in association with AMI, Pune Unit.
- MCC & NCCS jointly organized a science day program in February 28, 2016 for school children.
- Students from Moving academy of science visited MCC.
- City pride school, Nigdi, Pune (178 students) attended an event in MCC.
- Students from Welligton College, Satara visited MCC.

Talks / Teaching by MCC scientists

Dr. Praveen Rahi

- Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass-Spectrometry (MALDI-TOF MS) Based Identifications of Microorganisms of Non-Clinical Origin at III International Conference on Microbial Diversity: The Challenge of Complexity - MD 2015, Perugia, Italy (27-29 October 2015).

Dr. Dhiraj Dhotre

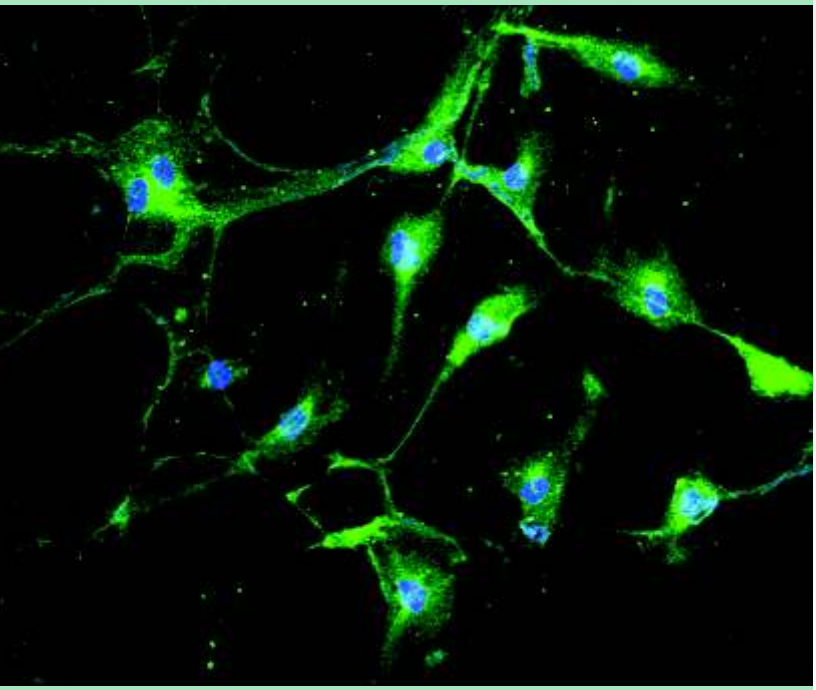
- Invited as a guest lecturer at Modern college, Ganeshkhind, Pune to teach bioinformatics and molecular phylogeny.

- Invited as a guest lecturer at the Department of Bioinformatics, Pune to teach genomics, metagenomics and transcriptomics.
- Invited as a resource person for "DBT training course on Transcriptomics" organized by department of Botany, SP Pune University (4 lectures).

Dr. Prashant Singh

- Invited lecture in Institute of Botany, Czech Academy of Sciences, Trebon, Czech Republic in October 2015 entitled "Systematics and taxonomy of cyanobacteria: Indian Perspectives".
- Invited lecture in Centre for Polar Ecology, University of South Bohemia, Ceske Budejovice, Czech Republic in October 2015 entitled "Phylogenetic and taxonomic evaluation of Indian cyanobacteria: A brief overview".

MCC scientists were engaged in teching courses in Microbiology, Microbial Ecology, and Bioinformatics for undergraduate, post graduate and doctoral students during the academic year, 2015-16.



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Book Chapters / Invited Reviews / Editorials

- Bapat S.A. Cancer Stem Cells: Perspectives beyond immunophenotypes and markers, In: Regenerative Medicine, 2016, Gustav Steinhoff (Ed.), Springer Verlag Publishers, (in press).
- Kumar, C.M.S. (2016). Molecular mechanism of drug resistance: common themes. In Antibiotic Resistance, V. C. Kalia, A. Sajid, and G. Arora, ed. (Springer Inc.).
- Kumar, C.M.S. (2016). Drug resistance in malaria. In Antibiotic Resistance, V. C. Kalia, A. Sajid, and G. Arora, ed. (Springer Inc.).
- Tumor exosomes: cellular postmen of cancer diagnosis and personalized therapy. Sharma A, Khatun Z, Shiras A. Nanomedicine (Lond). 2016 Feb;11(4):421-37. Epub 2016 Jan 19. (Invited review)
- Cancer stem cell-vascular endothelial cell interactions in glioblastoma. Sharma A, Shiras A. Biochem Biophys Res Commun. 2016 May 6;473(3):688-92. Epub 2015 Dec 12. (Invited review)
- Systems Biology Application in Synthetic Biology (Edited Volume 1). Singh, Shailza (Ed.) Springer Publishers. DOI: 10.1007/978-81-322-2809-7

Publications of MCC Scientists

1. Lavecchia, A., Curci, M., Jangid, K., Whitman, W.B., Ricciuti, P., Pascazio, S., and Crecchio, C. 2015. Microbial 16S gene-based composition of a sorghum cropped rhizosphere soil under different fertilization managements. Biology and Fertility of Soils 51: 661-672.
2. Nair AV, Joseph N, Krishna K, Sneha KG, Tom N, Jangid K, Nair S. A comparative study of coastal and clinical isolates of *Pseudomonas aeruginosa*. Braz. J. Microbiol. 2015 Jul 1; 46(3):725-34. doi:

10.1590/S1517-838246320140502.eCollection 2015 Jul-Sep. PubMed PMID: 26413053; PubMed Central PMCID:PMC4568853.

3. Polkade AV, Mantri SS, Patwekar UJ, Jangid K. Quorum Sensing: An Under-Explored Phenomenon in the Phylum Actinobacteria. Front Microbiol. 2016 Feb 10; 7:131. doi: 10.3389/fmicb.2016.00131. eCollection 2016. Review. PubMed PMID:26904007; PubMed Central PMCID: PMC4748050.
4. Singh Prashant, Shaikh Zaid Muneef, Gaysina Lira A., Suradkar Archana, Samanta Upasona. (2016) New species of *Nostoc* (Cyanobacteria) isolated from Pune, India using morphological, ecological and molecular attributes. Plant Systematics and Evolution, pp.1–14; doi:10.1007/s00606-016-1337-z.
5. Thorat Vipool, Bhale Udhav, Sawant Vijay, More Vijay, Jadhav Praveen, Mane S. S., Nandanwar R. S., Tripathi Savarni and Yadav Amit. Alternative weed hosts harbors 16SrII group phytoplasma strains associated with little leaf and witches' broom diseases of various crops in India. Phytopathogenic Mollicutes, 2016, 6 (1), 50-55.

Book Chapters / Invited Reviews / Editorials (MCC)

1. Chaudhuri, S.R., Sharmin, J., Banerjee, S., Jayakrishnan, U., Saha, A., Mishra, M., Ghosh, M., Mukherjee, I., Banerjee, A., Jangid K., Sudarshan, M., Chakraborty, A., Nath, R., Banerjee, M., Singh, S.S., Saha, A.K., and Thakur, A.R. 2016. Novel microbial system developed from low-level radioactive waste treatment plant for environmental sustenance. In Management of Radioactive and Hazardous Wastes. Intech Open. (In Press).
2. Ghosh, M., Saha, A., Jangid K., Joshi, A.A., and Chaudhuri, S.R. 2016. A polyphasic approach of species identification for genus *Bacillus*. In Chaudhuri, S.R. (ed), Life Science Recent Innovations & Research. International Research Publishing House, India. (In Press).
3. Prakash, O. Sharma, R, Singh, P. and Yadav, A. (2016). Strategies for taxonomical characterization of agriculturally important microorganisms. Singh, D. Pratap, S., Harikesh B., Prabha, R. (Eds.) (eds). In: Microbial Inoculants in Sustainable Agricultural Productivity. Volume 1: Research Perspectives. Springer India. Pp. 85- 101.
4. Rahi, P. and Vyas, P. Microorganisms associated with tea rhizosphere in the Indian Himalayan Region. ENVIS Centre on Himalayan Ecology, ENVIS Bulletin Himalayan Ecology, 2015, Vol. 23, pp. 48-54.
5. Sharma R. and Jangid K. Role of Quorum Sensing in Fungal Morphogenesis and Pathogenesis. In: Fungal Metabolites (eds, J.-M. Mérillon, K.G. Ramawat). Springer International Publishing Switzerland. 2016. DOI 10.1007/978-3-319-19456-1_38-1.
6. Sharma, R., and Jangid, K. 2015. Fungal quorum sensing inhibitors, p.237-257. In Kalia, V.C. (ed.), Quorum Sensing Vs Quorum Quenching: A Battle With No End in Sight. Springer, India.

Publications of other scientists

Dr. Debasri Mukherjee (DST-INSPIRE Faculty)

Publication numbers 42 & 51 (D. Mukherjee: corresponding author in the 'Publications of NCCS Faculty' list.)

Patents (filed / sealed) - NCCS faculty

Sharmila Bapat

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

  PCT and Indian Applications filed. Co-inventor : Naik R., NCCS, Pune.

- Development and applications of a cytotoxic monoclonal antibody that targets stem and progenitor cells in tumors

  Indian Application filed. Co-inventor : Naik R., NCCS, Pune.

Manoj Kumar Bhat

Protein based product from fenugreek seeds that regulates dyslipidemia and obesity, and a process for the preparation thereof.

European Patent issue No. EP2323676- dated: 15-07-2015 (Germany and France)

PCT Publication: PCT/IN2008/000877 dated: 30-09-2008. Patent filed by DBT & NCCS. (WIPO) Publication no. WO/2009/157013, dated: 30-12-09

Co-inventors: Pandey, V. and Vijayakumar, M.V., NCCS, Pune.

Anjali Shiras

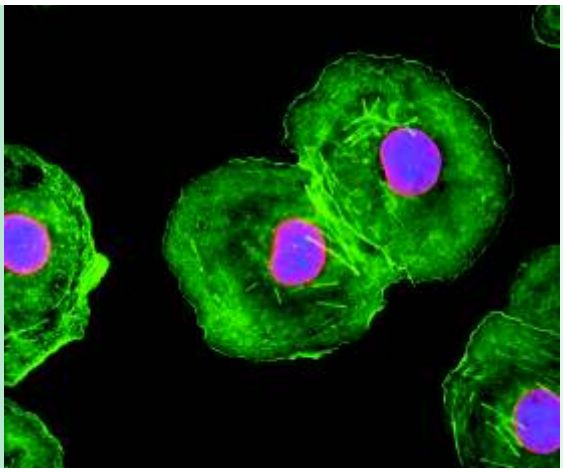
- Method of enrichment of GSCs and ECs from glioma and co-culturing interchangeably in 2D &3D

  Country: INDIA . Patent No: 4044/MUM2015. Date: 27.10.2015. Filed by: NCCS, Pune, India Co-inventor: Aman Sharma, NCCS, Pune.

- Identification and multi-parametric analysis of GSC-EC interacting subpopulations in glioblastoma

  Country: INDIA. Patent No: 4045/MUM2015. Date: 27.10.2015. Filed by: NCCS, Pune, India.

Co-inventor: Aman Sharma, NCCS, Pune.



Awards/ Honours/ Memberships/ Extramural Funding

Awards / Honours / Memberships

Faculty

Sharmila Bapat

Fulbright Fellowship 2015- 2016, USA.

May 2016 Outreach Lecturing Fund (OLF) Award from the Council for International Exchange of Scholars (CIES), Institute of International Education (IIE), USA

Group Award

RR Naik, Ak Singh, AM Mali, MF Khirade and SA Bapat - from Flow Cytometry Society, India at th 8th annual TCS meeting Tata Memorial Centre, Mumbai, 24th -28th Oct 2015, for published research work carried out using flow cytometry for novel applications and as a significant experimental too - Research article: A tumor deconstruction platform identifies definitive end points in the evaluation of the drug responses, Oncogene, 2016 Feb 11; 35(6):727-37.

Samit Chattopadhyay

Elected as a Fellow of 'The World Academy of Sciences (TWAS) for the advancement of science in developing countries'

Gopal Kundu

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

Girdhari Lal

- International Congress of Immunology 2016 (Australia) travel grant from the American Association of Immunologists.
- 4th European Immunology Congress 2015 travel grant from American Association of Immunologists, USA.

