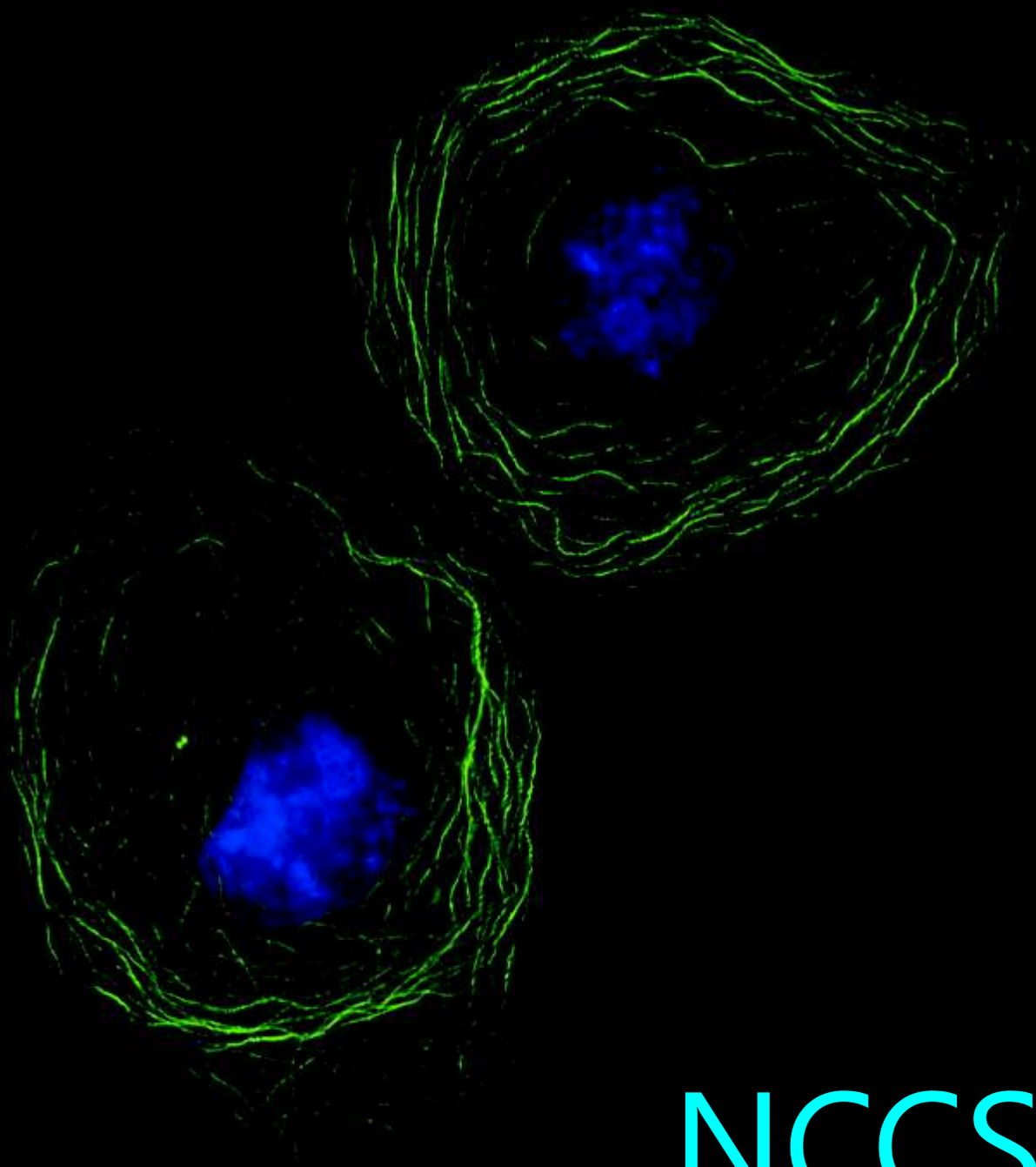
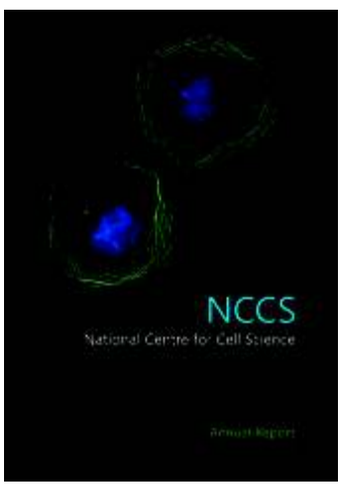


NCCS

National Centre for Cell Science

Annual Report

2013-2014



Cover page image

Cos-7 cells expressing GFP-tagged N terminal region (1-900 amino acids) of the nucleoporin Nup358 (green). DNA is stained with Hoechst dye (blue).
(Image courtesy: Dr. Jomon Joseph)

National Centre for Cell Science

Annual Report 2013-2014

Contents

Mandate of NCCS

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

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

- Research and development.

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

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

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

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

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

From the Director's Desk

I am very pleased and honoured to present this annual report in the year that NCCS has completed 25 successful years. NCCS has been carrying out cutting-edge research in frontline areas of modern cell biology, has been providing valuable services as a national cell repository and has been supporting human resource development through various programmes. The NCCS family enthusiastically celebrated the Silver Jubilee on its Foundation Day on the 26th of August, 2013. This day began with a formal inauguration of the new auditorium by Prof. P. Balaram, former Director of IISc, Bengaluru, who then delivered the Foundation Day lecture entitled, 'Chemical Diversity in Biology'. This was followed by felicitations of all NCCS employees who have completed 20 years of service, by Prof. W. N. Gade, Vice Chancellor, S. P. Pune University. These felicitations marked the beginning of a new tradition to recognize the valuable contributions made by the staff towards the growth, achievements and success of NCCS. The Foundation Day celebrations concluded with an enjoyable cultural programme organized by the staff and students of NCCS.

Various other activities were also organized over the past year and half to commemorate this special milestone. This included several Silver Jubilee Orations and other talks by eminent researchers and academicians, including Nobel laureates like Dr. Venkatraman Ramakrishnan and Prof. Jules Hoffmann. Furthermore, the research scholars at NCCS organized a special students' symposium for oral and poster presentations by Ph.D. students from different institutions in Pune. On the cultural side, staff and students joined in the celebrations by participating in events like debate, essay-writing, photography and T-shirt design contests organized over this period. We also used this special occasion to initiate collective public outreach through talks broadcast by All India Radio, with co-participation by faculty from NCCS and our colleagues from neighbouring research and academic institutions.

In keeping with the mandate and years' of tradition of serving as a national cell bank of animal cell lines, the NCCS repository supplied three thousand three hundred and thirty five cell lines to over a hundred and seventy research institutions all over India, during the year 2013-14. NCCS also offers different

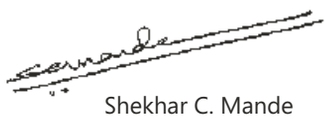
academic programmes to encourage the youth towards a career in science. NCCS admitted thirty one research scholars into its Ph.D. programme, following a multi-step selection process. Twenty five of our research scholars registered as Ph.D. students with the S.P. Pune University during this year, bringing the total number of registered Ph.D. students to a hundred and twenty. Twenty-six project trainees and fifteen summer trainees from all over the country also received training in research through multiple programmes run at NCCS over the year. Forty three research scholars at NCCS availed of opportunities to participate in several national and international conferences and workshops, where many of them also won accolades for their work.

Studying the nuances at the molecular level of diverse biological systems that are important in human health is the major focus of the research carried out at NCCS. A research team led by Dr. Arvind Sahu, for example, studies the complement system, an important component of the immune system. Proper regulation and functioning of this arm of the immune system is paramount in protecting the body from infections. Since the complement system forms an essential barrier against infection by viruses, the latter have evolved a series of counter-adaptations to subvert this system. The findings of this team have shown that one such counter-adaptation involves encoding structural and functional homologs of human complement regulators, by the viruses. These viral regulators inactivate complement by binding to complement proteins C3b and C4b and recruiting a protease (factor I) which cleaves C3b/C4b, thereby shutting off complement activation. The molecular mechanism of such inactivation was however, not clear earlier. Recently, Dr. Sahu's team has fine-mapped the sites of interaction on the viral regulators for C3b/C4b and the protease, leading them to propose a model for inactivation of C3b/C4b by viral/human regulators. They suggest that bridging of the MG2 and CUB domains of C3b/C4b by these regulators helps in stabilization of the CUB domain with respect to the core of the C3b/C4b molecule and that this, in turn, enables the protease to cleave the scissile bonds in the CUB domain. Since the viral regulators are structural and functional mimics of human complement regulators, a similar mechanism may hold true for the latter also. Further, they have also demonstrated how N-linked glycans affect the interaction of viral regulators with C3b and thereby complement regulation. Studies to confirm their proposed model and to further understand finer details involved could help in designing better control measures and treatment regimens against viral infections.

While a healthy immune system protects the body from infections and disease, faulty functioning of this system can lead to the body's healthy tissue being targeted and destroyed, resulting in autoimmune disorders. Dr. Mohan Wani's research group at NCCS is involved in studying one such autoimmune disease, Rheumatoid arthritis (RA), which is characterized by the presence of inflammatory synovitis, resulting in the destruction of cartilage and bone at the synovial joints. They have observed that adipose tissue-derived mesenchymal stem cells prevent bone loss in collagen-induced arthritis mouse model of RA. Their studies thus suggest that these cells exert a direct effect on the prevention of bone loss, in addition to dampening of ongoing inflammatory responses. These promising findings with potential for application in preventive and regenerative medicine are being explored further.

NCCS also has many strong research groups that work towards deciphering the molecular basis of complex disease like cancer. One of these, a team led by Dr. Samit Chattopadhyay, has demonstrated some of the intricate mechanisms involved in cancer cell death and survival. Their research involves taking a close look at the functions of one of the tumor suppressors and master regulator of global gene transcription, SMAR1. They have recently demonstrated the role of SMAR1/BANP in modulating the functions of DNA damage repair proteins like Ku70, gamma- H2AX etc. They also study the regulation of alternative splicing of the CD44 receptor protein. They are now trying to identify a new regime of small molecules that can activate the tumor suppressor, p53, and also prevent the accumulation of CD44 variables, using breast cancer and other cancers as a model. Towards this, they have demonstrated for the first time that SMAR1 exerts negative control on miRNA 373, downmodulating cancer cell metastasis. Their findings have led to an advancement in our understanding of cancer cell metabolism, with implications for improving the treatment regimens used for this disease.

The NCCS team is proud to have triumphantly brought the Centre to the Silver Jubilee landmark through its concerted efforts. While rejoicing, we further resolve to use this occasion as a spring board to launch NCCS to even greater heights of excellence in research, research-related services and promotion of science for the benefit of society.



Shekhar C. Mande
Director

Human Resource Development

During the year 2013-14, thirty one 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 120, as on 31st March, 2014. Thirteen students submitted their theses to the University and sixteen students were awarded the Ph.D. degree during the said year.

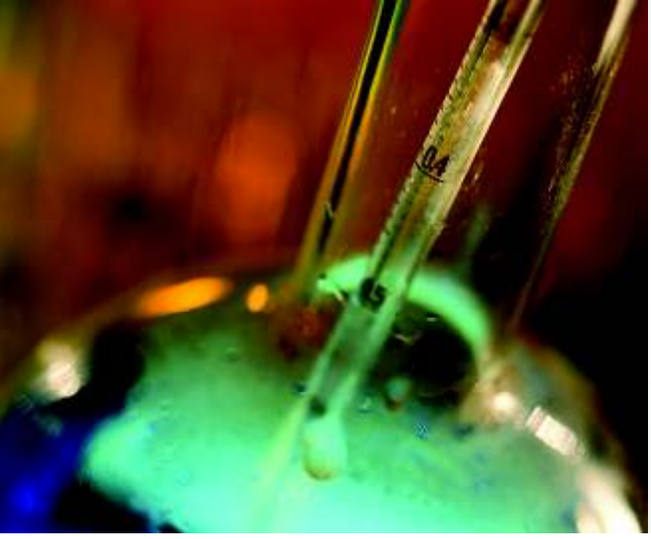
NCCS also conducts training programmes for students every year, as given below:

(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 2013-14 is as follows:

Project trainees	:	26
Summer trainees	:	15



Repository

NCCS serves as a national cell bank for animal cell lines. The repository manages the expansion, cryo-preservation and distribution of cell lines to research and academic institutions throughout the country. In the year 2013-14, three thousand three hundred and thirty five cell lines were supplied to more than a hundred and seventy research institutions.

Scientists

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Research Reports

Index of Research Reports

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Identification of epigenetic biomarkers in epithelial ovarian cancer

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Collaborator

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Background

Aberrant gene expression, credited to genetic and epigenetic changes are reportedly responsible for disruptive molecular behavior, cancer occurrence and progression. Several earlier studies have shown that DNA hypermethylation associated with CpG islands is a widespread occurance in cancer cells, wherein it represses transcription and in some instances has been considered to be functionally equivalent to gene deletion. Towards resolution of a better perspective of its underlying mechanisms, that could generate possible avenues for treatment, it becomes relevant to study differential methylated genes associated with tumorigenesis.

High-grade serous ovarian cancer (HGSC) remains the most lethal gynecological cancer. The present study is based on differential promoter methylation of an in vitro progression model of HGSC established earlier in the lab *(Bapat et al. Cancer Research. 2005)* wherein one of 19 single cell spontaneously immortalized clones derived from a grade IV patient underwent transformation. Briefly, initially this A4 clone is slow-cycling and non-tumorigenic, but around passage 20–25 it transforms into an aggressively tumorigenic clone with metastatic capabilities. This data suggests that early A4 cells, although lacking tumorigenecity had already acquired some of the features of transformation. Hence we referred to these as being pre-transformed (A4-P), while the cells with tumorigenic potential were termed as A4-T. This paired set of cell lines provided us a suitable progression model of two functionally discrete cell groups derived from a single cell clone. We have earlier carried out extensive gene expression and proteomic analysis of the

same that is reported (Bapat et al. 2010; Kalra and Bapat 2013). The present study comprises of firstly, a genome-wide identification of differential methylated genes in the A4 progression model that are validated at a basic level through profiling of their expression patterns; a caveat being that hypermethylated genes are repressed while hypomethylated genes are expressed. Similar differential profiles were also generated using early and late stage HGSC datasets in The Cancer Genome Atlas (TCGA). An overlap of these profiles identified a concise set of genes for our analysis. The extent of promoter methylation in these identified genes was initially characterized through bisulfite sequencing; further, realizing a potential application as predictive biomarkers for epigenetic drugs, we demonstrate the same for evaluation of the efficacies of a DNA demethylating agent 5-Aza-dC and HDAC inhibitor Trichostatin A (TSA).

Aims and Objectives

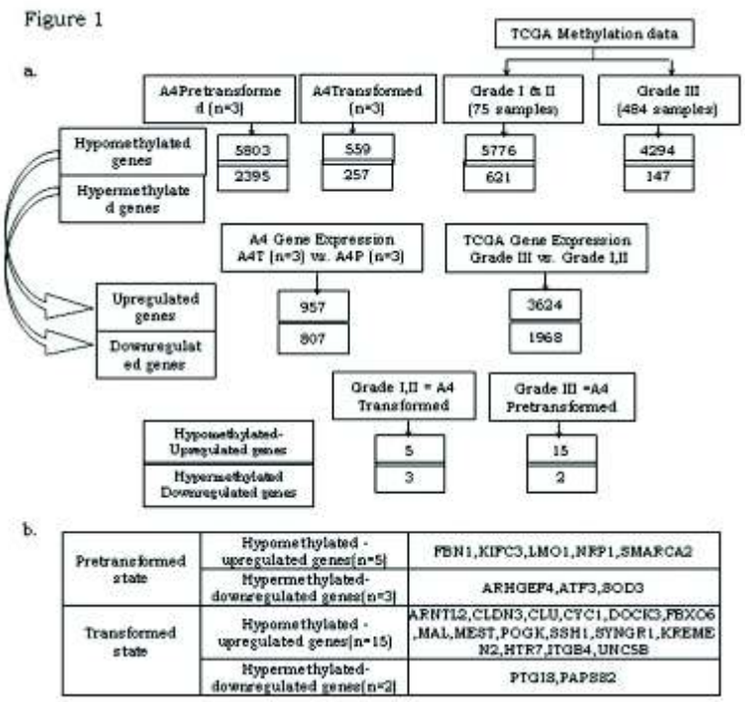
1. Identification of candidate genes with altered methylation patterns
2. Validation of candidate genes with altered methylation patterns
3. Evaluation of PTGIS as a predictive marker for epigenetic therapy

Work Achieved

Identification of candidate genes with altered methylation patterns

Genome-wide methylation profiles of the A4 progression model were established using MeDIP combined with Agilent Methylation microarray (27,800 CpG islands region probes in the human genome). Enrichment analyses was performed at $p<0.05$, with enrichment ratios being represented as a function of probe intensities, and calculated between $\log_2$ ratio and signal intensity besides specifying the distribution of probe sets. Hypomethylated and hypermethylated genes were identified on the basis of positive and negative enrichment respectively between the two cellular states in association with transformation. Thus, 1474 hypo methylated and 492 hyper methylated genes were associated with transformation (Fig.1). Similar analyses with the TCGA methylation dataset with grade wise stratification of data identified 5776 hypo- and 621 hyper- methylated genes for low Grade 1 & 2 tumors and 4294 hypo- and 147 hyper- methylated genes for high Grade 3 tumors (Fig.1). Correlation studies of the methylation profiles with corresponding A4 and TCGA gene expression datasets resulted in the identification of two groups of conformers, *viz*. hypo methylated-upregulated genes and hyper methylated-downregulated genes. An overlap of these enriched targets revealed commonly regulated genes in the two datasets. This resulted into 5 hypo- and 3

Fig. 1: Analytical pipeline for biomarker identification



hyper- methylated candidate genes in early stage and 15 hypo- and 2 hyper-methylated genes in high-grade tumors (Fig.1). Overall, hypomethylation was more prevalent than hypermethylation as a mechanism of gene expression regulation in ovarian cancer progression.

Validation of candidate genes with altered methylation patterns

Bisulfite genomic sequencing (BGS) confirmed the above association of differential promoter methylation with transformation (Fig.2a). An average of 2-4 fold promoter CpG demethylation was observed in predicted hypomethylated target genes; while the methylation index of *PAPSS2*, *PTGIS* and *FBN1* was higher in A4T cells as compared to A4P cells, suggesting these to be methylated during transformation (Fig.2b). Such differential promoter methylation was functionally validated in driving altered gene expression; thus hypomethylated genes *CYC1*, *POGK*, *TYMS*, *MCM2*, *MAL* and *MEST* were upregulated while hypermethylated genes like *PTGIS* and *FBN1* were downregulated in transformed A4T cells (Figs.2c-i, 2c-ii). Further analyses of BGS data combined with expression analyses confirmed that random CpG methylation/demethylation of a few CpG sites could be effective in differential gene regulation. Interestingly, concurrent methylation and demethylation of different CpG sites in the *POGK1*, *PAPSS2*, *PTGIS* & *FBN1* promoter was observed; such effects have been reported earlier.

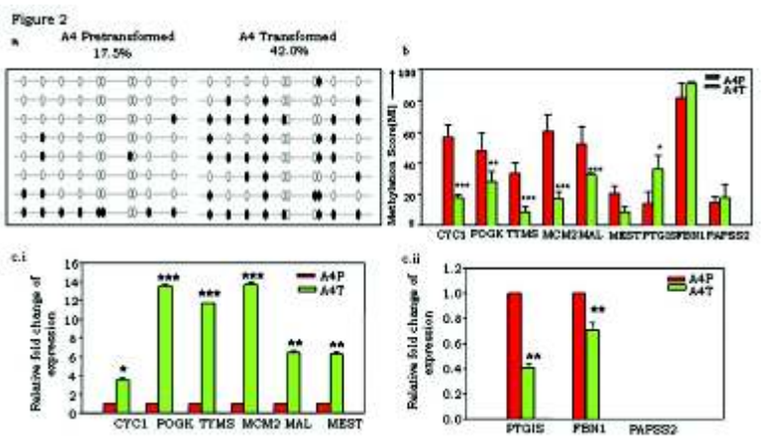


Fig. 2: **(a)** Bisulfite genomic sequencing (BGS) of PTGIS promoter indicating increased DNA methylation during transformation. **(b)** Graphical representation of methylation index of BGS validated differentially methylated genes. **(c)** qRT-PCR analysis of hypomethylated-upregulated and hypermethylated-downregulated genes. (* P value ≤ 0.05;** P value ≤ 0.01;*** P value ≤ 0.001)

Evaluation of *PTGIS* as a predictive marker for epigenetic therapy

In an earlier report from our group, *PTGIS* was identified to be associated with the repressive histone mark H3K27me3 (trimethylated lysine 27 on Histone 3) at its promoter region, while the present study indicates this tumor suppressor to be regulated through promoter DNA methylation. In untreated tumors *PTGIS* expression was very low; together this suggested that *PTGIS* could well be an important biomarker for the evaluation of epigenetic drugs. The same was thus undertaken to study the efficacy of the epigentic drug 5-Aza-dC which is a known DNA demethylating agent and Trichostatin A (TSA) a HDAC inhibitor.

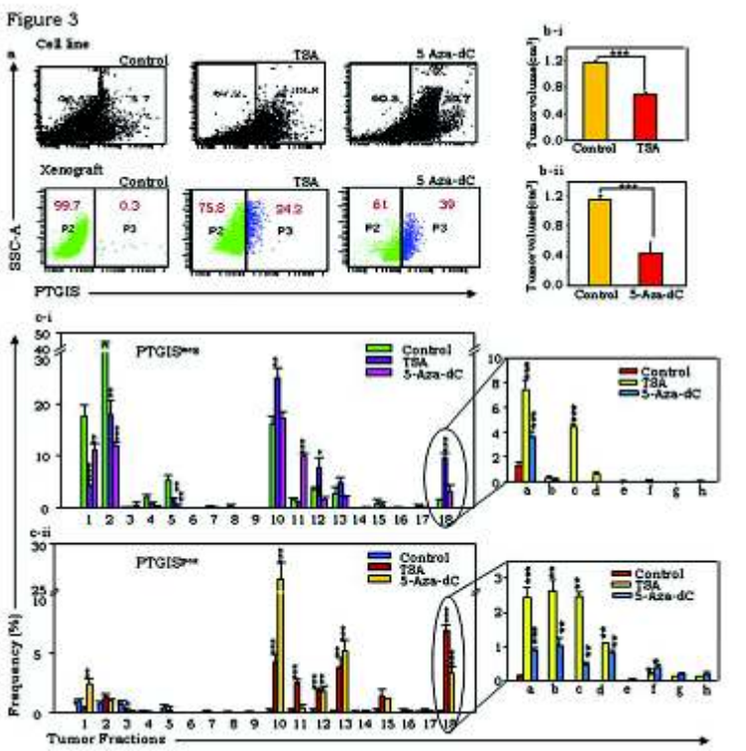


Fig. 3: **(a)** Representative FACs images of PTGIS expression in A4 cell line (upper panel) and A4 Xenograft (lower panel) after TSA and 5Aza dC treatment with respective controls; Graphical representation of tumor volume after TSA **(b-i)** and 5Aza dC **(b-ii)** treatment, with respective controls; **(c-i)**, **(c-ii)**: Frequency of tumor fractions (±SEM) based on proliferative hierarchy, ploidy and cell cycle analysis of $PTGIS^{neg}$ and $PTGIS^{pos}$, respectively, of control vs. TSA and 5Aza dC treatment, wherein 1- host, 2-PKH^{neg} Eu G0, 3-PKH^{neg} Eu G1,4-PKH^{neg}EuS, 5-PKH^{neg} Eu G2/M,6-PKH^{neg} Aneu G0,7- PKH^{neg} Aneu G1,8- PKH^{neg} Aneu S, 9-PKH^{neg} Aneu G2/M, 10-PKH^{lo} Eu G0, 11- PKH^{lo} Eu G1, 12-PKH^{lo} Eu S, 13- PKH^{lo} Eu G2/M, 14- PKH^{lo} Aneu G0, 15-PKH^{lo} Aneu G1, 16-PKH^{lo} Aneu S, 17- PKH^{lo} Aneu G2/M, 18-PKH^{hi}, Inset shows frequency of cycling PKH^{hi} tumor fractions a- PKH^{hi} Eu G0, b- PKH^{hi} Eu G1, c- PKH^{hi} Eu S, d-PKH^{hi} Eu G2/M, e- PKH^{hi} Aneu G0, f- PKH^{hi} Aneu G1, g-PKH^{hi} Aneu S, h- PKH^{hi} Aneu G2/M. (*, P < 0.05; **,P< 0.01; ***, P<0.001).

PTGIS expression increased and tumor volumes decreased drastically following both 5Aza-dC and TSA treatments (Figs.3a-i, a-ii, 3b), with the progenitor (PKH^{lo}) and CSC (PKH^{hi}) fractions being mostly $PTGIS^{pos}$. These exhibited anti-correlative behavior in terms of cycling status with a large fraction of $PTGIS^{pos}$ progenitors having exited the cell cycle or being trapped in G2M and euploid and aneuploid CSCs being in a cycling state that could lead to the emergence of drug resistant hierarchies. Surprisingly, both treatments also largely depleted the differentiated $PTGIS^{neg}$-PKH^{neg} tumor fractions, did not trigger a re-entry of PKH^{neg} CSCs into the cell cycle, but significantly increased the quiescent $PTGIS^{neg}$ progenitor fractions (Fig.3c). This data also confirmed epigenetic drug treatment inhibit the generation of aneuploidy through genetic instability in the residual cells; however cycling $PTGIS^{pos}$ CSCs and $PTGIS^{neg}$ progenitors could result in refractory tumor behavior. However, *PTGIS* appears to be an excellent biomarker for evaluating the efficacy of epigenetic drugs.

Future Research Plans

Intial studies of histone modifications associated with the EOC transformation led us to epigenetic regulation of co-repressive complex, exploration of this novel finding would led us to another dimension of epigenetic regulation of an essential machinery in transformation.

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Cancer, Chemotherapy, and Metabolic disorders

Background

Cancers of all forms account for about 12 per cent of total deaths throughout the world. In India, cancer has become one of the ten leading causes of death. It is estimated that there are nearly 1.5-2 million cancer cases at any given point of time. As per recent reports, approximately, 217 million people worldwide have diabetes and this number is expected to increase to at least 366 million by 2030. By the year 2030, India is expected to be the home of 79.4 million diabetic people as compared to 35 million diabetic people at present.

Both cancer and diabetes are a major concern for the health of adult population in India. Several studies have suggested that diabetes mellitus may alter the risk of developing variety of cancers, and the associations are biologically plausible. Though, an interrelationship between diabetes and cancer was found over 100 years ago, it is only modern, prospective, epidemiological cohort and case-control studies conducted in several countries that have provided reliable evidence of an increased cancer risk in diabetic patients, mainly in those with type 2 diabetes. The greatest risk has been reported for primary liver cancer, moderately elevated for pancreatic cancer, and relatively low for colorectal, endometrial, breast, and renal cancer. However, risk of developing other cancer types in diabetics is obscure. Even though it is proposed that India will have large incidences of both diabetes and cancer, which will have serious ramifications on socio-economical status, no study addressing the association of these diseases has been reported from India. Since, the magnitude of both these health problems in India are growing at an alarming rate, effective management of these health problems warrants studies related to the

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prevalence of various cancer in diabetic population. The study designed is a retrospective observational study of deceased population of Pune city, excluding suburbs, by probing into one-year death certificate data provided by the State Bureau of Health Intelligence and Vital Statistics (HIVS), Pune, India. Death certificate gives information about the cause of death, age, sex and identity as well as the diseases the patient suffered from.

Aims and Objectives

1. To evaluate the percentage occurrence of various cancer types in diabetic and non-diabetic deceased population.
2. To evaluate occurrence in male and female specific manners.

Work Achieved

Death certificates of 14838 reported cases from Pune city excluding the suburbs were analyzed of which 1212 deceased adults were suffering from diabetes and 1012 deaths were due to cancer. These diabetes and cancer decedents represented 8% and 6.8% respectively of total medically certified deaths (1212/14838, 1012/14838). Fifty seven percent (694/1212) of deceased diabetics were males and forty three percent were (518/1212) females. Eighty-five deaths, which constitute 0.6% of total medically certified deaths, were patients who suffered from both diabetes and cancer. (85/14838) . The percentage of males and females with both diabetes and cancer in total deceased population was 0.3% and 0.27% respectively. Data analyses revealed that 7.6% (1127/14838) of the deceased suffered from diabetes related disorders only and 6.2% (927/14838) of the deceased suffered from cancer only. Of the 1212 diabetic patients, 85 patients had cancer that corresponds to 7%, whereas 6.8% of the non-diabetics (927/13626) had cancer (Figure 1).

Thirty two forms of cancer were reported in deceased population (Table 2). Twenty three forms of cancer were detected in males and 29 forms of cancer were detected in females. Of total 1012 cancer related mortalities, breast (11.3%), lung (7.4%), colon (7.4%), oral (6.9%), esophageal (6.6), liver (6.4%), cervix (5.9%), leukemia (5.5%), non-Hodgkin's lymphoma (5.3%), ovary (5.2%), CNS tumor (4.4%), pancreas (4.0%), stomach (3.5%), prostate (3.4%), larynx (3.0%), urinary bladder (2.3%), multiple myeloma (1.9%), skin (1.4%), gall bladder (1.4%) and uterus (1.3%), were relatively more prevalent cancer types. Breast, cervical, ovary, colon, esophageal, oral, lung and liver cancer were

predominant cancer types in females whereas lung, liver, colon, oral cavity, leukemia, esophageal, prostate and non-Hodgkin's lymphoma were the major forms of cancer in males (Table 1).

Percent occurrence of various cancers in total population of males and females were 3.2 and 3.6% respectively. Sex specific cancer in male and female accounted for 0.27% (40/14838) and 1.7% (245/14838) respectively of total population. Breast, cervix, ovary, uterine and vaginal cancer accounted for 0.8%, 0.4%, 0.4%, 0.1% and 0.03% of the total deceased population respectively. They contribute 11.3%, 5.9%, 5.2%, 1.3% and 0.5% respectively, towards total deceased population with cancer. These results indicate that breast, cervix, ovary, uterus and vaginal cancer caused death in about 25% cancer patients though breast cancer remained a major killer in females. Similarly penis, prostate and testicular cancer accounted for 0.03%, 0.2%, 0.01% respectively of the total deceased population and 0.4%, 3.4%, 0.2%, respectively, of the deceased population with cancer suggesting that prostate cancer is the major killer in males. Surprisingly, in diabetic females occurrence of only breast and ovarian cancer was recorded. These cancers constitute approximately 1% and 0.2% respectively of total diabetic population (12/1212 and 2/1212). The mean survival age of 102 non-diabetics with breast cancer was 57.98 whereas the mean survival age of 12 deceased diabetics having breast cancer was 70.33.

Occurrence of ovarian cancer in diabetic females accounted for 0.2% of the total diabetic patients and that in non-diabetic females it was 0.4% (51/13626). Moreover, cancer of cervix, vagina and uterus were not recorded in diabetic females. Cervical cancer constituted 0.4% of the non-diabetic population and low occurrence of vaginal and uterine cancer were recorded in non-diabetic females. Cancer of penis and testis were not recorded in diabetic males. The percent occurrence of prostate cancer was identical (0.2% and 0.22%) in diabetic and non-diabetic population respectively. Levels of certain hormones, which have significance in prostate and ovarian cancer, could not be ascertained and alteration in the levels of these hormones could affect the outcome of the study (Table 2).

These findings, despite being preliminary observations, certainly call for more thorough and detailed studies for deciphering the association between the occurrence of cancer and diabetes in Indian population. Death certificate-based research, while potentially providing valuable data, is full of possibilities

for bias. Though, significance of various confounding factors on the results presented in this study could not be overlooked, none-the-less the percent occurrence of liver, prostate, pancreatic and lung cancer in diabetics is much higher, as compared to their occurrence in non-diabetics. These findings are in accordance with the observations in certain other large cohort studies, suggesting that abnormal glucose metabolism is a general risk factor for cancer development.

In conclusion, this study indicates that, though the occurrence of certain cancer types increase in diabetics, at the same time there seems to be an inverse relationship between occurrence of other cancer types and diabetes. Similarly, diabetes seems to be associated with decreased survival rate in case of patients with certain types of cancer, whereas in others, it prolongs survival of the patient. Our observations clearly suggest the need to explore the inter relationship between diabetes and cancer. A thorough investigation involving basic research studies as well as long term studies in association with clinicians will be helpful in ascertaining this relationship.

Future Research Plans

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

To study the hyperglycemia induced alterations at gene level as well as signalling cascade in solid tumors cells.

To study the role of glucose lowering drugs on the growth, proliferation and survival of solid tumors.

To study the regulation of glucose metabolism in the tumor cells.