Memberships

- Member, Society of Leukocyte Biology (SLB), USA.
- Life Member, The Indian Science Congress Association, India.
- Member, American Association of Immunologists (AAI), USA.
- Member, American Society of Transplantation (AST), USA.

Nibedita Lenka

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

Memberships

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

Lalita Limaye

Life member of

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

Srikanth Rapole

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

Arvind Sahu

- Elected as a Fellow of the Indian National Science Academy (INSA), New Delhi (14 Oct, 2015)

Anjali Shiras

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

Shailza Singh

- Life Member-Indian Biophysical Society (IBS)
- Life Member -Biotechnology Society of India (BSI)
- Life Member-Society of Biological Chemists, India (SBC)
- Life Member-Association of Microbiologists of India (AMI)
- Life Member-Association for DNA Fingerprinting and Diagnostics

Sandhya Sitaswad

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

Deepa Subramanyam

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

Memberships

- Member of the Indian Society for Developmental Biology.

Mohan Wani

- Academic Editor, PLOS ONE (2015-16).
- Editorial Board Member, Journal of Laboratory Animal Science (April 2015).
- Member, Stem Cell Task Force, DBT (2014-2017).
- Member, Scientific Advisory Committee, Krishna Institute of Medical Sciences, Karad (August 2015).
- Felicitated by KNP College of Veterinary Science, Shirwal, Maharashtra during "Veterinary Orthopedic Foundation Workshop" (October, 2015).

Memberships

- Life Member, Laboratory Animal Scientist's Association (LASA), India (October 2015).

Students' Awards & Honours

Amruta Barhanpurkar-Naik (DBT Bio-CARe Early Career Scientist): Travel Grant Award from the International Society for Stem Cell Research (ISSCR) to attend its 13th Annual Meeting held at Stockholm, Sweden during June 24-27, 2015.

Jitendra Kumar: Best poster award at the 15th European Meeting on Complement in Human Disease held at Uppsala, Sweden (June 27-30, 2015).

Pranav Pande: 'Best Poster Award' at the workshop on 'Insights in Biology 2025' organized by the Maharashtra Academy of Sciences and CSIR-NCL on 29th Oct, 2015.

Rutika Naik: 'TCS 2015 Award in Basic Sciences'
The paper published by Dr. Bapat's group (Naik, et al, Oncogene 130; 27 April 2015) was selected for this award in recognition of the novel and creative use of flow cytometry in research.

Sagar Varankar: Best poster award for the poster, 'Slug and Tcf21 govern modes of metastasis in high grade ovarian cancer', presented at the 1-day workshop on 'Insights in Biology 2025' organized by the Maharashtra Academy of Sciences and CSIR-NCL, 29th October 2015.

Swapnil Kamble: 2nd prize in poster presentation at International Conference of Cancer Research: New Horizons 2015, NCCS, Pune.

Sandip Sonar: The International Congress of Immunology 2016 (Australia) travel award from the American Association of Immunologists.

Venkatesh Chanukuppa: Second prize in the poster competition at the 'Targeted Proteomics Workshop & International Symposium' at IIT-Bombay, 13-14 Dec, 2015.

Srinadh Choppara: Best poster award at the 'Conference of new ideas in cancer-challenging dogmas', TATA THEATRE, NCPA, Mumbai, 26 – 28 February, 2016

Srinadh Choppara: Best poster award at the 'International Conference on Cancer Research: New Horizons, National Centre for Cell Science, 19 – 21 November, 2015

Extramural Funding

Sharmila Bapat

- DBT funded Indo-Finnish collaborative project titled 'Prostate and ovarian cancer detection from innovative use of antibody libraries, human lectins, exosomes and simple affordable assay platforms - PROVATECT' : 2015-2018; Role: Principal Investigator; Co-Investigator - Dr. Shekhar Mande; Collaborators - Dr. Urpo Lamminmaki, Dr. Pietterssen, Prof. Ollie Carpen (Turku, Finland)

Manoj Kumar Bhat

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

Radha Chauhan

- Structural and functional studies on Nup93 subcomplex of the nuclear pore complex. 2012-2017. (DST-SERB Ramanujan fellowship)
- Reconstitution and structural studies on Nup93Nup205 complex of the nuclear pore complex. 2013 - 2016. (DST-SERB EMR funded)
- Reconstitution and structural studies on Nup93Nup62Nup54Nup58 quaternary complex 2015-2018. (DBT-basic sciences EMR funded)

Jomon Joseph

- Exploring the functional connection between Par polarity proteins and Nup358 in cell polarity. 2012-2015. (DBT, India)
- ? Role of Nup358 in the regulation of cytoplasmic mRNP granules. 2016-2019 (DST, India)

Janesh Kumar

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

Gopal Kundu

- Chitosan nanoparticle mediated Andrographolide and/or Raloxifene delivery in breast cancer and its implication in multi-targeted therapy. (2016-) (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).

Girdhari Lal

- Role of chemokine and its receptors in the pathogenesis and regulation of autoimmunity (2011-2015) Ramalingaswami Fellowship from Department of Biotechnology, Government of India.
- Cellular and molecular mechanism of CD4 T cell and brain endothelial cell interaction to control inflammation and tolerance (2012-2015) from Department of Biotechnology, Government of India.

Nibedita Lenka

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

Lalita Limaye

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

Amitabha Majumdar

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

Shekhar Mande

- SYS TB: A Network program for Resolving the intracellular Dynamics of Host pathogen interaction in TB infection 2012-2017, DBT, India

- Metabolomic applications to the identification of new therapeutic targets for tuberculosis treatment. 2012-2015, DST.
- TBomics: An omics approach for diagnosing tuberculosis. 2013-2016, DBT, India
- Elucidation of gene regulatory in *Mycobacterium tuberculosis* from the available high-thoughtput data and prediction of transcription regulation (Indo-Russian) 2014-2016, DST, India.

Debashis Mitra

- Identification of novel cellular targets and new lead molecules to inhibit HIV-1 infection.
  (2012-2017) Tata Innovation Fellowship grant, Department of Biotechnology, India

Srikanth Rapole

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

Bhaskar Saha

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

Arvind Sahu

- Soluble mediators of the immune system against Aspergillus fumigatus. 2014-2016 (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.

Vasudevan Seshadri

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

Anjali Shiras

- Studies on Exosome mediated regulation of Angiogenesis in Glioblastoma (GBM) 2015-18; Department of Biotechnology (DBT), Govt. of India, India.
- Cis-acting pair of novel non-coding RNAs - Ginir and Giniras in cell growth regulation and transformation of mouse and human cells. 2015-18; Department of Biotechnology (DBT), Govt. of India, India.
- Altered microRNA and its targets in Glioblastoma cell lines; 2016-19; Department of Biotechnology (DBT), Govt. of India, India.
- A novel strategy for reprogramming of somatic cells to induced pluripotent stems (iPS) cells by a single non-coding RNA – Ginir for applications in regenerative medicine. 2014-16; DST-UKIERI, Govt. of India, India.

Yogesh Shouche

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

Shailza Singh

- Systems and Synthetic Biology for Leishmania, funded by Department of Science and Technology (2013-2016)
- Molecular motors as nanocircuits in Leishmaniasis: System cues guiding synthetic biology device construction (2016-19), funded by Department of Biotechnology

Sandhya Sitaswad

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

Deepa Subramanyam

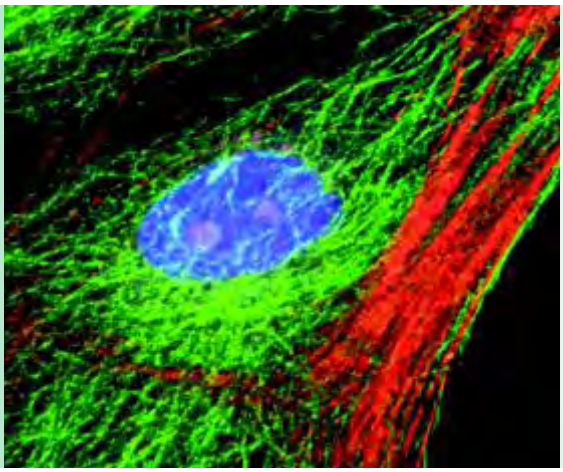
- The role of endocytosis and vesicular trafficking in regulation of stem cell functions and cell fate decisions during early development.' 2013- 2018 (Wellcome-Trust DBT India Alliance Intermediate Fellowship).

Vidisha Tripathi

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

Mohan Wani

- National Facility for Laboratory Model Organisms (a collaborative initiative between DBT-IISER-NCCS, Pune and UAB, USA), 2016-2021; Funded by DBT, New Delhi, India.



Conferences / Symposia / Workshops / Training Programmes / Talks / Meetings

Participation by Faculty

Sharmila Bapat

- Tumor Deconstruction identifies Definitive End-points in Drug Screening, Invited Talk during Indian Science Congress 7th January, 2015 at University of Mumbai, Mumbai.
- Recent concepts in Cancer Biology - Cancer Stem Cells, Invited Talk during ISSRF Annual Meeting from 14th - 17th February, 2015 at Nehru Science Centre, Mumbai.
- Ovarian Cancer Stem Cells: Implications for effective treatment, Invited Talk during IACR Annual Meeting from 19th - 21st February, 2015 at Jaipur.
- Ovarian Cancer Stem Cells: Implications for effective treatment, Invited Talk during Cancer Stem Cell Workshop on 24th April, 2015 at Curtin University, Perth Australia.
- Heterogeneity: Not just noise in tumors, Invited Talk during on 29th April, 2015 at Queensland University of Technology, Brisbane, Australia.
- Present Day Knowledge in Ovarian Stem Cell & Cancer Biology - on 29th April, CABAL Talk at TRI, , Brisbane, Australia.
- Cancer Stem Cells: Basic concepts and Therapeutic opportunities, Invited Talk during DDS - Uncover 2015 on 5th June 2015 at Lupin Research Labs, Pune.
- Cross-talks between molecular and cellular heterogeneity determines drug efficacy in cancer therapy, Invited Talk at the11th Indo-Australian Biotechnology Conference 6-8 September 2015 at Sydney.
- Tumor Heterogeneity and its implications in evaluation of drug responses. Invited Talk at the 2-4 October 2015 at the 6th International Conferece on Stem Cells and Cancer, Pune.
- The Theme of Stem Cell Biology in Cancer, Invited Talk at the one day symposium titled "Insights into biology 2025" on 29th October 2015 at the National Chemical Laboratory, Pune.
- Cancer Stem Cells and stress induced evolution - understanding the drug recalcitrance phenomenon, Invited Talk at the IAS half-year meeting from 6 - 8th November 2015 at IISER, Pune.

- The 2015 Nobel Prizes in Science: A curtain-raiser', celebrating 30 years of DBT at NCCS, Pune.
- Cancer - A Stem Cell Disease ? Invited to inaugurate and deliever a talk at 'CHIMERA' the departmental festival of Biotechnology Department , Fergusson College, on 29th December 2015.
- Network Approaches to molecular stratification and therapeutic prediction in high-grade serous ovarian adenocarcinoma, Invited Talk at the Medical Sciences Seminar in Cancer Biology, Indiana University, Bloomington, US on 22nd February, 2016.
- Identification and relevance of fusion transcripts in a novel in vitro progression model of high-grade serous ovarian cancer, Invited Talk at the 3rd Annual Genomics & Sequencing Data Integration, Analysis and Visualization Symposium in Molecular Medicine Tri-conference, San Francisco, US, on March 10-11, 2016.
- Cellular and Network Approaches to molecular stratification and therapeutic prediction in high-grade serous ovarian adenocarcinoma (HGSC), Invited Talk at the National Cancer Institute, US, on 5th April, 2016.
- Varying Levels of Tumor Heterogeneity in Ovarian Cancer, Invited Talk at the Department of Molecular an Medical Genetics, Oregon Health and Science University, Oregon, US, on 9th May, 2016, supported through an award from the Outreach Lecturing Fund (OLF) of the USIEF.
- Tumor Heterogeneity - not just noise! Invited Talk at the Ovarian Cancer Workshop: Challenges and Provocative Questions Penn State Hershey Cancer Institute, Philadelphia, US on May 16, 201Talk at a one day workshop on Insights in Biology 2025, organized by Maharashtra Academy of Science and CSIR-National Chemical Laboratory at CSIR-NCL, Pune on October 29, 2015.