Table 1: Percentage occurrence of different cancer types in total cancer population (1012)

	Cancer Type	Male (%)	Female (%)	Total (%)
1	Liver	4.3	2.2	6.4
2	Lung	5.0	2.4	7.4
3	Pancreas	2.4	1.5	4.0
4	Oral	4.0	3.0	6.9
5	Leukemia	3.8	1.8	5.5
6	Colon	4.1	3.4	7.4
7	Stomach	2.4	1.1	3.5
8	Non-Hodgkins lymphoma	3.3	2.1	5.3
9	Gall bladder	0.5	0.9	1.4
10	Renal cell	0.7	0.4	1.1
11	Urinary bladder	1.7	0.6	2.3
12	CNS tumor	2.5	2.0	4.5
13	Larynx	2.1	0.9	3.0
14	Small intestine	0.6	0.1	0.7
15	Skin	0.9	0.5	1.4
16	Soft tissue	0.3	0.6	0.9
17	Multiple myeloma	0.8	1.1	1.9
18	Esophageal	3.5	3.2	6.6
19	Bone	0.5	0.2	0.7
20	Hodgkins lymphoma	0.4	0.2	0.6
21	Thyroid	0.0	0.2	0.2
22	Lachrymal duct	0.0	0.1	0.1
23	Metastatic PNET	0.0	0.1	0.1
24	Adrenal neuroblastoma	0.0	0.1	0.1
25	Breast	0.0	11.3	11.3
26	Ovary	0.00	5.2	5.2
27	Cervix	0.0	5.9	5.9
28	Vagina	0.0	0.5	0.5
29	Uterus	0.0	1.3	1.3
30	Prostate	3.4	0.0	3.4
31	Penis	0.4	0.0	0.4
32	Testis	0.2	0.0	0.2

Table 2 : Sex-specific cancer

Male

Cancer Type	Diabetic	Non-Diabetic
	Mean age (no) Range	Mean age (no) Range
Prostate	76.33 (03)	73.38(31)
	59-87	50-90
Penis	0	67.25(04)
		55-78
Testis	0	35.50(02)
		25-46
Total	3	37

Female

Cancer Type	Diabetic	Non-Diabetic
	Mean age (no) Range	Mean age (no) Range
Breast	70.33 (12)	57.98(102)
	42-88	40-97
Ovary	59.20(02)	59.70(51)
	55-64	22-81
Cervix	0	60.63 (60)
		40-97
Vagina	0	74.00(05)
		62-84
Uterus	0	72.00(13)
		55-83
Total	14	231

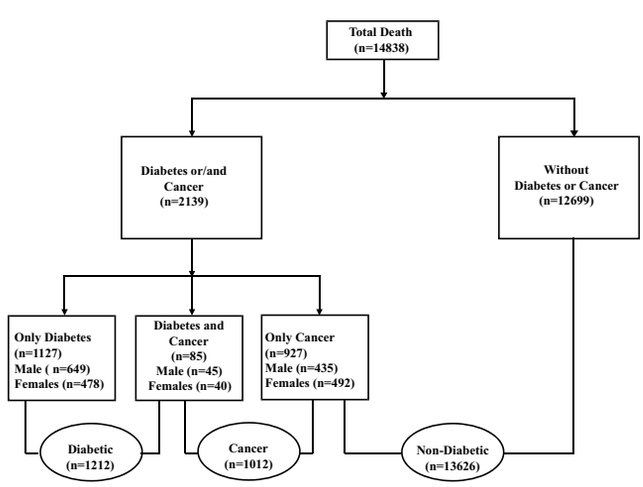
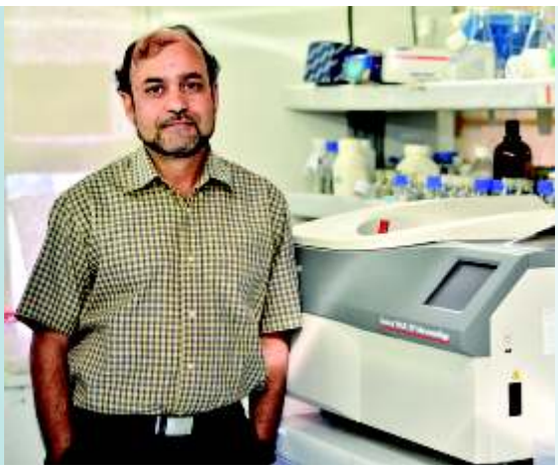


Fig. 1: Total number of decedents and occurrence of diabetes and cancer in deceased population



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Global gene regulation by tumor suppressor SMAR1

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Background

Scaffold/Matrix attachment region binding protein 1 (SMAR1) is a MAR-binding transcription factor which tethers chromatin to the nuclear matrix and modulates the architecture of the chromatin by forming loops. SMAR1 acts as a docking site for chromatin modifiers like HDAC1 and modifies histones over the range of 5 kb on the Cyclin D1 promoter (Rampalli et al., 2005). SMAR1 was first identified in mouse double positive thymocytes. It was found attached to the 400 bp region upstream of Eβ enhancer in the T cell receptor locus (TCR) which was characterized to be a nuclear matrix/ scaffold-associated region, referred to as MAR. This region was also reported to associate with the known MAR binding proteins SATB1 and Cux. SMAR1 shares 21.4 % identity and 64.3% similarity with the MAR binding domain of human and mouse SATB1, 23.9% identity and 56.5% similarity with a region of Bright that contains the tetramer domain (Chattopadhyay et al., 1998). SMAR1 transgenic mice show abnormal V(D)J recombination at specific Vβs (Kaul-Ghanekar et al., 2004). Further studies revealed that SMAR1 is a potent tumor suppressor protein, interacts with p53 and delays tumor progression in mouse melanoma model by imposing cell cycle arrest (Kaul-Ganekhar et al., 2003). SMAR1 serine-arginine rich domain specifically interacts with p53 and stabilizes it (Jalota et al., 2005). SMAR1 was also found to inhibit the expression of various cell cycle regulatory genes like Cyclin D1 (Rampalli et al., 2005), IκB (Singh et al., 2009), Bax, PUMA (Sinha et al., 2011) and many other genes. In the higher grades of cancer, SMAR1 levels were found to be drastically downregulated. The stress responsive nature of SMAR1 is also characterized as its levels were found to be elevated upon DNA Damage (Singh et al., 2007).

Aims and Objectives

1. To study the regulation of CD44 gene splicing and its implication in cancer
2. To study DNA damage repair by SMAR1 through Ku70 deacetylation
3. To study the control of cytokine genes for T_H1-T_H2- T_H17 and T-reg differentiation

Work Achieved

SMAR1 mediated alternative splicing of CD44

During the multistep process of cell cycle modulation, a minor alteration in gene expression which is regulated at different stages like transcription, pre-mRNA processing and translation can cause cellular transformation. Transcription and pre-mRNA splicing have emerged as highly coordinated processes. Alternative pre-mRNA splicing is indispensable for post transcriptional gene regulation. The spatial and temporal generation of splice variants of pre-mRNA demands that alternative splicing is subjected to stringent regulation. It has also been proposed that chromatin could play a role in chaperoning of the neosynthesized pre-mRNA with consequences on splicing. However, it remained unclear how the chromatin modulators and especially nuclear matrix proteins regulate transcription coupled constitutive and alternative pre-mRNA splicing.

The role of alternative splicing in the production of oncogenes and tumor suppressors has attracted considerable interest, as variants (v) of same set of genes are often found specifically and distinctly in tumors from diverse tissues. For example, the CD44 family of receptors includes multiple variant isoforms, several of which have been linked to malignant properties including migration, invasion and metastasis. A large variety of alternatively spliced CD44 variants (CD44v) are expressed in different tumors with possible implication for tumor progression, formation of metastasis and survival. The signal transduction activators of RNA (STAR) family proteins, like Sam68, ASD-2 are reported to regulate the incorporation of the variable exons by directly binding to the RNA elements.

We have found that SMAR1 interacts with Sam68 and the ERK-MAPK activation leads to the abrogation of SMAR1 and Sam68 interaction. This has led us to believe that SMAR1 maintains Sam68 in a repressive state. As SMAR1 has the tendency to maintain its interacting proteins in a repressive state by interacting with deacetylases, we checked the interaction of SMAR1 with different histone deacetylases. We found that both SMAR1 and Sam68 interact with HDAC6, thus Sam68 is maintained in a deacetylated state by forming a triple complex with

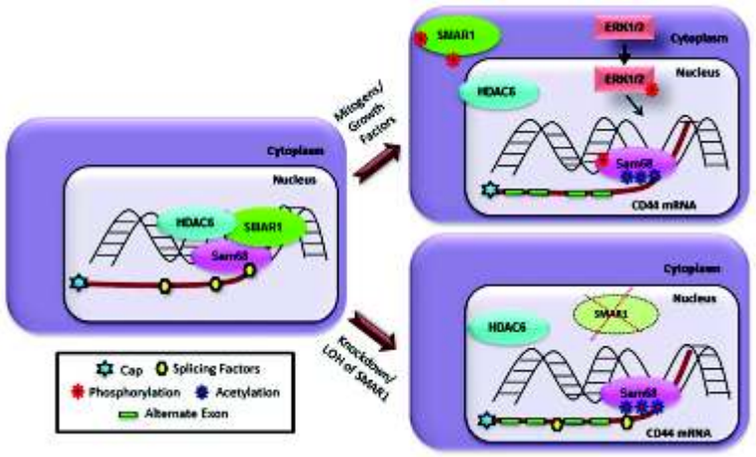


Fig. 1: A model representing the regulation of alternative splicing through SMAR1 phosphorylation and Sam68 acetylation. Endogenously, SMAR1 in consort with HDAC6 maintains Sam68 in a deacetylated state (left panel). In a signal-dependent mode of regulation, ERK-1/2 kinase phosphorylates SMAR1 and Sam68, thus disrupting SMAR1-Sam68-HDAC6 complex, resulting in SMAR1 export from the nucleus. SMAR1 translocation to cytoplasm induces Sam68 acetylation, and enhances *CD44* gene alternative splicing (right upper panel). Whilst, in a signal-independent mode, as observed upon knockdown of SMAR1 or LOH at Chr.16q24.3 locus, HDAC6 fails to maintain Sam68 in deacetylated state with a concomitant increase in *CD44* gene variant exon inclusion (right lower panel).

HDAC6. *In silico* docking studies of SMAR1 and Sam68 further revealed that SMAR1, through its C-terminal domain (CTD; residues 248-371), associates with Sam68 and shares a complimentary shape with the lysine-enriched KH-domain of Sam68. Knockdown of SMAR1 and/or ERK-mediated phosphorylation of SMAR1, favours the acetylation of Sam68, enhancing the affinity for pre-mRNA and enhances the incorporation of the variable exons. Using RNA immunoprecipitation and chromatin IP, we found that SMAR1 is accumulated endogenously into the coding region along the variable exons of CD44, which inversely correlates with the RNA Pol II Ser 5 phosho-CTD upon ERK activation, favoring the inclusion of variable exons. These results conclude that the low levels of SMAR1 in the higher grades of tumors, confers the metastatic potential through favorable inclusion of the variable exons. Our data for the first time suggests the ERK-MAPK-dependent phosphorylation and translocation of SMAR1 from the nucleus to the cytoplasm that ultimately leads to the acetylation of Sam68. Our work also highlights the ERK-MAPK activation-mediated phosphorylation of SMAR1 that favors the acetylation of RNA binding protein Sam68, which successively regulates the alternative splicing events (Figure 1). [Cell Death & Disease, 2014]

SMAR1 mediates DNA damage repair through deacetylation of Ku70

Matrix attachment region-binding proteins (MARBPs) are unique class of proteins that bind to specific non-coding sequences in the genome termed as scaffold/matrix attachment regions (S/MARs), and globally modify the topology of chromatin. Previous studies have established the importance of SMAR1 in helping DSB repair. Further we extended our study and reveal for the first time that NM-associated proteins play a key role in cellular response upon IR-induced DNA damage. SMAR1 imparts a critical role in the cell fate decision upon DNA damage by maintaining Ku70 in a deacetylated state via HDAC6. Deacetylated form of Ku70 is enriched in the damage-associated chromatin

fraction for efficient repair and also controls mitochondrial translocation of Bax. Furthermore, SMAR1 is a novel target of ATM kinase upon IR and regulates G2/M checkpoint. Phosphorylation of SMAR1 at Ser 370 residue increases upon IR in an ATM-dependent manner and such post translational modification increases the activity of SMAR1. Recruitment of SMAR1 on chromatin was also studied as chromatin-bound fraction contains all the repair associated proteins. SMAR1 gets recruited to chromatin upon DNA damage and this recruitment is ATM dependent as found by decreased recruitment when cells were pretreated with ATM inhibitor KU55933 and PI3K inhibitor caffeine. Acetylation status of Ku70 decides the cell's fate and it was found that SMAR1 modulates the acetylation of SMAR1 by favoring the deacetylation of Ku70 through its interaction with HDAC6. *In silico* analysis showed that SMAR1 binding interactions with Ku70 are predominantly dependent on several key salt bridge interactions, such as (A) VAL-157(Ku70) : ARG-335(SMAR1), (B) ASP-156(Ku70) : LYS-322(SMAR1), (C) LYS-114(Ku70) : ASP-185(SMAR1), (D) SER-96(Ku70) : ARG-316(SMAR1), and (E) SER-155(Ku70) : ARG-335 (SMAR1). The trimeric model of SMAR1 bound to HDAC6 and Ku70 revealed that 240-350 residues of SMAR1 interact to the N-terminal region of Ku70 through various inter residual salt bridge formation. *In silico* analysis of HDAC6-SMAR1-Ku70 docked model revealed that Ku70 is bound to SMAR1 adjacent to HDAC6-binding site. It was observed that C-terminal domain (CTD; residues 248-371) of SMAR1 is sandwiched between Ku70 and HDAC6. Deacetylated Ku70 interacts with pro-apoptotic protein Bax and inhibits the translocation of Bax from cytoplasm to mitochondria. Interaction studies between Bax and Ku70 were done and it was discovered that SMAR1 inhibits the release of Bax from Ku70. Knockdown of SMAR1 causes weak interaction between Bax and Ku70. Localization of Bax was also studied upon SMAR1 over expression and knockdown. SMAR1 favors the Bax localization in the cytoplasm and thus inhibits apoptosis. By inhibiting apoptosis, SMAR1 regulates the cell survival also. It was found that SMAR1 causes better cell survival, both endogenously and post IR. All such results strongly suggest the crucial role of SMAR1 in DNA damage repair and cell's fate decision making (Figure 2) [Cell Death & Disease, 2014].

Control of cytokine genes for T_H1-T_H2- T_H17 and T-reg differentiation

Scaffold/matrix attachment region proteins play important role in the orchestration of chromatin architecture, crucial for the regulation of transcription. S/MAR proteins have been implicated in transcriptional regulation, either activating or repressing the gene function. We have deciphered the role of SMAR1 in the differentiation of T helper cells. SMAR1 is selectively expressed in Th2 cells under the control of lineage specific master regulator GATA-3. Expression of SMAR1 in Th2 cell lineage elicits transcriptional

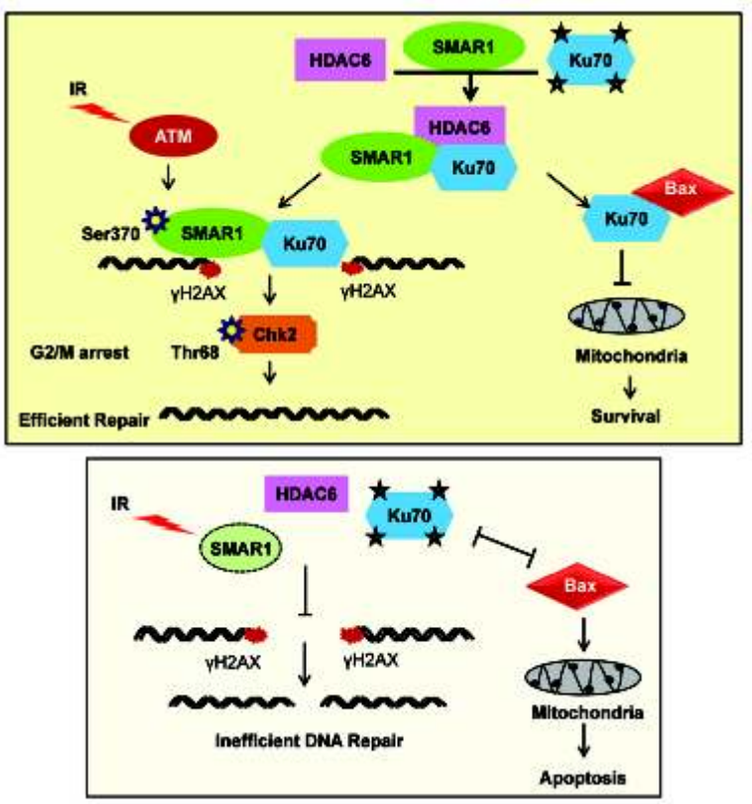


Fig. 2: A Simplistic model showing the involvement of SMAR1 in Ku70 mediated DNA damage repair. The upper panel shows the dual role of SMAR1 in Ku70 interaction cellular survival through prevention of entry of Bax to the mirochondria. The lower panel shows impairment of DNA damage repair and as a consequence signalling for cellular death in the absence of SMAR1. The model indicates that SMAR1 is involved in major repair pathways that in turn dictates cell survival od death.

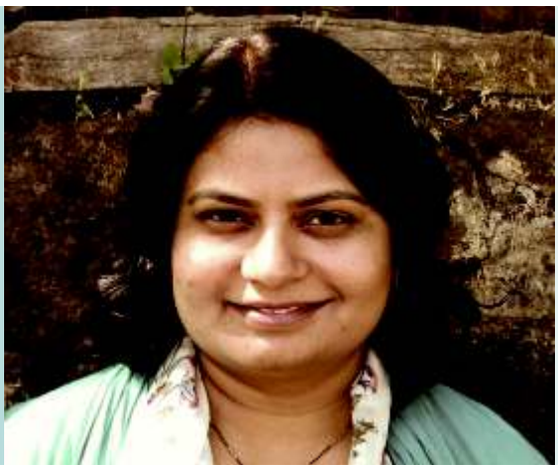
repression of both Th1 and Th17 lineage differentiation. We thereby propose the relevance of chromatin associated protein SMAR1 for the functional establishment of Th2 cells by GATA-3. Exogenous induction of SMAR1 causes reduced Th1 and Th17 cell formation, with a concomitant Th2 biasness of naïve T cell differentiation. SMAR1 regulates the Th1 differentiation by repressing the Notch mediated transactivation of T-bet and formation of repressive complex with HDAC1, SMRTe etc. It can also inhibit the Th17 differentiation by binding to the IL17 gene and mediating the epigenetic modifications of the region. SMAR1 deficiency in T cells caused compromised Th2 cell differentiation both *in vitro* and *in vivo*. We also show that T cell specific conditional knockout mice of SMAR1 were resistant to airway inflammation and asthma disease with low IgE production and Th1 biasness. These mice were further found to be susceptible for Th17 mediated encephalitis with MOG administration and increased Th17 production. Thus, taken together, we propose SMAR1 to be a critical regulator of T cell differentiation and investigate novel targets of immuno-modulation through nuclear matrix proteins.

Asthma is a complex airway allergic disease involving the interplay of various cell types, cytokines and transcriptional factors. Though many factors contribute to the disease etiology, what dictates the disease phenotype and

responsiveness is not well understood. Here we report the essential role of a MAR binding protein SMAR1 in the lung homeostasis during asthma disease progression. Conditional knockout of SMAR1 in T cells render the mice resistant to eosinophilic asthma against OVA allergen with low IgE and IL-5 levels. Moreover, a lower IgE/IgG2a ratio and higher IFN-γ response suggested an aberrant skewing of T cell differentiation towards Th1 response. Interestingly, we noticed neutrophilia and enhanced production of Th1 and Th17 cytokines in the lungs of these mice, indicative of deregulated T cell responses. Further, at molecular level, we show that SMAR1 functions as a negative regulator of Th1 and Th17 differentiation by interacting with two potential and similar MAR regions present on the promoters of T-bet and IL17. Thus, we show that SMAR1 plays a critical factor necessary for the lung homeostasis through modulation of T cell differentiation and we propose a possible therapeutic intervention by utilizing the immune-modulatory functions of SMAR1.

Future Research Plans

1. One of major targets of SMAR1 is miR 371-373 cluster where it acts as a repressor. Further, we are looking into the anti-metastatic properties of SMAR1 through regulation of miRNA 373. Since, miR 371-373 cluster is also involved in neuronal differentiation; we are looking more closely if such observation has any relevance taking ES cell differentiation.
2. Wnt pathway plays a critical role in regulating cellular proliferation and metastasis of cancer cells. Earlier we have shown that SMAR1 acts as repressor of Cyclin D1 promoter function by recruitment of HDAC1-Sin3A complex (Rampali et al, MCB, 2005). Recently it is shown that β Catenin acts as an activator of Cyclin D1 promoter. Using colorectal cancer model we now show a reverse correlation between SMAR1 and β Catenin for fine tune regulation of Cyclin D1 promoter. As a continuation of this work we have now identified peptides derived from Mycobacterium that induces SMAR1 and downmodulate Cyclin D1 expression in colorectal cell lines.
3. Recently from fish database we found that SMAR1 is expressed in Zebra fish. We will knock down SMAR1 in fish embryos and look at its role in development.
4. We have identified new compounds that induce SMAR1. These are derivatives of some other known compounds isolated from plant systems. We are testing anticancer properties of these synthetic compounds. Interestingly, 3 of these synthesized compounds can inhibit CD44 variable alternate splicing by inducing SMAR1. Thus, some of these can target cancer cell proliferation and metastasis.



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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 5 Nups, Nup93, Nup205, Nup188, Nup155 and Nup35. Among them Nup93 is key to anchor central channel (Nup62•Nup54•Nup58 complex). 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. Initial results indicate that full length Nup93 interact with 674-1609 region of Nup205. Work is in progress to co-express and purify the complex in large scale for structural studies. Also, one of the channel Nups, Nup62 can interact with either Nup54 or Nup88. We have crystallized Nup62 helical region and x-ray diffraction data is collected at ELETTRA synchrotron, Italy. Its 3D structure is determined by molecular replacement and the model building work is under progress. The comparative study of Nup62 homo-trimeric structure to its hetero-oligomeric assemblies would shed light on the molecular mechanisms of nucleoporin dynamics in the central channel.

Future Research Plans

1. X-ray diffraction structure determination of Nup62 and Nup93 of the nuclear pore complex.
2. Native isolation of the Nup93Nup205 and Nup62 subcomplex from HEK cell lines.

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Mechanisms of global gene regulation by SATB1

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Background

TCR signaling is critical for appropriate positive and negative selection of thymocytes to ensure appropriate tolerance and immune function both through proper positive and negative selection processes as well as the development of natural regulatory T cells (nTregs). While the sequence of events during T cell development is well defined, the molecular mechanisms including the role of transcription factors are just beginning to emerge. Previously, we have studied the role of Special AT-rich binding protein 1 (SATB1) during T cell activation and differentiation. SATB1 is a global regulator and chromatin organizer. In absence of SATB1 function thymocyte development is blocked at the DP stage. SATB1 null DP thymocytes ectopically express IL-2Rα and IL-7R and undergo activation induced cell death. However, the molecular mechanisms governing the role of SATB1 in T cell development remain to be determined. As a first step towards unraveling the role of SATB1 in T cell development and function, we monitored the expression profile of SATB1 in various thymocyte subsets and observed that SATB1 is differentially expressed. Our results further indicate that induction of SATB1 is dependent on TCR signaling and might in turn regulate the development of CD4 and CD8 thymocytes. Our results suggest that induction of SATB1 in developing thymocytes is transcriptionally regulated by the TCF1 - Gata3 axis followed by TCR signaling.

Aims and Objectives

1. To study the role of SATB1 in diffrentiation of CD4 T cells into T helper lineages
2. To elucidate the molecular mechanism(s) of regulation of SATB1

Work Achieved

1. TCR signaling positively regulates SATB1 in peripheral CD4$^+$ T cells

Peripheral CD4$^+$ T cells differentiate into various T helper lineages in response to TCR signals, cytokine milieu and other ligands in the microenvironment. We wished to determine the expression levels of SATB1 in response to TCR signals in vitro. Mouse CD4$^+$ T cells were activated in presence of plate-bound anti-CD3 or anti-CD28 or together with anti-CD3 plus anti-CD28. Immunostaining of SATB1 in CD4$^+$ T cells activated in presence of anti-CD3 or anti-CD3 plus anti-CD28 revealed the characteristic cage-like nuclear substructure as seen in thymocytes (Fig. 1A). In contrast, cells activated in the presence of only anti-CD28 exhibited poor induction of SATB1 expression (Fig. 1A & 4B). In activated CD4$^+$ T cells highly compacted chromatin is seen as pockets of densely DAPI stained heterochromatic regions in the nucleus. SATB1 is localized exclusively in the lightly stained euchromatic regions and is virtually absent in the heterochromatic regions. In naïve CD4$^+$ T cells SATB1 expression is almost undetectable and the prominent heterochromatic blobs as seen in activated

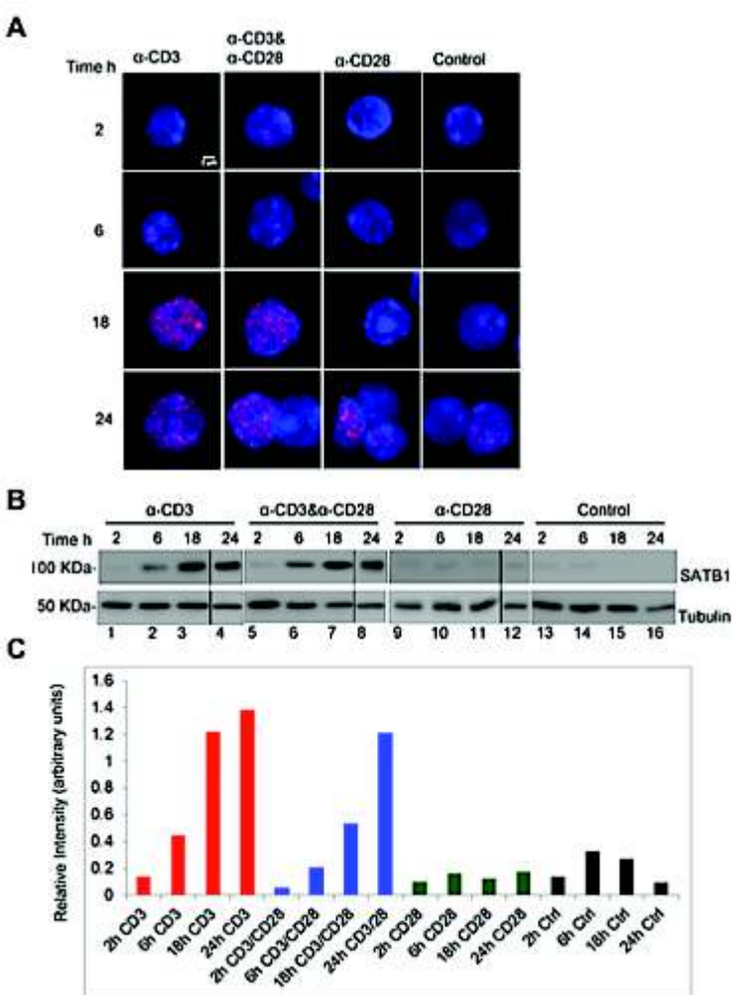

Fig. 1: SATB1 is induced in peripheral CD4 T cells upon TCR signaling.

nuclei are also not observed indicating that the gross chromatin organization could be very different (Fig. 1A). Immunoblot analysis indicated that SATB1 expression was induced within 2 h post anti-CD3 or anti-CD3/CD28 treatment and was sustained until 24 h of activation (Fig. 1C). In contrast, activation with only anti-CD28 antibody did not induce the expression of SATB1 in $CD4^+$ T cells (Fig. 1C). Notably, kinetics of SATB1 expression is steep in cells activated by TCR (anti-CD3) alone in comparison to cells activated with TCR and co-stimulatory signals (anti-CD3 and anti-CD28). Further, $CD4^+$ T cells cultured under control conditions do not exhibit significant induction of SATB1 expression (Fig. 1B and 1C). These results suggested that SATB1 expression is induced in peripheral $CD4^+$ T cells in response to TCR signaling.

2. TCF-1 and GATA-3 regulate SATB1 expression

Thymocyte development from early precursors to mature cells and further differentiation into effector cells depends on array of transcription factors that are expressed temporally at distinct stages of development. Transcription factors TCF1 and GATA3 are indispensable for thymocyte development. Overexpression of either TCF1 or GATA-3 in thymic precursors has been shown to initiate T lineage program. Therefore, we used TCF1 knockout (KO) mice and available data from GATA3 KO mice to investigate whether SATB1 expression is regulated by these core transcription factors during thymocyte development. In TCF1 KO mice most of the thymocytes were blocked at the DN stage and exhibited increased population of CD8 SP thymocytes that correspond to immature single positive (ISP) CD8 stage. We observed that SATB1 is downregulated in TCF-1 null thymocytes compared to wt B6 mouse thymocytes (Fig. 2A). Since most of the TCF1 KO thymocytes were blocked at DN stage, we further compared SATB1 expression in individual subsets of thymocytes between TCF1 KO and control mice (Fig. 2B). We observed that SATB1 is downregulated in DN thymocytes of TCF1 KO thymocytes and TCF null CD4 thymocytes do not exhibit the characteristic bimodal distribution as observed in the control B6 mice (Fig. 2C). Collectively, these data indicate that SATB1 is regulated by TCF1 in thymocytes and is a downstream target of TCF1.

Recent studies have shown that TCF1 transcriptionally regulates Gata-3 expression. Gata-3 has been shown to regulate key factors involved in the thymocyte development and differentiation. Gata-3 regulates key genes by facilitating histone modifications such as H3K4me1, H3K4me2 and H3K27me3 on the enhancer regions of the target genes. Gata-3 is known to play a critical role during differentiation of DN to $CD3^{Lo}$, $CD3^{Hi}$ to CD4 and CD4 to Th2 phenotypes. Therefore, we tested whether Gata-3 exerts a direct effect on SATB1 expression. Using the data from Wei et al. (GEO database GSE20898), we compared the expression of SATB1 in control and Gata3 null mice thymocytes at $CD3^{Hi}$ stage of thymocytes (Fig. 2D) and observed that SATB1 expression is

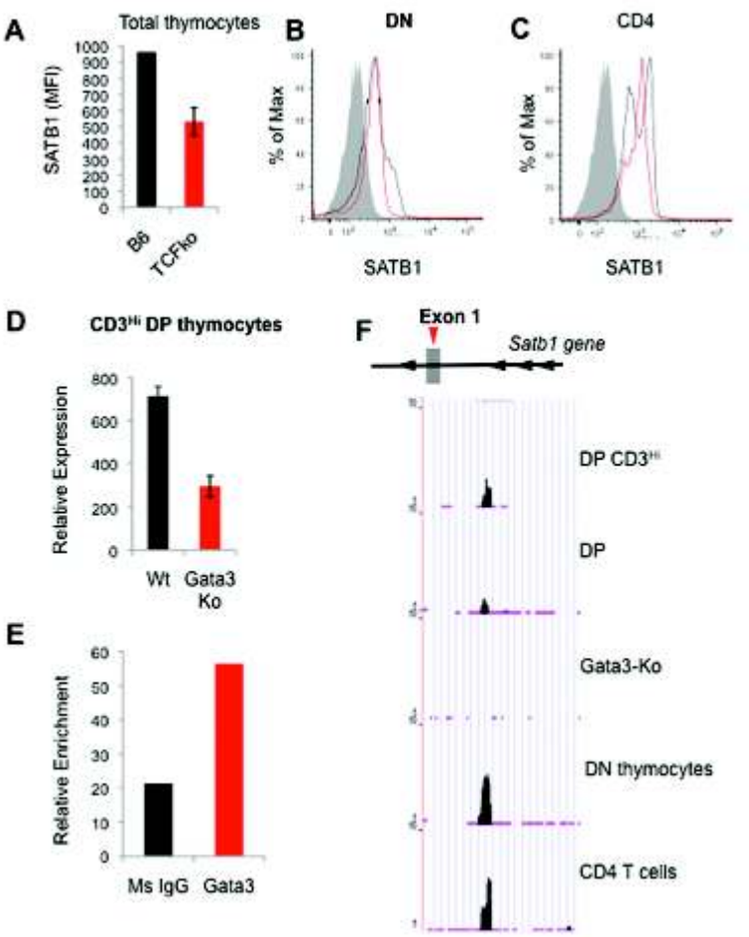

Fig. 2: Transcriptional regulation of SATB1 by TCF1-GATA3 axis.

downregulated in Gata-3 null mice in $CD3^{Hi}$DP thymocytes. We further monitored if Gata-3 directly regulates SATB1 expression. In silico analysis revealed presence of Gata-3 consensus 'WGATAA' motifs on *Satb1* promoter. We therefore performed ChIP assay to monitor the occupancy of Gata-3 at these sites and found out that Gata-3 binds to *Satb1* promoter in mouse thymocytes (Fig. 2E). Analysis of the ChIP-seq data sets for Gata-3 available in the public database (GEO20898) using the peak identification software 'MACS' corroborated the findings from our ChIP analysis (Fig. 2E). The comparative occupancy analysis revealed that *Satb1* promoter is bound by GATA3 in DN, DP and CD4 stages of thymocyte development (Fig. 2F). CD8 thymocytes from Gata-3 KO mice served as negative control, wherein Gata-3 occupancy on *Satb1* promoter was virtually absent (Fig. 2F). Together, these data suggest that TCF-1 and Gata-3 regulate SATB1 expression during thymocyte development.

Future Research Plans

We would like to further dissect the molecular mechanism(s) of regulation of SATB1 during T cell development and differentiation.

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Cytoplasmic Nup358: structure and function

Background

Nup358, a nucleoporin present on the cytoplasmic side of the nuclear pore complex (NPC), also is present in the cytoplasm as punctate structures. Earlier studies have characterized a cytoplasmic structure called annulate lamellae (AL), as stacked endoplasmic reticulum with pore complexes (ALPCs), which were structurally similar to the NPCs on the nuclear envelope (NE). Purification and characterization studies suggested that ALPCs contain many nucleoporins. However the functional significance of this structure or accumulation of nucleoporins in the cytoplasm as components of AL has not clearly been understood.

We had previously shown that Nup358 associates with interphase microtubules through its N-terminal regions and regulate microtubules and microtubule-dependent process such as directed cell migration. The role for Nup58 was confirmed to be conserved, as we showed that Nup358 plays a role in neuronal polarity as well.

However, we are more interested to characterize the molecular mechanism by which Nup358 would regulate cell polarity. We set out to study the cytoplasmic structure Nup358 localises to and to gain evidence for the cytoplasmic role of this nucleoporin. Our studies suggested that Nup358 is targeted to AL along with other known nucleoporins. Further characterization of AL suggested that the cytoplasmic Nup358 is often present in juxtaposition to two mRNA containing structures; P bodies and stress granules (SGs). Further studies indicate a role for Nup358 in miRNA pathway.

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Aims and Objectives

1. Characterize cytoplasmic Nup358 containing structures
2. Understand the dynamics of cytoplasmic Nup358 positive structures
3. Investigate the functional significance of cytoplasmic Nup358 structures

Work Achieved

Nup358 localizes with other nucleoporins in AL

To investigate if cytoplasmic Nup358 structures represented AL, we co-stained Nup358 with other nucleoporins known to be present in AL. Nup358 clearly co-localized with Nup214 (Fig.1A) and Nup62 (data not shown). Furthermore, as AL define a structure on the ER, we co-stained Nup358 with an ER marker, protein disulphide isomerase (PDI). As expected the Nup358 positive cytoplasmic structures are present on the ER network (Fig. 1B). Based on these results we conclude that Nup358 positive cytoplasmic structures represent the ALPCs.

Nup358 positive ALs physically associate with P bodies and SGs

To characterize the AL structures in relation to other cytoplasmic structures, we

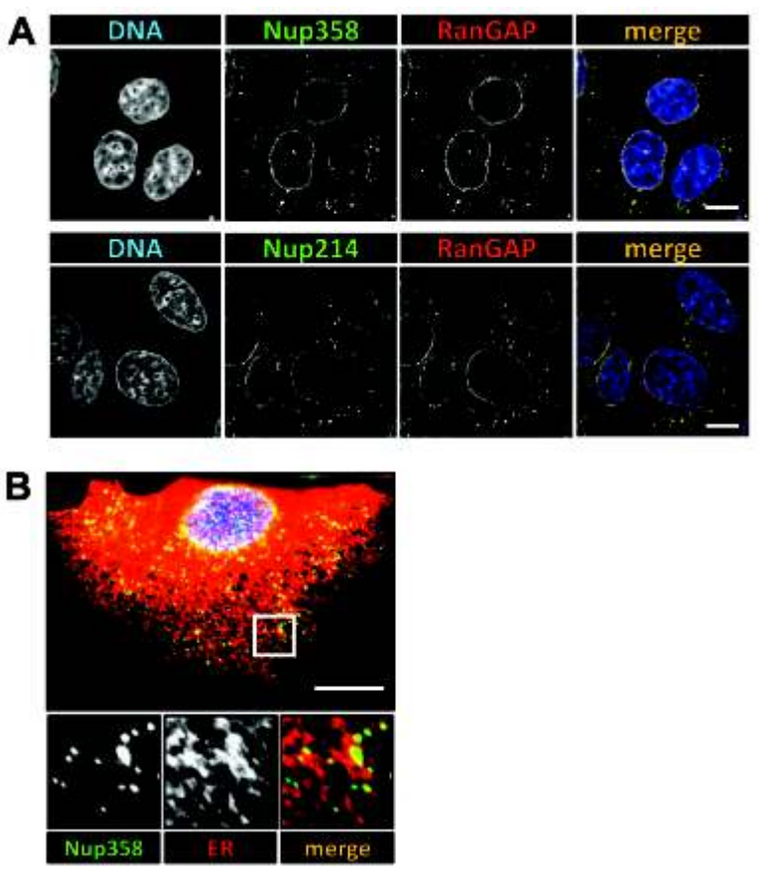


Fig. 1: Cytoplasmic Nup358 represents annulate lamellae. (A) HeLa cells were fixed and stained for Nup358 (green, upper panel) or Nup214 (green, lower panel) and RanGAP (red) using specific antibodies. Note the co-localization of Nup358 and Nup214 with RanGAP in the cytoplasmic structures. **(B)** HeLa cells were fixed and stained with Nup358 (green) and ER marker PDI (red) specific markers. DNA is stained in blue.