Manoj Kumar Bhat

- Cancer Chemotherapy: Enhancement in Cell Killing. A Symposium on Recent Trends in Biology, Department of Zoology, SPPU, Pune, March 28th 2016.
- Metabolic disorders: an unexpected role in cancers. School of Regenerative Medicine, Manipal University, GKVK Post, Bellary Road, Bangalore, July 30th, 2015.
- Career Opportunities in Biotechnology, Nutrition and Dietetics. Symbiosis School of Biomedical Sciences, Symbiosis International University, Lavale, Pune, July 10th , 2015.
- Metabolic disorders: an unexpected role in cancers. Science on the Swan-Post conference workshop held by the Faculty of Health Sciences, Curtin University, Perth, Australia, April 20th-24th, 2015.

Jomon Joseph

- Nup358 in miRNA pathway: Identification of a new conserved interaction motif for AGO family of proteins (Invited talk). 8th RNA Group Meeting, CSIR-Centre for Cellular and Molecular Biology 8-10 January, 2016, Hyderabad, India.

Janesh Kumar

- The twist in the tale of Glutamate receptor activation and desensitization, Indo-French conference on Application of Structural Biology in Translational Research Structure- Guided- Drug- Design at Advanced Centre for Treatment, Research and Education in Cancer, 19-20, November, 2015, Kharghar, Navi Mumbai, India.
- The twists and turns of Glutamate receptor activation and desensitization, 13th conference of Asian Crystallographic Association, 5-8, December, 2015, Science city, Kolkata, India.
- Insights into Glutamate receptor structure and function: Recent developments and their implications to design of novel therapeutics, International Conference entitled Scenario of Biotechnology in 21st Century, Devi Ahilya University, 10-12, March, 2016, Indore, India.

Gopal Kundu

- Functional characterization of CD133+ cancer stem cells in melanoma progression and angiogenesis, Curtin University, Perth, Australia, 24th April, 2015.
- Osteopontin, a chemokine like protein acts as therapeutic target covering all hallmarks of cancer, National University of Singapore, Singapore, 29th April, 2015.
- Therapeutic potential of Osteopontin in cancer, IIT, Guwahati, 29th May, 2015.
- Therapeutic targeting of Osteopontin in breast and other cancers, Cancer Science, 2015, Dubai, 27-29th August, 2015.
- Osteopontin, a chemokine like pro-angiogenic protein acts as therapeutic target covering all hallmarks of cancer, 3rd Intl. Conference on Angiogenesis Research, Sastra University, Trichi, 23-25th September, 2015.
- Therapeutic targeting of Osteopontin in cancer and cancer stem cells, Intl. Conf on Cancer and Cancer Stem Cells, Yashada, Pune, 2-5th October, 2015.
- Therapeutic implication of Osteopontin, a chemokine like protein in cancer and cardiovascular diseases, Northwestern University, Chicago, USA, 6th October, 2015.

- Therapeutic targeting of Osteopontin in breast and other cancers, The Intl. Osteopontin and Other SIBLING Protein Conference, Chicago Hilton Resort, Oak Brook Hills, Illinois, USA, 11th October, 2015.
- Osteopontin, a potentially important therapeutic target in cancer, Amity University, Gurgaon, 17th November, 2015.
- Inaugural address and Session Chair, Intl. Conference on Cancer Research: New Horizons, NCCS, Pune, 19-21st November, 2015.
- Application of selective food supplement in Health Industry, South China University of Technology, Guanzhou, China, 24th November, 2015.
- Therapeutic implication of CD133+ cancer stem cells in melanoma progression, Guha Research Conference (GRC)-2015, Bodhgaya, Bihar, 28th Nov-2nd Dec, 2015.
- Discussion Meeting on Stroma-Tumor interaction with Prof. Bob. Weinberg, Whitehead Institute for Biomedical Research, 12th January, 2016.
- Session Chair and Discussion, 1st MOSCON Conference, Four Points by Sheraton, Pune, 29-30th January, 2015.
- Therapeutic implication of Osteopontin in cancer, Rajiv Ghandhi Centre for Biotechnology (RGCB), Trivandrum, 29th February, 2016.
- Osteopontin, a potential therapeutic target in cancer, Devi Ahilya University, Indore, 10th March, 2016.
- Chitosan mediated drug delivery in cancer, 3rd Natl. Seminar on Nanotechnology, ITM, Vadodara, 11th March, 2016.
- Novel therapeutic approach targeting cancer and cancer stem cells, NCCS-Curtin University Workshop, 18-19th March, 2016, NCCS, Pune.

Girdhari Lal

- Sonar S and Lal G (2015) IFN-□ regulates transendothelial migration of CD4 T cells at blood-brain barrier (BBB) during inflammation and autoimmunity. MCB75:From Molecules to Organisms organized by Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore held on December 11-14, 2015.
- Sonar S and Lal G (2015) Changes at blood-brain barrier (BBB) during inflammation and autoimmunity. XXXIII Annual Conference of Indian Academy of Neurosciences (IAN2015) on theme 'Neuroscience Research from Mechanisms and Applications' held at Punjab University Chandigarh on October 31-November 2nd, 2015.
- Sonar S and Lal G (2015) IFN-□ regulates transendothelial migration of CD4 T cells at blood-brain barrier (BBB). Infection and molecular epidemiology conference held at Udaipur, India on 29-31st October, 2015.
- Paul S and Lal G (2015) Role of NK cells in the regulation of CD4 T cell Differentiation in the tumor microenvironment. 6th International Conference

on Stem Cells and Cancer (ICSCC-2015): Proliferation, Differentiation and apoptosis held at Pune India on 2-5th October 2015.

- Lal G and Paul S (2015) Natural killer (NK) cells in tumor microenvironment inhibit the differentiation of Th1 and Th17. 4th European Congress of Immunology, held on September 6-9th, 2015 in Vianna, Austria

Nibedita Lenka

- Efficacy of Perivitelline Fluid from Indian Horseshoe Crab in Promoting Cardiomyogenesis. UGC sponsored National seminar on "Biology, Biotechnology and Conservation of Indian Horseshoe Crabs", Balasore, Odisha, November, 2015 (Invited Speaker).
- Emerging Trends in Stem Cell Research – The Hypes & Hopes. Annual Biotech Alumni Meet, Utkal University, Bhubaneswar, Odisha, December, 2015 (Chief Speaker).
- Emerging Trends in Stem Cell Research – The Hypes & Hopes. Annual Biotech Alumni Meet, Ravenshaw University, Cuttack, Odisha, January, 2015 (Chief Speaker).

Lalita Limaye

- Fergusson College Zoology Department : Zoology Association's lecture series-lecture on "Cord blood banking", 13 January, 2016.
- Fergusson College Microbiology Department : DBT Star programme for UG students on 28th Jan.2016 on "Haematopoietic stem cells".
- Fergusson College Microbiology Department: Late Dr.G.K.Kamath memorial oration series on "Possible use of DCs derived from Umbilical cord blood in Cancer immunotherapy" on 8th Feb.2016
- Marathi Vidnyan Parishad: Nale Til Raktachi Pedhi on Science day 28th Feb.2016 in Maharashtra Sahitya Parishad Hall.
- Marathi Vidnyan Parishad : "Stem cells che jatan" in Science Park ,Pimpri Chinchwad,on 1st April 2016.

Amitabha Majumdar

- Using *Drosophila* to study circuit neuroscience, Invited talk in IBRO-NBRC APC school, Manesar, March15-30 2016.
- Role of a self-sustaining amyloidogenic protein Orb2 in persistence of memory, Invited talk in IBRO-NBRC APC school, Manesar, March 15-30 2016.

Shekhar Mande

- Crystallographers and their non-crystallographic activities, Plenary, NIMHANS, Bangalore April 2015.

- Computational aspects of analysis of large-scale data, Talk delivered at the Science on the Swan post-conference workshop on Cancer and Cancer Stem Cells Biology: Molecular Targets Curtin University, Perth, Australia 24-April-2015.
- Networks in Biology, Talk delivered at IISER, Bhopal, 21-Aug-2015.
- The role of structures in understanding function, JB Science College, Wardha, 22-Sept-2015.
- Structural analysis of *M. tuberculosis* redox enzymes, Indo-French seminar, ACTREC, Mumbai, 19-Nov-2015.
- Context dependent regulatory networks: motifs and combinatorial searches, CoE workshop, NCBS Bangalore, 25-Nov-2015.
- The role of structures in understanding *Mycobacterium tuberculosis*. Gitam University, 27-Nov-2015.
- Understanding protein flexibility in M. tuberculosis redox enzymes. International workshop on structure-based drug-designing and applications to infectious diseases, IIIT, Hyderabad, 4-Feb-2016.
- Biotechnology and infectious diseases, HS Gaur University, Sagar, 21-Jan-2016.

Debashis Mitra

- Cellular Stress Proteins in HIV-1 infection, Invited talk; Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India, 5th May 2015.
- Novel molecules and strategies in the fight against HIV/AIDS, Invited talk, CSIR, Indian Institute of Chemical Biology, Kolkata, India, 18th December 2015.
- HspBP1 restricts HIV-1 by inhibiting NF-κB mediated activation of viral gene expression,Molecular Immunology Forum 2016, 19-21st February 2016, ACTREC, Navi Mumbai and Lonavla, India.

Srikanth Rapole

- ISMAS-Summer School on Proteomics and Metabolomics organised by CCMB, June 15-20, 2015 in Hyderabad.
- HCV-meeting cum exchange visit under New Indigo project organised by CQM, July 19-26, 2015 at Madeira, Portugal.
- Targeted proteomics workshop and international symposium organized by Indian Institute of Technology, Bombay from 10-14 December, 2015.
- HR LC-MS/MS workshop organized by Indian Institute of Technology, Bombay from 10-12 December, 2015.
- Identification of novel potential biomarkers for breast cancer using mass spectrometry based proteomic and metabolomic approaches. Invited talk

at ISMAS-Summer School on Proteomics and Metabolomics organised by CCMB, June 15-20, 2015 in Hyderabad.

- Volatile Organic Compounds as Potential Biomarkers for Breast Cancer Diagnosis: A Non-Invasive Metabolomics Approach. Invited talk at HCV-meeting cum exchange visit under New Indigo project organised by CQM, July 19-26, 2015 at Madeira, Portugal.
- Investigation of metabolomic and lipidomic alterations towards potential biomarkers in breast cancer. Invited talk at Targeted proteomics workshop and international symposium organized by Indian Institute of Technology, Bombay from 10-14 December, 2015.
- Introduction to basics of mass spectrometry principles. Invited talk at HR LC-MS/MS workshop organized by Indian Institute of Technology, Bombay from 10-12 December, 2015.

Arvind Sahu

- Role of locally produced C3a and C5a in viral infection', 23rd Molecular Immunology Forum, 19th - 20th February, 2015, Lonavala.
- An introduction to the innate immune system', J. B. College of Science, Wardha, 9th September, 2015.
- Complement - A true multitasking system' 13th FIMSA Advanced Immunology Course, Department of Immunopathology, PGIMER, Chandigarh 17th March, 2016.

Manas Santra

- 14th FAOBMB Congress and 84th Annual Meeting of SBC (I) Centre for Cellular and Molecular Biology, Hyderabad, India, 27 - 30th November 2015.
- A conference of new ideas in cancer - challenging dogmas TATA THEATRE, NCPA, Mumbai, 26 - 28th February, 2016.
- Ubiquitin and ubiquitin like modification: National Centre for Biological Science, January 28, 2016.
- Bhabha Atomic Research Centre, Mumbai, September 7, 2015.
- Indian Institute of Technology Guwahati, Assam, April 27, 2015.
- Agarkar Research Institute, Pune, Maharashtra, March 3, 2016.

Vasudevan Seshadri

- Role of Human Phosphatidyl Inosital 5 Phosphate 4 Kinase in Plosmodium Falciparum Gene Regulation Arya Vindu, Vishal Dandewad, Abdul Khalique, Dhanasekaran Shanmugam, Jomon Joseph and Vasudevan Seshadri, 8th RNA Group Meeting, 8-10 Jan 2016, CCMB, Hyderabad

Padma Shastry

- Oncostatin-M mediated-STAT-3 signaling regulates mesenchymal and proneural genes in gliomas - New lessons from old chapters, scientific meeting on Inflammation, ChanRe Rheumatology and Immunology Center and Research, Bangalore. 22nd and 23rd August 2015.
- Prostate Apoptosis response (Par-4) for cancer therapy in Glioblastomas: Challenging the limitations, Guest lecture, Amala Cancer Centre, Thrissur, 27th October 2015.
- The expanding potential of Mammmalian Target of Rapamycin (mTOR) pathway as a therapeutic target for glioblastoma (GBM),National Conference on Drug Discovery & Therapy (DDT-2016), Annamalai University- Chidambaram. 7 - 8 January, 2016.
- In vitro models for studying drug resistance in glioblastomas, Guest lecture CSIR -Indian Institute for Integrated Medicine (IIIM), Jammu. - 13th April 2016.