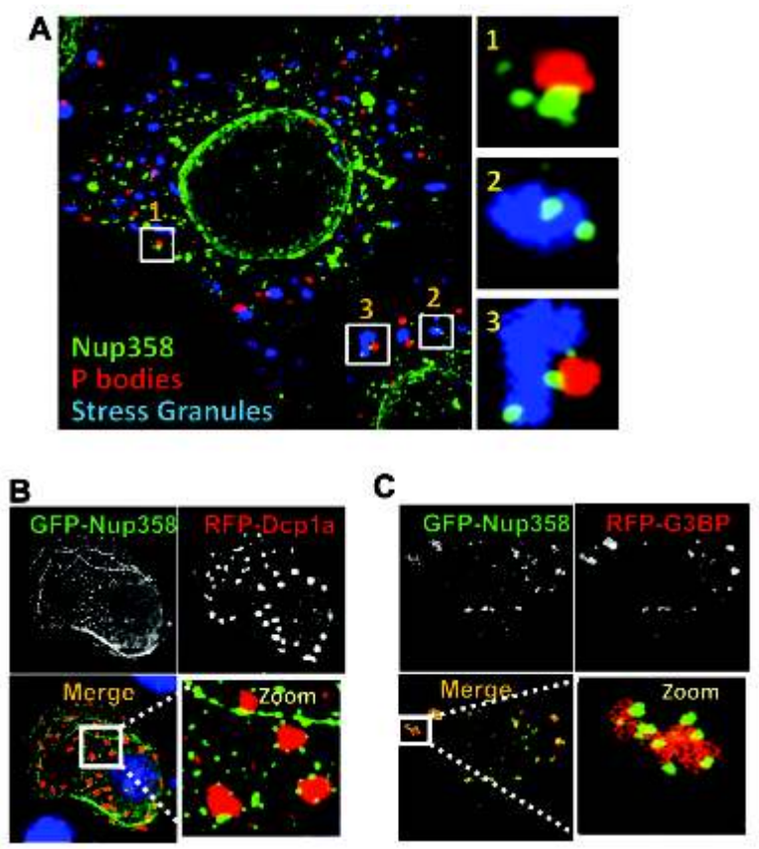


Fig. 2: Nup358 positive AL physically associate with P bodies and stress granules (SGs). **(A)** HeLa cells were treated with 0.5 mM sodium arsenite for 30 min to induce SGs and stained for Nup358 (green), P body marker Dcp1a (red) and SG marker eIF3η (blue) using specific antibodies. Note that the Nup358 structures are often present juxtaposed to P bodies (1), SGs (2) or both (3). **(B)** COS-7 cells were co-transfected with GFP-Nup358 (green) and RFP-Dcp1a (red, P body marker), fixed and observed with fluorescence microscopy. DNA is stained in blue. **(C)** COS-7 cells were co-transfected with GFP-Nup358 (green) and RFP-G3BP (red, SG marker), fixed and observed with fluorescence microscopy.

co-stained Nup358 with different markers representing different cellular structures. Interestingly, we found Nup358 physically associates with cytoplasmic mRNA containing structures called P bodies and stress granules (SGs) (Fig. 2 A). This association was more dramatic when Nup358 and P body (Fig. 2B) or Nup358 and SG marker was overexpressed (Fig. 2C).

Nup358 depletion affects P body, but not AL or SG, assembly

The physical association of Nup358 positive AL with the mRNP structures (P bodies or SGs) prompted us to investigate the functional significance of such an association. Initially, we depleted Nup358 using specific siRNA and monitored the formation of P bodies as well as SGs. We found that depletion of Nup358, but not Nup214 (another nucleoporin present on the cytoplasmic side of the NPC as well as in ALPCs, resulted in complete disruption of microscopically distinguishable P bodies (Fig. 3A). However, Nup358 depletion did not have any discernible effect on SG assembly (Fig. 3B). This result indicates that Nup358 is specifically important for the assembly of P bodies. As P bodies are intimately involved in miRNA mediated mRNA regulation, it would be interesting to investigate if Nup358 could play a role in miRNA function.

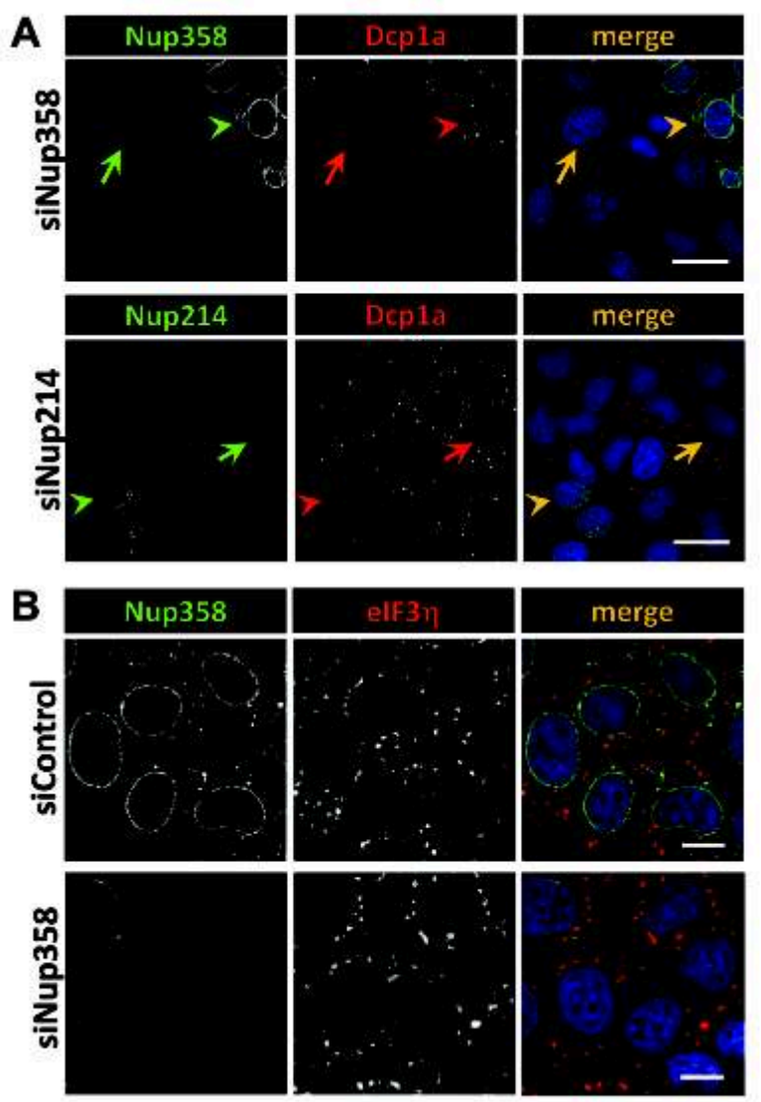
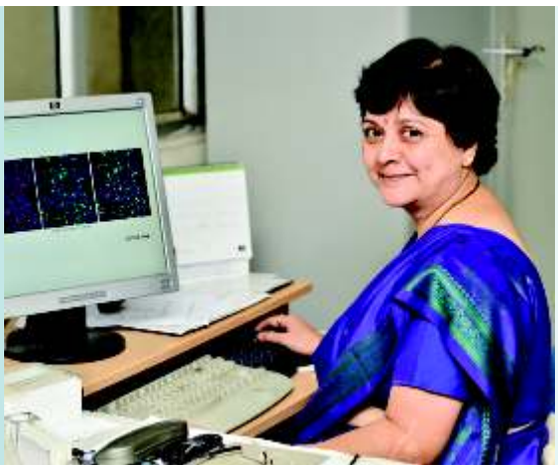


Fig. 3: Nup358 depletion leads to P body, but not SG, disassembly. (A) HeLa cells were transfected with Nup358 (siNup358) and Nup214 (siNup214) specific siRNAs for 72 h, fixed and stained for Nup358 (green, upper panel) or Nup214 (lower panel) and P bodies (red). DNA is stained in blue. Arrows indicate nucleoproin depleted cells. Arrowheads indicates cells in which siRNA treatment has not worked. (B) HeLa cells were transfected with control (siControl) or Nup358 (siNup358) specific siRNAs for 72 h, treated with 0.5 mM sodium arsenite to induce SGs, fixed and stained for Nup358 (green) and SG marker eIF3η (red). DNA is stained in blue.

Future Research Plans

To study the potential role of Nup358 in P body assembly and miRNA regulation



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Niche Biology: Identification of signaling mechanisms operative in the "engraftment window"

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Background

In an adult mammal, the bone marrow forms the micro-environment or niche wherein the hematopoietic stem cells (HSCs) reside. The HSC-niche is a multi-cellular entity comprising osteoblasts, adipocytes, mesenchymal cells, pericytes etc. These cells along with their extra cellular matrix (ECM) molecules and the cell-bound and/or secreted growth factors regulate the HSC fate. The most striking feature of the HSC niche is the presence of a hypoxia gradient, ranging from ~1% O_2 near the bone lining to ~6% in the sinusoidal cavity. Most of the quiescent or the slow cycling HSCs are present at the lowest end of this gradient, clearly indicating the importance of hypoxia in maintaining the stem cell pool *in vivo*.

In a bone marrow (or stem cell) transplantation (BMT/SCT) setting, the normoxic HSCs present in the graft home to the marrow cavity via an SDF1α-CXCR4-mediated chemotactic migration. These homed HSCs then interact with the hypoxic niche and proliferate rapidly to produce stem cells as well as committed progenitors, leading to the reconstitution of a multi-lineage hematopoiesis. Once the normal hematopoiesis is achieved, the HSCs undergo hibernation, and a steady-sate hematopoiesis is established. This period, the "engraftment window", is a very critical phase in the transplant scenario, when the transplant recipients are in the most susceptible condition. Thus, minimizing this window would lead to a significant reduction in the morbidity associated with the BMT/SCT procedures.

Although the role of hypoxia in the regulation of hematopoiesis is recognized, the signaling mechanisms operative in the "engrafment window" have not been

identified. We initiated our study with a goal to elucidate these signaling mechanisms. To achieve this goal, it was necessary to establish an experimental system that recapitulates the in vivo "engraftment window", wherein the stromal cells are in hypoxic conditions while the infused HSCs are normoxic. We accomplished this by using BM-derived mesenchymal stromal cells (BMSCs) treated with a hypoxia-mimetic compound, $CoCl_2$. Cells incubated under 1% O_2 (atmospheric hypoxia) were used as the comparator. Lineage negative (Lin-) fraction of the bone marrow-derived mononuclear cells (BM MNCs) was co-cultured on the $CoCl_2$-/hypoxia-primed BMSCs, and the output hematopoietic population was phenotypically characterized using polyvariate flow cytometry. Western blot experiments were performed to identify the signaling mechanisms evoked by various treatments.

Aims and Objectives

1. Creation of a hypoxic niche *in vitro*
2. Assessment of the effect of the hypoxic niche on the regeneration of hematopoiesis
3. Identification of signaling pathways evoked by hypoxia in the BMSCs

Work Achieved

Creation of hypoxic niche *in vitro*

As stated earlier, we created a hypoxic niche in vitro using BMSCs treated with $CoCl_2$. After a 48 hour-treatment, the cells were immuno-stained with an antibody to HIF1α to ascertain the stabilization of HIF1α in the nucleus. We observed that the $CoCl_2$-treated BMSCs showed a clear nuclear localization of HIF1α (Data not shown), confirming the induction of hypoxia in these cells. We then performed western blot experiments to examine whether this hypoxic condition remains stable after the removal of $CoCl_2$. The BMSCs were exposed to $CoCl_2$ or atmospheric hypoxia (1% O_2) for 48 hours, and then were lysed immediately or after incubation for additional 7 days after the removal of the stimulus. Equal protein samples were subjected to western blot analysis. We found that the cells treated with $CoCl_2$ showed a higher expression of HIF1α even after the stimulus was removed (Data not shown) while the HIF1α levels in the hypoxia-treated cells dropped after the cells were incubated under normoxia. The data show that a transient exposure of BMSCs to $CoCl_2$, but not to hypoxia, evokes a sustained HIF1α-mediated signaling in the BMSCs.

Hypoxic niche increases HSC pool

We then examined the effect of the hypoxic niche on the proliferation of the

HSCs. Lin- fraction of the BM MNC was co-cultured with the BMSCs pre-treated with $CoCl_2$ (48h hrs) for 7 days under normoxia ($CoCl_2$ 48 hrs.). Lin- cells grown on the BMSCs pre-incubated under 1% O_2 (hypoxia) for 48 hours (Hypoxia 48 hrs.) were used for comparison. In a set of co-cultures, $CoCl_2$ was continued in the medium till 7 days of co-culture ($CoCl_2$ continuous). A set of co-cutlure established on the BMSCs pre-exposed to hypoxia (48 hours), was further incubated for 7 days under hypoxia (Hypoxia continuous). In all the co-cultures a cocktail of early acting hematopoietic growth factors was added (SCF and IL-6, 25 ng/ml; IL-3, 10 ng/ml). After 7 days of co-culture, the output hematopoietic cells were phenotypically analyzed by a polyvriate flow cytometry using stem cell-specific markers. It was observed that the BMSCs treated with $CoCl_2$ for 48 hours ($CoCl_2$ 48 hrs.), but not the hypoxia-treated (hypoxia transient), supported a high output of hematopoietic cells (Fig 1A). Continuation of $CoCl_2$ in the medium ($CoCl_2$ continuous) negatively affected the cell yield compared to the $CoCl_2$-transient set. The proliferation of cells was

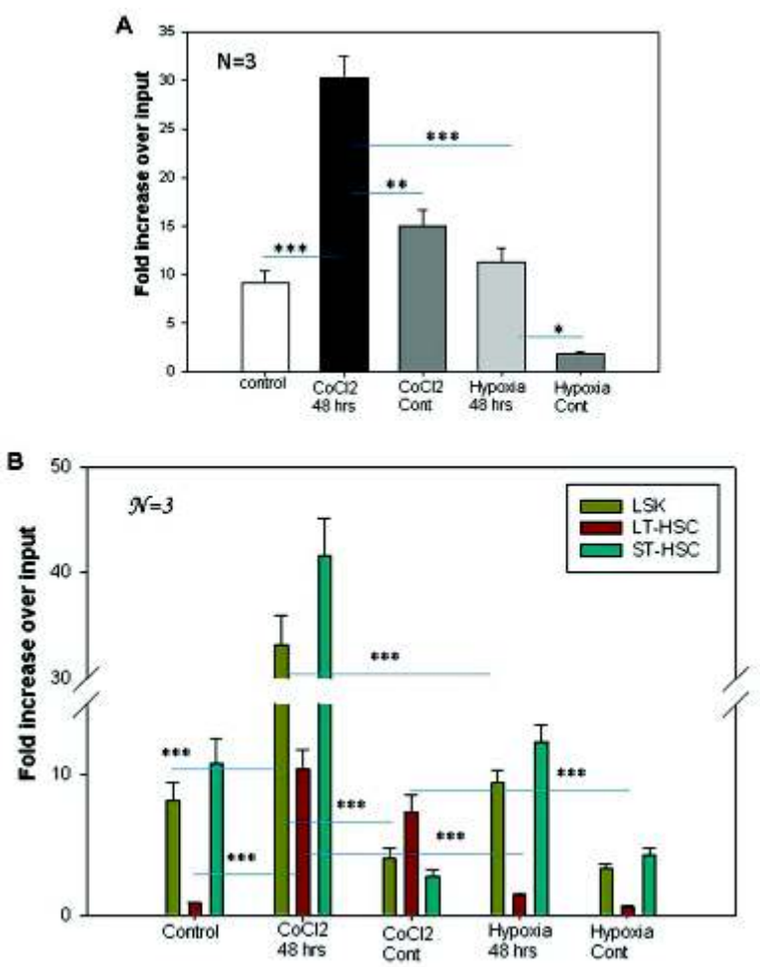

Fig. 1: A. Hypoxic niche stimulates HSC proliferation. Co-culture of Lin- hematopoietic cells with the BMSCs pre-treated with $CoCl_2$ for 48 hours yielded the maximum number of hematopoietic cells. B. A polyvariate flow cytometry analysis showed that the yield of LSK, LT-HSC as well as ST-HSC was also the highest in this set.

strongly suppressed in the co-cultures incubated under hypoxia (Hypoxia Continuous).

When the output population was analysed for LSK stem cell population(Lin-Sca1$^+$cKit$^+$), it was observed that the BMSCs transiently treated with $CoCl_2$ ($CoCl_2$ 48 hrs.) yielded the highest number of LSK stem cells compared to all other sets. Likewise, the yield of long-term- and short-term-engrafting stem cells (LT-HSC and ST-HSCs; LSK-CD34$^-$ and LSK-CD34$^+$ respectively) was the highest in this set (Fig1B). These data show that the BMSCs treated with $COCl_2$ for 48 hours gave the strongest proliferative signal to the HSCs co-cultured with them under normoxic conditions, and hence further experiments were performed using these cells as the model of hypoxic niche ($CoCl_2$-treated BMSCs), and the results were compared with the cells incubated under hypoxia for 48 hours as controls (hypoxia-treated BMSCs).

$CoCl_2$ evokes FAK signaling in the BMSCs

The differential hematopoietic support given by the $CoCl_2$-treated vs. hypoxia-treated BMSCs prompted us to investigate the down-stream signaling events happening in these cells. Since HIF1α is known to modulate the focal adhesion kinase (FAK)-mediated signaling mechanisms in various systems, we examined whether this pathway is operative in the $CoCl_2$- or hypoxia-treated BMSCs. The results of the western blot experiments showed that a 48-hour treatment with $CoCl_2$ resulted in a persistent tyrosine phosphorylation of FAK that lasted till 7 days even after the removal of the stimulus (Figure2). Hypoxia also evoked the FAK phosphorylation, but the signal did not persist after removal of the

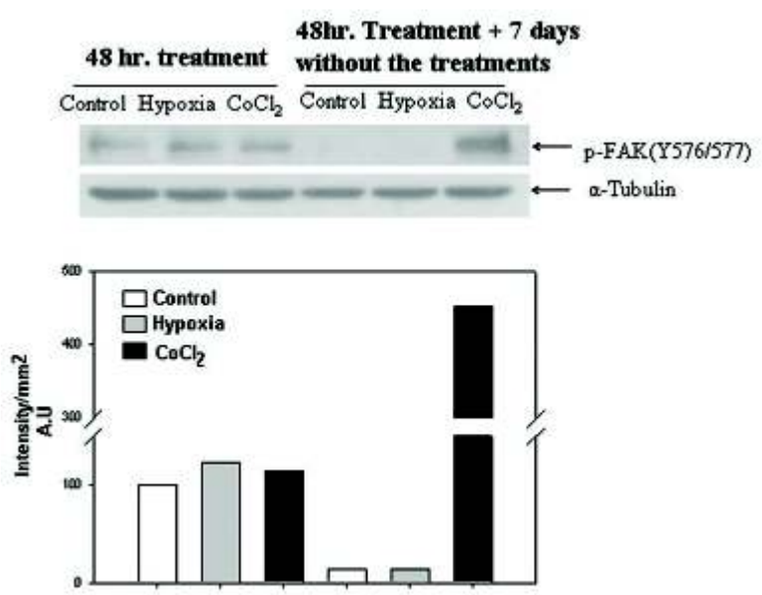

Fig. 2: Persistent activation of FAK signaling by $CoCl_2$. Western blot analysis of BMSCs treated with $CoCl_2$ and Hypoxia showed that both stimuli induce phosphorylation of FAK at Y576/577 residue. High level of phosphorylated FAK is seen till 7 days even after the removal of $CoCl_2$. The p-FAK level declined in the hypoxia-treated cells when they were incubated under normoxia.

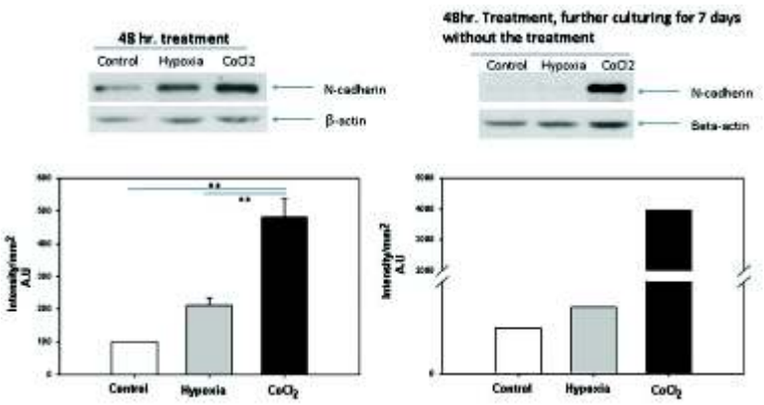

Fig. 3: N-Cadherin expression is regulated by hypoxia. Left hand panel shows the expression level of N-Cadherin at 48 hours of treatment (left hand panel) and at 7 days after the stimulus is removed (right hand panel). Both chemical as well as atmospheric hypoxia induced the N-Cadherin expression in the BMSCs; but in the $CoCl_2$-treated BMSCs the expression remained high even after the stimulus was withdrawn. The expression of N-Cadherin in the hypoxia-treated cells got completely down-regulated when these cells were incubated under nomoxia.

stimulus. The data suggest the role of persistent FAK signaling in the proliferative stimulus given by the $CoCl_2$-treated cells seen in the previous experiments.

Persistent up-regulation of N-Cadherin by $CoCl_2$

In vivo the niche cells express N-Cadherin, a molecule known to play an important role in the maintenance of the HSC pool. However, it is not known whether the N-Cadherin expression is an inherent property of the niche cells or the in situ hypoxia regulates the N-Cadherin expression. Here we examined whether the $CoCl_2$-treatemnt of the hypoxia-exposure regulates the N-Cadherin expression in the BMSCs. We found that the $CoCl_2$-treated BMSCs showed a very high expression of N-cadherin and this level persisted till 7 days of culture period even after the stimulus was removed (Figure 3). The hypoxia-treated BMSCs showed a high expression of N-Cadherin as long as they were under 1% O_2, but the levels declined once the cells were shifted to normoxia. These data clearly show that hypoxia regulates the expression of N-Cadherin in the BMSCs.

Collectively, these data demonstrate that the hypoxic niche supports HSC proliferation via activation of HIIF1α-, FAK- and N-Cadherin-mediated signaling processes. Further experiments are in progress to identify the contribution of the individual signaling pathway in the regeneration of hematopoiesis.

Future Research Plans

1. To identify the contribution made by each signaling pathway in the regengeration of hematopoiesis.
2. To perform *in vivo* competitive repopulation experiments to validate the results obtained *in vitro*.

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Site specific modification of Cys residues of mycobacterial TlyA reveals their essential role in oligomerization and lysis.

Background

The *tlyA* gene products of *Mycobacterium tuberculosis* (Rv1694; MtbTlyA) and *Helicobacter pylori* (Hp1086; HpTlyA) have been annotated as S-Adenosylmethionine-dependent rRNA methylases possessing 'non-conventional hemolytic activity'. The TlyA of *M. tuberculosis* (28kDa) and *H. pylori* (26kDa) consists of 268 and 235 amino acid residues, respectively. The purified MtbTlyA has also been shown to: (i) possess hemolytic activity through formation of stable oligomers on model target membranes (RBCs of both rabbit and human) (ii) be present at the cell surface of *E. coli* which exhibit contact-dependent hemolysis. These studies have also shown that the purified protein also exhibited S-Adenosylmethionine-dependent rRNA methylytion activity, making it a dually active protein. In the case of HpTlyA, no detailed studies are available on either of these aspects, with the exception of an unconfirmed observation which suggested the presence of a hemolytic molecule in *H. pylori*, which was restricted to membrane fractions. Both proteins have Cys residues, whose involvement in their function is unclear to date.

The below-mentioned studies were initiated to investigate the possible regions of both TlyAs that play an important role during oligomer formation and haemolytic activity. Our earlier observations indicated that oligomers of MtbTlyA dissociated in the presence of thiol-reducing agents (i.e. under reducing conditions) with 90% of the hemolytic activity being abolished, while they remained associated under boiling conditions. This suggested that MtbTlyA has the ability to resist heat and SDS, but is susceptible to reducing agents (e.g. β-mercaptoethanol). This indicated that the hemolytic activity of

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MtbTlyA was due to the formation of stable oligomers on target cell membranes only, with a possible role of a disulfide bond in oligomerization.

Aims & Objectives

To determine:

1. The role of TlyA in the host bacterium.
2. The role of Cys residues in oligomerization and lysis.

Work Achieved

We investigated the topology, at gross level, of the cysteine residues necessary for oligomer formation and lysis in MtbTlyA and HpTlyA. MtbTlyA and HpTlyA proteins have two and five cysteine residues respectively. The topology of a membrane protein can be studied with the help of a membrane -impermeant, Cys-specific reagent, iodoacetylstilbene disulfonic acid. This reagent cannot penetrate and modify Cys residues embedded inside the membrane. The primary sequence of MtbTlyA has two predicted transmembrane domains (67-87 and 150- 170), whereas the HpTlyA has one transmembrane domain (146-166) as determined from hydropathy plot analysis.

By measuring the reactivity of cysteine towards the IASD, it is possible to measure the accessibility of Cys residues of proteins. Modification with IASD was done after the addition of DTT to ensure that protein sulfhydryl(s) was completely reduced. The covalent attachment of IASD with cysteine was detected by a rapid gel-shift assay utilizing the standard SDS-PAGE. The labelled protein shows some magnitude of retardation in its mobility compared to unlabeled protein. The magnitude of the shift varies considerably with the modification and directly proportional to the number of additions i.e. number of Cys residues present in the protein. Hence, we have selected four distinguishable stages: (I) Protein present in solution (monomer), (II) Protein present on the membrane as a membrane-bound monomer state, (III) *E.coli* expressing protein and (IV) the protein associated with the outer membrane vesicle (OMV) state.

The TlyAs in soluble monomer stage have shown an unambiguous shift in their mobility in comparison to the unlabeled protein as shown in Figure 1 while the HpTlyA has shown the maximum shift owing to higher number of Cys residues. At membrane monomer stage, MtbTlyA showed partial shift as it is possible that one of the cysteine was embedded in lipid bilayer and might be inaccessible to the IASD, whereas the HpTlyA showed very clear shift (Fig 1b).

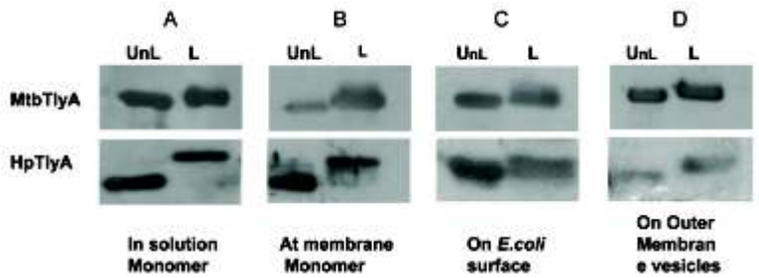

Fig. 1: IASD modification induces gelshift: (A) Protein present in solution (monomer), (B) Protein present as a Membrane-bound monomer, (C) *E.coli* expressing Protein and (D) Protein present on the outer membrane vesicles (OMV) were incubated in PBS, containing 0.1-1.0 mM DTT, for 5 min at room temperature. IASD was added to 10 mM. After 120 min, reaction was stopped by adding DTT to final concentration of 50 mM. The samples were resolved and analyzed by electrophoresis in 40-cm long 12% SDS-PAGE. The lane labels UnL and L respectively represent unlabelled and labeled TlyA with IASD.

This suggests that all the cysteine residues of HpTlyA (at membrane monomer stage) were accessible to IASD. In *E.coli* expressing protein, both TlyAs did not exhibit clear shift indicating that the Cys residues might not be accessible or may be located in vicinity of lipid bilayer (Fig 1c). In case OMV associated TlyAs, both TlyAs have exhibited shift in their mobility (Fig 1d).

Premodified TlyA do not oligomerize on rRBC membranes

Both TlyAs lyse the RBCs by forming oligomers on cell membrane which may form a functional pore, but it is still not clear how these proteins orchestrate themselves to form a pore. Therefore, we investigated the whether oligomer formation is possible or not after site specific modification of the monomer. In this the monomers were first modified with IASD and allowed to lyse the RBCs,. The RBC membranes were then examined for highest order of the oligomer as well as hemolysis. We noticed that both the labeled TlyAs were unable to forming oligomers and lyse the RBC (Fig 2a & 2b). IASD is impermeable to membrane and when it modifies the cysteines of TlyA, the modified Cys residues fail to insert into the lipid bilayer and hence no oligomer formation. It indicates that embedding of cysteine residues into the lipid bilayer is essential for oligomer formation and lysis. Accordingly, the labeled TlyAs were showed 80% decrease in hemolytic activity (Fig 3a). This was in agreement with the observation that labeled proteins were not forming oligomer as they fail to get embedded in lipid bilayer.

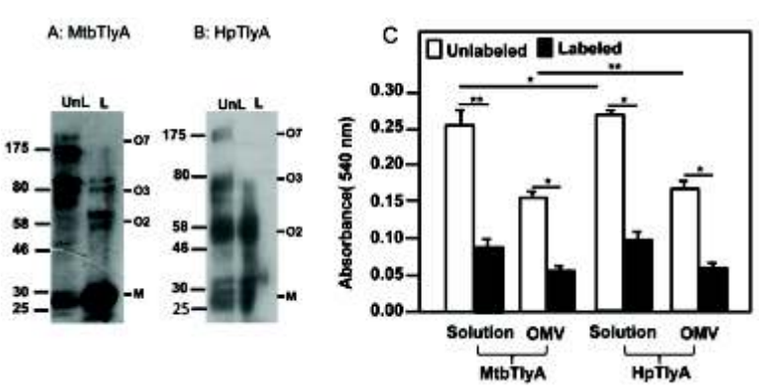

Fig. 2: IASD modification abolishes oligomerization and lysis: Both unlabeled (UnL) and labelled (L) (A) MtbTlyA and (B) HpTlyA were incubated with 10% rabbit red blood cells for 30 min at 37°C and the membranes were then recovered by centrifugation and the oligomers were visualized on SDS_PAGE. (C) Modification abolishes hemolytic activity: Both unlabeled and labelled insolution monomers and the TlyAs part of OMV were mixed with 1.5% rabbit red blood cells and incubated at room temperature for 24 hrs. The absorbance of the supernatant is an indication of hemolysis.

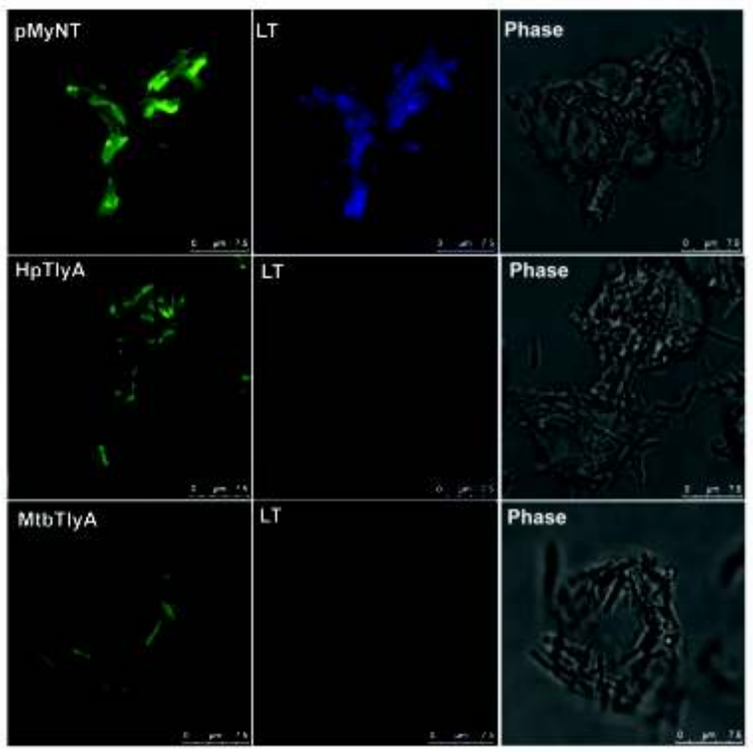

Fig. 3: TlyA expression abolishes acidification: RAW264.7 macrophages were infected with *M. smegmatis* constructs expressing the indicated TlyA at MOI:10 for 60 minutes. All bacteria were labelled with FITC (left panels). Lysotracker Blue was used for visualization of the acidification (middle panels). The right panels are phase contrast images of the infected RAW264.7 cells.

Phagocytosis results in internalization of microorganisms where non pathogenic bacteria are eliminated through acidification of phagosomes. These mature phagolysosomes are highly acidic compartments and can be visualized with pH sensitive dyes such as LysoTracker which has been extensively used by many investigators. However, *M. tb* has evolved mechanisms to subvert the uncongenial environment of macrophages. Consistent with our notion the *M. smegmatis* (Fig. 3) expressing both the TlyA did not exhibit any positive staining. We have noted that >65% of *M. smegmatis*-TlyA did not show any staining for LysoTracker blue whereas, more than 85% of non-TlyA expressing *M. smegmatis* (*M. smegmatis*/pMyNT) were positive for the LysoTracker. This supports our hypothesis that the TlyA expressing bacteria can compromise the integrity of this compartment, resulting in loss of acidification.

Summarily, these observations indicate that the orchestration of oligomers by the TlyA protein involves a possible burial of its cysteine containing region in the target membranes upon its contact and the cysteine residues play an important role in oligomerization and conformational changes necessary to form transmembrane pore and avoid acidification of the compartments by both human pathogens *M.tb* and *H.pylori*.

Future Research Plans

Further studies are underway to understand at which stage, the oligomer formation can play a role in maturation of phagolysosomes to prevent acidification of the engulfed bacteria and whether or not it can aid to survival strategies for the host bacterium.

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Class 3A Semaphorin suppresses breast tumor growth and angiogenesis through FOXO 3a-dependent MelCAM expression

Background

Semaphorins have been initially recognized as a family of evolutionary conserved secretory or membrane bound molecules implicated in diverse developmental processes like axon guidance during nervous system development. Semaphorins are known to mediate short- and long- range attractive and repulsive activities during embryonic development. Apart from shaping the development of central nervous system, they are implicated in a wide range of biological functions such as regulation of apoptosis, cell migration, adhesion, angiogenesis and modulation of tumor progression. These versatile and multifaceted roles can be accredited to involvement of various semaphorin-mediated signaling pathways. Semaphorin 3A (Sema 3A), a putative tumor suppressor of class 3 family is known to inhibit angiogenesis. The role of Sema 3A as a tumor suppressor is highlighted in several cancers including breast and prostate. Sema 3A triggers diverse signaling processes by binding with co-receptor molecule, neuropilin-1 (NRP-1). This Sema 3A/NRP-1 complex binds with plexin-A1 to serve as signal transducing unit for Sema 3A-mediated pathway.

In this study, we have demonstrated the in-depth molecular mechanism by which Sema 3A abrogates breast tumor growth and angiogenesis. The data suggested that Sema 3A regulates NRP-1-mediated PTEN-dependent FOXO 3a activation leading to MelCAM expression which ultimately suppresses breast tumor growth. The *in vivo* data revealed that overexpression of Sema 3A attenuates whereas silencing endogenous Sema 3A augments breast tumor growth and angiogenesis. Clinical specimen analyses revealed that reduced

expression of Sema 3A and p-PTEN are correlated with enhanced breast cancer progression, further strengthening our *in vitro* and *in vivo* findings. In summary, our study demonstrated a novel mechanism of regulation of tumor suppression by Sema 3A in synchronization with a chain of tumor suppressor genes which in turn impedes breast cancer cell migration and angiogenesis tumor growth (Mishra R---Kundu GC et al, Oncogene, 2014).

Aims and Objectives

1. To investigate the role of Sema 3A in NRP-1-mediated PTEN dependent FOXO 3a and MelCAM expression and cell migration in breast cancer cells.
2. To study whether Sema 3A regulated signaling controls breast tumor growth and angiogenesis in NOD -SCID mice models.
3. To study the expression pattern of Sema 3A and other Sema 3A regulated genes in various grades of breast cancer clinical specimens and their correlation with breast cancer progression and how Sema 3A regulated signaling may act as potential therapeutic target/strategy for management of breast cancer.

Work Achieved

Class 3A Semaphorin (Sema 3A), a member of semaphorin family is known as a candidate tumor suppressor which attenuates breast tumor progression by binding with its co-receptor, neuropilin-1 (NRP-1). However, the mechanism by which Sema 3A suppresses breast tumor growth is still unexplored. In this report, we have shown that Sema 3A regulates phosphorylation and nuclear translocation of PTEN and FOXO 3a (Fig. 1). Moreover, Sema 3A controls NRP-

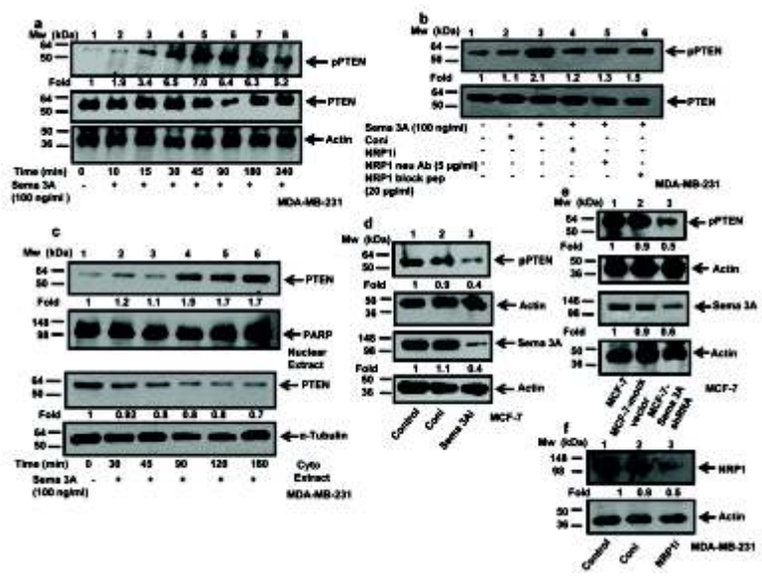

Fig. 1: Sema 3A stimulates NRP-1-mediated PTEN phosphorylation and nuclear translocation in breast cancer cells. **(a)** Serum starved MDA-MB-231 cells were treated with rh Sema 3A (100 ng/ml) for 0-240 min, total protein was extracted and level of p-PTEN was examined by western blot. PTEN and actin served as loading controls. **(b)** MDA-MB-231 cells were either treated with Sema 3A alone or NRP-1 expression was inhibited with NRP-1 neutralizing antibody (5 µg/ml) or NRP-1 blocking peptide (20 µg/ml) or NRP-1 siRNA and then treated with Sema 3A (100 ng/ml) and level of p-PTEN was analyzed by western blot. The blots were reprobed with anti-PTEN antibody. **(c)** Nuclear and cytoplasmic extracts isolated from Sema 3A treated MDA-MB-231 cells were examined using anti-PTEN antibody. PARP and α-tubulin were used as loading controls. **(d, e)** Sema 3A in MCF-7 cells were silenced with Sema 3A siRNA or Sema 3A shRNA and levels of p-PTEN and Sema 3A were analyzed by western blot. The blots were reprobed with anti-actin antibody. **(f)** Expression of NRP-1 in MDA-MB-231 cells transfected with NRP-1i was analyzed by western blot. Actin was used as loading control.

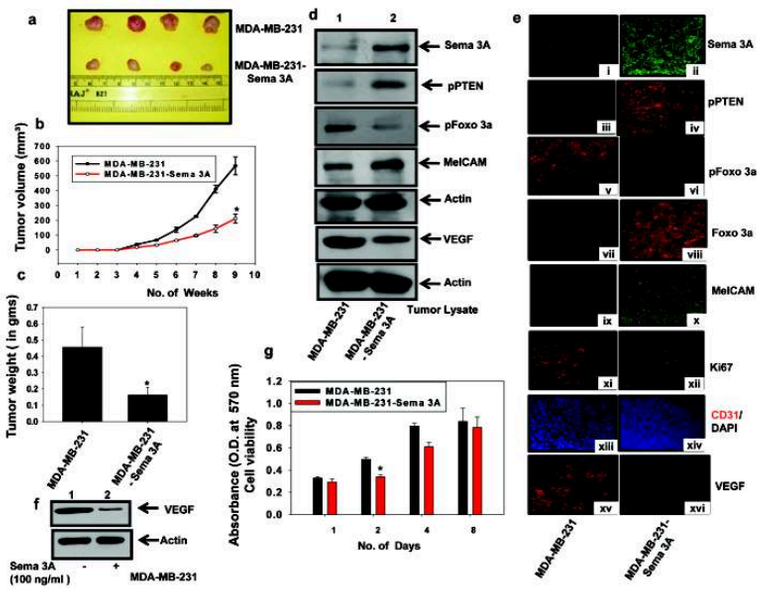

Fig. 2: Sema 3A overexpression suppresses breast tumor growth in NOD-SCID mice model. **(a)** Equal number of MDA-MB-231 or MDA-MB-231-Sema 3A stable cells were injected orthotropically into female NOD-SCID mice. Mice were sacrificed and photographs were taken. Six mice were used in each set of experiments. **(b)** Tumor growth kinetics of the above mice for 9 weeks is represented graphically. Error bars, s.e.m, changes in 9th week.*p<0.005. **(c)** Tumor weights were estimated and compared. Error bars, s.d,*p<0.005. **(d)** Sema 3A, p-PTEN, p-FOXO 3a, MelCAM and VEGF expressions were checked in tumor lysates by western blot. Actin served as loading control. **(e)** Immunofluorescence analyses of tumor specimens for Sema 3A (green; i, ii), p-PTEN (red; iii, iv), p-FOXO 3a (red; v, vi), FOXO 3a (red; vii, viii), MelCAM (green; ix, x), Ki67 (red; xi, xii), CD31 (red; xiii, xiv) and VEGF (red; xv, xvi) expressions. **(f)** VEGF expression was detected in MDA-MB-231 cells treated with Sema 3A (100 ng/ml). Actin was used as loading control. **(g)** Growth kinetics of MDA-MB-231 and MDA-MB-231-Sema 3A stable cells were determined *in vitro* and represented in the form of bar graph. Error bars, s.e.m.,*p<0.05 versus MDA-MB-231.

1-mediated PTEN-dependent FOXO 3a activation. Overexpression of PTEN and FOXO 3a enhances Sema 3A-induced attenuation of breast cancer cell migration. Chip and EMSA data revealed that FOXO 3a regulates MelCAM at transcriptional level. Sema 3A induces NRP-1-mediated MelCAM expression through PTEN and FOXO 3a. The data also showed that VEGF-induced angiogenesis is inhibited by Sema 3A. Loss of or gain in function study revealed that Sema 3A modulates phosphorylation of PTEN and FOXO 3a and expression of MelCAM leading to suppression of tumor growth and angiogenesis using *in vivo* mice model (Fig. 2). Clinical specimen analysis revealed that reduced expression of Sema 3A and p-PTEN are correlated with

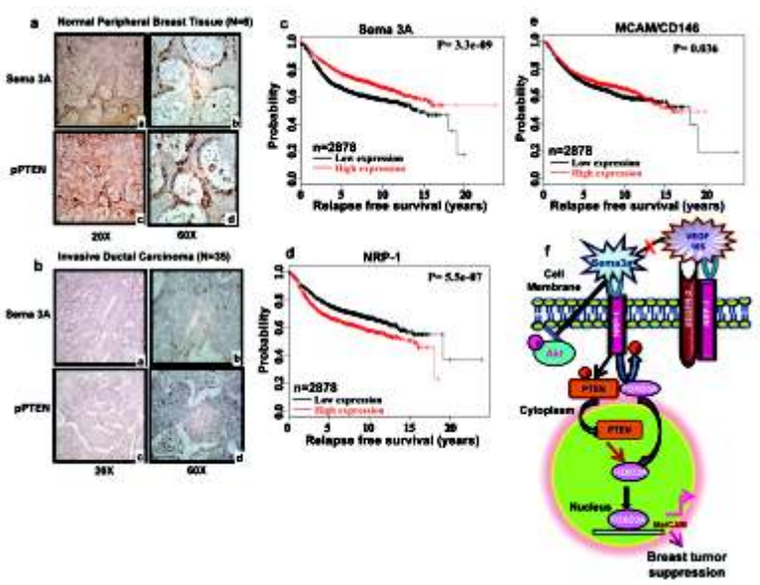

Fig. 3: Correlation of expressions of Sema 3A, p-PTEN, NRP-1 and MelCAM with breast cancer progression and patient's survival. **(a and b)** Immunohistochemical analyses of Sema 3A and p-PTEN expressions in peripheral normal (n=6) and breast cancer specimens (n=35). **(c-e)** Kaplan-Meier (KM) analysis of relapse-free survival in breast cancer patients (n=2878) using KM plotter. Patients were divided according to the expression of three gene signatures (Sema 3A, NRP-1 and MelCAM) **(f)** Schematic representation of Sema 3A-induced NRP-1-mediated PTEN/FOXO 3a dependent MelCAM expression leading to suppression of breast cancer growth and angiogenesis.

enhanced breast cancer progression, further strengthening our *in vitro* and *in vivo* findings (Fig. 3). Correlation of relapse free survival of breast cancer patients (n=2878) with expressions of Sema 3A, NRP-1, FOXO 3a and MelCAM were studied by Kaplan-Meier analysis (Fig. 3). Statistical analysis revealed a close association between reduced expression of Sema 3A and MelCAM with that of poor patient's survival. Our study demonstrated a novel mechanism of regulation of tumor suppression by Sema 3A in coordination with a chain of tumor suppressor genes which in turn inhibits breast cancer cell migration, tumor growth and angiogenesis. (Mishra R---Kundu GC et al, Oncogene, 2014).

Future Research Plans

To study the role of CD20 and CD133 positive cancer stem cell in control of melanoma growth, angiogenesis and metastasis.

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

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Background

Inflammation is the complex set of reaction involves a set of cytokines, chemokines and adhesion molecules. Several chemokines, cytokines and its specific ligands expressed into the inflamed microenvironment. A joint venture of pro- and anti-inflammatory functions are initiated together by infiltrating leukocytes, tissue fibroblasts, epithelial and endothelial cells during the pathogenesis of autoimmunity. 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, do intrinsic signaling from these receptors perturbs the cell differentiation and function is not well characterized.

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

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

Aims and Objectives

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

Work Achieved

Treg and Th17 cells are known to express CCR6 on their surface during physiological and inflammatory conditions. We cultured C57BL/6 mice purified $CD4^{+}CD25^{-}CD44^{-}CD62L^{+}$ cells in presence of IL-2, anti-CD3ε, TGF-ε and in presence or absence of recombinant purified CCL20 for 4 days. Our result showed that CCR6-CCL20 signaling inhibits the expression of Foxp3 and induces the differentiation of $ROR\gamma\tau^{+}$ Th17 cells. Inhibition of Foxp3 or differentiation of Th17 cells was blocked in $CCR6^{-/-}$ CD4 T cells. The CCL20 induced differentiation of Th17 cells was p38MAP kinase, AKT and pSTAT3 signaling dependent. Blocking of p38MAP kinase with specific inhibitor prevents the CCL20 induced differentiation of Th17 cells. However, CCR6-CCL20 axis did not show any effect on the differentiation of Th1 and Th2 cells.

We are currently investigating the further detailed molecular mechanism of CCR6 signaling and its role on Th17 and Treg cell differentiation and function. Understanding the CCR6 signaling in CD4 T cells will help us to develop novel therapeutic strategies to control the autoimmune diseases.

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Signaling cues guiding Mesoderm induction and Cardiomyogenesis

Background

Mesoderm is one of the three embryonic germ layers during gastrulation that further gives rise to cells of various vital systems including hematopoietic, cardiovascular, reproductive, excretory, urogenital etc. during early development. Hence, it is essential to understand the guiding cues dictating mesoderm specification and the downstream developmental events. The *Brachyury* gene, a well-known *T-box* transcription factor is required for mesoderm and the anterio-posterior axis formation during early development and its expression is detectable as early as 6.5 dpc in mouse embryo in mesoderm cells of the early primitive streak. During gastrulation Brachyury expresses with Tbx6 and Wnt3a in the primitive streak, thereby hinting at the plausible role of canonical Wnt signalling in specifying mesoderm. In fact, Brachyury has been reported as the direct target gene of Wnt/β-catenin signalling. It has also been proven that, β-catenin recruits Smad2/3, which is a downstream effector molecule of Nodal Signalling, to induce Brachyury expression. Using murine embryonic stem (ES) cells as a model system we have also demonstrated Wnt-BMP synergy to underlie mesoderm induction. However, canonical Wnt activation also led to attenuation in cardiomyogenesis. Since cardiomyogenic cells are the mesodermal derivatives, the question arises regarding which cell fate the Wnt activation induced mesodermal cells might be promoting, in place of the cardiac ones. Conflicting reports do exist regarding the role of Wnt/β-catenin signaling in cardiac differentiation and haematopoiesis. Accordingly, we have tried to address the cell fate decision machinery exploring how different signalling mechanisms integrate and crosstalk in a spatio-temporal manner during mesoderm induction and

Participants

Varun Haran M, *JRF*

subsequent differentiation into its derivatives with special focus on cardiomyogenic lineage.

Aims and Objectives

1. Establishment and characterization of stable transgenic ES cell clones expressing live reporter under the regulatory control of Brachyury promoter.
2. Exploring the cell fate decision machinery underlying mesoderm specification and subsequent cardiomyogenesis.
3. Elucidating the Chromatin dynamics during mesoderm Induction

Work Achieved

About 6 kb of the murine Brachyury promoter along with LacZ reporter (3Kb) (a kind gift from Dr. Stott) was subcloned into a promoter-less IRES-EGFP vector. A number of stable transgenic Brachyury ES cell clones (Bry-LIG) were established by transfecting D3 ES cells with Bry-LacZ-IRES-EGFP vector with the rational that, the stable clones having EGFP reporter gene under the regulatory control of mesoderm specific Brachyury gene promoter would specifically demarcate the mesodermal population during mesoderm induction from ES cells. Some of the representative Bry-LIG clones were assessed for their retention of undifferentiated ES cell characteristics during maintenance, similar to that in the parental D3 ES cells. Immunocytochemical investigation in these cells revealed the expression of pluripotency associated markers like Oct4 and Nanog in them during maintenance (Fig. 1A). Further, the authenticity for mesoderm specific EGFP expression in these clones, and their differentiation into various mesoderm derivatives was determined. The Bry-LIG clones were observed under the fluorescence microscope to monitor the Brachyury driven EGFP

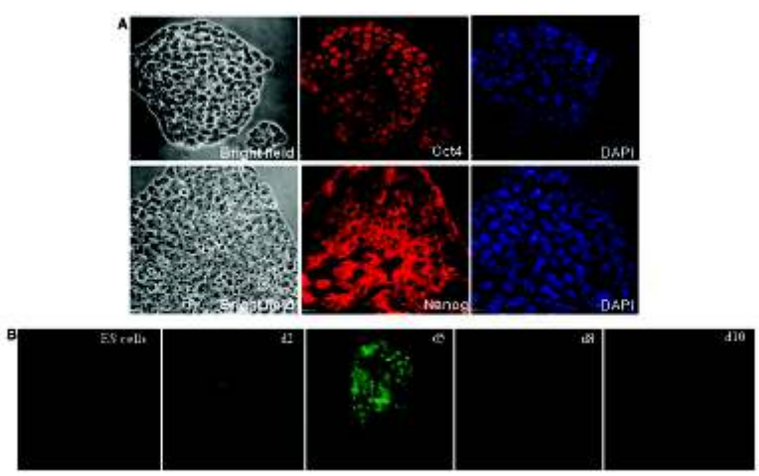

Fig. 1: **(A)** Bry-LIG stable ES cell clones showing Oct4 and Nanog expression during their maintenance in culture. **(B)** Brachyury promoter driven EGFP expression profile during differentiation of Bry-LIG ES cell clones.

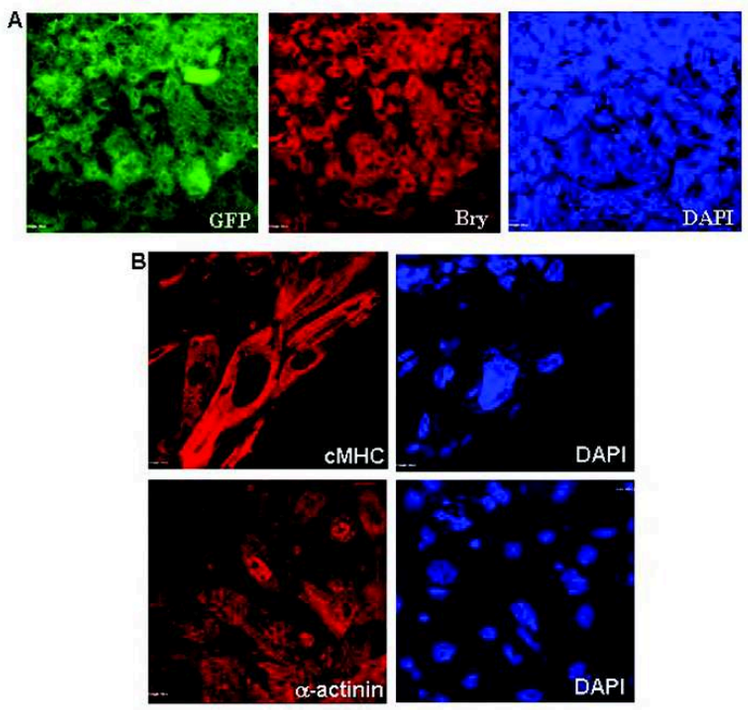

Fig. 2: **(A)** Expression of Brachyury promoter driven EGFP corresponding to endogenous Brachyury authenticates the Bry-LIG clones and validates our approach to demarcate the mesodermal cells during differentiation of ES cells. **(B)** Differentiation of Bry-LIG clones into cardiomyocytes as shown by cardiac MHC and a-actinin positivity.

expression profile at various time points during differentiation (Fig. 1B). The EGFP expression was seen in discrete regions from day 2 (d2) of differentiation, thereby reflecting to the mesoderm induction. This peaked at d5 and diminished at subsequent days during differentiation. This indicated the Brachyury driven EGFP to be maximum at d5 of ES cells differentiation that corroborated well with the earlier findings from our group showing maximum expression of Brachyury transcript at d5. The co-localisation of EGFP and the endogenous Brachyury in these clones validated the Brachyury driven EGFP to be mesoderm specific (Fig. 2B) and hence authenticated our approach of mesoderm demarcation during ES cells differentiation. Further, differentiation of Bry-LIG clones (d12) yielded mesodermal derivatives which stained positive for cardiac (cMHC), hematopoietic (CD45) and muscle specific (Sarcomeric α actinin) markers (Fig. 2B). These findings suggested that EGFP expression faithfully recapitulated Brachyury expression in differentiating EBs and hence facilitated discrimination of mesodermal cells among the heterogeneous population of differentiating EBs. To assess the influence of Wnt signalling during mesoderm specification in ES cells and the further diversification of mesodermal cells to both hematopoietic and cardiomyogenic lineage, Bry-LIG clones were subject to Wnt activation by GSK3 inhibition during the initiation of differentiation. Our preliminary observation on the same suggests the preference of mesodermal cells to give rise to hematopoietic than cardiac

lineage in response to Wnt activation during differentiation. To decipher further the epigenetic regulation of Brachyury gene during mesoderm induction following Wnt activation, we have considered the -404 region of Brachyury promoter in order to assess the binding of β-catenin and CTCF to the same. Interestingly, chromatin immunoprecipitation analysis confirmed the binding of β-catenin and CTCF at the Brachyury promoter specifically during d2 of ES cells differentiation and with lack of binding in the undifferentiated ES cell state. Thus the results suggest that CTCF, which is a well-known chromatin remodeling factor, may be influencing Brachyury expression by recruiting β-catenin, which is important for Wnt signal induced mesoderm formation. Further investigations are underway to decipher the role of Wnt signalling in modulating the binding affinity of these proteins.

Future Research Plans

We would like to investigate the hematopoietic or the cardiogenic potential of differentiating mesodermal cells and delineate the temporo-spatial role of Wnt signalling during the same. Moreover, determining the various genetic and epigenetic modulators underlying mesoderm induction and further differentiation would shed light on this critical developmental event during embryogenesis.

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

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Background

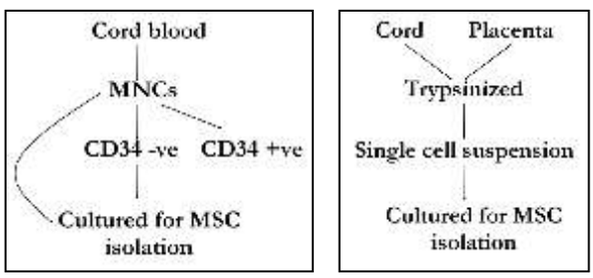
Out of the five different on-going projects from the laboratory, the project on differentiation of MSCs to neural cells is described in detail below. Mesenchymal stem cells, the non hematopoetic stem cells, isolated from bone marrow have been shown to gain wide popularity in regenerative medicine. In order to have a non-invasive source of MSC isolation, placenta, umbilical cord tissue and cord blood - all being clinical wastes - are being harnessed as alternative sources for MSC isolation. MSCs have been known to replenish cells of mesodermal origin, thereby acting like reservoirs. Because of this multilineage differentiation potential, MSCs are sought after in transplantation settings. An aspect considered to be important in transplantation is the graft versus host disease. MSCs are known to have an immunomodulatory ability where they can modulate the immune reactions *in vitro* and are able to escape the immune system *in vivo*. Whether this ability of the MSCs varies according to the tissue source is also being studied. Adult stem cells are known to have lineage specific differentiation ability. The mesenchymal stem cells are shown to have trans differentiation ability wherein they may acquire an ability to differentiate into cells of endo - or ectodermal lineage thereby showing stem cell plasticity. On these lines we are trying to differentiate MSCs into neural cells (belonging to the ectodermal lineage). This differentiation will help us to define a new source of MSCs which has neural differentiation potential, which can be looked into for use in various neurological disorders.

Aims and Objectives

1. Isolation of MSCs from the sources like cord blood, cord tissue and placental tissue; their phenotypic and functional characterization.
2. To explore ways to induce mesenchymal stem cells (MSCs) to differentiate into neural cells in vitro and to characterize them phenotypically and functionally.

Work Achieved

MSCs have been cultured from various sources such as umbilical cord tissue, placenta and umbilical cord blood.



The frequency of isolation of MSCs varied with the source used for MSC isolation .From cord blood the frequency was very low (5-10%) whereas from cord and placenta it was very high (80-100%) (Fig.1 a)

In another project from our lab, we have come across an interesting observation that PMSCs are more potent feeders as compared to CMSCs for expansion of HSCs . In order to check whether any such differences are seen in their neural differentiation ability, we derived P and C MSCs from the same donor mother and compared them by morphology and phenotype. There were no significant differences between morphology (Fig. 1b & 1c) and phenotype (Fig.1d &1e) of CMSCs vs PMSCs respectively. The figures show data from one representative sample. However we got reproducible results with 3 more samples.

Further characterization of P and C MSCs was done by checking their immunomodulatory ability. The immunomodulatory ability of MSCs on the immune cells was assessed by co-culturing MSCs derived from cord and placenta with dendritic cells. Briefly, DCs were generated from cord blood as per our previously published method . The cord blood derived DCs showed typical DC morphology (Fig 2a) and phenotype like high expression of markers like CD80, CD83, CD11c, CD80, HLA ABC, HLA DR in a range of 80-90% and low expression of CD3, CD14. Mature dendritic cells (DCs) have an ability to

a Sample type	No. of samples processed	MSCs generated
Cord blood MNCs	60	2
Cord blood CD34 -ve fraction	93	1
Umbilical cord	10	8
Placenta	6	5

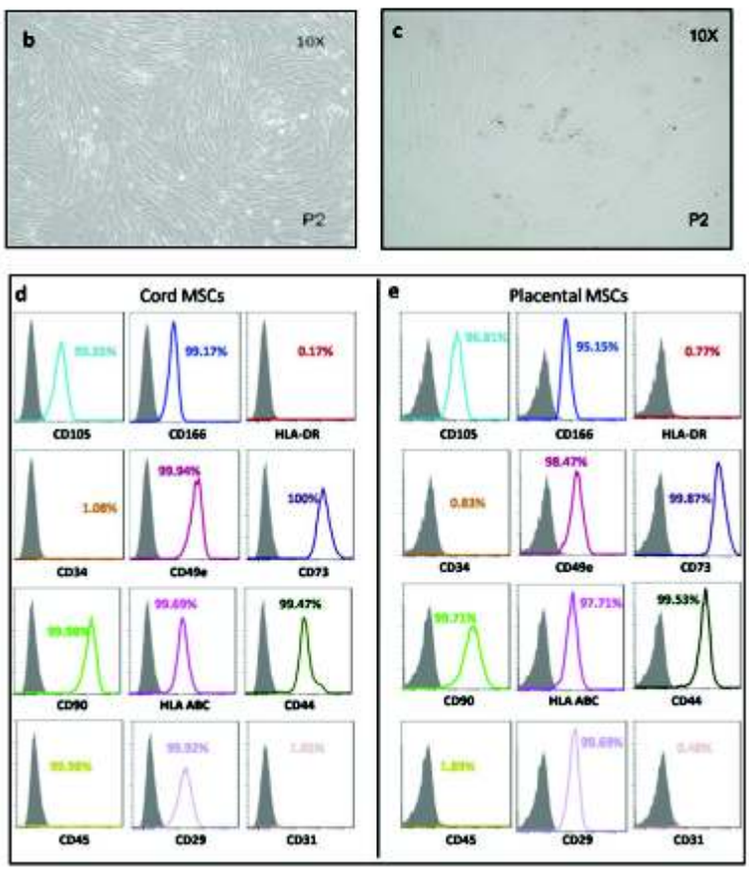


Fig. 1: MSC isolation and characterization: a) Frequency of isolation of the MSCs from different sources differs. b & c) Typical fibroblastic morphology of MSCs derived from cord and placenta, d & e) Marker expression profile of cord and placental MSCs analyzed by flow cytometry.

stimulate the proliferation of T-cells . To observe the effect of MSCs on the proliferation of T cells induced by DCs, DCs were co-cultured with MSCs (Fig 2b) . The proliferation of T cells , as assessed by Thymidine uptake, was hampered in case of MSC-DC co-cultures as compared to only DC control (Fig. 2c). However, among the two MSCs, placental derived MSCs were more potent in reducing the T cell proliferation (Fig. 2d). Thus though the P and C MSCs were obtained from the same donor they showed differences in immunomodulatory abilities.

The transdifferentiation ability of placental and cord MSCs to neural lineage was assessed by culturing them in a suitable differentiation medium. The cells were harvested on 10 and 21 days and studied on the basis of their morphology and surface marker expression.The PMSCs exhibited neural morphology on 10th

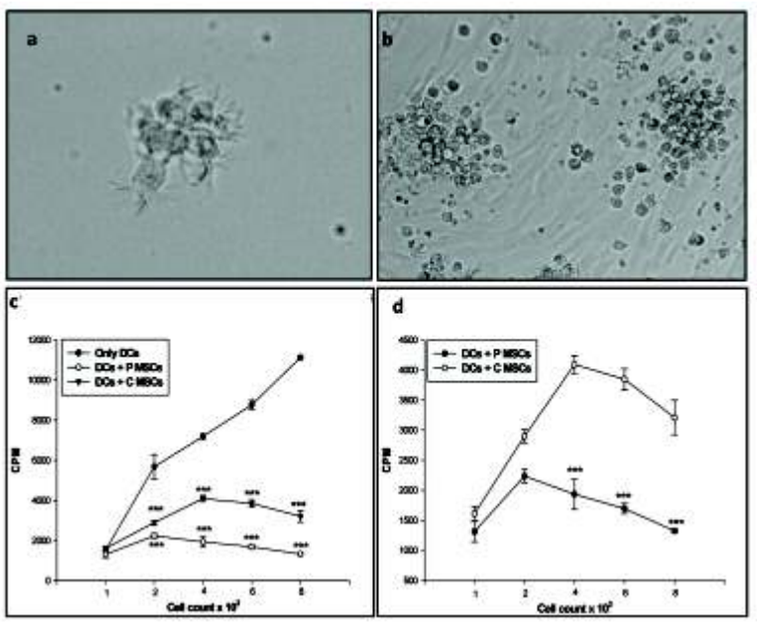


Fig. 2: Immunomodulatory ability of MSCs: a) Mature DCs were derived from cord blood b) DCs were cocultured with MSCs. c) The effect on T-cell proliferation was analyzed by thymidine uptake after 72 hrs . Both the cord and placenta derived MSCs showed reduced T-cell proliferation as compared to DCs alone. d) the reduction by placental MSCs was more significant as compared to cord derived MSCs.

day (Fig. 3 a & b) and also on 21 day (Fig. 3 c & d). The differentiated cells showed increased expression of neural lineage markers like MAP2, βIII tubulin, NF200 and S100β as compared to control undifferentiated MSCs. (Figs.3 e-h) Cord MSCs however showed poor neural differentiation, the cells came out in the suspension thus making quantitation of data difficult (data not shown). The experiments are being repeated to make conclusive statements and to get reproducible data.

Our results indicate that MSCs could be isolated and expanded from different cord tissue sources. They have a multilineage differentiation potential where they were differentiated into mesodermal cell types such adipocytes, osteocytes and chondrocytes. These MSCs also modulate the immune system by affecting the antigen presenting ability of dendritic cells, analyzed by the reduced proliferation of T-cells. Here, placental derived MSCs showed a better reduction as compared to cord tissue derived MSCs. The plasticity of these stem cells was confirmed by their transdifferentiation ability where the placental MSCs, when cultured in presence of specific factors, showed neurite like extensions and positive expression of neural-specific markers. Thus, MSCs have the ability to differentiate into cells of ectodermal lineage.

Future Research Plans

Neural differentiation of MSCs will be confirmed by gene expression profiling. Docosahexanoic acid is an important component of the brain. Hence, enhancement in the neural differentiation ability using agents like DHA is being

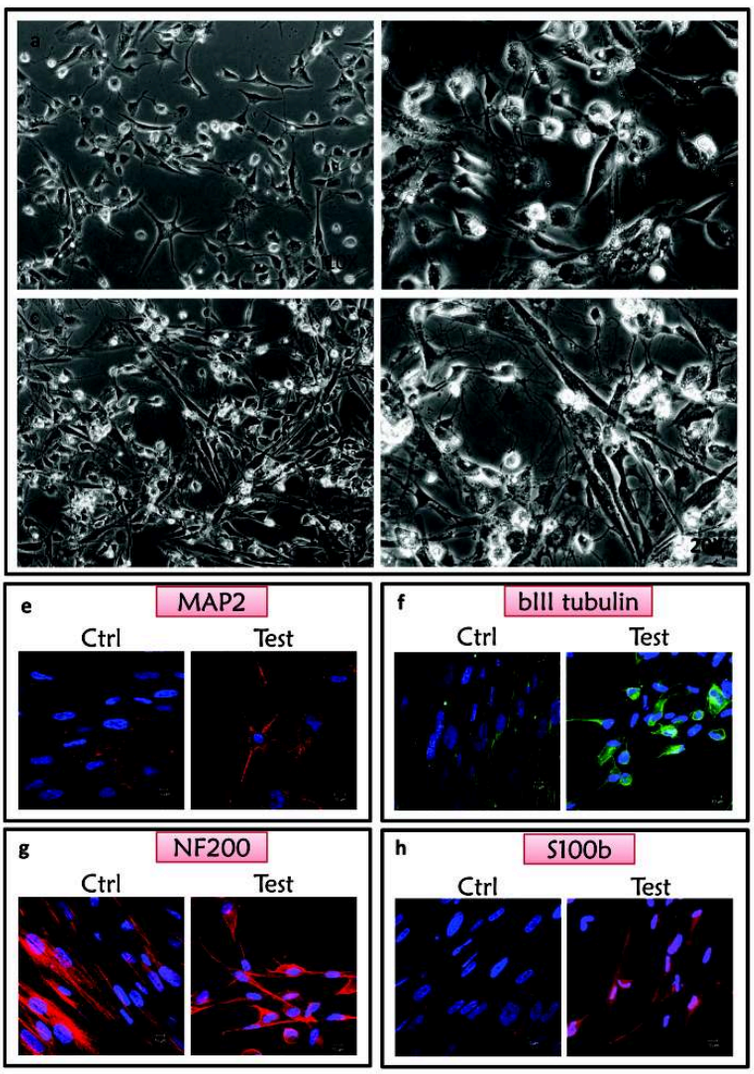


Fig. 3: Neural differentiation of placental MSCs.: a - d) P-MSCs subjected to neural differentiation analyzed by morpholoy after 10 days (a - 10X & b - 20X) and 21 days (**c** - 10X & **d** – 20X) showing neurite like extensions and expression of markers such as **(e)** MAP2, **(f)** βIII tubulin, **(g)** NF200 and **(h)** S100β.

planned. As MSCs from cord and placenta have shown difference in their immunomodulatory ability, the comparative differentiation potential of cord and placental MSCs towards neural lineage will be studied in depth.