Anjali Shiras

- Role of non-coding RNA- Ginir in Generation of Induced Pluripotent Stem Cells: International Society for Stem Cell research (ISSCR), June, 2015, Stockholm; Sweden.
- Long non-coding RNA Ginir functions as an oncogene by causing mitotic dys-function of mouse cells: Keystone Symposia on Noncoding RNAs in Health and Disease- Feb 21-14, 2016. Santa Fe, New Mexico, USA.
- Elucidating the Role of linc-RNA Ginir as an Oncogene in Mouse Cells: 8th RNA group Meeting: CSIR - Centre for Cellular and Molecular Biology 8- 10 January 2016. Hyderabad, India.
- What We Know & What We Ought to Know in Glioma: Indian Society of Neuro-Oncology Meeting; April 2015; Kochi, India.
- Niche Biology in Glioma; Novel Therapeutic Approaches Targeting Cancer and Cancer Stem Cell: Indo-Australian Meeting. 18th March, 2016; NCCS, Pune; India.

Yogesh Shouche

- Species identification of birds involved in bird strikes using DNA barcoding. First National Conference on Wildlife Hazard Management in Aviation, May 18, 2016, Mumbai.
- Microbial Repositories and Biotechnology: MCC Experience. National Seminar on Conservation Biotechnology and DNA Barcoding. Gujarat State Biotechnology Board 18-19 May, Ahmedabad.

- Diversity Metabolic Potential of Bacterial Communities in the Continental Shelf of Agatti Island as revealed by genomic and metagenomic studies. Next-GenGenomics, Biology, Bioinformatics and Technologies (NGBT) Conference, October 1-3, Hyderabad
- Human microbiome: Indian perspective. Insights into Biology 2025" CSIR-National Chemical Laboratory October 29, 2015, Pune
- Molecular characterization of gut microbial communities of Indian subjects: an insight in to Indian Gut Microbiome. 56th Annual conference of Associationof Microbiologists of India held in JNU, 7 -10 December, 2015 New Delhi .
- *In Silico* Comparative analysis of 16S rRNA gene sequences. Short course on metagenomics, October 26 - November 15, 2015. Anand Agricultural University, Anand, India
- *In Silico* Comparative analysis of 16S rRNA gene sequences. Short course on metagenomics, February 9-18, 2016. Anand Agricultural University, Anand, India.
- Human Microbiome: Indian perspective. International Symposium on Microbiome in Health and Disease 2016 Feburary 23-25, Bangalore

Shailza Singh

- Talk on "Rational Drug Design in the Bioinformatics Era: Navigating the translational challenges through an integrated approach" in the Bioinformatics Workshop, ACTREC, Navi Mumbai, Kharghar, 23rd-24th April 2015.
- Talk on "Computational System Biology and Synthetic Biology : The Future of Infectious Disease" at Jamia Millia Islamia, 14th October 2015, Hall No.202, S Ramanujan Block, Mujeeb Bagh, JMI, New Delhi
- Talk on "Systems and Synthetic Biology Approaches in Infectious Disease: Two sides of the same coin dealing with Validation and Variability" at TERI, New Delhi, 13th October 2015.
- Talk on "Paradoxical components in Biological circuit and negative autoregulation of transcriptional factors: A systems theoretic model in Leishmaniasis" at 14th FAOBMB Congress and 84th Annual Meeting of SBC (I), Hyderabad, 27th-30th November 2015.
- Talk on "Anti-leishmanial activity of coumarin derivatives: Network based drug design using mechanistic systems biology" at IIT Guwahati, Bioinformatics and Computer aided drug design conference, 7th December 2015.

Sandhya Sitaswad

- Mitochondrial dysfunction, oxidative damage and apoptotic cell death stress mechanisms in diabetic cardiomyopathy' at Dept. of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad, August 2015.

Deepa Subramanyam

- Regulation of embryonic stem cell pluripotency by clathrin-mediated endocytosis (Narayana YV, Ridim Mote, Revati Dewal, Kriti Chopra, Jayashree Jagtap, Raghav Rajan and Deepa Subramanyam), Annual Fellows meet of the Wellcome Trust - DBT India Alliance, 5-6 November, 2015, Hyderabad, India.
- Regulation of mouse embryonic stem cell pluripotency by clathrin-mediated endocytosis; NCCS-Maastricht University Medical Centre Workshop; March 10-11, 2016; NCCS, Pune.

Mohan Wani

- Development of Laboratory Animal Models for Biomedical Research' during International Conference on "Promotion of Animal Research, Welfare, and Hormonization of Laboratory Animal Science" at ACTREC, Mumbai, October 15-16, 2015.
- Adult stem cell therapy in animal model of human rheumatoid arthritis' in The 12th Annual Meeting of Society for Regenerative Medicine and Tissue Engineering "STEM 2016" at School of Regenerative Medicine, Bangalore, February 1, 2016.
- Mesenchymal stem cells prevent pathological bone loss and promote immune tolerance in mouse collagen-induced arthritis' during joint workshop of NCCS, Pune and Maastricht University Medical Centre, Netherland conducted at NCCS, Pune, March 10-11, 2016.
- Participated in Discussion Meeting of Dept. of Biotechnology on "Transgenic Animal Facility" at University of Alabama, Birmingham, USA, August 5-8, 2015.
- Guha Research Conference (GRC) 2015 meeting at Bodhgaya-Nalanda, Bihar, November 28-December 2, 2015.
- ? Molecular Immunology Forum (MIF) at ACTREC, Mumbai, February 19-21, 2016.
- Attended "DBT JRF Biotechnology Eligibility Test (BET) 2016 - Question paper setting committee meeting at BCIL, New Delhi, February 22-24, 2016.

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

- Poster presentation: "Slug and TCF21 govern modes of metastasis in high grade ovarian cancer". A one day workshop on Insights in Biology 2025, organized by Maharashtra Academy of Science and CSIR-National Chemical Laboratory at CSIR-NCL, Pune on October 29, 2015.
- Swapnil Kamble - Poster presentation: "Validation of in silico Classifier for High Grade Serous Ovarian Adenocarcinoma to identify putative markers" at International Conference on Cancer Research: New Horizons. 19-21st November, 2015. NCCS, Pune.
- Sagar Varankar - Poster presentation: "Slug and TCF21 govern modes of metastasis in high grade ovarian cancer" at International Conference on Cancer Research: New Horizons. 19-21st November, 2015. NCCS, Pune.
- Ancy Abraham - Poster presentation: "Epigenetic Regulation of Transcription Factors in Ovarian Cancer Metastasis" at International Conference on Cancer Research: New Horizons. 19-21st November, 2015. NCCS, Pune.
- Madhuri More - Poster presentation: "Study of Cancer Stem Cell Heterogeneity in Ovarian Cancer" at International Conference on Cancer Research: New Horizons. 19-21st November, 2015. NCCS, Pune.
- Chouhan, S and Bhat M.K: SIRT-2/p53 regulates glucose induced canonical wnt signaling in hepatocellular carcinoma. Metabolic Signaling & Disease: From Cell to Organism, August 11th -15th, 2015, Cold Spring Harbor Laboratory, New York, USA.
- Singh, S. and Bhat, M.K.: Resistin; Travelling a Pathway from Insulin Resistance to Chemoresistance", at 40th Symposium on Hormones and Cell Regulation organized by European Society of Endocrinogy (ESE) at Mont Ste Odile, France, Nov 8th-11th, 2015. (Oral and poster)
- Suprita S. Ghode; Context-dependent Effect of Neuropilin-1 on the Regulation of HSC Fate. Suprita Ghode, Manmohan Bajaj, Lalita Limaye and Vaijayanti P. Kale 44th International Society for Experimental Haematology (ISEH) Annual Scientific Meeting, 7th September to 19th September 2015, Kyoto, Japan
- Sapana Jalnapurkar; Nitric oxide regulates CD34 expression in murine hematopoietic stem and progenitor cells. Sapana Jalnapurkar, M. Ranjia Devi, Shweta Singh, Lalita Limaye and Vaijayanti Kale, IFOM-inStem Conference on Inflammation and Tissue Homeostasis, 3rd Feb – 5th Feb 2016, The Institute for Stem Cell Biology and Regenerative Medicine (inStem), NCBS, Bangalore, India

- Dhiraj Kumar: 'Notch1-MAPK Signaling Axis Regulates CD133+ Melanoma Initiating Cell Growth and Metastasis' (Dhiraj Kumar, Santosh Kumar, Mahadeo Gorain and Gopal C. Kundu) Conference on Cancer Research: New Horizons. 19th-21st November, 2015, NCCS, Pune, India.
- Pompom Ghosh: Attended '12th Science Communication Workshop' organized by the Wellcome Trust/ DBT India Alliance from 14th-15th September, 2015
- Pompom Ghosh: Poster entitled 'Stroma derived CCL5 promotes breast tumor progression through PI3'-kinase/NFκB pathway' (Pompom Ghosh and Gopal C. Kundu) presented at the International Conference on Cancer Research: New Horizons held at NCCS, Pune from 19th - 21st November, 2015.
- Pompom Ghosh: Poster entitled 'Mesenchymal Stromal Cell Derived Chemokine CCL5 Regulate Melanoma Progression' (Pompom Ghosh and Gopal C. Kundu) presented at the Keystone Symposia Conference on 'Cancer Pathophysiology: Integrating the Tumor and Host Environments' held at Colorado, USA from 28th March to 2nd April, 2016.
- Amit Yadav: Poster entitled 'Chitosan nanoparticles mediated raloxifene delivery in breast cancer' (Amit Yadav and Gopal C. Kundu) presented at the International Conference on Cancer Research: New Horizons held at NCCS, Pune from 19th - 21st November, 2015.
- NNV Radha Rani: Poster entitled 'Tumor Associated Macrophages (TAMs) Regulates Tumor Angiogenesis and Cancer Stem Cell (CSC)-mediated Tumor Progression' (NNV Radha Rani, Smita Kale and Gopal C. Kundu) presented at the International Conference on Cancer Research: New Horizons held at NCCS, Pune from 19th - 21st November, 2015.
- NNV Radha Rani: Oral talk entitled 'Role of Tumor Associated Macrophages (TAMs) in Regulation of Tumor Angiogenesis and Cancer Stem Cell (CSC)-mediated Tumor Progression' (NNV Radha Rani, Smita Kale and Gopal C. Kundu) presented at the 3rd International Conference in Angiogenesis: Technology and Therapeutics held at SASTRA University, Thanjavur from 23rd - 25th September, 2015.
- Totakura V S Kumar: Poster entitled 'Role of breast cancer stem cells in regulation of tumor progression and angiogenesis in response to hypoxia' (Kumar V S Totakura, Remya Raja and Gopal C. Kundu) presented at the 3rd International Conference in Angiogenesis: Technology and Therapeutics held at SASTRA University, Thanjavur from 23rd - 25th September, 2015.
- Totakura V S Kumar: Poster entitled 'Hypoxia regulates cancer stem cell mediated breast tumor growth and angiogenesis' (Kumar V S Totakura, Remya Raja and Gopal C. Kundu) presented at the International Conference

on Cancer Research: New Horizons held at NCCS, Pune from 19th - 21st November, 2015.