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Structure function properties of *M. tuberculosis* proteins

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Background

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

Aims and Objectives

1. To identify important proteins of *M. tuberculosis* involved in redox reactions and in heat shock response. Purify these recombinant proteins, as well as relevant protein complexes and undertake structure-function analyses on them.
2. Analyze large scale data arising from studies such as gene expression, deep sequencing, proteomics, available in literature to find common patterns, if any.
3. Evolve methodologies for analysis of protein: protein interactions, especially the dynamics of changes in these interactions and their relevance on biological processes.

Work Achieved

During the period under review, we have initiated work on an important redox complex from *M. tuberculosis*. These proteins are encoded by the Rv0081-Rv0088 genes present on a multi cistronic operon. Rv0081 encodes a

transcription factor, which belongs to ArsR/Smtb family and Rv0082 is a hypothetical oxidoreductase. Both Rv0081 and Rv0082 are up regulated during dormancy and are regulated by DosR (Dormancy survival regulator). Further, it has been predicted that Rv0082 might interact with NADH ubiquinone oxidoreductase (NDH-1) plays an important role in redox sensing. Genes on the Rv0081 - Rv0088 operon are predicted to form components of potential Formate hydrogenlyase (FHL) complex involved in formate metabolism. By sequence analysis it is predicted that FHL (Rv0081-Rv0088) operon is unique to certain mycobacterium species and has been demonstrated to be associated with respiratory disease or granuloma formation in their respective hosts. The ability to metabolise formate might be one of the processes evolved to enhance the survival of M. tuberculosis in the dormant phase. However, how *M. tuberculosis* metabolises formate during the dormant phase is yet to be demonstrated. To address these questions, we have initiated structure-function analyses on the proteins of the Rv0081-Rv0088 operon. The Rv0081 protein was cloned, expressed and purified. Crystallization trials were performed using a mosquito (TTP LabTech) crystallization robot with a total drop volume of 300 nl (at 3 different protein/precipitant ratios: 1:1, 1:2 and 2:1). Different screens of Hampton, Qiagen, molecular dimention and JBS classic were used for initial crystallization trials at two temperatures, i.e. room temperature and at 4 ºC. Crystals were obtained in 0.1 M Tris-HCl pH 8.5, 0.2M Li_2SO_4, 20% w/V PEG 4000. X-ray diffraction data were recorded from flash-frozen crystals at -178 °C

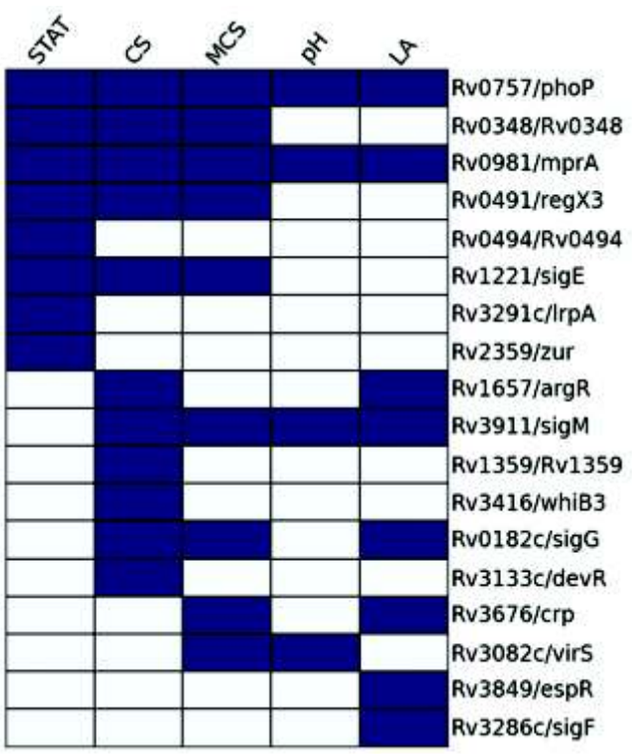

Fig. 1: Diffraction image of Rv0081 crystals. The diffraction data were collected at the Elettra synchrotron using Pilatus detector.

first in IISER, Pune rotating anode source on a MAR image plate detector and later using a Pilatus detector at Elletra synchrotron, Tristy Italy (Figure 1). The data were processed using XDS software. Structure determination is in progress.

Our laboratory is also involved in characterizing the 60kDa chaperonins of *M. tuberculosis*. *Mycobacterium tuberculosis* encodes two copies of *groELs*, of which *groEL1* is arranged in an operon with *groES* and is dispensable whereas *groEL2* is located separately on the genome and is indispensable. Unusually, *M. tuberculosis* GroELs exist as lower oligomers when the recombinant proteins are purified from *E. coli*. Intriguingly, biochemical, genetic and structural studies have demonstrated *M. tuberculosis* GroELs as inefficient chaperones owing their lower oligomer status. Yet abundance of GroEL2 by means of enhanced expression or by chemical collocation has been shown to recover *in vivo* and *in vitro* function indicating GroEL2 as a naturally stunted chaperone which can function as a canonical chaperonin when present in higher concentrations. The reasons for its reduced chaperonin activity are likely to arise out of weakened substrate interactions or slower rates of ATP hydrolysis or potential defects in allostery. The Mycobacterial chaperonins therefore offer an interesting system to dissect individual components of chaperonin function without affecting the other components. To address this important problem we created chimeras of *M. tuberculosis* GroEL2 and *E. coli* GroEL, by mutually exchanging either apical or equatorial domains. Domain boundaries in the resulting chimeras were altered to study influence of domain boundaries and hinge residues in chaperonin function. All the mutants generated we tested for activity in a conditional E. coli mutant of *groEL*. These studies reveal important aspects of GroEL function and the relation between different interdomain hinges and the chaperonin ability of GroEL.

We had also undertaken work on GroEL1 protein to understand its biological function. To understand the effect of its absence in *M. tuberculosis*, we have measured bacterial survival under in vitro stress conditions and in macrophages. Additionally, genes expression analysis for wild type H37Rv (WT) and groEL1 knockout (KO) was done under stationary phase, cold shock, low aeration, mild cold shock and low pH. Interestingly, the absence of GroEL1, did not affect the survival of bacteria in in vitro log phase conditions (in 7H9 media). However, the survival under stress conditions: low aeration and cold shock was significantly compromised. Our experiments using large-scale differential gene expression analyses of the groEL1 mutant show changes in expression of several key virulence factors like whiB3, sigma factors sigB, D, E, G and H; two component system PhoP/R, SenX3/RegX3 and MprA/B. When the differential

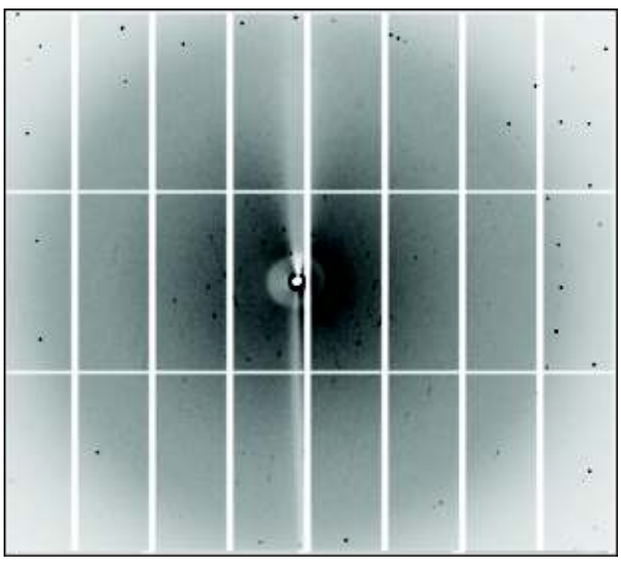

Fig. 2: The transcription factors responsible for differential regulation of a large number of genes in the *groEL1* knock out strain of *M. tuberculosis*. The five experimental conditions are stationary phase, low aeration, cold shock and mild cold shock and low pH. The method to identify these transcription factors involved superposing the data from differential gene expression with a regulatory network. As can be seen transcription factors PhoP and MprA appear to be involved in gene regulation under all the experimental conditions.

gene expression was overlayed on transcriptional regulatory network, we were able to identify a small set of transcription factors that exhibit control of such differential expression (Fig. 2).

Future Research Plans

In the coming year, we aim to complete the structure determination of the Rv0081 transcription factor. We also aim to obtain crystals of complex between Rv0081 and cognate oligonucleotides and determine the structure of the complex.

The work describing chimeras of GroEL2 and *E. coli* GroEL is in the publication stage. A few remaining experiments such as prevention of model substrate aggregates, refolding assays, and ATPase activity measurements of the various mutants will be undertaken.

We also propose to start reconstituting the ribonucleotide reductase NrdE:NrdF2 complex. These two genes have already been cloned. Their copurification might be expected to yield the complex of the two proteins in correct stoichiometry.

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

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Background

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

Aims and Objectives

1. Role of viral regulatory proteins Tat and Nef in HIV pathogenesis.
2. Differential gene expression studies to identify molecular mechanism of HIV-1 induced T-cell apoptosis.
3. 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

The Human Immunodeficiency Virus Type 1 encodes a 27 KDa protein, Nef, which has come a long way from being termed as a negative factor to being one of the most important proteins of HIV-1. However, its role in HIV-1 replication and gene expression remains to be clearly understood. Although

involvement of heat shock proteins in viral pathogenesis has been reported earlier, a clear understanding of their role in viral replication and infectivity remains to be elucidated. We have earlier shown that Hsp40 and Hsp70 reciprocally regulate HIV-1 gene expression and replication. We have also shown that HSF-1 positively regulates HIV-1 gene expression and replication by two distinct pathways. Firstly, along with Nef it increases HSP40 expression that promotes viral gene expression and replication. Secondly, HSF1 directly interacts with a novel HSF-1 binding sequence in the HIV-1 LTR promoter and induces viral gene expression and replication. We have now initiated a comprehensive study of all the HSP protein family members during HIV infection. Each family is represented by different HSP members and their isoforms, encoded by different genes. In order to identify various HSP family members that are modulated during HIV-1 infection, we have performed PCR array analysis for HSP family members using RNA isolated from HIV-1 infected CEM-GFP cells. Our results indicate that a significant number of genes belonging to HSP40 and HSP70 family are differentially expressed during infection. While expression of most of the HSP60 members remains unchanged during infection, few members of HSP90 and HSP100 family seem to be modulated. Further characterization of the individual role of these isoforms in HIV-1 infection is currently in progress. We have also looked at the role of different HSPs in viral replication and infectivity by knocking down individual HSPs and analyzing viral production and infectivity. Our preliminary results suggest that several HSPs play an important role in viral replication and infectivity. Further analysis of their role is currently in progress.

We have also initiated studies on an HSP70 binding protein; HspBP1. 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 HspBP1 can regulate various HSP70 activities; so it's worth studying the role of HspBP1 during HIV-1 infection, if any. Expression of HspBP1 gets down-modulated during HIV-1 infection in T-cells and PBMCs. Furthermore, silencing of HspBP1 seems to increase HIV-1 gene expression whereas its over-expression leads to inhibition of HIV-1 replication. Our results also indicate that over-expression of HspBP1 significantly reduces LTR-driven gene expression while silencing enhances it. Our recent studies also suggest that HspBP1 restricts the translocation of HSP40 in to the nucleus and thereby inhibits viral replication. Further studies are in progress to elucidate the mechanism.

The major problem in defining Nef function has been its possible involvement in multiple pathways and its pleiotropic role in HIV-1 life cycle. Nef directed regulation of transcription can be best visualized when HIV infection is allowed to progress in presence or absence of Nef. Definite role of Nef in enhancing viral replication is evident from our results where Δnef HIV infected cells produced less virus as compared to WT infected cells. When we compared the gene expression profile of WT and Nef deleted virus infected cells using microarray, we found a large number of genes differentially modulated in these cells. Gene expression profiling comparison shows differential regulation of genes belonging to most of the biological processes including receptor activity and cell signaling, regulation of gene expression, TCR pathway, targets of miRNA etc. Nef induces all these changes to favor HIV-1 infection progression. We are currently trying to elucidate the mechanism of Nef mediated regulation of transcription.

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 responsive region, which results in recruitment of positive transcription elongation factor b (P-TEFb) complex to the LTR promoter. The P-TEFb 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. Earlier we have shown that direct binding of Tat to the NFκB enhancer sequences on the LTR promoter as one of the mechanisms underlying TAR independent transactivation. We have also studied the genome wide occupancy of Tat protein on host cell chromatin by ChIP-on-chip analysis in HIV-1 infected T cells to look for a potential role of Tat on cellular gene expression. We have identified a number of genes, which show a significantly high localization of Tat protein in HIV-1 infected T-cells, a majority of the recruitment being observed on gene promoters. Tat was identified as a repressor of c-Rel in the present study as it down regulates expression of c-Rel in HIV-1 infected cells. We have shown that Tat down regulates c-Rel promoter activity by interacting with its specific NFκB sites. 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. We are currently trying to study mechanism of regulation of other cellular genes by Tat protein.

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. Although evidences pointing towards the importance of mitochondrial energy generating system in apoptosis exist, its exact role remains to be clearly understood. Our earlier results show differential regulation in activities of OXPHOS system complexes leading to modulation of host cell energy generating system during HIV infection that ultimately leads to T cell apoptosis. Furthermore, HIV-1 infection is known to be associated with the hijacking of a number of cellular factors including the cell cycle associated molecules. The subversion of the host cell cycle during HIV-1 infection progression includes arresting of the normal cell cycle at mitosis, specifically at the boundary of the G2-M phase. Although many studies validate that Vpr and Vif is involved in causing the G2-M arrest associated with HIV-1 infection, not much information is available related to other viral and cellular protein interactions that might be crucial in the background. We have now initiated studies intending to look into the role of cell-cycle associated proteins in HIV-1 pathogenesis. In this direction, we have performed differential gene expression analysis using PCR array specific for cell cycle associated genes. Our results show differential gene expression of a significant number of cell cycle associated genes. A number of these modulations have been validated and a few of them are being currently studied in detail to identify their role in HIV-1 pathogenesis and T-cell depletion.

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 involved in identification of novel anti-HIV molecules and study of their potential use as microbicides. We have also been working on structure based development of new Integrase inhibitors in collaboration with Birla Institute of Technology, Mesra and seem to have identified a couple of novel inhibitors, which are being characterized at present. Finally, we have also started screening of a library of pharmacologically active bio-molecules which are known to target cellular pathways for identification of

novel anti-HIV molecules, with ultimate objective to identify novel cellular targets for inhibition of HIV-1. 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 family members in HIV-1 replication and pathogenesis, which will provide us a comprehensive knowledge about the role of different HSPs during HIV infection. We are also planning to identify the specific role of different HSP isoforms during HIV-1 infection. We are continuing characterization of several new Nef interacting host cell proteins identified previously by yeast two hybrid screening for their functional relevance in HIV lifecycle. Furthermore, we also plan to characterize the mechanism of Tat mediated regulation of gene expression of some of the cellular genes identified by genome wide recruitment analysis of Tat protein in HIV-1 infected cells. 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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Host Parasite Interaction: Genome annotation of *Leishmania donovani*

Background

Leishmania donovani is a kinetoplastid protozoan parasite that causes a deadly disease visceral leishmaniasis or kala-azar and it is transmitted by the phlebotomine sand fly vector. Sequencing efforts to elucidate the genome of kinetoplastid protozoan parasites started off with the sequencing of complete genome of *Leishmania major* in 2005. In 2007, two other *Leishmania* parasites namely *L. braziliensis* and *L. infantum* were sequenced. Sequence comparison of the genomes of *L. major*, *L. infantum* and *L. braziliensis* species show that they have roughly similar number of genes, protein coding regions and these genes show high degree of conservation in terms of content and gene synteny across the genus. *L. donovani* causes genome was sequenced much later in 2011 as compared to other *Leishmania species*. The sequencing of *L. donovani* genome resulted in the identification of 8,195 genes of which 8,032 are protein coding genes and 51 are pseudo genes. Upon availability of *L. donovani* genome sequence, we have searched our *L. donovani* proteomic data against the newly available protein and six-frame translated genome data of *L. donovani*. This study resulted in identification of 3,999 proteins (50% of *L. donovani* proteome). Additionally, we also identified 20 novel genes previously missed by the genome sequencing group and corrected annotations of 40 genes in *L. donovani*.

Aims and Objectives

Annotation of "partial genes" from *L. donovani* genome.

Participants

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Collaborators

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Work Achieved

A preliminary analysis of the 8032 protein coding genes resulted in the identification of 334 partial protein coding genes which were truncated mostly due to existence of gap resulting from missing nucleotide information arising during genome sequencing. Each of the partial protein coding gene, was individually analyzed for presence of gaps downstream or upstream of the coding sequence. In this analysis, we have excluded previously identified 26 C-terminal extensions and have used a bioinformatics approach to refine annotation of remaining 308 partial genes. Among the 308 partial genes identified in *L. donovani*, we categorized them into C-terminal extensions which account for major fraction and remaining were categorized as overlapping genes.

Our previous comparative proteogenomic study mapped the *L. donovani* proteome based on homology with related *Leishmania* species (*L. infantum*, *L. major* and *L. braziliensis*). Various peptides, derived from our mass spectrometry study, were identified and sorted out, which did not map to the current protein database of *L. donovani*. Further analysis was carried out by searching these peptide data set against the orthologues of these partial *L. donovani* genes. We found out that these peptides map to the C-terminal extended region in *L. infantum* or *L. major* orthologs. This goes on to prove that full length proteins should be expressed and exist in *L. donovani*. The next major category into which the partial genes in *L. donovani* grouped was overlapping genes. We identified 66 overlapping genes of the 308 partial genes in *L. donovani*. We also refined genome annotations in *L. donovani* by identifying longer full length ORFs that had been wrongly annotated as two separate genes in the current genome annotation of *L. donovani*.

Future Research Plans

To confirm the annotation, performing sequencing of the gaps present in the genome of *L. donovani*.

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Studies on Experimental Cerebral Malaria

Background

Malaria caused by *Plasmodium falciparum* is a major cause of morbidity and mortality in tropical and subtropical regions of the world. Despite intense research efforts, there is currently no vaccine that protects against the blood stage of malarial infection. The parasite causes different clinical manifestations including mild malaria, severe malaria, cerebral malaria, multi-organ failure, placental malaria and asymptomatic malaria. The outcome of the pathophysiology depends upon several factors, including the strain of the parasite and the infected person's immune status. The sequestration of parasite infected erythrocyte to endothelial cells of brain blood capillaries, rosetting of p-RBC and infiltration of CD8+ cells in the brain are implicated in experimental cerebral malaria (ECM). Cerebral malaria (CM), a severe manifestation of *Plasmodium falciparum* infection, can lead to neurological complications and death. Murine models of malaria have become important tools for studying the pathogenesis of malaria, with numerous clinical and histopathological similarities between human and murine malaria having been described. A number of significant changes in the murine brain that characterize CM have been described, including breakdown of blood-brain barrier, apoptosis of endothelial cells and metabolic changes.

Aims and Objectives

1. Studies on host-parasite interactions
2. Studies on murine malaria pathogenesis
3. Studies on effect of immune modulators on ECM
4. Studies on parasite-macrophage interaction

Participants

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Collaborators

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Work Achieved

We have investigated the expression of certain important molecules (CXCL-4, CXCL-10, VEGF, PGDF, IL-1Ra, IL-8, MIP-1B, sFAS-L, FAS-L, sTNF-R1) at mRNA levels in the brain tissue of C57BL/6 mice infected with 10^6 *P. berghei* ANKA parasites, at different stages of infection (asymptomatic, symptomatic non-ECM and ECM). The brain tissue was isolated from different stages of ECM developing mice and RNA was extracted from it. The expression of different molecules at mRNA levels was investigated by RT-PCR.

CXCL4 was found to be upregulated in ECM mice. The expression of this molecule was not seen in other animals. A gradual increase was observed in the expression of CXCL10 mRNA from the stage of symptomatic malaria to that of ECM-developed mice. The VEGF-2 expression was not prominent in any group, with the mRNA expressed at moderate levels in all the samples tested (asymptomatic, symptomatic, non ECM & ECM mice). MIP-1 expression was observed from the asymptomatic stage and increased in ECM-developed mice. The IL-1R molecule was found to be expressed from (control) asymptomatic to ECM developed mice. Though i-NOS was expressed in asymptomatic mice, it was not so significant, and it was not expressed at all in the animals at all the other stages. TNF R-2 expression was observed in all the samples, including controls. Soluble FAS showed a gradual increase in the brain from the asymptomatic to the ECM stage. The FAS-L ligand was also expressed in all the samples.

The expression of CXCL-10, MIP-1, sFAS and IL-1R molecules gradually increased from asymptomatic through symptomatic non-ECM and ECM mice, while CXCL4 was upregulated only in ECM mice and not in the other animals. We also found increased levels of IgM autoantibodies against normal brain antigen in ECM mice sera.

Future Research Plans

1. Studies on serum-level cytokines and chemokine levels in ECM developing mice.
2. Studies on interactions of parasitized RBC with macrophages.

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

Background

In women, breast cancer is the most common malignancy and the second most common cause of cancer-related mortality. It accounts for 23% of all the cancer cases and 14% of all the cancer deaths. Breast cancer incidences in India increasing at a rate of 3% per year and by 2020 the cases may increase to 100,000. Three subtypes of breast cancer are identified ER and PR positive, HER2 positive and triple negative. As yet very few drugs are available for breast cancer, which remains a major cause of morbidity and mortality in women. Early diagnosis of breast cancer improves the likelihood of successful treatment and can save many lives. But, it requires successful strategies for early detection and screening of the disease. However, current techniques like mammography to detect breast tumor has intrinsic limitations. Thus early diagnostic biomarkers are critically important for detection, diagnosis, and monitoring disease progression in breast cancer. There is an urgent need to discover novel biomarkers of breast cancer for early detection and diagnosis. Quantitative proteomic and metabolomic profiling of body fluids, tissues, or other biological samples to identify differentially expressed proteins/metabolites represents a very promising approach for discovering novel potential biomarkers. Proteins and metabolites associated with breast cancer identified through proteomic and metabolomic profiling technologies could be useful as biomarkers for the early diagnosis, assessment of prognosis, prediction of therapeutic effect and treatment monitoring.

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Mass spectrometry (MS) based proteomic and metabolomic technologies have experienced major improvements in recent years for simultaneous analysis of thousands of proteins and metabolites on the basis of differences in their expression levels which are actually involved in cancer progression. Such rapidly developing technology enables us to discover novel cancer biomarkers to a greater extent. Such biomarkers may have broad applications, such as early indication of disease, monitoring of disease progression, and monitoring of drug targets.

Already, several putative breast cancer biomarkers with potential clinical applications have been reported using proteomic technology. Estrogen receptor, progesterone receptor, Her-2/neu, CA125, CEA, CA15.3 are among reported biomarkers and have been widely accepted for routine clinical use. These biomarkers have been serving as prognostic and predictive markers for targeted therapy. However, their detection sensitivity, and specificity to predict metastasis potential is limited. With reported breast cancer biomarkers, much is left to be desired in terms of clinical applicability. We need novel breast cancer biomarkers that will further enhance our ability to diagnose, prognoses, and predict therapeutic response. Since biomarkers can be analyzed relatively noninvasive methods and are economical as compared with other expensive techniques, hence it is worth to investigate novel biomarkers. In this work, we plan to identify novel potential biomarkers for breast cancer using high throughput mass spectrometry based proteomic and metabolomic approaches in Indian scenario. In addition, identified biomarkers will be subjected to bioinformatic tools to understand the various physiological pathways.

Aims and Objectives

1. Identifying novel potential biomarkers for breast cancer using proteomic approaches.
2. Identifying novel potential biomarkers for breast cancer using metabolomic approaches.
3. Understanding the various physiological pathways and disease pathogenesis of the identified novel potential biomarkers using bioinformatic tools.

Work Achieved

Comparison of breast cancer tissue proteome with its controls using complementary 2-DIGE and iTRAQ proteomic approaches

In this study, we investigated to identify breast cancer involving proteins using high-throughput quantitative proteomic approaches such as 2-D DIGE (gel-based approach) and iTRAQ (gel-free approach). The advantage of using two complementary techniques gives us more confidence about their involvement in breast cancer progression. Comparative proteome analysis was performed on 56 samples with different early grades and stages to identify grade specific and stage specific markers. 2-D DIGE data of tissue samples using discriminant extended data analysis shown 182 proteins and 200 proteins with 95% accuracy for grade-wise comparison and stage-wise comparison respectively which can be used for potential biomarkers (Fig. 1). Differentially expressed and statistically significant (Student's t-test; $p<0.05$) protein spots were identified by MALDI-TOF/TOF mass spectrometry. Quantitaive proteomic analysis based on iTRAQ ratio indicated 380 proteins and 524 proteins are significantly differentially expressed with 1% FDR for grade-wise comparison and stage-wise comparison respectively. In addition, the comparative data between two complementary techniques was also showed that most of the DIGE proteins were able to detect in iTRAQ data. The differentially expressed tissue proteins in breast cancer identified in this study were subjected to functional pathway analysis for better understanding of the biological context of the identified proteins and their involvement in various physiological pathways. Functional pathway analysis suggested the modulation of multiple physiological pathways including apoptosis signaling pathway, glycolysis, hormone receptor pathway,

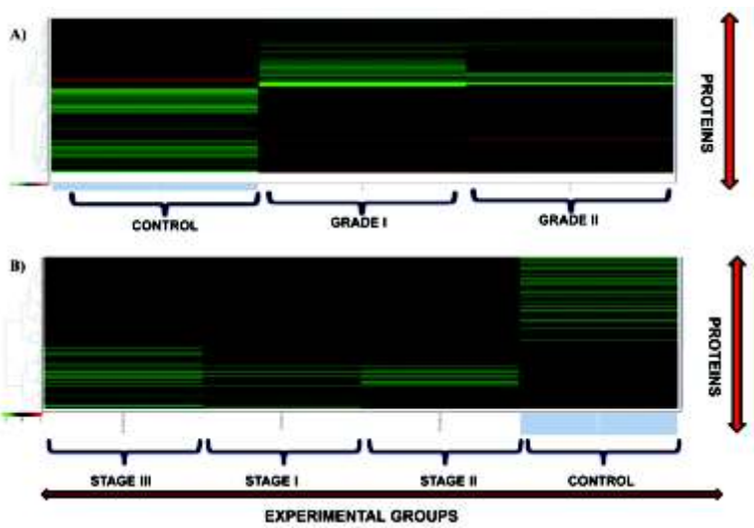

Fig. 1 : Hierarchical cluster analysis of breast cancer tissue proteome using decyder extended data analysis for protein vs. experimental groups A) grade-wise comparison, B) Stage wise comparison. Experimental groups are on X-axis.

and integrin signaling pathway. We are in the process of validation of these differentially expressed proteins to confirm as potential biomarkers.

Comparison of breast cancer serum proteome with its controls using 2-DIGE and iTRAQ proteomic approaches

The aim of this study was to perform a comparative serum proteome analysis of breast cancer patients and healthy subjects to identify the differential expression pattern of serum proteins in breast cancer. A comprehensive serum proteome analysis was performed on 56 samples (40 malignant, 8 benign and 8 control) using 2-D DIGE and iTRAQ. 2-D DIGE data of serum samples using discriminant extended data analysis showed 28 proteins and 18 proteins with 95% accuracy for grade-wise comparison and stage-wise comparison respectively which can be used for potential biomarkers (Fig. 2). Differentially expressed and statistically significant (Student's t-test; $p<0.05$) protein spots were identified by MALDI-TOF/TOF mass spectrometry. Similarly, iTRAQ data of serum samples showed 53 proteins and 34 proteins with 1% FDR at grade-wise comparison and stage-wise comparison respectively. Validation experiments for these differentially expressed proteins are in the process.

Identification of key metabolites associated with breast cancer using mass spectrometry and NMR

While genomic and proteomic alterations have been extensively studied in breast cancer, the changes in metabolite levels have not been analyzed in detail to date. LC-MS and NMR were found to be powerful techniques to identify metabolites involving in various diseases. Comparative analyses of the

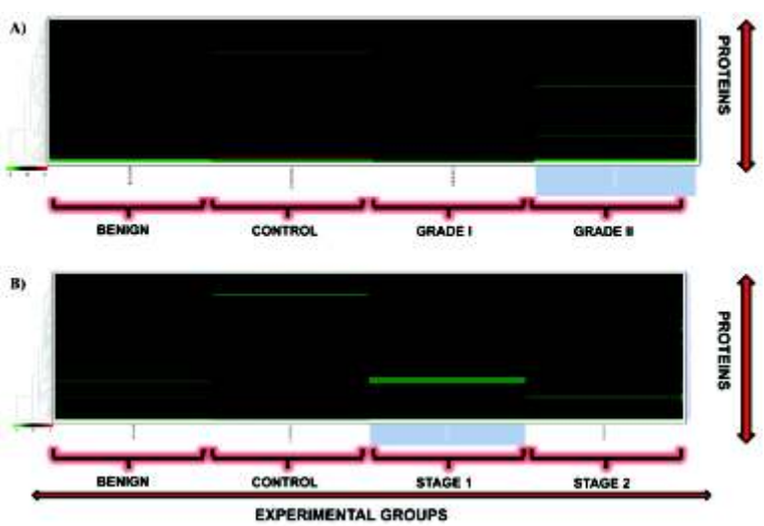

Fig. 2: Hierarchical cluster analysis of breast cancer serum proteome using decyder extended data analysis for protein vs. experimental groups A) grade-wise comparison, B) Stage wise comparison. Experimental groups are on X-axis.

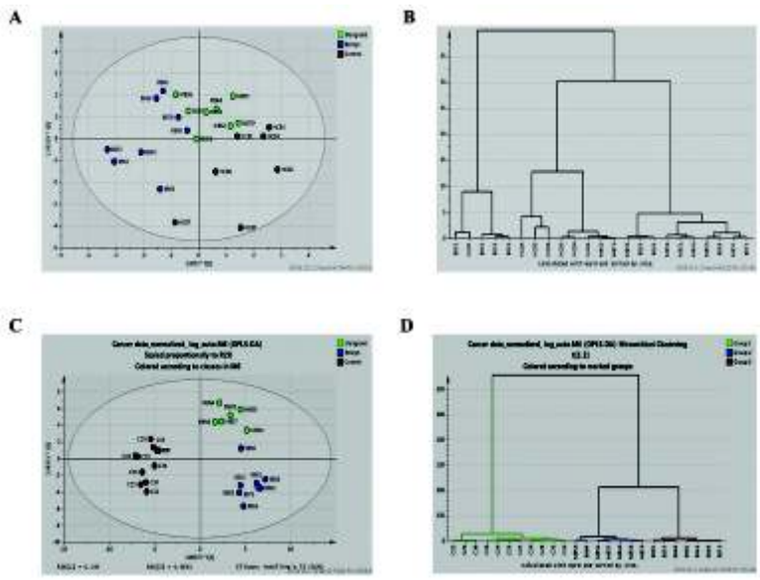

Fig. 3 : LC-MS and NMR based identification of metabolites involving in breast cancer. **A)** LC-MS based 2D scores scatter plot resulting after applying orthogonal-partial least squares discriminant analysis (OPLS-DA), **B)** LC-MS based hierarchical clustering model based on the basis of differences between metabolite expressions. **C)** NMR based 2D scores scatter plot resulting after applying orthogonal-partial partial least squares discrimination analysis (OPLS-DA), **D)** NMR based hierarchical clustering model based on the basis of differences between metabolite expressions.

metabolome of normal, benign and malignant serum samples can provide a better understanding of the molecular events involved in tumor development, and are essential for early detection, disease diagnosis and prediction of response to therapy and/or drug target discovery. In this study, we performed a comparative serum metabolome analysis of breast cancer patients and healthy subjects to identify the differential expression pattern of serum metabolites in breast cancer. In order to understand what type of metabolites involving various cancers, first we thoroughly searched the literature and made the list of metabolites involved in various cancers. From the list of metabolites, we purchased the standards (more than 100 metabolites) and made the in-house library in our quantitative mass spectrometer using MS spectrum and MS/MS spectrum. For these 100 metabolites, we developed MRM based methodology for identification and quantification using different chromatographic columns. LC-MS/MS data suggest that L-Homoserine, L-Tryptophan, Taurine, Inosine, L-Threonine, L-Citruline, and L-Serine were observed to be up-regulated significantly whereas L-Glutamine, Betaine, L-Tyrosine, Xanthine levels were significantly down-regulated in breast cancer samples as compared to benign and healthy controls. Several amino acids, organic acids and other molecules showing differential expression in serum of breast cancer were identified using LC-MS/MS metabolic profiling. Orthogonal-partial least squares discriminant analysis (OPLS-DA) of identified metabolites was employed to detect intrinsic clustering and possible outliers (Fig. 3). NMR data provides Phenylalanine, Glycerol, Lactic acid, Histidine, Tyrosine were observed to be increased significantly whereas Creatinine, L-Glutamine, Proline, Citric acid, Isopropyl

alcohol, Creatinine, Mannose levels were significantly decreased in breast cancer samples as compared to benign and healthy controls. Several amino acids, organic compounds, fatty acids and other molecules were identified in serum using 1H NMR metabolic profiling. Orthogonal-partial least squares discriminant analysis (OPLS-DA) of identified metabolites was employed to detect intrinsic clustering and possible outliers (Fig. 3). Multivariate statistical analysis revealed that the cancer group could be clearly distinguished from the control groups on the basis of their metabolomic profiles, even when the benign control group was included. We are in the process of further extensive metabolomic profiling with tissue samples to confirm as potential metabolite biomarkers.

Future Research Plans

1. Validating identified differentially expressed proteins using western blotting and MRM based LC-MS/MS.
2. Identification of metabolites involving in breast cancer using tissue samples.
3. Identification of volatile metabolites involving in breast cancer using GC-MS.
4. Integration of metabolomic data with proteomics data for understanding the various physiological pathways and disease pathogenesis using bioinformatics.

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Anti-VEGF antibody enhances the anti-tumor effect of CD40

Background

As its central immunomodulatory effects, activation of CD40 with agonistic anti-CD40 monoclonal antibody activated dendritic cells to secrete IL12, which in turn activated T cell-mediated anti-tumor immune response and tumor regression. As its local pro-tumor effects, CD40 induces the expression of vascular endothelial growth factor (VEGF) that promotes tumor angiogenesis and growth. Therefore, we examined if the anti-tumor functions of CD40 are self-limited by VEGF induction.

Aims and Objectives

To enhance the anti-tumour immune response of anti-CD40 agonistic antibody by co-treatment with anti-VEGF neutralizing antibody.

Work Achieved

We addressed the self-limiting anti-tumor effects of CD40 using a previously established mouse tumor model. We treated the tumor-bearing mice with the agonistic anti-CD40 antibody or neutralizing anti-VEGF antibody-alone or in combination. We found that anti-VEGF antibody significantly enhanced the anti-tumor effects of the anti-CD40 antibody, observed through increased survival of the mice, accompanied by reduced angiogenesis, higher T-cell proliferation in response to tumor antigens, increased IFN-γ production and tumor cell cytotoxicity.