- Ramesh Butti: Attended 1st International Conference on Cancer Research: New Horizons 2015 held at National Centre for Cell Science, Pune from 19th to 21st June, 2015.
- Ramesh Butti: Attended 1st Moscon: Conference of Molecular Oncology Society held at Hotel Sheraton, Pune from 29th to 30th January, 2016.
- Deepti Tomar: Attended 1st International Conference on Cancer Research: New Horizons 2015 held at National Centre for Cell Science, Pune from 19th to 21st June, 2015.
- Nimma Ramakrishna: Attended 1st International Conference on Cancer Research: New Horizons 2015 held at National Centre for Cell Science, Pune from 19th to 21st June, 2015.
- Nimma Ramakrishna: Attended 1st Moscon: Conference of Molecular Oncology Society held at Hotel Sheraton, Pune from 29th to 30th January, 2016.
- Sumit Das: Attended 1st International Conference on Cancer Research: New Horizons 2015 held at National Centre for Cell Science, Pune from 19th to 21st June, 2015.
- Sumit Das: Attended 1st Moscon: Conference of Molecular Oncology Society held at Hotel Sheraton, Pune from 29th to 30th January, 2016.
- Paul S and Lal G (2016) Tumor infiltrating natural killer T (NKT) cells express reduced Th1 and increased Th17 cytokine, and contribute to the tumor growth. Platinum Jubilee conference: New Ideas in cancer challenging dogmas organized by Tata Memorial Centre, Mumbai from 26-28 February 2016.
- Paul S and Lal G (2015) Intratumoral NK cells inhibit the differentiation of effector Th1 cells and promote tumor growth. International Conference of Cancer Research: New Horizons. Held at National Centre for Cell Science, Pune on 19-21st November, 2015 in Pune.
- Darshana Kadekar, Sonal Rangole, Vaijayanti Kale, Lalita Limaye. Inclusion of Conditioned medium of Mesenchymal Stem Cells in the freezing medium imparts cryoprotective effect on the ex vivo expanded Umbilical cord blood cells. Poster presented by DK in ISEH Conference held in Kyota, Japan in Sept 2015.
- Limbkar K.R., Kale V.P. and Limaye L.S.*'Oral feeding of mice with nutraceuticals -Omega3 and Omega6 fatty acids stimulates the bone marrow stem cell population' Poster presented by KRL in 39th All India Cell Biology Conference, held at Trivandrum, Kerala from 6th -8th December, 2015.

- Dhenge A.C , Kale V.P. and Limaye L.S.* 'Understanding the process of platelet biogenesis by Valproic acid mediated differentiation of MEG-01 cell line ' Poster presented by AD in 39th All India Cell Biology Conference, held at Trivandrum, Kerala from 6th - 8th December, 2015.
- Swastik Phulera, ICTP- JNCASR School on Imaging, Structural and Single Molecule Approaches to Biology, January 11 - January 16, 2016, Bangalore, India.
- Ashwani Kumar, Computational Biotechnology at the Nanoscale: CCP4 Workshop 2016, February 15 - February 20, 2016, Gurgaon, India.
- Vipul Nilkanth; MetaMod 2016 Workshop on Metabolic Modelling, March 14 - March 16, 2016, Pune, India.
- Tracy Augustine; Interplay of Cyclin F in the modulation of HIV-1 viral infectivity, Tracy Augustine and Debashis Mitra, Keystone Symposium on Mechanism of HIV Persistence: Implications for a Cure, April 26 to May 1, 2015, Boston, Massachusetts, USA.
- Tushar More presented a poster entitled "Investigation of phospholipid alterations in breast cancer using mass spectrometry" at proteomics conference and 7th annual meeting of Proteomics Society, India (PSI), 3-6 December, 2015 organized by VIT University, Vellore.
- Raju Dhabi presented a poster entitled "Identification of subtype specific serum potential protein biomarkers in breast cancer using complementary gel-based and gel-free quantitative proteomic approaches" at proteomics conference and 7th annual meeting of Proteomics Society, India (PSI), 3-6 December, 2015 organized by VIT University, Vellore.
- Venkatesh Chanukuppa presented a poster entitled "Quantitative proteomic analysis towards new targets and biomarkers for multiple myeloma" at proteomics conference and 7th annual meeting of Proteomics Society, India (PSI), 3-6 December, 2015 organized by VIT University, Vellore.
- Venkatesh Chanukuppa presented a poster entitled "Differential proteomic analysis of multiple myeloma towards thernostic biomarkers" at Targeted proteomics workshop and international symposium organized by Indian Institute of Technology, Bombay from 10-14 December, 2015.
- Jitendra Kumar presented a poster entitled 'Species selectivity in poxviral complement regulators: Negatively charged residues govern the specificity of VCP towards bovine classical pathway' at the 15th European Meeting on Complement in Human Disease, June 27-30, 2015, held at Uppsala, Sweden.
- Rucha Sarwade presented a poster titled "Delineating the role of PABP and PDI in translational regulation of insulin and insulin like UTR containing

mRNAs" Rucha Srwade, Poonam Pandey, Abdul Khalique and Vasudevan Seshadri. 8th RNA Group Meeting, 8-10 Jan 2016, CCMB, Hyderabad.

- Poonam Pandey delivered an oral talk entitled "HuD A interacts with PABP and regulate insulin translations", Poonam Pandey, Rucha Srwade, Abdul Khalique and Vasudevan Seshadri. 8th RNA Group Meeting, 8-10 Jan 2016, CCMB, Hyderabad.
- Poonam Pandey was selcted for national workshop on Science communication workshop held on 21-22 March, 2016 at Hyderabad, organized by The Wellcome Trust/DBT India Alliance
- Abdul Khalique - Poster entitled "Role of the insulin 5'UTR-binding factors in insulin gene regulation" presented at RNA 2015 twentieth annual meeting by RNA Society on 26th -31st May, 2015 at University of Wisconsin, Madison, USA.
- Suchismita Panda; A Novel lncRNA-Protein Interaction Drives Cellular Transformation, Tumorigenicity and Metastasis of Mouse Fibroblasts: 8th RNA Group Meeting, 8-10 January, 2016, Hyderabad, India
- Meenakshi Setia; Studies on a Novel Long Non-coding RNA in Cellular Growth and Development: 8th RNA Group Meeting, 8-10 January, 2016, Hyderabad, India
- Divya Kumari; 8th Annual Conference of Indian Society of Neuro-oncology, 1-3 April, 2016, Hyderabad, India
- Aman Sharma: Caner Stem Cell-Endothelial Cell Interactions in Glioblastoma; International Conference on Cancer Research: New Horizons; 19-21st November, 2015, NCCS, Pune.
- Mohasina Khan: International Conference on Cancer Research: New Horizons; 19-21st November, 2015, NCCS, Pune.
- Suchismita Panda: Long non-coding RNA-Ginir promotes cellular transformation, tumorigenicity and metastasis of mouse cells; The Non-Coding Genome EMBL Heidelberg, Germany Oct-2015
- Debasis Paul delivered a talk in "International Conference on Cancer Research: New Horizons, National Centre for Cell Science, 19 - 21st November, 2015"
- Srinadh Choppara: Best poster award in "A conference of new ideas in cancer -challenging dogmas TATA THEATRE, NCPA, Mumbai, 26 - 28 February, 2016"
- Srinadh Choppara: Best poster award in "International Conference on Cancer Research: New Horizons, National Centre for Cell Science, 19 - 21st November, 2015"
- Narayana YV; Regulation of mouse embryonic stem cells pluripotency by clathrin-mediated endocytosis; Narayana YV, Ridim Mote, Revati Dewal,

Kriti Chopra, Jayashree Jagtap and Deepa Subramanyam; Indian Society of Developmental Biology/ July 15-17, 2015, Hyderabad, India.

- Dr. Amruta Barhanpurkar attended "Hands on Training Workshop on Multicolor Flow Cytometry at Venture Centre, NCL Innovation Park, Pune, May 13-14, 2015.
- Dr. Amruta Barhanpurkar presented poster "Interleukin-3 has a potential to enhance the migration of human mesenchymal stem cells" at 12th Annual Meeting of International Society for Stem Cell Research (ISSCR), Stockholm, Sweden, June 24-27, 2015. She was awarded ISSCR Travel Grant to attend this conference.
- Dr. Amruta Barhanpurkar attended "Workshop on Confocal Microscopy" at Venture Centre, NCL Innovation Park, Pune, Feburary 2-3, 2016.
- Dr. Amruta Barhanpurkar attended International Conference on Innovative Trends in Chemical, Physical and Biosciences (ITCPB), Annasaheb Magar Mahavidyalaya, Hadapsar, Pune, February 9-10, 2016.
- Dr. Lekha Goyal attended "Hands on Training Workshop on Multicolor Flow Cytometry at Venture Centre, NCL Innovation Park, Pune, May 13-14, 2015.
- Dr. Lekha Goyal attended "Workshop on Confocal Microscopy" at Venture Centre, NCL Innovation Park, Pune, Feburary 2-3, 2016.
- Ms. Snehal Joshi attended "International Conference on Cancer Research: New Horizons" at National Centre for Cell Science, Pune, November 19-21, 2015.
- Mr. Vikrant Piprode presented poster "Anti-osteoclastic effect of interleukin-3 is conserved in rats" in Australian New Zealand Bone & Mineral Research Society Annual Scientific Meeting (ANZBMS) at Tasmania, Australia, November 1-4, 2015. He was awarded with DBT International Travel Support to attend this meeting.
- Mr. Satish Pote attended workshop on "Research Methodology for PhD Students'' at Maharashtra University of Health Sciences, Nashik, April 16-22, 2015.

Symposia / Workshops / Training programmes / Meetings Organized by NCCS

1) Pune Biologists' Meet (Meeting Focus: Model Systems)

5 June, 2015

Dr. Sandhya Koushika
TIFR, Mumbai

'Understanding biogenesis of synaptic protein transport carriers using the C. elegans model'

Dr. Anjan Banerjee
IISER, Pune

'Moss: An excellent bryophyte model for evolutionary crossroads in plant developmental biology'

Dr. Mahendra Sonawane
TIFR, Mumbai
& NCRA, Pune

'Zebrafish: A model to study cell biology of the epidermis development'

Dr. Surendra Ghaskadbi
ARI, Pune

'Evolutionary developmental biology: Hydra continues to hold it's Noggin high'

Dr. Amitabha Mazumdar
NCCS, Pune

'Of flies and persistence of memory'

2) Science Academies' Summer Research Fellows' Symposium

20 June, 2015

3) 'Planning and Funding Scientific Enterprises'

[Seminar organized in association with the Venture Center (NCL Innovation park, Pune)]

24 July, 2015

4) 'BIG 08 Mentoring Seminar'
(co-organized by Venture Center and NCCS)
4 Dec, 2015

5) India-EMBO Partnership Symposium
(organized by IISER-Pune jointly with NCCS)
5 Feb, 2016

6) Joint workshop by NCCS & Maastricht University, Maastricht, the Netherlands
10-11 March, 2016








7) International Mini-Conference on Novel Therapeutic Approaches Targeting Cancer and Cancer Stem Cell
(organized in association with Curtin University, Australia)
18 March, 2016



8) TRAINING PROGRAMMES

(including in-house training for the students & technicians of NCCS)

a) 'Basic course on Cytometry (BD FACS Canto II)'22-24 April 2015
Organized under the BD-NCCS Centre of Excellence in Flow Cytometry (COE) Program

b) 'Flow Cytometry Sorting Workshop'22-24 June, 2015
Organized by BD

c) **Lecture workshops** organized by Dr. Shekhar Mande jointly with the Indian National Science Academy, New Delhi; the Indian Academy of Sciences, Bangalore and the National Academy of Sciences, India, Allahabad &:

(i) The Gitam University, Visakhapatanam: 27-28 Nov, 2015

(ii) The HS Gaur Sagar University, Sagar:21-22 January, 2016

9) **The International Osteopontin and Other SIBLING Protein Conference** (Illinois, USA)
(Co-hosted by NCCS; Organizer from NCCS: Dr. Gopal Kundu)
8-11 October, 2015

10) 3rd **International Conference on Angiogenesis** (Sastra University, Trichi)
(Co-Convenor: Dr. Gopal Kundu, NCCS, Pune)
23-25 September, 2015

11) Fostering International Collaborations

- Meeting with a delegation from the Maastricht University (MU), The Netherlands (to explore possibilities for academic & research collaborations between NCCS & MU)
13 Oct, 2015

- Meeting with Dr. Sylviane Pied (Sr. Scientist, CNRS, Lille University & Institut Pasture de Lille, France) & Prof. Pierre Andre Cazenave (Institut Pasture de Lille, France) (to discuss the SIGID programme & explore possibilities for collaborative projects and funding opportunities between Indian and European agencies).
6 Nov, 2015

- NCCS signed a Memorandum of Understanding jointly with the Maastricht University & the Maastricht University Medical Center, Maastricht from The Netherlands, to enable academic and research collaborations.
11 Mar, 2016

Research Students of NCCS awarded with Ph. D. Degrees

(01.04.2015 – 31.03.2016)

No.	Research Scholar	Thesis Title	Month & Year of Award	Research Guide
1	Jeetendra Kumar	Functional characterization of dendritic cells generatedin vitro from umbilical cord blood with especial emphasison their role in adaptive immune responses.	November 2015	Dr. Lalita S. Limaye
2	Supinder Kour	Regulation of chondrocyte differentiation by interleukins	January 2016	Dr. Mohan R.Wani
3	Manmohan Bajaj	Alteration of adipoctyre:osteoblast ratio in the bone marrow niche and studies on its impact on the hematopoietic stem cells	January 2016	Dr. Vaijayanti P. Kale
4	Jayshree C Jagatap	Expression and role of prostate apoptosis response-4 (PAR-4) during drug-induced responses in gliomas.	February 2016	Dr. Padma Shastry
5	Moirangthem Ranjita Devi	Studies on regulation of HSC fate by hypoxia in bone marrow microenvironment	March 2016	Dr. Vaijayanti P. Kale
6	Aparajita Dasgupta	Evaluation of anti-angiogenic activity of novel triterpenoid AECHL-1 and or its synthetic analogs	March 2016	Dr. Sandhya Sitasawad

NCCS Foundation Day

(26th August, 2015)

Foundation Day Oration:

'The development of neural and muscle circuits required for complex behaviour'

Prof. K. VijayRaghavan, FRS

Secretary, Department of Biotechnology




'Einstein'

Play written by Gabriel Emanuel; Starring Naseeruddin Shah



Talks by Invitees

- **Dr. Sudhir Kumar Rai**
  National Institute of Child and Human Development (NICHD), NIH, Maryland, USA
  'Functional Genomic Screening of host genes required for Long Terminal Repeat retrotransposon integration in Schizosaccharomyces pombe'
  21April, 2015

- **Prof. Umesh Varshney**
  Dept. of Microbiology and Cell Biology, IISc, Bangalore
  'Initiation of translation in Escherichia coli by tRNAs lacking vital structural features needed for ribosomal P-site binding'
  7 May, 2015

- **Dr. Shamik Dasgupta**
  Centre for Neural Circuits and Behaviour, Univ. of Oxford, UK
  'Genes, Brain and Behavior'
  13 May, 2015

- **Dr. Amit Sharma**
  Group Leader, Mammalian Biology: Structural and Computational Biology, ICGEB, New Delhi
  'Insights into malaria parasite protein translation machinery and implications for drug discovery'
  5 August, 2015

- **Prof. Jayant Udgaonkar**
  Professor, NCBS, Bengaluru
  'Mechanism of misfolding of the prion protein'.
  6 August, 2015

- **Dr. Chetan Chitnis**
  Head, Malaria Parasite Biology and Vaccines Unit, Institut Pasteur, France
  'Entry and exit from red cells by malaria parasite'
  13 August, 2015

- **Dr. Sivakumar Vallabhapurapu**
  Lousiana State University, Health Science Center, Shreveport, the USA.
  'Balancing the Act: Regulation of the NF-kB signaling in normal homeostasis and disease'
  30 September, 2015

- Dr. Chandrama Mukherjee
  Department of Molecular and Cellular Biochemistry, Ohio State University, the USA
  'Exploring cytoplasmic capping; a new player in gene expression regulation'
  12 October, 2015

- Dr. Indrani Talukdar
  BITS Pilani, Goa
  'A tale of Tesk1 (and other barrier kinases) in somatic cell reprogramming'
  19 October, 2015

- Prof. Sen Pathak F.N.A.Sc
  University of Texas MD Anderson Cancer Center, the USA
  'Contamination and misidentification of cancer cell lines: Ghost in Biomedical Research'
  4 November 2015

- Dr. Rajan Raghavachari
  Senior Technical Advisor, Everstone Capital Advisors Limited
  'Financing startups and MSMEs (micro small medium enterprises)'.
  5 November, 2015

- Dr. Ashish Lal
  Investigator, Regulatory RNAs and Cancer Section, NCI, NIH, USA
  'Regulation and function of non-coding RNAs in colorectal cancer'
  17 November, 2015

- Dr. Avinash Pradhan & Dr. Anupama Joshi
  Consultant histopathologists, KEM Hospital, Pune
  'Histopathological analysis of neoplasms'
  27 November 2015

- Dr. Jugnu Jain
  Co-Founder and Chief Scientific Officer, Sapien Bioscience Private Limited, Hyderabad
  'A Biobank Enabling a Deeper Understanding of Diseases and Development of Novel Diagnostics and Targets: Case studies from Patient-derived Breast and Glial Cancers'
  30 November, 2015

- Dr. Anagh Sahasrabuddhe
  Department of Biotechnology, Pandit Ravi Shankar Shukla University, Raipur
  'Proteolytic regulatory mechanisms of epigenetic modulator polycomb group proteins'.
  1 December, 2015

- Dr. Ashish Misra
  University of Massachusetts Medical School, the USA
  'Mechanisms Regulating Alternative Splicing Networks'
  7 December, 2015

- Dr. Sagar Pandit
  Max Planck Institute for Chemical Ecology, Germany
  'Lepidopteran Reverse Genetics: The integration of molecular biology & classical ecology for the In Situ Analysis of tritrophic interactions and their infochemistry'
  10 December, 2015

- Dr. Bernhard Radlwimmer
  Deputy Head of Department & Team Leader, DKFZ, Heidelberg, Germany
  'Brain tumour metabolism'
  11 December, 2015

- Dr. Sonal Nagarkar-Jaiswal
  HHMI - Baylor College of Medicine, Houston, USA
  'Novel genetic approaches to explore neural stem cell homeostasis'
  14 December, 2015

- Dr. Rashmi Priya
  Institute for Molecular Bioscience, University of Queensland, Australia
  'Patterning Rho-GTPase signaling at the epithelial cadherin-junctions: a tale of feedback loops'
  4 January, 2016

- Dr. Manish Jaiswal
  Baylor College of Medicine, the USA
  'Genetic dissection of neuronal maintenance and demise'
  14 January, 2016

- Dr. Durba Sengupta
  CSIR-National Chemical Laboratory, Pune
  'Conformational dynamics of GPCR dimers is dependent on membrane components'
  11 February, 2016

- **Prof Iqbal Ahmad**
  University of Nebraska Medical Center, Omaha, the USA
  'Induced Pluripotency and Blinding Degenerative Diseases'
  11 February, 2016

- **Dr. Rukmini Banerji** (Director, ASER Centre) &
- **Dr. Madhav Chavan** (CEO Pratham Education Foundation)
  'Challenges of elementary education in India today'
  1 March, 2016

- **Dr. Anna George** (National Institute of Immunology, New Delhi)
  'Regulation of B cell differentiation choices'
  8 March, 2016
  (in celebration of women in science, on the occasion of the International Women's Day)

- **Dr. Neha Rani**
  Neuroscience Research Institute, Univ. of California Santa Barbara, USA
  'Non-Coding RNAs in Human Cortical Brain Development'
  23 March, 2016

TECHNICAL SEMINARS

- **'Flow Cytometry – Basics & Instrumentation'**
  Talk by BD application specialist.
  22 June, 2015

- **'Integrated multi-Omics Approach- OneOmics for System Biology using LC-MS/MS'**
  Dr. Aron Hudson (AB Sciex)
  29 Oct, 2015.

NCCS Celebrating 30 Years of DBT

1) 'Einstein'
(Play hosted by NCCS to commemorate 30 years of DBT + NCCS Foundation Day)
26 August, 2015

2) Public talk
'Past (Im)perfect, Present Tense, Future Bright: Biology & Society in the 21st Century'
Prof. K. VijayRaghavan
27 August, 2015

3) International Conference on Cancer Research: New Horizons
19-21 November, 2015

4) 'The 2015 Nobel Prizes in Science: A curtain-raiser'

(Talks about the 2015 Nobel prizes in science, organized for college students)

8 Dec, 2015

Prof. Naba Mondal
(Project Director, India-based
Neutrino Observatory, TIFR, Mumbai)

Dr. Sharmila Bapat
(Scientist, NCCS)

Dr. Vasudevan Seshadri
(Scientist, NCCS)

5) 'INNOVATION: Some myths and realities'

Talk by Mr. R. Gopalakrishnan

Former Executive Director, Tata Sons Ltd.

17 February, 2016

6) Online essay competition

Eight research scholars of NCCS participated in the inter-institution online essay competition on 'Biotechnology: Impacting Lives, Transforming Society', which was organized by the National Institute of Biomedical Genomics (NIBMG), Kalyani, on 18th September, 2015, to commemorate 30 years of DBT.

Outreach

1) '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. JBCS is supported under the 'Star College Scheme' by DBT.

'Edu-Bridge' was inaugurated on 22nd September, 2015

2) National Science Day (28 Feb, 2016)

Popular science talks & displays open to all

a) Talks

'The Unknown Einstein'

- Dr. Gajanan (Bal) Phondke
(Former: Scientist at BARC, Mumbai;
Editor of Science Today magazine;
Science Editor of The Times of India)

Q & A

'Climate Change in the Indian Context'

- Prof. R. R. Kelkar
(Member of the ISRO Advisory
Committee on Space Sciences.
Former: Director General of the
India Meteorological Department, New Delhi)

b) Displays

3) Public Talks

a) 'Past (Im)perfect, Present Tense, Future Bright: Biology & Society in the 21st Century'

This public talk delivered by Prof. K. VijayRaghavan organized at NCCS on 27th Aug, 2015, was subsequently also broadcast by All India Radio, Pune, on 9 Sep, 2015.

b) 'Molecules that make up living systems'20 Sep, 2015

Talk delivered by Dr. Shekhar Mande for school students, at the 'Popular Science Talks' series organized by the 'Exciting Science' group (Venture Center, Pune).

c) 'Stem Cells: Superheroes of the past, present and future'18 Oct, 2015

Talk delivered by Dr. Deepa Subramanyam for school students, at the 'Popular Science Talks' series organized by the 'Exciting Science' group (Venture Center, Pune).

d) A Curtain-raiser to the 2015 Nobel Prizes Award Ceremony8 Dec, 2015

(Talks about the 2015 Nobel prizes in science, organized for college students)

Speakers:

- Prof. Naba Mondal (Project Director, India-based Neutrino Observatory, TIFR, Mumbai)
  - Spoke about the Nobel prize in Physics.
- Dr. Vasudevan Seshadri (Scientist, NCCS) - Spoke about the Nobel prize in Physiology / Medicine.
- Dr. Sharmila Bapat (Scientist, NCCS) - Spoke about the Nobel prize in Chemistry.

e) Talks delivered in Marathi by Dr. L. S. Limaye for the Marathi Vidnyan Parishad:

- * "Naletil Raktachi Pedhi" - at the Maharashtra Sahitya Parishad Hall (Science Day- 28th Feb.2016)
- * "Stem cells che jatan" - at the Science Park, Pimpri-Chinchwad (1st April 2016)

4) Vigyan Rail

NCCS provided exhibits on the theme 'Drug Discovery and Stem Cell Biology' for display on the mobile science museum on the train, the 'Science Express'.

5) TV Programmes

- Dr. Bhat and his research team spoke about their research in the documentary, 'Wise Spice', made by Vigyan Prasar of DST for telecast on the DD National channel, DD Bharati, Lok Sabha TV & Rajya Sabha TV.
- Dr. Mande spoke about his research on 'Eureka', a TV programme produced by Rajya Sabha TV & CSIR-NISCAIR to showcase contributions of Indian scientists. It was telecast in February, 2016, by the Rajya Sabha TV Channel.

6) Radio programmes

(a) Radioscope

NCCS organized talks & discussions for this national science magazine broadcast by the All India Radio, New Delhi, to educate the general public about diverse topics in science. These were organized in association with scientists from other institutions. The programme was broadcast on 12th June, 2015.

- **Topics Featured:** 'The Ebola virus'; 'Astrobiology'; 'Understanding Earthquake and Seismic Safety'; 'Snippets from the world of science'.
- **Partcipants:** Dr. Atanu Basu (NIV, Pune); Dr. Preeti Nema (Blue Marble Space Institute of Science, USA); Dr. Arun Bapat (formerly with the Central Water & Power Research Station, Pune); Dr. Jyoti Rao (NCCS).

(b) 'Moon To Mars : A Space Odyssey'

Dr. Jayant Narlikar's interview conducted by Dr. Yogesh Shouche was broadcast by All India Radio, Pune, in July 2015.

7) Poular Science Articles

'War, Peace and Crystallographers'

An article by Dr. Shekhar Mande, was published in the IUCr Newsletter (2015, Vol. 23, No. 4).