Participants

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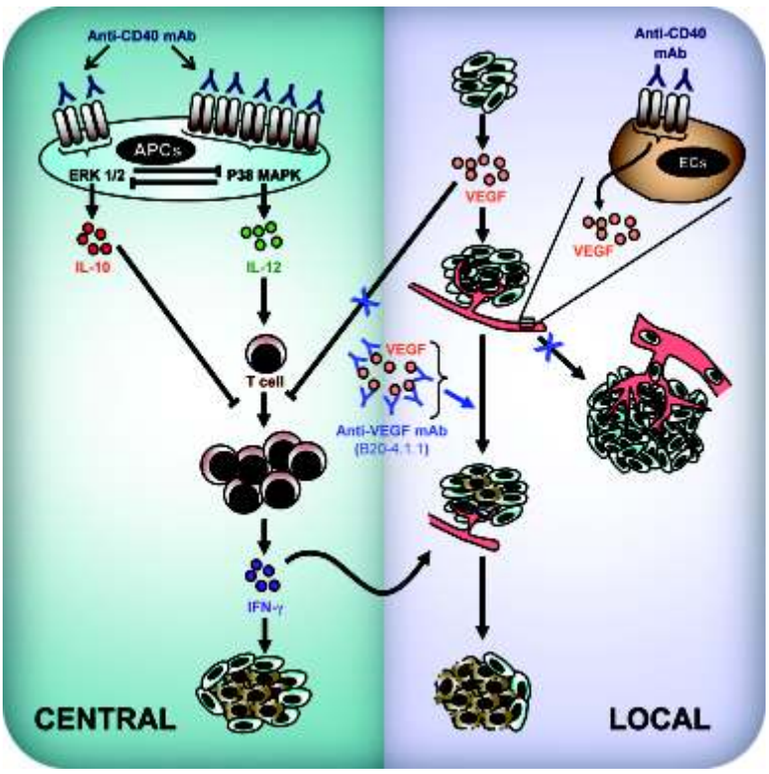
Fig. 1: Simultaneous augumenation of the CD40-induced anti-tumor immune response and neutralization of CD40-induced VEGF production is found to enhance the anti-tumor functions of CD40.



Future Research Plans

Our future plan is to decipher the detailed mechanisms involved in this counteractive function of CD40 in anti-tumor immune response.

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Understanding molecular basis of viral complement regulation

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Background

Given that viruses are obligate intracellular parasites they are exposed to constant host-induced pressures, which drive adaptations. On that account, viruses have developed mechanisms to subvert various key host immune barriers including humoral and cellular responses as well as the effector functions including the complement system and thus, are dubbed as "masterpieces of evolution".

It is often argued by immunologists that if viruses subvert a particular immune response, then this provides a stronger evidence of participation of that component in controlling these entities. This is clearly true for the complement system as studies performed thus far have decisively demonstrated that complement annuls viruses by direct as well as indirect mechanisms. The direct mechanisms include neutralization by aggregation, opsonisation, lysis, and promotion of phagocytosis through complement receptors, while the indirect mechanisms include boosting of the protective acquired immune responses. Concerning counteradaptations for evading the host complement system, both DNA as well as RNA viruses have devised a series of strategies which include encoding structural and/or functional mimics of host complement regulatory proteins, acquisition of host complement regulatory proteins, inhibition of complement synthesis, upregulation of host complement regulatory proteins on the infected cells and use of complement receptors for cellular entry. As expected, the mimics of complement regulatory proteins are found only in viruses with larger coding capacity like poxviruses and herpesviruses. These viruses have been found to express homologs of

regulator of complement activation (RCA) as well as non-RCA (CD59 and HSV gC) proteins. Our laboratory focuses on molecular characterization of viral homologs belonging to the RCA family (vRCA) with the expectation that it would not only provide new insight into host-virus interplay, but would also exemplify novel features of complement regulation that are central to the biology of complement.

Aims and Objectives

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

Work Achieved

Importance of N-linked glycosylation in vRCA

Earlier our laboratory has shown that Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) encodes a structural and functional homolog of the human RCA protein, which was named as Kaposica. It is composed of four complement control protein (CCP) modules, which are followed by a dicysteine motif, a serine/threonine (S/T)-rich region and a transmembrane domain. Functional characterization of the protein revealed that it inactivates C3-cleaving enzymes termed C3-convertases, which are the central enzymes of complement pathways formed during the initial steps of complement activation. Hence, inactivation of these enzymes results in the complete shutoff of the activation cascades, including the formation of the membrane attack complex and generation of the inflammatory peptides C3a and C5a.

Interestingly, like members of the human RCA proteins, Kaposica also contains potential N-linked glycosylation sites. The putative N-glycosylation sites however are restricted to and conserved in herpesviral RCA homologs, while the poxviral homologs lack these sites, except for the vaccinia virus complement control protein (VCP) from the smallpox vaccine strain LC16m8, which has only one N-glycosylation site. What role the N-glycosylations impart in the complement regulatory function of herpesviral complement regulators is still not clear. We therefore, employed Kaposica as a model protein to decipher the role of N-glycans in the function of herpes viral RCA regulators. Because viral complement regulators exist in both soluble and membrane-bound forms, we chose to examine the influence of N-glycans on the complement regulatory activities of soluble (CCPs 1-4) as well as on the membrane-bound (full-length) form of Kaposica. To generate the N-glycan deletion mutant, putative N-

glycosylation sites (N-X-S/T) located within CCP1 (N44), CCP2 (N92) and CCP4 (N236) of the Kaposica gene were mutated to glutamine (Q) using a commercially available site-directed mutagenesis kit (Stratagene) and then the soluble/membrane-bound forms were subcloned into the mammalian expression vector and expressed in desired human cells.

Kaposica inactivates C3-convertases owing to its decay-accelerating activity towards classical pathway C3-convertase (accelerated decay of C3-convertases) and cofactor activity (inactivation of C3-convertase subunit C3b and C4b with the help of protease factor I) towards C3b and C4b. We therefore assessed the role of N-glycans on these activities of soluble Kaposica. Further, we also probed if the N-glycans affect the target-binding ability of soluble Kaposica. The results showed that the N-glycan deletion mutant was as efficient as the wildtype protein in decaying the C3-convertase, inactivating C3b and C4b, and recognizing the targets. Thus, the data obtained indicate that N-linked glycans do not influence the complement regulatory activities of soluble Kaposica. Next, to evaluate the role of N-glycans in the complement regulatory functions of membrane-bound Kaposica, we expressed the wildtype transmembrane form, and its N-glycan deletion mutant, on the Chinese hamster ovary (CHO) K1 cells and compared the cytoprotective ability conferred by these molecules against complement-mediated lysis (Fig. 1). The wildtype Kaposica significantly protected the CHO cells from lysis, which was more apparent at higher serum dilutions. Intriguingly, transfectants expressing the N-glycan deletion mutant showed a significant loss in cytoprotective ability compared to the wildtype, suggesting that unlike the soluble form, the

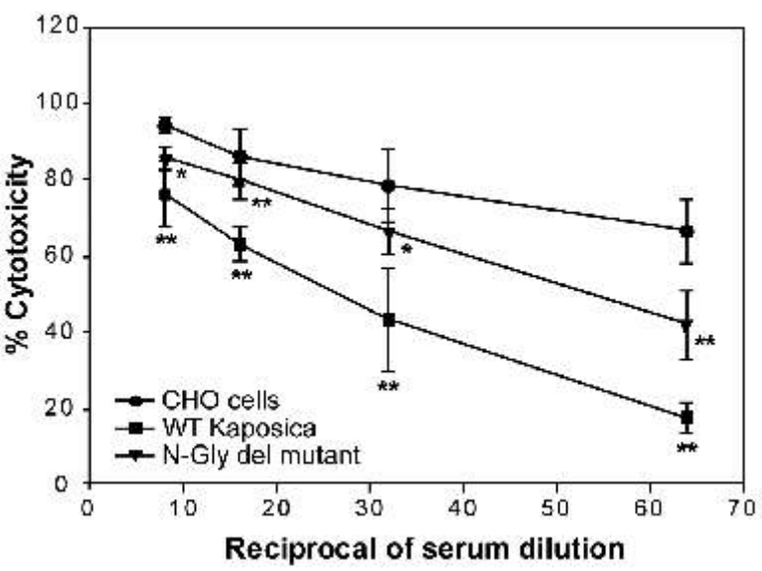

Fig. 1: Relative cyto-protective ability of Kaposica and its N-glycan deletion mutant. The experiment was performed three times in duplicate and results are expressed as mean + SD. CHO cells treated with antibody alone and serum alone were considered as background controls and untreated cells lysed with lysis buffer were considered as 100% for normalizing the data. Pairwise multiple statistical comparisons were performed between WT Kaposica and untransfected CHO cells, and between WT Kaposica and N-glycan deletion mutant transfected groups. *p < 0.05; **p< 0.001.

complement inactivation ability of the membrane form of Kaposica is greatly influenced by the presence of N-glycans (Fig. 1). We therefore suggest that the glycans in the membrane form of Kaposica interact with the cell membrane and orient the molecule in such a way that the glycan-free region is highly accessible for interaction with C3b and C4b, which results in increased complement inhibition at the cell surface.

Molecular basis of cofactor activity of vRCA

As described above, vRCAs inhibit C3-convertases owing to their cofactor activity for C3b and C4b as well as decay-acceleration activity for the classical/lectin pathway C3-convertase. Previously, we and others have dissected the relative contribution of each of the CCP modules of these proteins in imparting the above mentioned functional activities, but the precise structural determinants required for each of these activities have not yet been elucidated. In the present exercise, we therefore, utilized Kaposica as a model protein to fine map its structural determinants critical for imparting the cofactor activity against C3b and C4b. As a first step, we swapped each of its modules with the homologous modules of human regulator decay-accelerating factor (DAF) which possesses decay-accelerating activity, but lacks cofactor activity. Functional analysis of the mutants identified domains 2-3 as the critical domains for cofactor activity. Each CCP module is composed of about 60 amino acids and is characterized by four invariant cysteines. Thus to further narrow the region(s) necessary for cofactor activity, we swapped the inter-cysteine regions of domains 2-3 with the homologous regions of DAF. Characterization of these sets of mutants identified two inter-cysteine regions as crucial for this activity. Next, to identify the residues critical for this activity, we scanned the surface exposed residues of this region by site-directed mutagenesis. The results showed that two sets of residues are important for this activity: one that interacts with C3b, and the other that interacts with factor I (Fig. 2). This is consistent with the current model for cofactor activity which suggests that RCA bridges the target protein (C3b/C4b) and the protease factor I, and this bridging is essential for cleavage of the target molecule by the protease.

Recently, the structure of the N-terminal four CCP modules of factor H in complex with C3b has been solved. Thus to get a better insight into the likely regions of C3b, with which the first set of residues of Kaposica interact, we aligned the Kaposica sequence with that of human factor H (fH) and examined the C3b domains interacting with the corresponding residues of fH in the C3b-fH complex structure. The structure showed that these residues interact with the

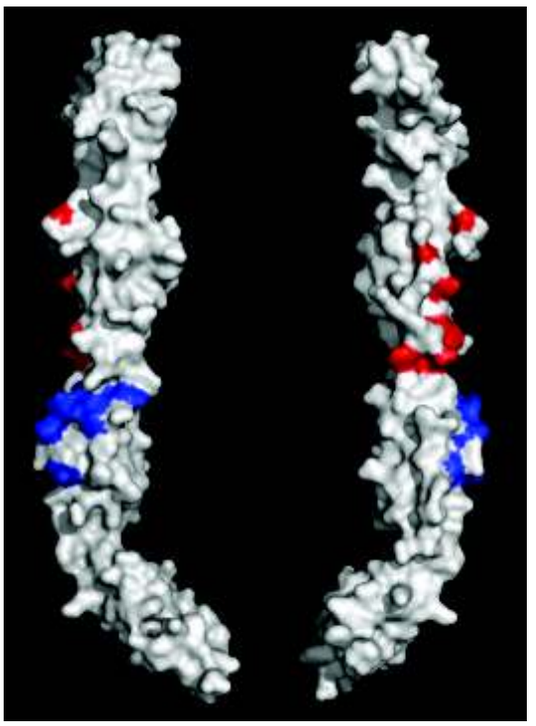

Fig. 1: Kaposica model showing sites important for its cofactor activity. Residues important for C3b/C4b binding are coloured in blue, while those critical for factor I binding are coloured in red. The left and right image of the protein represents 0° and 150° rotations about the vertical axis.

MG2 and CUB domains of C3b. Our data thus suggests that bridging of MG2 and CUB domains of C3b is essential for the cofactor activity of Kaposica. We propose that bridging of MG2 and CUB domains by Kaposica helps in stabilization of the CUB domain with respect to the core of the C3b molecule and this in turn enables the factor I to cleave the scissile bonds in this domain.

Future Research Plans

1. Fine mapping of functional sites in vRCA critical for its decay-accelerating activity.
2. Crystallization of vRCA molecules - alone as well as in complex with target proteins.
3. Designing pathway specific complement inhibitors.

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Protein phosphatase 1 regulatory subunit p90 functions as a tumor suppressor

Background

Cancer is one of the leading causes of death worldwide. It manifests due to uncontrolled proliferation of abnormal cells. Normal cells transform into cancer cells, as a result of activation of proto-oncogenes and/or inactivation of tumor suppressor genes. In addition, some of the kinases play a pivotal role in accelerating malignant transformation. Most of the kinases responsible for malignant transformation undergo activation through phosphorylation. Therefore, dephosphorylation of malignancy-promoting kinases by phosphatases would play an important role in controlling malignant transformation.

The serine/threonine protein phosphatase 1 (PP1) plays an integral role in the regulation of a number of major signaling pathways whose deregulation can contribute to cancer. The specificity and activity of PP1 are highly regulated through interaction with a number of regulatory subunits. These subunits determine the substrate-specificity for the complex. PP1 is ubiquitously expressed in all eukaryotic cells. PP1 is involved in a wide range of cellular processes, including meiosis and cell division, apoptosis, protein synthesis, metabolism, cytoskeletal reorganization, and the regulation of membrane receptors and channels. Each functional PP1 enzyme consists of 2 types of subunits: the catalytic subunit has the ability to dephosphorylate specific substrates, while the regulatory (R) subunits govern substrate-specificity. The regulatory subunits may be involved in targeting the catalytic subunit to specific subcellular compartments and may modulate substrate specificity, or serve as substrates themselves. Thus, the interactions between the catalytic subunits and specific R subunits are central to the function(s) of PP1.

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The existence of more than 100 regulatory subunits has been predicted in the human genome. Recently, CCDC8 / p90 has been shown to be an important regulatory subunit of PP1. It is deleted in human glioma and is found to be epigenetically silenced in renal cell carcinoma. Its expression is also associated with 3M syndrome. The above observations suggest that p90 may play a role in malignant transformation, though the exact role of p90 in cancer and its mechanism of action are yet unknown. In this study, we sought to determine the role of p90 in cancer using breast cancer as a model system.

Aims and Objectives

To determine:

1. Is p90 a potential Tumor SuppressorΔ
2. What is the molecular mechanismΔ
3. What is the physiological role of p90Δ

Work Achieved

Is p90 a potential Tumor Suppressor?

To check the tumor suppressor activity of p90, we overexpressed it in MCF7 cells and then tested its growth suppressive potential. A cell count assay was first performed to check the effect of p90 on cell viability. Cells were stained with trypanblue and counted with the help of a haemocytometer under the microscope. Less viable cells were observed upon overexpression of p90, as compared to control vector-transfected cells (Figure 1A). For example, p90 overexpression resulted in only 40% viable cells as compared to vector control on the fifth day (Figure 1A). To verify cell viability data obtained with the cell counting assay, the MTT assay was performed, which yielded similar results. For example, p90 overexpression showed 50% growth inhibition of MCF7 cells as compared to vector control transfected cells (Fig. 1B). The growth suppressive activity of p90 is further supported by colony formation assay (Fig. 1C). Collectively, the results suggest that p90 has growth suppressive activity, which

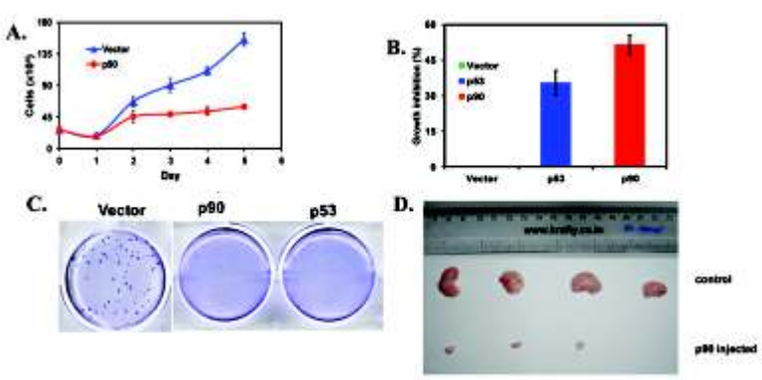

Fig. 1: Role of p90 in cell proliferation. A) Measurement of cell viability by cell count assay. Viable MCF7 cells were counted for both the vector and p90 transfected cells using trypan blue exclusion methods. B) Cell proliferation was determined using MTT after 48 hours of transfection. C) MCF7 cells form less colonies upon p90 overexpression. Colony formation assay was performed to evaluate potential growth suppressive activity of p90. D) p90 suppresses the tumor formation in Nude-SCID mice.

was further checked in vivo using Nude-SCID mice. Viable cells (5 X 10^6) with and without overexpressed p90 were injected in the right flank of mice and the progression of the tumor was monitored. We found that p90 overexpression lead to a significant reduction of tumor size as compared to control vector-transfected cells (Fig. 1D).

What is the molecular mechanism?

To understand how p90 suppressed cellular growth as well as retarded tumor growth in Nude-SCID mice, we asked whether the growth suppression effect of p90 was due to the induction of apoptosis. FACS was performed to analyse induction of apoptosis upon overexpression of p90. Our results demonstrated that p90 suppressed the growth of MCF7 by inducing apoptosis, at least in part (Fig. 2A). For example, 15% and 30% cells were found to be apoptotic upon overexpression of p90 for 48 and 72 hours, respectively (Figure 2A). Further, we found that p90 overexpression led to the induction of known proapoptotic markers like Bax, Puma etc. (Fig. 2B). We then asked why p90 overexpression led to the induction of apoptosis - was it due to an inhibition of activity of growth promotic kinasesΔIn order to find the pathway regulated by p90, we checked the activity of different kinases upon overexpression of p90. The results demonstrated that overexpression of p90 significantly reduced phosphorylation levels of components of the MAPK-Erk pathway. For example, phosphorylation levels of MAPK ½, ERK, and p38 were significantly reduced (Fig. 2C). A strong correlation was also observed between endogenous expression of p90 in higher grade breast cancer cell lines, which is highly suppressed, and upregulation of MAPK-ERK pathway components.

What is the physiological role of p90?

Our results demonstrated that p90 could be a tumor suppressor. Most of the tumor suppressors are found to have a role in maintaining genomic integrity. Therefore, we asked whether p90 too might have such a role. To find an answer, p90 stable knockdown MCF7 cells and scramble shRNA (NS)-mediated MCF7 cells were generated. The NS cells were used as control. Both these types of cells were exposed to gamma radiation and it was found that p90 KD cells were less

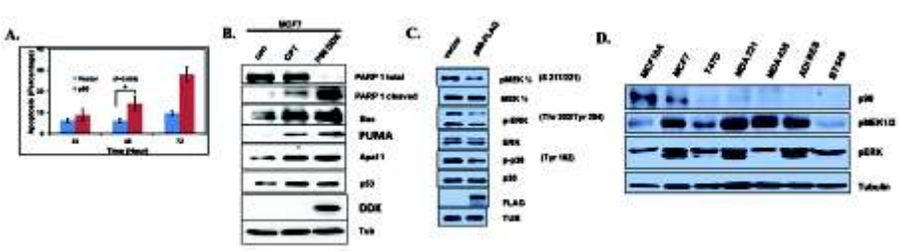

Fig. 2: p90 induces apoptosis through inhibition of MEK-ERK pathway. A) p90 significantly induces apoptosis . The Bar graph showing the relative percentage of dead/apoptotic cell populations upon p90 over expression compared to vector. B) Most of apoptotic markers are elevated in p90 transfected cells. C) p90 dephosphorylate the components of MAPK signalling pathway. D) Endogenous expression level of p90 in breast cancer cell lines along with MAPK ERK patheay components.

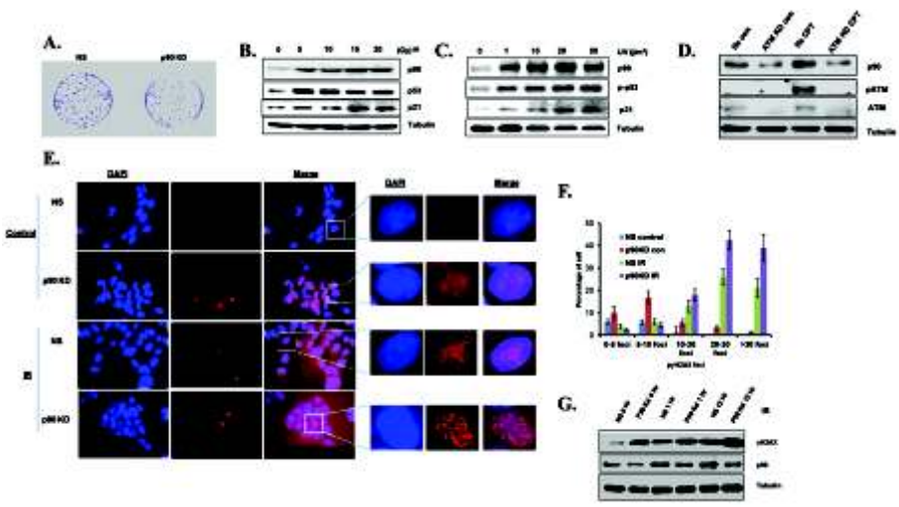

Fig. 3: Role of p90 in DNA damage response **A)** p90 stable knockdown cells are genomically unstable as compared to scrambled ShRNA knock down cells. p90 gets stabilized in both double stranded (IR) and single stranded (UV) DNA damage (panel **B** and **C**). **D)** ATM is necessary for p90's genotoxic stabilization, Stable cells lines of ATM knock down fails to show p90 stabilization. **E)** p90 stable knock down cells show more phospho-γH2AX compared to non-silencing cells. **F)** Graphical representation of differential apperance of pγH2AX foci in normal as well as DNA damage condition. **G)** Western blot showing phospho-γH2AX status in p90 KD cells compared to NS cells both in normal and DNA damaged conditions.

viable as compared to NS cells, indicating a probable role of p90 in genotoxic stress response (Figure 3A). Immunoblotting results showed that p90 was stabilized upon gamma irradiation (Fig. 3B), which induces mainly double-strand breaks in cells. To determine whether the stabilization of p90 is associated with double-strand breaks alone, the cells were exposed to ultra violet (UV) radiation, which mainly generates single-strand DNA damage. Interestingly, p90 was also found to be stabilized upon UV treatment, suggesting that p90 may play a role in both single- and double-stranded DNA damage response (Figure 3B &3C). In the classical DNA damage response pathway, most of the stabilized proteins are phosphorylated by two apical kinases, ATM and ATR. Therefore, we sought to investigate whether any of these kinases were involved in the stabilization of p90 upon exposure to genotoxic stress. For this purpose, stable knock down cells of ATM in MCF7 were generated and subjected to genotoxic stress. We found that in the absence of ATM, p90 failed to be stabilized upon gamma radiation, confirming that ATM is the upstream kinase regulating p90 stabilization following genotoxic stress (Fig. 3D). We then asked why p90 was getting stabilized upon genotoxic stress and whether it might have any role to play in the DNA damage repair process. One of the early events in DNA damage repair (DDR) is γH2AX phosphorylation, which is associated with the formation of nuclear foci containing factors that are essential for DNA damage repair, signalling and many other events. The appearance of p-γH2AX foci coincides with DNA double-stranded breaks and help in the recruitment of DNA damage repair machinery. As p90 stable knockdown cells were found to be less viable upon DNA damage, we examined whether p90-deficient cells were defective in DNA repair. Our results showed that cells with and without p90 showed significant differences in the appearance of p-γH2AX (Figure 3E), indicating a probable role of p90 in

maintaining genomic integrity through regulation of the DNA damage repair pathway. Furthermore, we quantified the γH2AX foci under normal and DNA-damaged conditions to analyse the kinetics of p-γH2AX appearance (Figure 3F), and found that p90 stable KD cells showed more foci as compared to NS cells under control and DNA-damaged conditions. p90 stable KD cells were also found to have more γH2AX foci as compared to NS cells in immunoblot studies (Fig. 3G), suggesting that p90 might be regulating the DNA damage repair machinery by controlling phospho-levels of γH2AX. Further experiments will be carried out to study this in detail.

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Translational Regulation of Insulin mRNA

Background

Insulin is a small peptide hormone secreted from the pancreatic beta cells and is important for glucose homeostasis in mammals. The deregulation of insulin biosynthesis leads to diabetes and subsequent abnormalities. Insulin expression begins at embryonic (e) 9.5 day in the gut endoderm. Insulin expression in β cells is regulated by many nutrients and glucose is the pre-eminent one controlling almost all cellular processes like transcription, splicing, translation, processing and secretion of insulin. Interestingly, insulin secretion is immediately followed by several fold specific increase in translation upon glucose stimulation. A number of studies have focused on the mechanism of glucose mediated insulin translation regulation and revealed the role the 5' and 3'un-translated regions (UTRs) and their trans-acting factors on insulin mRNA. In mouse, two non-allelic genes encode for insulin and specific splice variants from these genes have also been reported. Some splice variants have the altered 5'UTR and have differential translation efficiency and hence implicated to play some role in diabetes.

We have previously reported the expression of a novel splice variant that uses the splice site that is 12 bases downstream to the reported mouse insulin2 intron 1 resulting in shorter insulin 2 mRNA (named mIns2-S). This specific alternate splicing of insulin2 mRNA results in significant changes to the 5'UTR sequence and structure. The 5'UTR of the two mouse insulin2 mRNA isoform differentially bind to factors resulting in differential translation efficiency. We have also shown that PABP is one of the factors that preferentially associate with insulin 2L splice variant. In the present study we show that the middle region of

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the insulin 5'UTR associates with PABP. We further show that another protein HuD also associates with insulin mRNA. We further shows that isoform D of HuD was able to interact with insulin mRNA while isoform B was unable to do so. The exact role of such association is still under investigation.

Aims and Objectives

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

Work Achieved

Recombinant His-PABP associates with the middle region of insulin2 5'UTR

We had previously shown that recombinant PABP associates differentially with the long and short isoforms of the insulin 2 5'UTR. We wanted to identify the specific region in the 5'UTR region that associates with PABP. We synthesized 4 RNA that corresponds to the insulin mRNA 5'UTR divided into 4 equal parts (L1, L2, L3 and L4). We then used these fragments as competitors in RNA-EMSA that has the labeled longer 5'UTR and His PABP. The protein was incubated in presence of labeled probe and different molar excess of unlabeled RNAs. The specific complex that is formed with insulin long-5'UTR and His-PABP was very efficiently competed by the RNA corresponding to the region L2 (Fig 1). This region is common in both long and short 5'UTR thus suggesting that the differential binding of PABP to the long and short 5'UTR RNA observed is due to

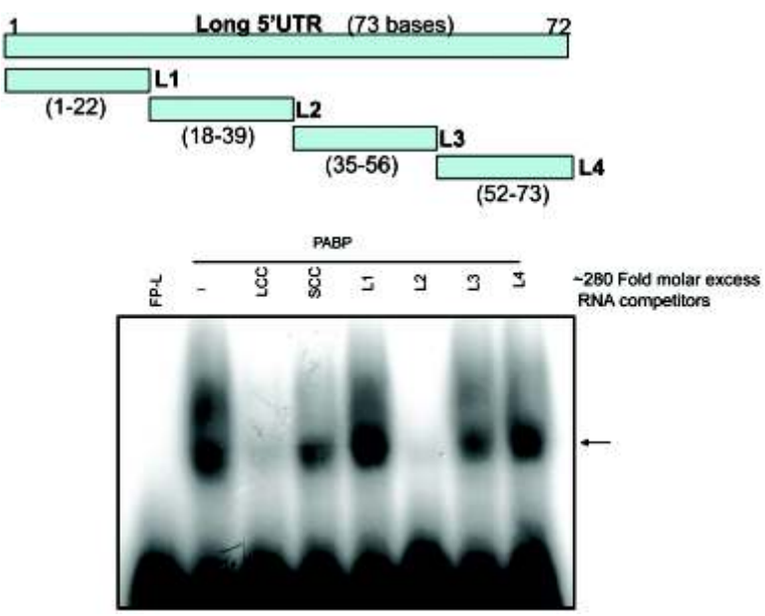

Fig. 1: Recombinant PABP associates with L2 Region of the insulin 5'UTR. Schematic representation of insulin2 5'UTR (Top). His tagged recombinant PABP was expressed in E. coli and purified using Nickel NTA column. 500 ng of the purified PABP was incubated with long insulin UTR probe in the presence of varying amount of the unlabelled competitor RNA (Lower). The specific competitor used is indicated.

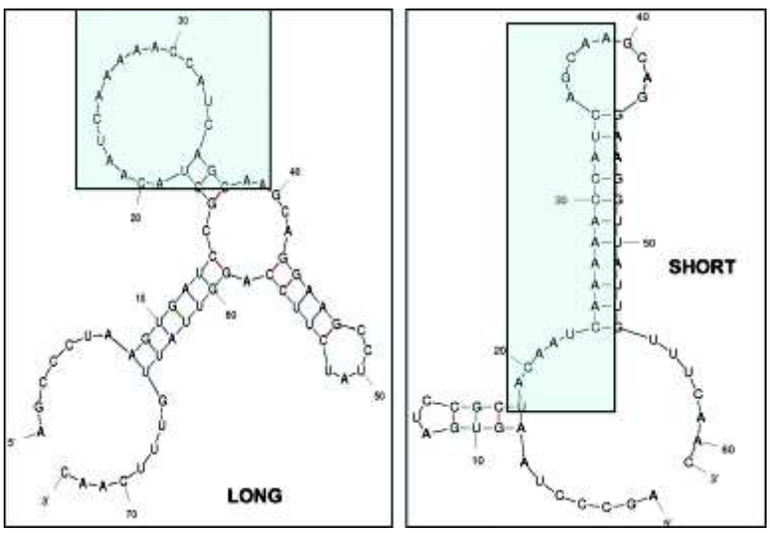

Fig. 2: Predicted secondary structure of the long and short insulin2 5'UTR RNA sequences. The region corresponding to L2 is shaded in blue color.

the secondary structure differences in the whole UTR context (Fig 2). We then confirmed the binding of L2 region with His-PABP by RNA-EMSA assay using radiolabel led L2 region as a probe (Fig 3)

Functional assessment of Recombinant His-HuD

We have previously shown that although PABP can specifically associate with insulin 5'UTR splice variants in a preferential manner but the binding of PABP alone is not sufficient to result in differential translation of the two isoforms. UV crosslinking experiments identified a predominant band about ~45kDa. We believed that the 45kDa protein is HuD. We cloned HuD from insulin producing cells. We cloned two different splice isoforms of the HuD (isoform B and D) and analyzed their insulin 5'UTR binding activity by RNA-EMSA. The B and C isoforms has different N terminal sequence compared to the A and D isoform. While the HuD B isoform did not interact with insulin 5'UTR but the D isoform

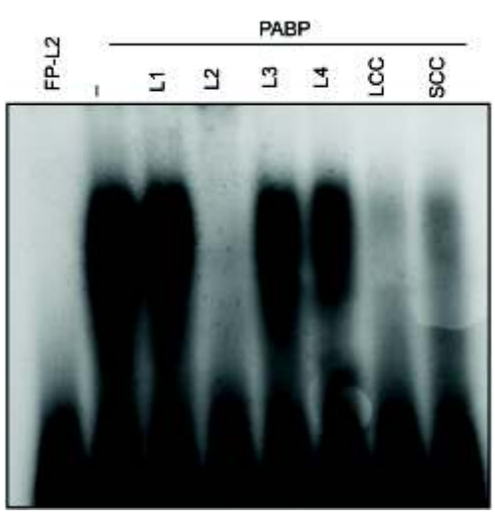

Fig. 3: Recombinant PABP associates with L2 Region of the insulin 5'UTR. His tagged recombinant PABP was expressed in E. coli and purified using Nickel NTA column. 500 ng of the purified PABP was incubated with labeled L2 region insulin UTR probe in the presence of varying amount of the unlabelled competitor RNA. The specific competitor used is indicated.

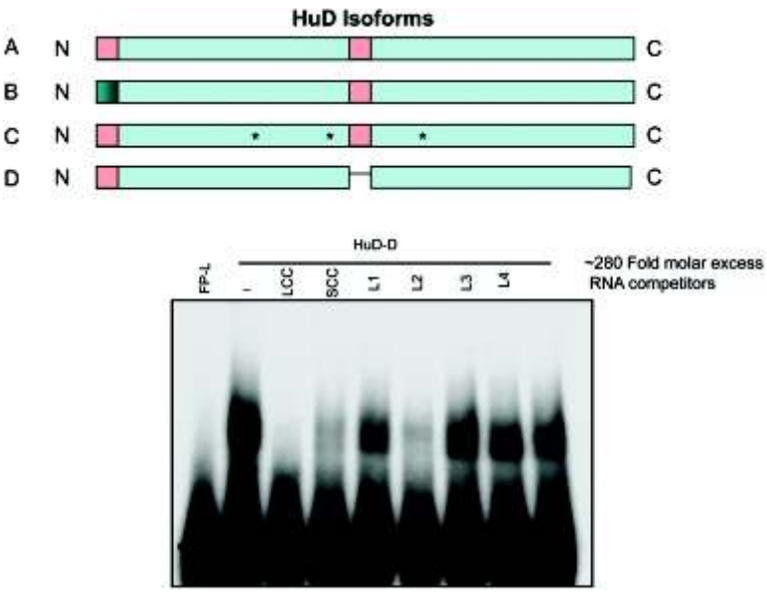

Fig. 4: Recombinant HuD-D associates with L2 region of the insulin 5'UTR. Schematic representation of the HuD isoforms (Top). His tagged recombinant Hud-D isoform was expressed in E. coli and purified using Nickel NTA column. 500 ng of the purified HuD-D was incubated with long insulin UTR probe in the presence of varying amount of the unlabelled competitor RNA (Lower). The specific competitor used is indicated.

specifically interacted with insulin 5'UTR (Fig 4). These results suggest that the N-terminal region of the HuD plays and important role in insulin mRNA recognition. Further this binding was to the L2 region of the insulin 5'UTR, however HuD also shows some interaction with the L4 region of the insulin mRNA. Thus our results indicate that HuD and PABP associate with the two splice isoforms of insulin mRNA in preferential manner and they may be responsible for the differential translation regulation of the splice isoforms.

Future Research Plans

Functional characterization of HuD and PABP and their interaction with insulin mRNA

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

Background

Glioblastoma multiforme (GBM) is the most common form of primary malignant gliomas and astrocytomas. As indicated by the name "multiforme", GBM is featured by marked cytological and histological differences and displays extensive genetic and biological heterogeneity. Extensive surgery followed by radiation therapy and chemotherapy offers hope of long-term survival for some patients with glioblastoma, but for most patients, the prognosis is poor. Extensive differences in gene expression are reported among GBMs, particularly in genes involved in tumor invasion, angiogenesis, immune cell infiltration, and extracellular matrix remodelling.

The mammalian Target of Rapamycin (mTOR) signaling network down-stream to EGFR/PI3K/AKT pathway regulates cell growth, proliferation, and survival. The central component of the pathway, the mTOR protein kinase, nucleates two distinct multi-protein complexes that regulate different branches of the mTOR network. The mTOR complex 1 (mTORC1) consists of mTOR, raptor (regulatory-associated protein of mTOR), PRAS40 and mLST8 (also known as GβL) and regulates cell growth translational machinery through effectors such as S6K1 and 4E-BP1. The mTOR complex 2 (mTORC2) contains mTOR, rictor (rapamycin-insensitive companion of mTOR), Sin-1 and mLST8, regulates actin cytoskeletal functioning (RhoA, Rac1), PKCα activity and the pro-survival kinase Akt/PKB by phosphorylating it on S473. The targeted inhibition of up-regulated mTORC1 pathway is efficient to some extent but is not sufficient to control growth and proliferation of cancer cells, therefore, mTORC2-mediated pathway and their mutual interaction should also be explored as target to control tumor growth.

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Rapamycin is an FDA approved drug that works through a gain-of-function mechanism in which it binds to the intracellular protein FKBP12 to generate a drug-receptor complex that binds to and inhibits the kinase activity of mTORC1. However, recently, it is demonstrated that on prolonged treatment, rapamycin suppresses the assembly and function of mTORC2 to inhibit Akt/PKB. Temisirolimus, FDA approved analog is the earliest developed water-soluble ester derivative of rapamycin. Both the drugs cross Blood Brain Barrier and are presently under phase III clinical trials to treat GBM. Torin-1 and PP-242 are potent and selective small molecule inhibitors that bind to ATP binding site of mTOR molecule. They inhibit mTORC1 and mTORC2 with equal efficiency and differ in the mechanism of rapalogs, as they can prevent cap dependent translational process.

Invasive capacity of tumors contributes to tumor recurrence and therefore is important to understand the biology of gliomas. Matrix Metallo-Proteinases (MMPs) play a significant role in regulating the invasion of tumors. MMP-2 and MMP-9 belong to gelatinases, most commonly attributed to invasive potential. They have differential mechanisms of regulation, so their expression varies in response to similar treatment. Paxillin is a member of focal adhesion proteins, which occurs at low levels in normal brain tissue and affects cell migration and motility in most cancers. Cross-talk between paxillin and Actin fibres causes reorganization of cytoskeletal networks and thus forms important components to understand mechanisms of invasion.

Aims and Objectives

To study the crosstalk between the mTOR complexes (mTORC1 and mTORC2) pathways using inhibitors- Rapamycin and its analogs in context with tumor micro-environmental factors such as TNF-α, IL1-β (cytokines), growth factors and MMPs.

To examine the potential of mTOR inhibitors on survival and invasion mediated by ECM components in human cell lines and primary cultures derived from GBM tumor.

Work Achieved

The study focuses on tumour micro-environmental factors such as cytokines (TNF-α and IL1-β) that induce PI3K/AKT/mTOR signalling and NFκB pathway. In our previous study, we reported that Rapamycin (RAP) and Temisirolimus (TEM) significantly reduced constitutive MMP-2 and TNF-α/ IL1-β - induced MMP-9

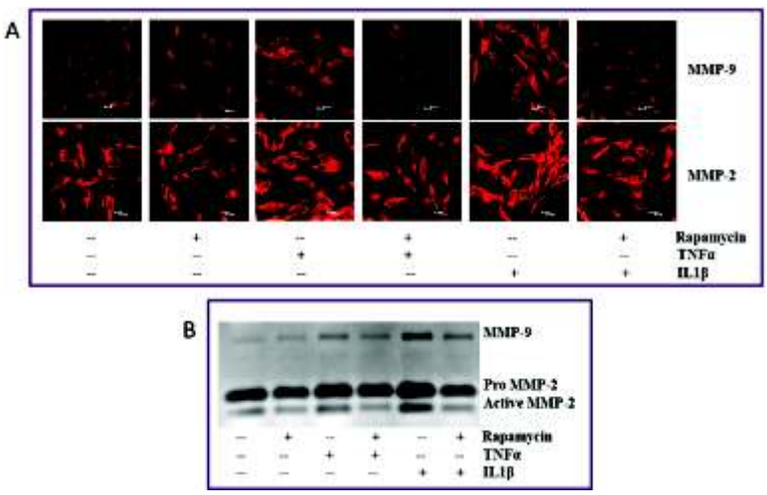

Fig. 1: Effect of Rapamycin (RAP) on MMP-9 and MMP-2 in primary cultures (GBM-1). Cells treated with RAP (10μM), were exposed to IL1β (10ng/ml) for 24h or TNFα (10ng/ml) for 12h before termination of time point. **A.** The cells were stained with primary antibodies to MMP-9 and MMP-2 and probed with fluorescent labelled secondary antibodies. (Mag.60X) and **B.** Gelatinolytic zymography analysis for MMP-9 and MMP-2 performed on conditioned media of cells.

in LN-18 cells. The study was extended to human primary cultures derived from GBM tumors. The response among the cultures was variable and could be attributed to different subtypes of GBM. Recent experiments done in one of the human primary cultures (GBM-1), showed that RAP caused reduction of protein expression and enzymatic activity of constitutive MMP-2 and TNF-α/ IL1-β -induced MMP-9 (Fig.1). In contrast to the human glioma cell line -LN-18 which showed MMP-2 as a single band, the conditioned medium of primary cultures showed two distinct bands representing pro- and -active forms of MMP-2. In addition to RAP and TEM we included other mTOR inhibitors Torin-1 (TOR) and PP-242. The findings revealed that TNF-α / IL1-β- induced MMP-9 was significantly reduced in LN-18 and GBM-1 (Fig.2). In addition to tumor micro-environmental factors, extrinsic signalling triggered by ECM components enhances invasive potential in cancer cells. mTORC2 is considered as a major mediator of Actin cytoskeletal regulation. Reduction of TNF-α-induced paxillin

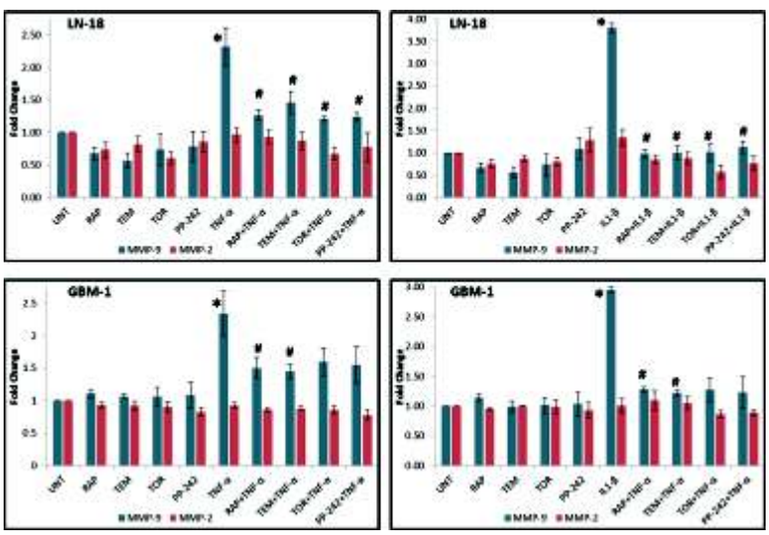

Fig. 1: Effect of m-TOR inhibitors on MMP-2 and MMP-9 activity. Gelatinolytic zymography analysis for MMP-9 and MMP-2 was performed on conditioned media from LN-18 (cell line) and GBM-1 (primary tumor culture) cells treated with RAP (10μM), TEM (5μM), TOR (100nM), PP-242 (100nM) for 3h and then exposed to TNF-α (10ng/ml) for 12h or IL1-β (10ng/ml) for 24h before termination of time point. Densitometric analysis using ImageJ software was performed to estimate the fold change. Data is represented as mean fold change +/- SEM of three independent experiments. *p<0.05 untreated vs. TNF-α/IL1-β. #p<0.05 TNF-α/IL1-β- induced MMP-9 in the presence and absence of drugs.

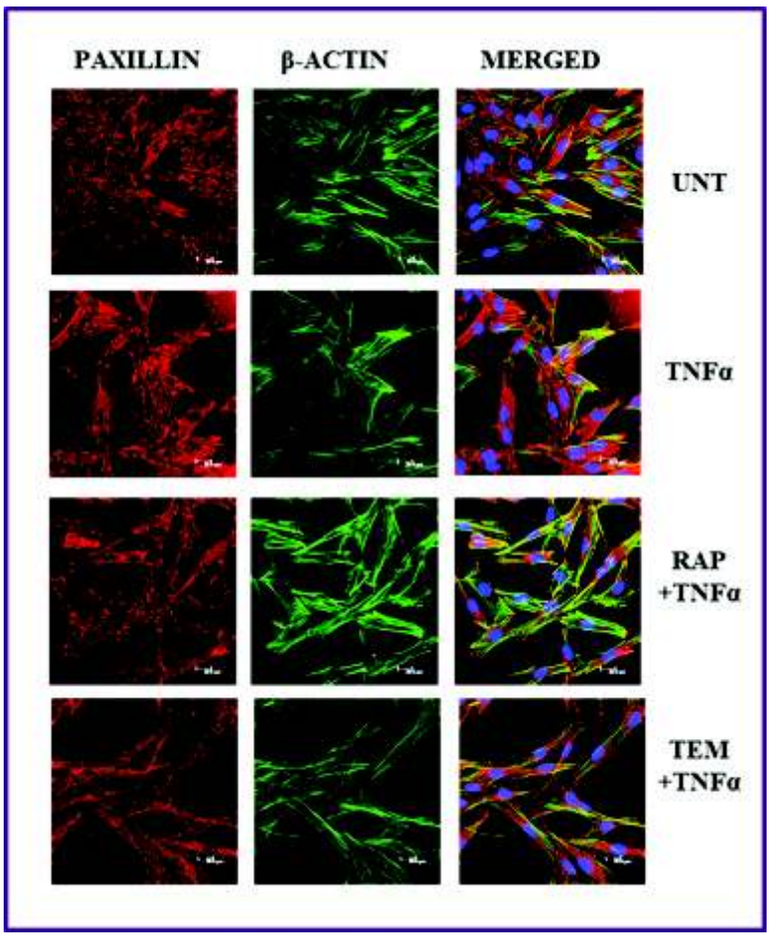

Fig. 3: Effect of Rapamycin and Temisirolimus on Paxillin and Actin re-organisation in GBM-1. Immunofluorescent staining for paxillin and actin re-organisation was performed on cells treated with RAP (10μM), TEM (5μM) for 48h and exposed to TNF-α (10ng/ml) for 12h before termination of time point. (Mag. 60X)

protein expression and regulation of β-actin organization were observed in GBM-1 cells treated with RAP and TEM (Fig. 3). Our results revealed that mTOR inhibitors- RAP, TEM, Torin-1 and PP-242 could effectively suppress TNF-α/ IL1-β-induced- invasive activity in cell lines as well as human primary cell cultures of gliomas. These findings implicate the involvement and interaction between mTORC1 and mTORC2 during invasion in gliomas.

Future Research Plans

Study the mechanism of action of the mTOR inhibitors in invasion of GBM tumors.

Since, ECM components are abundantly expressed in the microenvironment and play an important role in tumor progression, it is planned to examine the potential of mTOR inhibitors on survival and invasion mediated by ECM components.

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

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Background

The genetic program of complex organisms including human are shown to be transacted by non-coding RNAs that rival that of proteins. Rather, the extent of control exerted by non-protein-coding RNAs increases with increasing complexity of the organisms, perhaps exceeding that of proteins in well-developed organisms, reaching an estimated value of ~ 98.8% in humans. A majority of these ncRNAs are dynamically transcribed, with many showing specific expression patterns and sub-cellular localizations, but their exact functions in cells are not well defined. The emerging evidence indicates, however, that several of these RNAs control the epigenetic states that govern development and that many of them are dys-regulated in cancer and other complex diseases. Moreover, it appears that plasticity in these ncRNA functions might exist, especially in the brain. Thus, un-raveling the role of non-coding RNAs in specific tissue functions is likely to be crucial for understanding of growth, differentiation, development and overall function of the organs in higher animals.

Aims and Objectives

1. To investigate potential of ncRNA Ginir in inducing cellular transformation and metastasis in various mouse and human Ginir over-expressing cell systems.
2. To specify targets through which Ginir mediates its effects on cellular growth and their relationship to various signalling pathways.
3. To investigate the interaction partners of Ginir through which it mediates its functions associated with oncogenesis and embryo development.

Work Achieved

We identified a pair of non-coding, over-lapping transcripts- Ginir–sense transcript and Giniras- anti-sense transcript, which do not have protein coding potential. This pair of non-coding RNA is of 612 bp size and is localized to q arm of mouse X chromosome. Our studies indicate towards existence of homeostasis between this pair of cis-Natural Anti-sense Transcripts (NAT)- Ginir and Giniras expressed in mouse fibroblasts. However, when one of the members - Ginir is over expressed, it leads to de-regulated cell cycle progression, genomic instability, cellular transformation in vitro followed by tumorigenicity and high metastatic potential *in vivo*. In contrast, antisense RNA counterpart - Giniras is non-oncogenic in the same cells. Over-expression of Giniras RNA antagonizes Ginir function to significantly restore order into cell cycle progression in Ginir-induced oncogenic cells and attenuates *in vivo* tumor growth from them. The potential of Ginir to form tumors is outcome of its key role in inducing genomic instability (GI), the function from which it derives its name - "Ginir"- Genomic INstability Inducing RNA. Our study addresses role of transcripts - Ginir and Giniras in development, cell proliferation, transformation and metastasis.

Sequence of Ginir RNA is conserved across species and many of the mouse and human EST's involved in embryonic development share significant homology to Ginir sequence. Our data demonstrates an important role for Ginir in mouse embryonic development. Our localization studies for expression of Ginir and Giniras during development by LNA-FISH exhibits its prominent expression in the telencephalon region of 14.5 days mouse embryonic brain. A significant

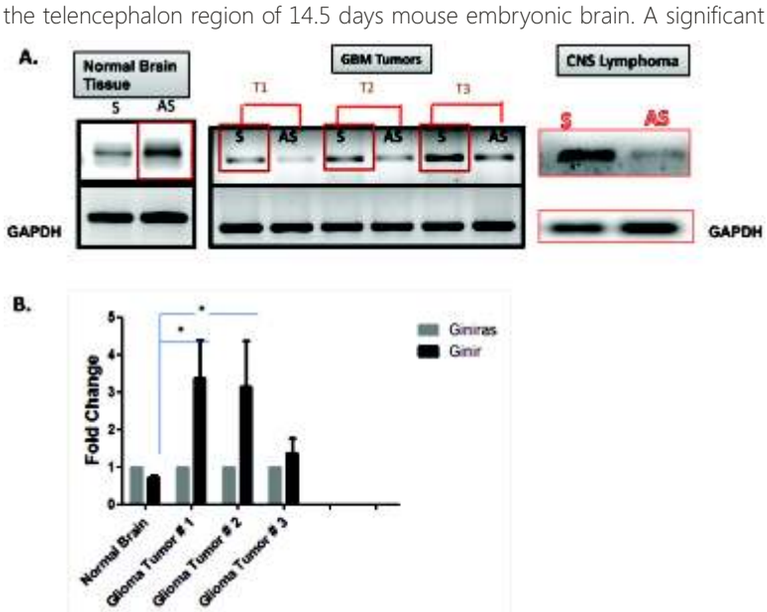

Fig. 1: Expression analysis of Ginir & Giniras in Glioblastoma tumor samples. A) Semi Quantitative PCR showing comparative expression of Ginir (S) & Giniras (AS) in GBM tumors (n=3) and lymphoma sample. **B)** Real time PCR showing comparative expression of Ginir & Giniras in GBM tumor samples. CDNAs were prepared using strand specific primers. GAPDH was used as internal control. Experiment was done with three independent tumor samples & in triplicates. Graph B represents average +/- SEM.

Fig. 2: Schematic Analyses of putative Protein Targets of Ginir determined by MALDI-TOF analysis of proteins obtained in RNA affinity pull down experiment. The protein score in the result report from an MS/MS search is derived from the ions scores. It is the sum of the highest ions score for each distinct sequence (Protein score > 50% Confidence index (C.I) is significant).

decrease in Ginir levels occurs as development proceeds from neonatal to adult stages with Giniras levels remaining constant. Similar pattern of Ginir expression is found in human brain wherein we find that Ginir and Giniras levels are very low in human adult brain. In contrast, in tumors of CNS like Glioblastoma (GBM), the levels of Ginir RNA increases significantly in a graded manner indicating its function in tumorigenicity and suggesting its role as a RNA Bio-marker (Fig.1). Recently NC-RNA's like MALAT 1 (1), ANRIL (2) are associated with several malignancies with nc-RNAs like HOT AIR considered as predictor of eventual metastasis and death (3). HOTAIR RNA in epithelial cancers was shown to induce genome-wide re-targeting of Polycomb repressive complex 2 (PRC2) to an occupancy pattern more resembling embryonic fibroblasts, leading to altered histone H3 lysine 27 methylation, gene expression, and increased cancer invasiveness and metastasis in a manner dependent on PRC2.

To investigate the transforming and metastatic nature of Ginir, we over-expressed Ginir, Giniras and Ginir + Giniras RNA in wide range of cell-lines and generated multiple stable clones of Ginir, Giniras and Ginir + Giniras over-expressing cell-lines. Interestingly, while Ginir was able to form tumors in immuno-deficient mice within two to three weeks, Giniras was non-tumorigenic. Mice injected with cells double positive for Ginir and Giniras (Ginir + Giniras: G418 + Hygromycin positive clones) showed intermediary sized tumors indicating the dominant nature of Ginir as an oncogene. Interestingly, the tumors generated through Ginir over-expression were found to be enriched with a cancer stem cell population also suggesting novel role for Ginir in stemness.

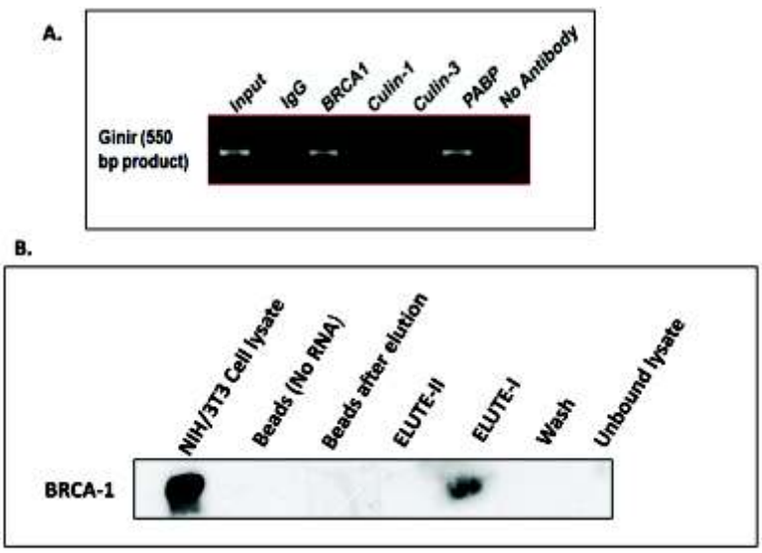

Fig. 2: **A)** RNA Immuno-precipitation with BRCA-1, Culin-1 & Culin-3 respectively for binding with Ginir RNA by PCR using gene specific primers. IgG & no antibody controls are used as negative controls whereas PABP is used as a positive control for the RIP experiment. **B)** Biotinylated RNA affinity pull-down assay followed by immune-blotting for BRCA-1.

In a mechanistic study to investigate Ginir function, our data indicated that Ginir over-expression caused de-repression of satellite repeat elements like LINE-1, MIN Sat and IAP-1, including dys-regulation of genes involved in DNA replication, chromosome segregation, cell-cycle checkpoints pre-disposing cells towards tumorigenesis and cancer. Our data exemplified that only Ginir but not Giniras caused defects in pathways associated with degradation of Cyclins D and E mediated through Culins 1 and 3 leading to faster cycling of cells through G1/S phase causing error-prone DNA synthesis leading to tumor development followed by metastasis.

In a study to identify targets for Ginir, we performed mass spectrometry studies with Ginir over-expressing NIH3T3 cells and identified multiple putative targets using RNA affinity pull down assay (Fig. 2). Further, these targets of Ginir in mouse cells were validated by performing RNA-protein interaction studies that included RNA immune-precipitation studies (RIP) and affinity IP studies. One of the prominent Ginir interacting partners was found to be Brca1 (Fig. 3). Interestingly, Cells that over-express Giniras have higher levels of Brca1 and show minimal binding to Brca-1. The identification of these targets through which the oncogenic functions of Ginir are manifested have provided a mechanistic insight into role of Ginir in development, cell growth, differentiation, regeneration and cancer.

In summary, our study shows that Ginir over-expression causes genome-wide changes that lead to cancer, whereas the Giniras RNA is not only required to maintain cellular homeostasis but also suppresses cancer *in vivo*. A similar effect

is also manifested by loss of another NCR- Xist localized on the X-chromosome that results in X reactivation and consequent genome-wide changes that lead to cancer, thereby causally linking the X chromosome to cancer in mice (4).

Future Research Plans

We aim to construct a network model that highlights critical interacting partners for Ginir and identify candidate targets for action of Ginir in mouse and human cells.

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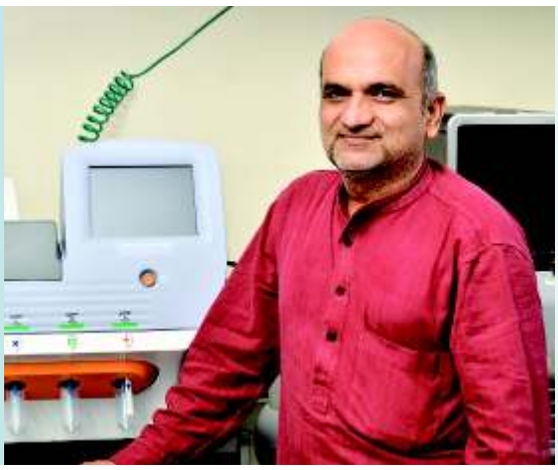

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Human Microbiome Indian perspective

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Background

Human body is host to large diversity of microbes which inhabit various locations on human body such as skin, gastrointestinal tract and vagina. Human gut harbours a dense population of 10^{14} bacterial cells which is ten times more than human cells in our body. Gastrointestinal tract is inhabited by *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. It is now believed that bacteria residing in the gut have a major impact on human wellbeing. The gut microbiome plays beneficial role in extracting nutrients from the diet, regulating host fat storage, stimulating intestinal epithelium renewal, and directing the maturation of the immune system. At the same time, some of these microbes are associated with some disbioses like obesity and cardiovascular disease, inflammatory bowel disease. It is therefore important to understand the microbiome structure in the gut for the development of personalized strategies of healthcare, as well as potentially providing new targets for drug development.

Aims and Objectives

Till now, most of the studies on human gut flora are carried out on European or American populations neglecting Indian subjects. Hence, there is lack of knowledge about composition of gut flora of Indian population. The physiology of Indian population is different from that of western population as suggested by YY- paradox and it is expected that composition of gut microbes would also be different. With this background the studies on the gut flora of Indian population to understand the genetic and environmental factors that contribute to its development and also the changes that are associated with diseases were initiated.

Work Achieved

Effect of diet and geographical location

The study included strict vegetarian and omnivorous individuals. The 16S rRNA tagged amplicon sequences generated using Ion torrent PGM platform were compared with publicly available sequences of healthy individuals from different geographic locations like Bangladesh, Korea, Spain, Africa, US and Europe. Several phyla have been found to be altered based on geographic location and dietary habits. In this analysis, seven different populations were divided in four continents (Europe, USA, Africa and Asia) as shown in figure 1a. It was observed that atleast 27 bacterial genera were shared in all the samples from four continents and may be a part of core human microbiome. Very few genera were found to be shared in samples from Western (US and Europe) and Asian population (Korean, Bangladesh and India). Also, 8 bacterial genera were found to be specific for samples from Asian population and 5 were found to be specific in samples from European population.

To study the effect of dietary habits, sequences from Indian individuals were compared with sequences from hind gut fermenting herbivores, foregut fermenting herbivores, omnivores, carnivores and human samples from western population. It was observed that families like Prevotellaceae, Lachnospiraceae, were dominant in Indian population (p-value < 0.05). Weighted UniFrac PCoA plot based on abundance of 1635 taxa showed a significant microbiome difference based on diet (ANOVA p-value 0.01) (Figure 1b). Also based on the abundance of the 1635 rarified taxa it was evident that

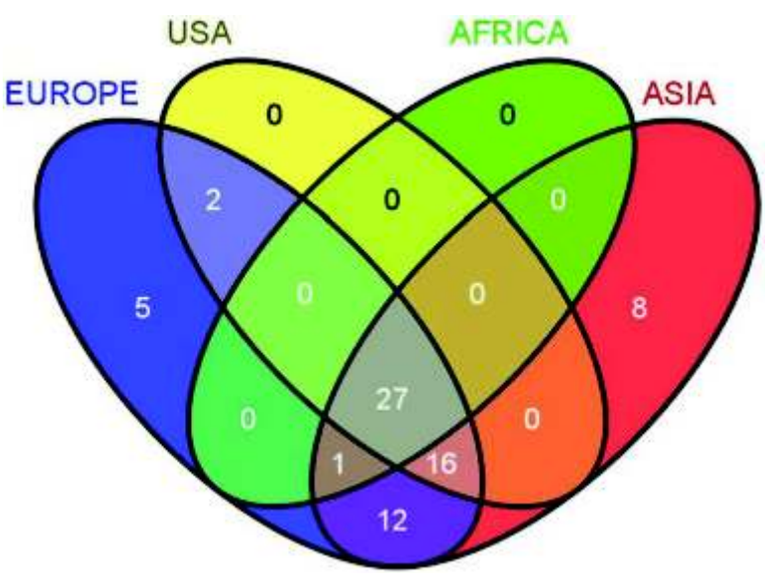
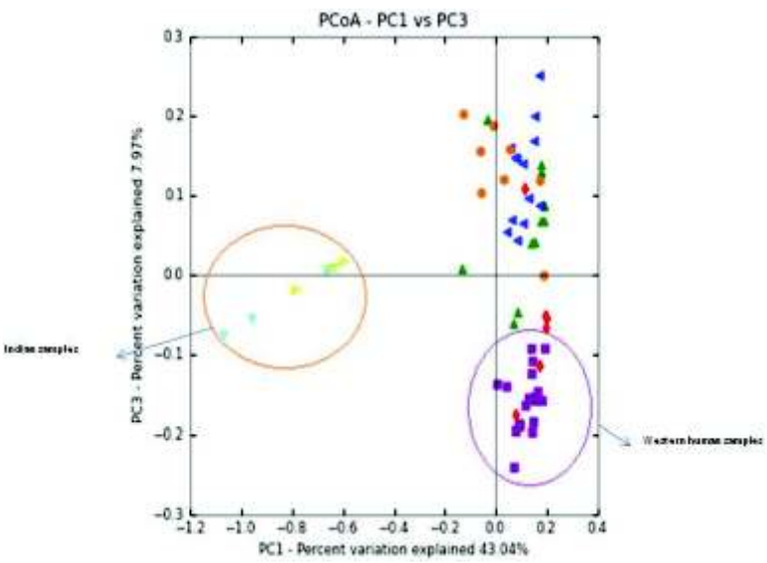


Fig. 1a: Venn diagram showing distribution of bacterial genera across different continents

Fig. 1b: Showing Weighted UniFrac PCoA plot on abundance of 1635 taxa compared based on their dietary habits



gut microbiome of Indian individuals in significantly different as compared to the other samples in the study.

In another study mode of delivery related variations in Indian and Finnish cohorts was studied using quantitative PCR for *Bifidobacterium* group which plays a key role in human health, as it carries a wide range of functions like improving intestinal integrity, development of infants' immune system and harboring genes for utilizing the complex oligosaccharides which are prime source of nutrition (breast milk) during the early stages of the infant. The results indicate that the birth mode has a marginal effect on the *Bifidobacterial* population, especially with the *B. Catenulatum* and *B longum*, however large population of the cohort showed observed counts above the median in normal delivery as compared to C-section born children. *Clostridium spp*. observed to be highly abundant in C-section born 13-14 year old children as is *Akkermancia muciniphila*. The secretor studies carried out within the same cohort, show that the secretor status (*fut2* gene) of the individual affects the Bifidobacterial population in the gut, especially of *B. bifidum* and *B. adolecentis* population in the individuals with non-secretor genotype. Similar results were obtained for other bacteria like *C. leptum*, suggesting the possibility of fut2 secretor status of the individual modulating the population of certain important gut microbes.

Small intestinal and whole gut metagenome of celiac disease patients, their first-degree relatives and controls

Celiac disease (CeD) is an autoimmune enteropathy caused by interaction between host genetics and environmental factor gluten. A comprehensive

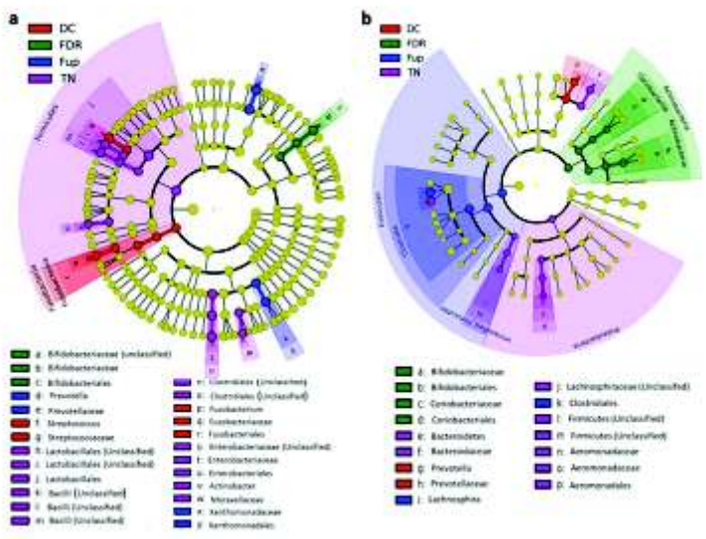


Fig. 2: Differentially abundant microbial clades. **a)** Differentially abundant microbial clades in the small intestine microbiome where TN (purple) is treatment naïve celiac; Fup (dark blue) is celiacs after six months of GFD; DC is the dyspeptic control (red); and FDR is first degree relative (green). **b)** Differentially abundant microbial clades in the fecal microbiome where TN (purple) is treatment naïve celiac; Fup (dark blue) is celiacs after six months of GFD; DC is the dyspeptic control (red); and FDR is first degree relative (green).

understanding of the community level dysbiosis and dysfunction in gut microbiome remains obscure.

Using high-throughput bacterial community profiling, we identified a dysbiotic gut microbiome in adult treatment naïve celiacs (TN) which is enriched in OTUs affiliated to Proteobacteria and depleted of Bifidobacteriaceae members when compared to first-degree relatives (FDRs) and controls. Longitudinal assessment of celiacs after six months of gluten free diet indicated that the gut microbiome of celiac patients was depleted in OTUs affiliated to Enterobacteriaceae (Fig. 2). The imputation of metagenomes from amplicon data revealed enriched virulence genes and anti-oxidative stress response pathways in celiacs compared to FDRs and controls. Additionally, metabolic pathways for metabolism of cofactors and vitamins which are important for immune homeostasis were depleted in small intestine of TN and enriched in FDRs. Results of our study indicates a pro-inflammatory gut microbiome in celiacs harbor which lacks microbial metabolic traits required for immune homeostasis in host that are enriched in FDRs. This association suggests that gut microbiome may influence intestinal homeostasis in individuals genetically susceptible to development of celiac disease.

Gut microbiome of diabetic individuals in Indian population

Diabetes is one of the complex multisystem disorders and most common non-communicable disease worldwide that results from pancreatic β-cell dysfunction and/or insulin resistance. Studies on characterization of gut

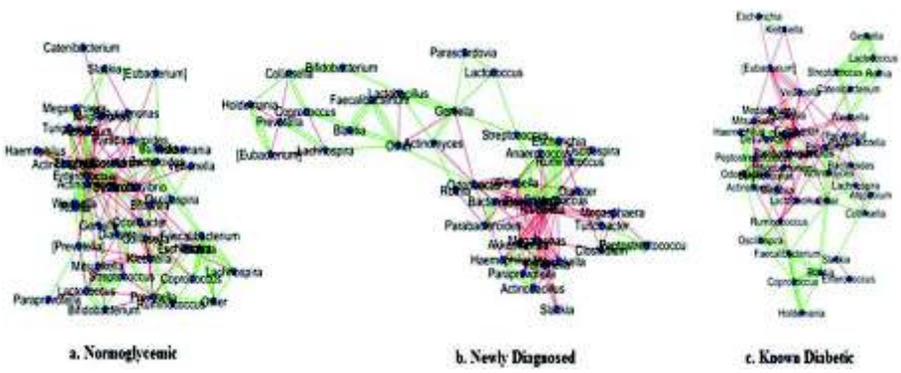


Fig. 3: Significant co-occurrence and co-exclusion observed among the three different categories of samples. Each node (blue coloured spheres) represents a genus and each edge represents a significant co-occurrence/co-exclusion relationship. Colour of the edges indicates the sign of the association (red negative, green positive).

microbiome from diabetic individuals of Indian origin was initiated in collaboration with the Diabetic Unit, KEM hospital, Pune.

A total of 49 subjects were enrolled in the study and 2.1 million good quality reads were generated by sequencing V3 region of 16S rRNA gene using Ion Torrent PGM sequencing technology. Operational taxonomic unit (OTU) analysis was conducted to obtain detailed structural overview of microbiome and yielded 11,781 OTUs with a 97% similarity cutoff. *Firmicutes* and *Bacteroidetes* were found to be the dominant phyla of the overall structures of the microbiome at phylum level. The relative abundance of *Bacteroidetes* in the diabetic subjects was lower than that in the normoglycemic subjects, while relative abundance of *Firmicutes* and *Actinobacteria* were higher in the diabetic subjects than that in the normoglycemic subjects .The co-occurrence and co-exclusion analysis on genus abundance data (Figure3) showed that the normoglyceminc network (fig 3a) was much condensed and stable and at the early stages of diabetes development this network was more spread (fig.3b) and later as the disease progressed the network became stabilized again (fig.3c).

The positive co-occurrence association types could include nutritional cross-feeding, coaggregation, co-colonization, signaling pathways, and co-survival in similar environments. Negative exclusion interactions likewise might span toxin or small molecule production, environmental modification (to the detriment of microbial neighbors), immunomodulation, or gross overpopulation of a niche.