Teaching

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

Scientist	Topic / Symposium	Class / Department	Institution	Date
Rahul Bankar	Biology and Husbandry of Laboratory Animals & Injection Techniques	Project Staff	NIV, Pune	09/12/2015
Sharmila Bapat	The Theme of Stem Cell Biology in Cancer At the Symposium: "Insights into biology 2025"	Undergraduate students	NCL, Pune	29/10/2015
Sharmila Bapat	Nobel Prize in Chemistry 2015 At : 'The 2015 Nobel Prizes in Science: A curtain-raiser'	College students	NCCS, Pune	8/12/2015
Sharmila Bapat	Cancer - A Stem Cell Disease? At: 'CHIMERA' the departmental festival.	B.Sc. Dept. of Biotechnol.	Fergusson College, Pune	29/12/2015
Manoj K. Bhat	Career Opportunities in Biotechnology, Nutrition and Dietetics	M.Sc.	Symbiosis School of Biomedical Sciences, Symbiosis International University, Pune	10/06/2015
Manoj K. Bhat	Metabolic disorders: an unexpected role in cancers.	M.Sc.	School of Regenerative Medicine, Manipal University, Bangalore	30/06/2015
Manoj K. Bhat	Cancer ChemotherapyEnhancement in Cell Killing	Zoology (Department of Zoology)	Department of Chemistry, Savitribai Phule Pune University, Pune	28/03/2016
Jomon Joseph	Looking through the microscopic eyes (use of microscopy in biology)	XII and B.Sc	J. B. College of Science, Wardha	04/03/2016
Jomon Joseph	Microscopic analysis of cell cycle	XII and B.Sc	JBSC, Wardha	05/03/16
Musti Krishnasastry	1. Basics of Electrophoresis & demonstration 2. Basics of Game theory 3. Evolution of defection 4. Inside class simulation on evolution of cooperation and defection.	XII & B.Sc.	J. B. College of Science, Wardha	18/03/2016 19/03/2016
Janesh Kumar	Structures of tRNA and tRNA synthatase	M.Sc. Biotechnology	Dept, of Biotechnology, SPPU, Pune	04/04/2015
Janesh Kumar	Functional Insights from Ribosome structures	M.Sc. Biotechnology	Dept, of Biotechnology, SPPU, Pune	07/04/2015
Janesh Kumar	Synaptic receptors and their regulation	Neurobiology	NCL, Pune	08/09/2015
Janesh Kumar	Structural aspects of membrane proteins	M.Sc. Biotechnology	Dept, of Biotechnology, SPPU, Pune	22/03/2016
Janesh Kumar	Structure of RNA polymerase and its role in transcription	M.Sc. Biotechnology	Dept, of Biotechnology, SPPU, Pune	26/03/2016

Scientist	Topic / Symposium	Class / Department	Institution	Date
Gopal Kundu	Cancer Awareness in India (INSPIRE Lecture)	Junior College	KIIT University	21/08/2015
Girdhari Lal	Biology of Adaptive immune cells: a great responsibility from the best education.	XIIth and B.Sc.	J. B. College of Science, Wardha	23/09/15
Girdhari Lal	Discussions on various immunological assays and troubleshooting	XII and B.Sc	Jankidevi Bajaj College of Science, Wardha	22/09/2015
Girdhari Lal	Skin transplantation and discussion	XII and B.Sc	Jankidevi Bajaj College of Science, Wardha	23/09/2015
Girdhari Lal	Induction of Experimental autoimmune diseases	XII and B.Sc	Jankidevi Bajaj College of Science, Wardha	23/09/2015
Girdhari Lal	Discussion on various immunological diseases	XII and B.Sc	Jankidevi Bajaj College of Science, Wardha	23/09/2015
Girdhari Lal	A story of T cells in transplantation & autoimmunity. (*Invited talk*)	M.Sc.	School of Regenerative Medicine, Manipal University, Bangalore	25/04/2015
Nibedita Lenka	Science: Explore the possibilities	12th Std., Science	Modern Public School, Balasore, Odisha.	30-11-2015
Nibedita Lenka	The Biology and Implications of Stem Cells	B.Sc., Botany & Zoology	Dr. Jadunath College, Balasore, Odisha	01-12-2015
Nibedita Lenka	The Biology and Implications of Stem Cells	M.Sc., Biotech, Botany & Zoology	North Orissa University, Baripada, Odisha	02-12-2015
Nibedita Lenka	Emerging Trends in Stem Cell Research	B.Sc. & M.Sc. Zoology	Ravenshaw University, Cuttack, Odisha	04-01-2016 06-01-2016
Nibedita Lenka	Emerging Trends in Stem Cell Research: The Hypes & Hopes At: Annual Biotech. Alumni Meet	UG and PG	Utkal University, Bhubaneswar, Odisha	22-12-2015
Nibedita Lenka	Emerging Trends in Stem Cell Research: The Hypes and Hopes At: Annual Biotech. Alumni Meet	UG and PG	Ravenshaw University, Cuttack, Odisha	06-01-2016
L. S. Limaye	Cord blood banking	Zoology Dept.	Fergusson College, Pune	13/1/2016
L. S. Limaye	Haematopoietic stem cells	Microbiology Dept.	Fergusson College, Pune	28/01/2016
L. S. Limaye	Possible use of DCs derived from umbilical cord blood in cancer immunotherapy	Microbiology Dept.	Fergusson College, Pune	08/02/2016

Scientist	Topic / Symposium	Class / Department	Institution	Date
Shekar Mande	Introduction to structural biology	XIIth & B.Sc	J. B. College of Science, Wardha	22/09/15
Debashis Mitra	Anti-HIV Drug Discovery: Past, Present and Future	BSc Zoology/ Biotechnology/ Microbiology	Vivekanda College, Thakurpukur, Kolkata	16/12/2015
Debashis Mitra	Anti-HIV Drug Discovery: Past, Present and Future	BSc Zoology/Botany/ Microbiology/ Chemistry	Maulana Azad College, Kolkata	17/12/2015
Debashis Mitra	Anti-HIV Drug Discovery: Past, Present and Future	BSc Zoology/ Biotechnology/ Microbiology students	Charuchandra College, Kolkata	18/12/2015
Debashis Mitra	An introduction to Viruses	XII & BSc Biotechnology/ Microbiology	J. B. College of Science, Wardha	18/03/2016
Debashis Mitra	HIV/AIDS	XII and B.Sc	JBSC, Wardha	19/03/16
Debashis Mitra	Protein electrophoresis techniques	XII and B.Sc	JBSC, Wardha	18/03/16
B.Ramanamurthy	Biosafety, Bioethics and Animal Ethics	Ph.D.	Zoology Dept. S.P. Pune University	12-09-2015 19-09-2015
Arvind Sahu	An introduction to the innate immune system	XIIth and B.Sc	J. B. College of Science, Wardha	22/09/15
Arvind Sahu	Ouchterlony technique	XII and B.Sc	JBSC, Wardha	22/09/15
Arvind Sahu	ELISA technique	XII and B.Sc	JBSC, Wardha	22/09/15
Manas Santra	Protein structure, function	XII and B.Sc	J. B. College of Science, Wardha	9/10/15
Manas Santra	Molecular Biology-cloning	XII and B.Sc	J. B. College of Science, Wardha	10/10/15
Manas Santra	Preparation of Bradford reagent, Protein estimation	XII and B.Sc	JBSC, Wardha	09/10/15
Manas Santra	PCR, agarose gel electrophoresis	XII and B.Sc	JBSC, Wardha	10/10/15
Vasudevan Seshadri	Basic concepts in protein synthesis	XII and B.Sc	J. B. College of Science,	04/03/16 Wardha
Vasudevan Seshadri	Protein purification: Isolation of recombinant Taq DNA polymerase and PCR assay	XII and B.Sc	JBSC, Wardha	05/03/16
Anjali Shiras	Recent Advances in Molecular Biology	B.Sc. Zoology & Biotechnology	S.P. College, Pune	06/02/2016 07/02/2016

Scientist	Topic / Symposium	Class / Department	Institution	Date
Anjali Shiras	National conference on Convergence of Stem cells and Medical Nanotechnology	MBBS and MSc Biotechnology)	D.Y. Patil College of Medicine	02/09/2015 03/09/2015
Sandhya Sitasawad	'Mitochondrial dysfunction, oxidative damage & apoptotic cell death stress mechanisms in diabetic cardiomyopathy'	Dept. of Biochemistry	School of Life Sciences, University of Hyderabad	03/08/2015
R Srikanth	Proteomics and applications	XII and B.Sc	J. B. College of Science, Wardha	09/10/2015
R Srikanth	Mass spectrometry based proteomics	XII & B.Sc.	J. B. College of Science, Wardha	10/10/2015
R Srikanth	Analytical methods for chemical analysis and drug discovery	XII & B.Sc	JBSC, Wardha	10/10/15
R Srikanth	Mass spectrometry instrumentation and applications	XII and B.Sc	JBSC, Wardha	10/10/15
R Srikanth	GIAN Proteomics course	B. Sc. & M. Sc	IIT-Bombay	17/12/2015 18/12/2015
R Srikanth	Separation of DNA, RNA and Protein from cell lysate	XII and B.Sc	JBSC, Wardha	09/10/15
Deepa Subramanyam	Stem Cells : Superheroes of the past, present and future	Class XI and XII (Biology)	Delhi Public School, Pune	20/8/2015
Deepa Subramanyam	Stem Cells : Superheroes of the past, present and future	Class X (Science)	Gurukul School	22/28/2015
Deepa Subramanyam	Stem Cells : Superheroes of the past, present and future	Class IX (Science)	Gurukul School	22/28/2015
Deepa Subramanyam	Stem Cells : Superheroes of the past, present and future	Class V to Class XII	NCL Outreach Centre	18/10/2015
Deepa Subramanyam	Experiments demonstration	Class XII (Biology)	Delhi Public School	31/10/2015
Deepa Subramanyam	Stem Cells : Superheroes of the past, present and future	Class IX (Science)	Hutatma Balaveer School, Shivajinagar	20/1/2016
Deepa Subramanyam	Stem Cells : Superheroes of the past, present and future	Class VII	KC Thackeray Vidyaniketan, Shaniwarvada	23/1/2016

Classes taught by NCCS scientists for the JRF coursework (2015)

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

Scientist	Subject
Rahul Bankar	Demonstration : Handling & Restraining of Laboratory Animals
Rahul Bankar	Biology & Husbandry of Laboratory Animals
Rahul Bankar	Injection & Blood Collection in Rodents
Sharmila Bapat	Cancer biology
Manoj K. Bhat	Cancer therapies
Jomon Joseph	Microscopic Techniques
Jomon Joseph	Non-coding RNAs and miRNA
Musti Krishnasastry	Basics of Protein Chemistry
Janesh Kumar	Membrane protein crystallization and strategies
Janesh Kumar	Examples of membrane protein structures
Janesh Kumar	Tools and techniques
Gopal Kundu	Application of Nanomedicine in Cancer
Gopal Kundu	Tumor Immunology and Targeted Therapy
Girdhari Lal	Transplantation Immunology
Girdhari Lal	Tumour Immunology
L. S. Limaye	Flow cytometry
Amitabha Majumdar	Stem cell biology
Shekhar Mande	Structural Biology
Debashis Mitra	Immunology - B cells & antibodies
B. Ramanamurthy	Laboratory Animal Experimentation and Ethics Course
Arvind Sahu	Proteomics
Manas Santra	Transcription, cancer biology
Vasudevan Seshadri	Biosafety
Anjali Shiras	Stem Cells (also conducted paper presentations by students)
Anjali Shiras	Hallmarks of Cancer
Shailza Singh	Computer Applications and Bioinformatics
R. Srikanth	Proteomics Strategies
R. Srikanth	Proteomics applications & 2-D analysis
R. Srikanth	MALDI mass spectrometry
R. Srikanth	LC-MS. GC-MS and Metabolomics
R. Srikanth	Quantitative proteomics
Deepa Subramanyam	Stem Cell Biology
Vidisha Tripathi	Tools and techniques
Mohan R. Wani	Autoimmunity
Mohan R. Wani	Animal models of immune disorders

Other Happenings at NCCS

1) Visit by Dr. Harsh Vardhan, Hon'ble Union Minister for Science & Technology & Earth Sciences.
3rd February, 2016








2) Visit to Australia
19-26 Apr, 2015

NCCS faculty members with Nobel laureate, Barry Marshall

3) Dr. Gopal Kundu visited the Whitehead Institute for Biomedical Research, MA, USA
12 Jan, 2016

Dr. G.C. Kundu with Dr. Robert Weinberg

4) NCCS signed an MoU with the Venture Center, Pune

(for scanning and showcasing the technology & expertise of NCCS to entrepreneurs and potential partners in the industry)

18 Sep, 2015

5) International Yoga Day

21 June, 2015

6) Digital India week

NCCS organized an essay writing competition on the topic:

"Digital India: The digital future I want to see"

1-7 July, 2015

Rucha Sarwade, winner of the competition

7) 'Stress management & understanding depression'

(Talk and activities conducted by psychologist Aparna Thakar, for the staff & students)

13 April, 2016

8) Sports

15 Aug, 2015:The prize distribution for the 2015 sports events was done by the Arjuna awardee, Mr. Shantaram Jadhav, Captain of the Indian Kabaddi team at the Asian Championships in 1980-81.



Sports 2016






NCCS in the News

The research findings of Dr. Mohan Wani and his team on the protective role of IL-3 in mouse osteoarthritis (Kour et al, J. Immunol, 2016, doi: 10.4049/jimmunol.1500907) were featured under 'Research Highlights' in Nature Reviews Rheumatology (doi: 10.1038/nrrheum.2016.96).

The research findings of Dr. Manas Santra & his group and Dr. Samit Chattopadhyay & his team (Nakka et al, PNAS, 2015 Jun 30;112(26):E3374-83; Malonia et al, PNAS, 2015 Jul 14;112(28):8632-7) were featured in articles in the news papers, Sakal Times (15 July, 2015), The Telegraph (Calcutta, 6 July, 2015), Loksatta (15 July, 2015), Sakal (17 July, 2015).

The research findings of Dr. Arvind Sahu & his group (Gautam et al, PNAS, 2015 Oct 13; 112(41):12794-9) were featured in an article in the news papers, Loksatta (17 Oct, 2015)

An article about the novel bacterial species which was named after Pune, Clostridium punense, [Lanjekar et al, IJSEM, 2015 Dec;65(12):4749-56], was featured in Maharashtra Times (Oct, 2015). Discussions on C.punense by Dr. Ranade and his team from ARI, Pune, and Dr. Shouche & his team from NCCS, who isolated, identified and named this species, were also recorded & broadcast by the All India Radio, Pune.

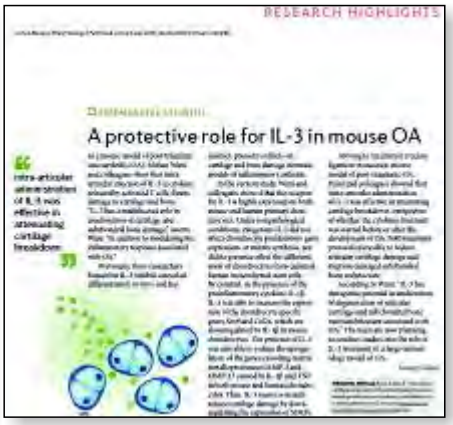
The research findings of Dr. Manoj Bhat and his team on the influence of fenugreek seeds on metabolism was featured in Sakal Times (29 July 2015).

The research carried out at NCCS was featured in the Golden Sparrow
(7 Aug, 2015: http://thegoldensparrow.com/news/pune-is-science-central/;
11 Mar, 2016: http://thegoldensparrow.com/news/to-see-a-world-in-a-grain-of-sand/)


'एनसीसीएस'मधील शास्त्रज्ञांच्या संशोधनाला अमेरिकेतील मानाच्या नियतकालिकात स्थान

कर्करोगावरील उपचारासाठी नवीन आशा!


विषाणूरोधक औषधाच्या निर्मितीसाठी 'एनसीसीएस'च्या शास्त्रज्ञांचे संशोधन!


RESEARCH HIGHLIGHTS

A protective role for IL-3 in mouse OA


कर्करोगावर मात करण्यासाठी संशोधन

डॉ. समित चटोपाध्याय व त्यांच्या सहकाऱ्यांचे ठराविक वनस्पतींवर प्रयोग सुरू

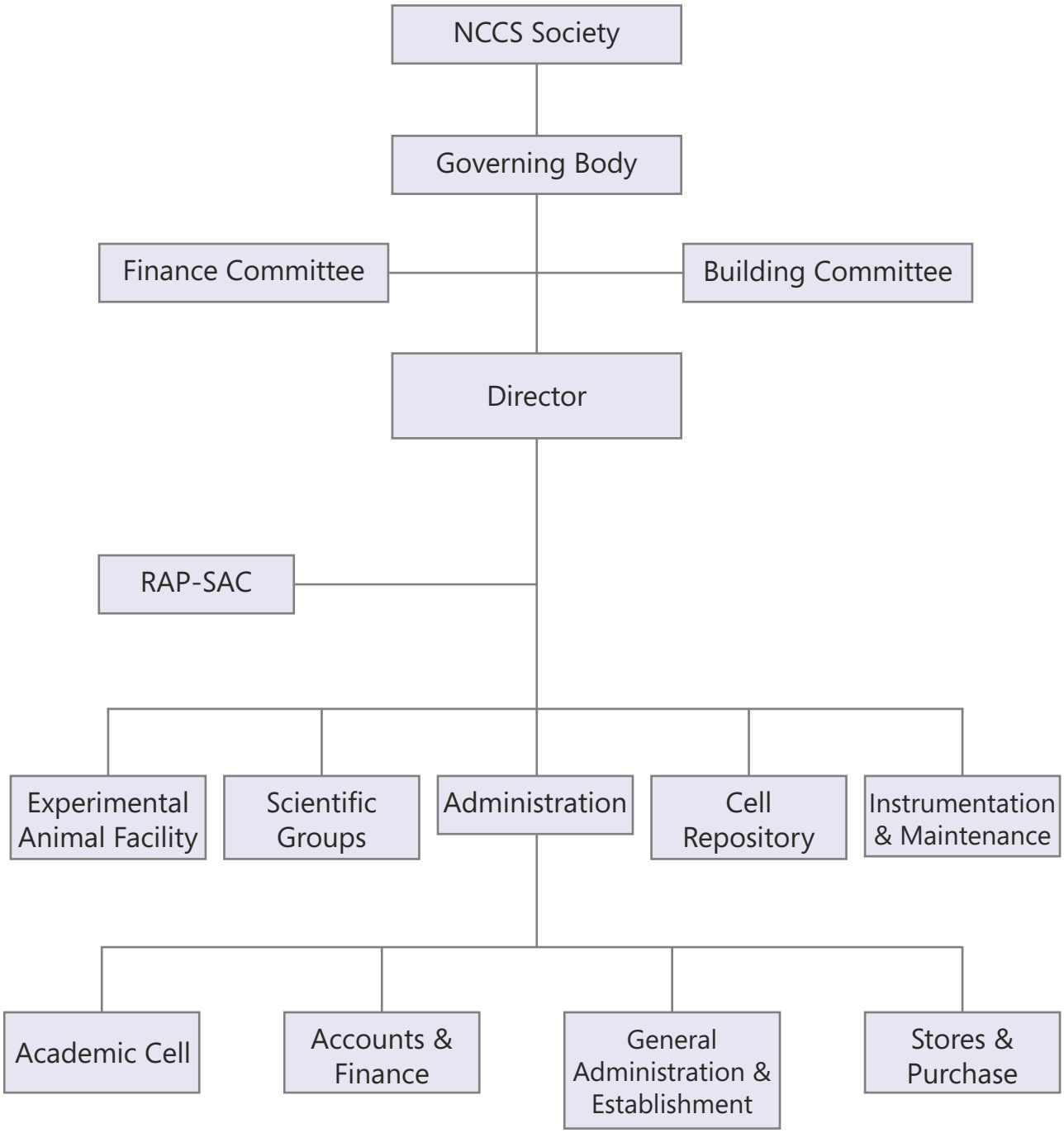

Sakal Times

city

NCCS scientists may develop compound with few side effects

PLANT SOURCES CAN CONTROL CANCER CELLS, SAY SCIENTISTS

NCCS Organization



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    Pashan, Pune 411021, India

15. **Dr. Arvind Duggal** — Member
    Adviser
    Department of Biotechnology
    11 Lodi Road, CGO Complex,
    7-8th floor, II Block
    New Delhi 110 003, India

Administration

The NCCS Administration consists of the following sections: General Administration & Establishment, Civil Maintenance, Accounts & Finance, and Stores & Purchase. The centre also has an Instrumentation & Maintenance unit. All these sections provide support services to the main scientific activities of the centre.

The NCCS staff strength (as on 31st March, 2016):

Scientists	:	33
Administrative Staff	:	42
Technical Staff	:	71
Total	:	146

Reservation Policy

NCCS follows the Government of India orders on reservation matters. For direct recruitments, respective rosters are followed, with reservation as follows: 15% for SC, 7.5% for ST and 27% for OBC, on an All India Basis by Open Competition. Liaison officers have been nominated to ensure compliance with the reservation orders issued in favour of SC/ST/OBC.

Reservation for persons with disabilities:

NCCS also follows the Government of India rules for Persons with disabilities (Equal Opportunities, Protection of Rights and Full Participation Act 1995). NCCS have five employees with disabilities. NCCS has provided double Transport Allowance to these employees and the salaries of such employees are exempted from deduction of Professional Tax.

Right to Information Act 2005

As per the requirement of the RTI Act 2005, NCCS has nominated Shri. V. S. Shinde, Officer 'B' (Administration) as the CPIO and Dr. Jomon Joseph, Scientist 'E', has been nominated as the First Appellate Authority.

Security

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

Committees

The Centre has formed the following committees as required under various statutes and guidelines for smooth functioning of the institute:

1. Grievance Committee
2. Committee for prevention of sexual harassment of working women employees
3. Animal Care and Use Committee
4. Biosafety Committee
5. Institutional Ethics Committee

Disciplinary Matters

The Centre follows CCS (CCA) rules 1965 and NCCS bye-laws for monitoring disciplinary matters at the Centre.

Vigilance Matters

Quarterly reports on vigilance-related matters at NCCS were sent to the Department of Biotechnology (DBT), Ministry of Science & Technology, Government of India. Dr. M. R. Kadole, Head of the CBI, Pune region, Akurdi, had accepted the invitation extended by NCCS and was scheduled to deliver a lecture on the occasion of the vigilance week. However due to an unforeseen emergency meeting of the CBI in New Delhi this lecture was cancelled.

Implementation of Official Language

The Director, NCCS, strongly supports the use of the Official Language in official work, and other related activities carried out at the Centre. NCCS has constituted the Official Language Implementation Committee to implement the Government of India orders to use the Official Language in day-to-day official work. Meetings of this committee are held quarterly, where various ways to implement the Official Language at NCCS are discussed.

Towards ensuring implementation of the Official Language, most of the official documents that are used regularly have been made bilingual (Hindi + English). Also, almost all the official correspondence carried out within the country is in the bilingual format.

The 'Hindi week' was celebrated with much enthusiasm in 2015 by holding 'Hindi essay writing', 'Hindi Letter Writing' and 'Antakshari' competitions for the staff & students of NCCS. Each competition was organized separately for 'Hindi

Bhashi' & 'Non-Hindi Bhashi' participants in 2015, with the aim of encouraging greater participation. Taking into consideration the consequent enthusiastic and overwhelming response received, this practice will be continued henceforth. Cash prizes and certificates were awarded separately for the 'Hindi Bhashi' and 'Non-Hindi Bhashi' winners of each competition. Participation certificates were also given to all participants to ensure continued zeal towards being involved in the Hindi week celebrations as well as in Official Language Implementation overall. Shri. R. M. Vishwakarma, Chief Manager (Official Language), Bank of Maharashtra, Head Office, Pune, was invited as the Chief Guest for the Hindi Day Function held on 18th September, 2015. On this day, the third issue of 'Meemansa' (Hindi Patrika) was released at the hands of Shri. R. M. Vishwakarma, Dr. Dr. G. C. Mishra (former Director of NCCS), Dr. Shekhar C. Mande (Director, NCCS) and Dr. Shailja Singh (Scientist & Chief Editor-Meemansa). Dr (Mrs). Saroj Ghaskadbi and Dr. Surendra Ghaskadbi's interview on kidney transplant and other scientific articles are the main attraction of this year's magazine.

National Centre for Cell Science
An autonomous institution aided by the Department of Biotechnology, Govt. of India

Photographs courtesy : Mahavir Rangole, Sunil Kachare, Mandar Rasane, Prithviraj Baijugam, Dhiraj Kumar, Jeetendra Kumar, Jyoti Rao

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