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

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

Background

Synthetic circuits have long been regarded as 'plug and play' devices; recent trend in the circuit design also incorporates the principles of evolutionary genetics. Biological parts are analogous to 'offspring' where mutations arising in the parent population can either improve or decrease the fitness of a particular phenotype. Estimating the evolvability of synthetic devices is essential as modeling approaches that do not incorporate fitness differences will lead to biased parameter estimates and misinterpretation of intracellular dynamics. Design principles of synthetic biology have incorporated similar concepts where evolvability is being considered to obtain maximum functionality of the circuit. We have reported the construction of tristable circuit for the parasite *'Leishmania'* which exhibits tristability and robustness to external perturbations (Mandlik *et al*, 2012, Figure 1). The basic aim for which the circuit was constructed was to increase the production of a protein proSLS4. Enhancing the production of proSLS4 is expected to alter the levels of IPC. IPC is the most important sphingolipid present in the membrane rafts of the parasite, adequate levels of IPC have been shown to be important for the infectivity and viability of the parasite. (Sutterwala *et al*, 2007) In order to improve the extent of proSLS4 production and to achieve optimum functionality, the evolvability of the circuit has been studied. Systematic exploration of parameter space could elucidate principles for circuit design illustrating composability, performance, robustness and efficiency.

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Aims and objectives

1. To understand robustness trade off against adaptability of the circuit.
2. To understand how natural selection deal with environmental disturbances or noises in the circuit.
3. To lay an insight into the emergence of synchronization in the IPCS synthetic circuit designed and its further implications for robustness with an aim to understand the significance of co- operativity.

Work done

Synthetic circuits are now programmed based on 'modularity', 'scalability' and 'evolvability' by incorporating the appropriate biological parts and further optimizing the parameters involved. Incorporation of such circuits in the host involves a tradeoff between the cost of fitness and the function of the synthetic circuit. Biological systems exhibit evolvability and are robust in nature. The robustness of the synthetic circuit can be defined in terms of the robustness of the dynamical attractors to external perturbations. Directed evolution of such circuits *in silico* involves manipulating the behavior of the components involved, systematically perturbing and characterizing individual components. This provides additional insight into functionality of the constructed circuit and the cooperativity between the components involved. The evolutionary search space for both the tristable and bistable circuit of *Leishmania* was subjected to conditions like a) detuning components and parameters like reducing the effect of repressilator over the toggle switch b) altering independent properties like altering strength of activator and the concentration of inducer. In comparison to the bistable circuit, the tristable circuit based on the principle of autoactivation

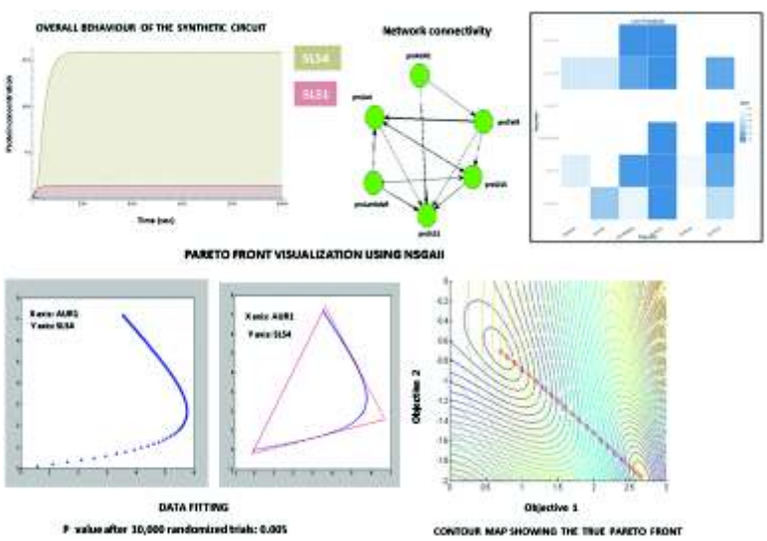

Fig. 1: Evolutionary trajectories defined for IPCS synthetic circuit. Performance contours generating straight edged fronts.

Objective function expressions used for minimization:

```
function Y = inducer(x)
Y(1)=(141.66*x(1))*(0.19*sqr t(x(2)))+(141.66*x(1))*(14.
28*sqrt(x(3)));
Y(2)=(141.66*x(1))*(3.64*sin(x(2)))+(141.66*x(1))*(4.56
*sin(x(3)))+(141.66*x(1))*(5.74*sin(x(4)));
end
```

exhibited a better functionality with respect to the concentration of the protein SLS4. There was no effect of the parameter variation and hence it was observed that the parameter space was large and the system remained unperturbed to variations. Thereby the circuit maintained its performance under a broad range of random perturbations and this explains the robustness of the system.

To further tune the circuit such that it exhibits the highest functionality, the evolvability of the circuit has been considered. Estimating the evolvability of synthetic devices is essential as modeling approaches that do not incorporate fitness differences will lead to biased parameter estimates and misinterpretation of intracellular dynamics. Tradeoffs lead to an unexpected simplicity in the range of optimal phenotypes- they fall on low dimensional shapes in trait space such as lines, triangles and tetrahedrons. At the vertices of these polygons are phenotypes that specialize at a single task accounting for phenotypic plasticity in variable environments. A system is generally considered to be evolvable if it acquires novel functions when subjected to perturbations. This can lead to the emergence of phenotypes with higher fitness than the original parent population. Models simulated dynamically are often analyzed for their steady state behavior. The stochastic switching between phenotypes evolved due to competition may suppress the emergence of regulated phenotypes and may serves as crucial viable intermediates on route to regulated phenotypes. The next goal would be to optimize the circuit. This is often a multiobjective problem and there exists tradeoff's between the

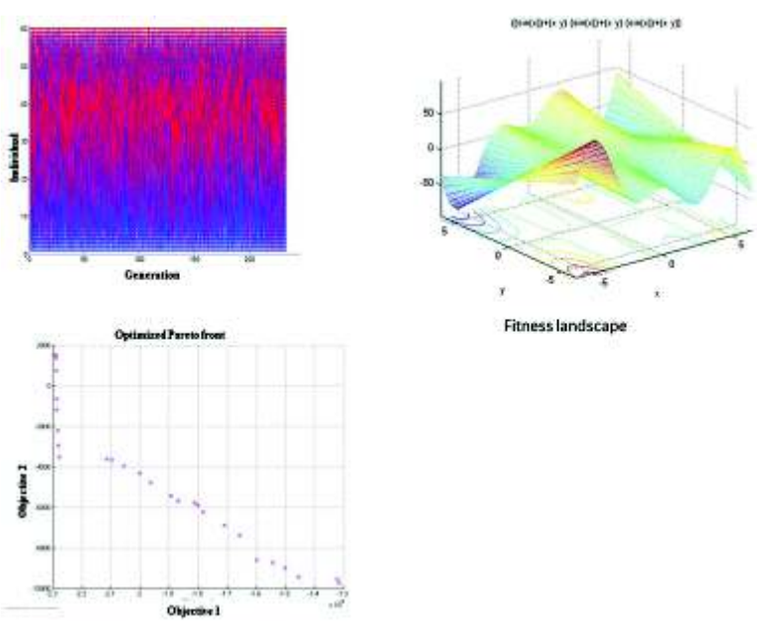

Fig. 2: Accumulated evolutionary experience of the parental lines experiencing wide range of habitats and as a result evolved developmental mechanisms that can be tuned to optimize phenotypes across the range defined. Constraints evolve and encode the desired Pareto front in the shape of an allometric curve. Good spread of solutions at local Pareto front is obtained.

proposed objectives. Multiobjective optimization using evolutionary algorithms such as NSGAII deal with the large set of solutions obtained, eliminate the set of dominating solutions, retaining only the set of non-dominated solutions that couldn't be improved further without compromising on some of the objectives. (Deb *et al*, 2002) The selection is performed by means of an objective function (or fitness that measures), for instance how close the dynamics of a biological systems is to a desired behavior. In our current study, we are concerned with obtaining any solution that may fulfill the designed specifications of the IPCS synthetic circuit. Thus, the pareto optimal solutions for the tristable circuit were obtained and represented on the pareto front. (Figure 2) The search space was reduced and the optimal points with the highest fitness score were obtained on the pareto front. It was seen that there were no points off the pareto front and all the solutions obtained after pareto optimization fitted well on the true pareto front. Considering the robustness of the model and its evolvability, the model appears to capture the underlying objectives behind the circuit designed and serves as a benchmark for further experimental studies.

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Future Research Plans

1. Parts present in the synthetic circuit will be assembled using DNA assembly techniques.
2. Directional assembly of the customized DNA molecules into series of linearized plasmids to construct the toggle switch and repressilator of the tristable circuit.

In future also study the evolutionary pathway mechanism for transcription factor target gene network in leishmaniasis.

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Role of Monoamine Oxidase-A (MAO-A) in hyperglycemia-induced oxidative stress mediated apoptosis in diabetic cardiomyopathy

Background

Diabetic cardiomyopathy characterized by cardiac dysfunction with subsequent heart failure in patients with diabetes mellitus in the absence of coronary atherosclerosis is a major cause of morbidity and mortality in diabetic patients. Several studies have shown hyperglycemia as an independent risk factor and apoptosis playing an important role leading to diabetic cardiomyopathy. The mechanisms of hyperglycaemia-related damage of the cardiovascular system are multi-factorial and still not known in detail. However, there is considerable evidence that hyperglycaemia results in the generation of reactive oxygen species (ROS), leading to increased oxidative stress and cellular damage. Over the last decades numerous studies are investigating the underlying pathological mechanisms of diabetic cardiomyopathy using animal models of diabetes mellitus as well as clinical data from diabetic patients. Mitochondria are the major source of reactive oxygen species (ROS) because these organelles continuously generate superoxide ($O_2^{\cdot-}$), as a byproduct of electron transport, so it is not unexpected that mitochondria have been shown to be a primary target of damage in diabetes. Despite the presence of various antioxidants and detoxifying enzymes, the mitochondria appear to be the most powerful intracellular source of ROS; according to one estimation the steady-state concentration of $O_2^{\cdot-}$ in the mitochondrial matrix is approximately five- to tenfold higher than that in the cytosol or nuclear space.

The mitochondrial apoptotic pathway appears to play an important role in diabetes-induced myocardial cell apoptosis, and among the apoptotic stimuli, ROS and/or reactive nitrogen species (RNS) play a critical role in the mitochondrial cytochrome c release and caspase-3 activation.

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In addition to the respiratory chain, monoamine oxidase (MAO), a flavoprotein localized on the outer mitochondrial membrane, is another important mitochondrial source of ROS, in particular of H_2O_2. MAO catalyzes the oxidative deamination of primary aromatic amines along with long-chain diamines and tertiary cyclic amines and is a quantitatively important source of H_2O_2. Because H_2O_2 easily passes through mitochondrial membranes, MAO can contribute to an increase in the steady state concentrations of ROS within the mitochondrial matrix and cytosol. Based on their substrate preference and inhibitor specificity, 2 functional isoenzymes, MAO-A and MAO-B, have been identified In the heart, MAO-A is a predominant enzyme involved in the deamination of endogenous or exogenous amines. Recently, stimulation of oxidative stress by MAO-A was demonstrated to play a major role in reactive oxygen species-dependent cardiomyocyte apoptosis and post-ischemic cardiac damage, however, the role of MAO-A and the link between MAO-produced ROS and apoptosis in diabetic cardiomyopathy is not well defined. Because ROS are involved in hyperglycemia induced cardiac cell death, we will investigate whether MAO-A is a potential source of ROS.

Aims and Objectives

To investigate the potential role of ROS generated by MAO-A during hyperglycemia induced cardiomyocyte death and the role of MAO in cardiac contractility in vitro and in vivo.

Work achieved

Our previous work showed that exposure of H9c2 cardiomyoblast cells to high-glucose (HG) resulted in apoptosis. Molecular mechanism of apoptosis indicated increase in ROS levels, loss of mitochondrial membrane potential and activation of caspase cascade. We hypothesized that MAO A-produced ROS could contribute to high glucose induced cardiac cell apoptosis. Hence we examined the effect of High glucose on MAO A expression and activity. HG did not alter MAO A protein expression but showed significant increase in MAO A activity. To determine whether increased MAO A activity contributes to HG induced oxidative stress, we used a specific inhibitor of MAO A clorgyline and the levels of ROS were measured. MAO A inhibition showed decrease in HG induced ROS levels (Fig.1A). High glucose induced ROS (Fig. 1B) perturb mitochondrial membrane potential and MAO A inhibition restore it to the normal level indicating its role in loss of mitochondrial membrane potential under high glucose condition (Fig. 1C). Disturbance of mitochondrial membrane potential under high glucose condition leads to activation of

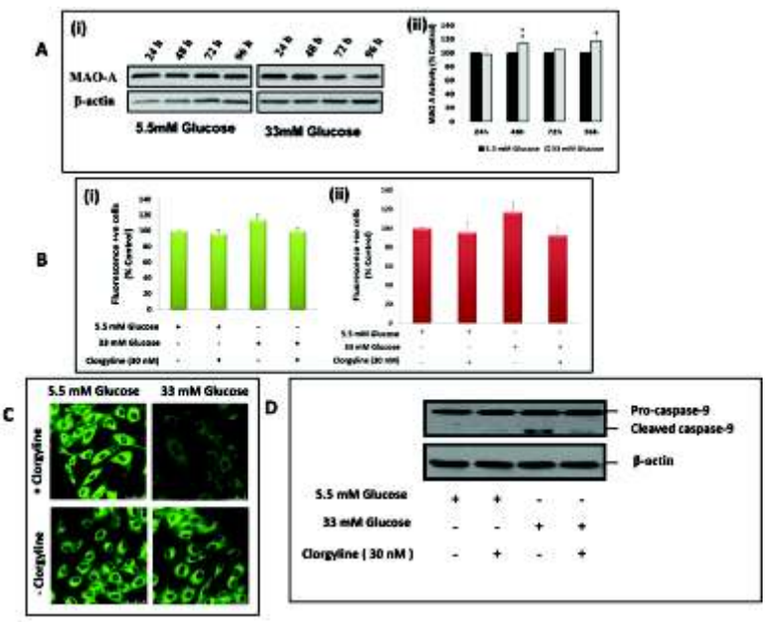

Fig. 1: HG induced MAO A activity contributes to ROS production, loss of mitochondrial membrane potential (Δ? m) and activation of caspase 9. H9c2 cells were treated with 5.5mM glucose and 33mM glucose for 24-96 hours. MAO A inhibition was carried out using MAO A specific inhibitor clorgyline **(A)** Western blot analysis was performed for MAO-A protein expression (i). MAO A activity assay was carried out (ii). **(B)** Intracellular levels of H_2O_2 (i) and O_2^- (ii) were measured after 48hrs of treatment using DCF-DA and DHE by flow cytometry **(C)** Δψm was assessed after 48hrs of treatment by $DiOC_6$ staining. **(D)** Caspase cleavage was examined after 72hrs of treatment by western blot method. Data represents mean ± S.E. of three experiments. *P < 0.05,
** P < 0.005 compared to 5.5 mM glucose treatment.

caspase cascade resulting in apoptosis. Since MAO A inhibition could reduce caspase -9 cleavage indicating it has an important role in caspase 9 activation under high glucose condition (Fig. 1D).

To validate our *in vitro* findings *in vivo* we induced diabetes in male Wistar rats by single Intraperitoneal (ip) injection of streptozotocin (55mg/kg body weight). MAO A inhibition was carried out by clorgyline (1mg/kg body weight/day, ip) for 2 months. Induction of Diabetes in STZ treated animals was confirmed by increase blood sugar levels, decline in serum insulin levels. Diabetic cardiomyopathy was characterized by declined systolic and diastolic performance, increased heart weight to body weight ratio and Biochemical Markers of Myocardial Injury in serum. No change in MAO A protein expression was observed but MAO A activity was increased significantly in hearts from STZ induced diabetic rats compared to that of controls (Table 1). Clorgyline treatment for 2 months did not alter MAO A protein expression but caused

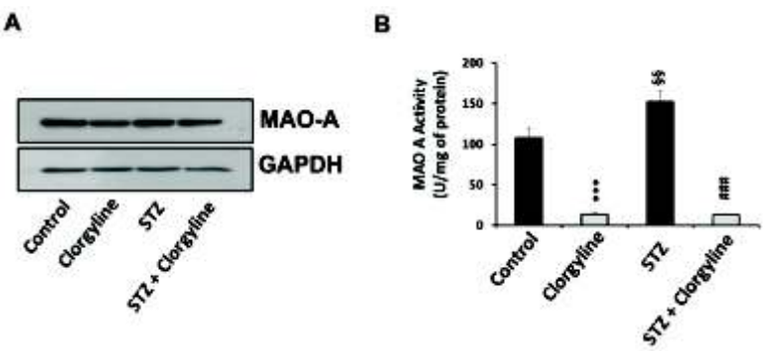

Fig. 2: MAO A Activity was enhanced in hearts from diabetic rats' and was inhibited by clorgyline treatment without affecting its protein expression. **(A)** Western blot for MAO A **(B)** MAO A Activity in hearts from control and diabetic rats treated with or without clorgyline. Values are means ± SE *** P<0.001 vs control,
§ § P < 0.01 vs control, ### P<0.001 vs STZ

Table 1: Physiological parameters after MAO A inhibition.

HW/BW : heart weight to body weight ratio, LV + dP/dT : LV systolic pressure rise, LV - dP/dT : LV systolic pressure decay, creatine kinase (CK-MB and CK-NAC)
* Between control and STZ, **Between STZ and STZ + Clorgyline

Parameters	Control	Clorgyline	STZ *	STZ + Clorgyline **
Blood glucose (mg/dl)	95.29 ± 7.09	88.83 - 12.91	541.60 ± 13.85 (P<0.001)	550.71 ± 9.57 (NS)
serum insulin (ng/ml)	0.30 ± 0.08	0.49 - 0.08	0.16 ± 0.03 (P<0.01)	0.15 ± 0.01 (NS)
HW/BW (mg/g)	2.93 ± 0.08	3.33 ±0.15	3.61 ± 0.12 (P<0.05)	3.91 ± 0.12 (NS)
LV + dP/dT (mm Hg/ s)	824.38 ± 67.72	744.90 ± 41.15	682.55 ± 34.88	529.78 ± 39.39
LV – dP/dT (mm Hg/ s)	-368.16 ± 61.68	-409.98 ± 22.98	-293.27 ± 10.33	-256.58 ± 22.32
Troponin I (ng/ml)	0.09 ± 0.03	0.05 ± 0.02	0.31 ± 0.06 (P<0.001)	0.09 ± 0.03 (P<0.05)
CK-Nac (IU/L)	181.83 ± 47.95	182.80 ±35.78	225.43 ± 27.07	138.60 ± 27.63
CK-MB (IU/L)	99.83 ± 24.49	70.40 ± 3.98	121.13 ± 12.48	108.83 ± 13.43

significant reduction in MAO A activity (Fig. 2). MAO A inhibition normalized Biochemical Markers of Myocardial Injury in serum but did not show significant change in other physiological parameter (Table 1). In conclusion, the present data support MAO-A as an important source of ROS that contributes to hyperglycemia induced myocardial dysfunction in hearts. Whether inhibited ROS production is the only key to interpret the beneficial impact of MAO-A inhibition in diabetic cardiomyopathy needs further investigation. However, present findings add MAO-dependent signaling as a cause of stress-induced diabetic cardiomyopathy.

Future Research Plans

Further, efforts are under way to further investigate the role of MAOs and the mechanisms involved in the hyperglycemia-induced cardiac cell death injury *in vivo*.

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

Aims and Objectives

Our lab is interested in aspects of cellular trafficking that differ between embryonic stem cells and differentiated cells. We are primarily focusing on understanding the role of endocytosis during the differentiation of embryonic stem cells and during the process of reprogramming differentiated cells to a pluripotent state. This work is important for understanding cell fate decisions during early mammalian development.

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Role of IL-3 in regulation of bone metastasis

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Background

Bone homeostasis is maintained by integrated activity of bone resorbing osteoclasts and bone forming osteoblasts. Imbalance between bone resorption and bone formation processes leads to bone loss in important diseases such as osteoporosis, osteopetrosis and rheumatoid arthritis. Massive bone loss also occurs in bone metastases of breast and prostate cancers. Bone metastases cause severe bone pain, pathological fractures, life threatening hypercalcemia, spinal cord compression and other nerve compression syndromes that lead to severe morbidity. The presence of metastatic tumor cells in the bone microenvironment leads to dysregulation of normal bone remodeling that results into either increased bone destruction or increased bone formation. Bone metastases of breast cancer mostly present with osteolytic phenotype whereas prostate cancer bone metastases are of osteoblastic phenotype. The dysregulated bone remodeling releases many growth factors from bone matrix that support the tumor growth and promote bone destruction. Breast cancer cells produce factors that directly or indirectly induce the formation of osteoclasts. This results in formation of a vicious cycle wherein bone destruction and cancer growth are supported by each other. In osteolytic bone metastases osteoclasts rather than tumor cells mediate bone destruction. Breast cancer cells produce several growth factors including IL-1, TNF-α, receptor activator of NFB ligand (RANKL), and IL-6, which play an important role in inducing osteoclastogenesis and bone resorption. Therefore, understanding the pathophysiology underlying bone metastases and identification of new therapeutic targets to treat bone metastases is very important.

IL-3, a cytokine secreted by activated T lymphocytes is known to regulate proliferation, differentiation, and survival of hemopoietic stem cells. IL-3 has also been suggested for the treatment of different states of bone marrow failure in hematologic malignancies, and also to mobilize or expand hematopoietic progenitor cells for transplantation and to support engraftment after bone marrow transplantation. Previously, we have demonstrated that IL-3 is a negative regulator of osteoclastogenesis and potently inhibits mouse and human osteoclast differentiation induced by both RANKL and TNF-α. IL-3 also inhibits pathological bone resorption induced by TNF-α and other pro-inflammatory cytokines such as IL-1, IL-6, TGFβ and PGE2. We have also demonstrated that IL-3 enhances osteoblast differentiation and bone formation from human mesenchymal stem cells. Importantly, IL-3 prevents the development of inflammatory arthritis, and protects cartilage and bone destruction in mice. These studies suggest that IL-3 plays an important role in regulation of pathological bone resorption. However, the role of IL-3 in regulation of bone metastases of breast and prostate cancers is not yet known.

Aims and Objectives

1. To evaluate the role of IL-3 on growth properties of breast cancer cells.
2. To evaluate the in vivo role of IL-3 on regulation of bone metastasis.

Work Achieved

Expression of IL-3 receptor on breast cancer cells varies with invasive phenotype

IL-3 exerts its biological activities by binding to specific high-affinity receptors expressed by hemopoietic stem cells, endothelial cells, and monocytes. However, the presence of IL-3 receptor (IL-3R) on breast or prostate cancer cells is not fully delineated. To investigate the role of IL-3 in regulation of bone metastasis, we first evaluated the expression of IL-3Rα on breast cancer cells using MDA-MB-231 and MCF-7 cell lines that are known to induce osteolytic bone metastases. Cells at 80% confluency were harvested and analyzed for expression of IL-3Rα (CD123). Both these cells showed expression of IL-3Rα at transcript and protein levels (Fig. 1A and B). Expression of IL-3Rα was also confirmed by immunofluorescence and flow cytometry. There was 40.78% and 87.72% expression of IL-3Rα on MDA-MB-231 and MCF-7 cells respectively (Fig. 1C and D).

MDA-MB-231 cells are known to be highly invasive cells whereas MCF-7 cells are less invasive. As shown in Fig. 1D the expression of IL-3Rα was almost two

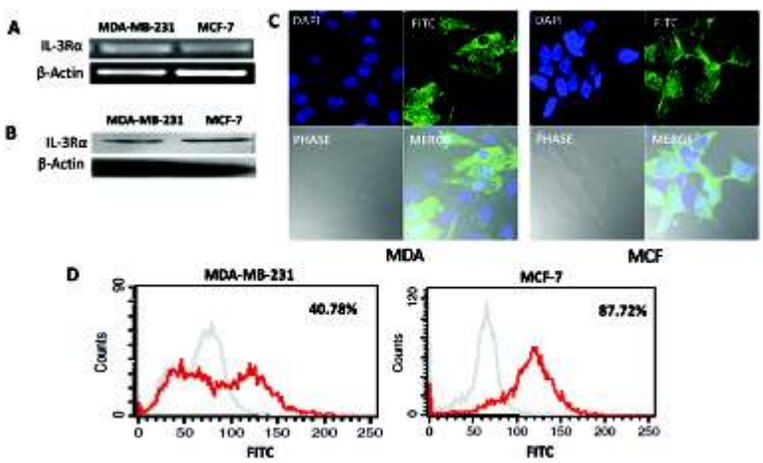


Fig. 1: Breast cancer cells express IL-3Ra : MDA-MB-31 and MCF-7 cells at 80 % confluency were harvested and analyzed for expression of IL-3Rα (CD123) at gene and protein levels. A) mRNA expression by RT-PCR and B) Protein expression by Western blotting. IL-3Rα expression was also assessed by C) Immunofluorescence and D) FACS.

fold higher on MCF-7 cells than MDA-MB-231 cells. This indicates that less invasive breast cancer cells show high expression of IL-3Rα. To confirm this we further evaluated the expression of IL-3Rα on a panel of five breast cancer cell lines, which differ in their estrogen receptor status (Fig. 2A) and invasiveness. In general the absence of estrogen receptor renders the cells to a more invasive phenotype. We used highly invasive MDA-MB-231 and SK-BR-3 cells which do not express estrogen receptor, and three less invasive cell lines, MCF-7, ZR-75-1 and T47D that express estrogen receptors. By Western blot analysis we demonstrate that the expression of IL-3Rα was higher in less invasive cells and comparatively low in highly invasive cells (Fig. 2B and C). Thus, our results demonstrate that breast cancer cells express IL-3Rα at both gene and protein levels and its expression may correlate with the invasiveness of cancer cells.

Development of murine models of bone metastasis

To evaluate the role of IL-3 in regulation of bone metastases we first checked the dose-dependent effect of IL-3 on proliferation of MDA-MB-231 and MCF-7 cells by MTT and BrdU incorporation ELISA. We observed that IL-3 had no effect on in vitro proliferation of breast cancer cells at all the concentrations ranging from 1 to 100ng/m. We then examined the effect of IL-3 on RANKL-inuced osteoclast formation in presence of conditioned medium of breast cancer cells. We observed that RANKL-induced osteoclast formation was

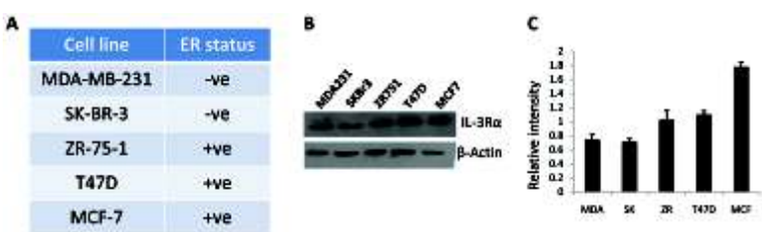


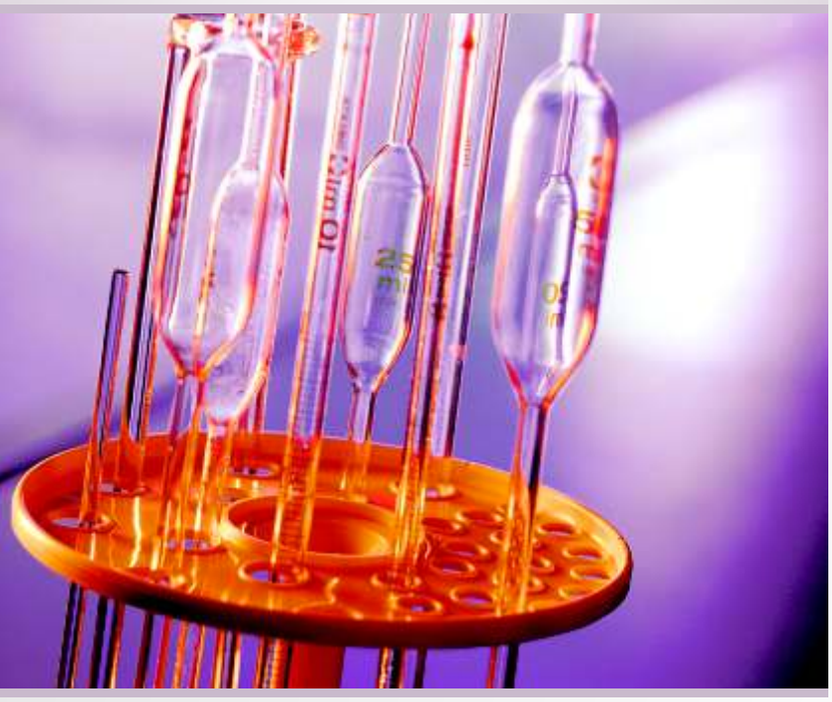
Fig. 2: IL-3Rα expression on breast cancer cells varies with invasive phenotype: A) A panel of breast cancer cell lines was selected based on their estrogen receptor status and reported invasiveness. B) Cells at 80% confluency were assessed for IL-3Rα by Western blotting. C) Densitomeric analysis of 2 independent experiments.

significantly increased by the conditioned medium, and IL-3 completely inhibited the increased osteoclast formation.

To investigate the in vivo role of IL-3 on bone metastasis we developed xenograft and syngeneic mouse models of experimental bone metastases. For xenograft mouse model, human MDA-MB-231 cells (10^5) were injected intra-tibially in 6-8 weeks old immunocompromised SCID mice. After six weeks mice were radiographed by soft x-rays and analyzed for skeletal tumor burden and tumor induced osteolysis. Femur and tibia bones were harvested and analyzed by histomorphometry for measurement of various bone structural parameters using micro computed tomography (μCT). We observed significant bone destruction and skeletal tumor burden in long bones of mice injected with tumor cells. For syngeneic mouse model we injected mouse breast cancer 4T1-tdtomato-Luc2 cells (10^5) intra-cardially in 6-8 week old immunocompetent Balb/c mice. The metastatic spread in vivo was tracked at different time points by in vivo bioluminescent imaging. We observed initiation of bone metastasis after 8 days. These mice are further being characterized by μCT. After complete characterization of bone metastases parameters these two murine models will be used in all further experiments.

Future Research Plans

To study the in vivo effect of IL-3 on bone metastases mice will be treated with breast cancer cells and different doses of recombinant IL-3. Tumor burden and lytic bone lesions will be assessed radiographically by soft x-ray analysis. To study the osteolytic and osteoblastic phenotype cancellous and cortical bone parameters of femur and tibia bones will be analyzed by μCT. Analysis of bones for osteoclastogenesis, and different soft tissues for presence of cancer metastases will be done by histology. We will also analyze the effect of IL-3 expressing 4T1-tdtomato-Luc2 cells on bone metastases.



Support Units & Other Facilities

Experimental Animal Facility

Ramanamurthy Boppana

Rahul Bankar

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

MICE:

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

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

* BALB/c with cataract mutation # Outbred

RATS:

- WISTAR

RABBITS:

- NEWZEALAND WHITE

The Team

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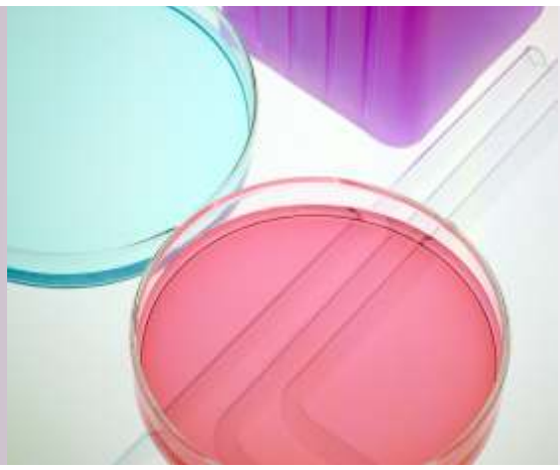
Mr. Mahavir Rangole
Mr. Rahul B. Kavitake
Mr. Ganesh B. Yadav
Mr. Sanjay Gade
Mr. Harshal G. Gaonkar
Mr. Dilip B. Thorat

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

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

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

Complete scientific support and advice is extended regularly to scientists and their group members for conducting experiments under IAEC-approved projects. The facility also conducts training/course work for the research fellows of the Centre in the area of Laboratory Animal Experimentation and Ethics.



Proteomics Facility

Srikanth Rapole

Technical Staff

Mrs. Snigdha Dhali

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 list of various instruments available at the facility is given below:

4800 LC-MALDI TOF/TOF system (AB Sciex) - This 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 molecular ions of interest and measuring the fragment ion masses. From April 2013 to March 2014, approximately 1020 samples were analyzed, including 130 external samples.

4000 Q-Trap LC-MS/MS system (AB Sciex) - This is a hybrid triple quadrupole/linear ion trap mass spectrometer. The system is ideal for proteomic applications including the discovery of post-translationally modified proteins, protein identification, and biomarker validation. From April 2013 to March 2014, approximately 89 samples were analyzed, including 13 external samples.

Eksigent Tempo Nano MDLC system - This 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 - This is an advanced micro-LC technology with pneumatic pumps, integrated autosampler, ultra-sensitive, full-spectral UV detector, and temperature-controlled column oven. With

EKSIGENT NANO-LC and SPOTTER

EKSIGENT MICRO-LC

AGILENT GC-MS

Eksigent's intuitive software, users have full system control as well as complete analysis and reporting capabilities. The advantages of Eksigent's state-of-the-art micro-LC are fast analysis with excellent reproducibility, with only a small amount of solvent required.

Eksigent EKSpot MALDI Spotter - This couples the Nano MDLC to the 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.

2-D DIGE proteomics set-up - This includes 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.

Bioinformatics and High Performance Computing Facility

Shailza Singh

Technical Staff

Mrs. Virashree Jamdar, *Technician*

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

Hardware Infrastructure

SGI Altix XE 1300 Cluster

Head Node:

SGI Altix XE 270 Serve.

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

Compute Nodes:

SGI Altix 340 Servers

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

SGI Cluster Software Stack:

SLES Ver 11

SGI ProPack 7

SGI Foundation Software Ver 2.0

Interconnect:

24-Ports Gigabit Ethernet Switch

GPU Computing HP Proliant SL6500

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

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

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

Integrated Graphics ATI RN50/ES1000 with 64 MB memory

2x NIVIDIA Tesla 2090 6 GB GPU computing module Specialized Workstations:

HP Elite 8200 CMT PC

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

Intergrated 4 port SATA 6GBs controller

Intergrated Intel HD graphics

HP Z800 High End Work Station (2 in number)

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

5.86GTs QPI, DDR3 1333 MHz, HT Turbo

NVIDIA Quadro FX380 Graphics with 256MB memory

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

19" LCD wide Display with Windows OS

HP Z820 High End Work Station

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

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

NVIDIA Quadro 4000 Graphics with 2GB DDR memory

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

22" LCD wide Display with Windows OS

High End Desktop (2 in number)

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

Desktop Computers

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

iMAC: For running specialized software like Biojade

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

APC UPS 10 KVA for supporting the HPCF

Software infrastructure

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

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

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

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

Pharmacophore Modeling: Auto Pharmacophore generation, Receptor-ligand pharmacophore generation,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, Recursive Partioning Model, Multiple Linear Regression Model, partial least squares model, genetic function approximation model, 3D QSAR model (Discovery Studio 3.0). Intelligent QSAR using molecular fragments of interest and their features, evaluation of descriptors from template scaffold to form relationship with the activity.

Molecular Dynamics: CHARMM, GROMACS, NAMD, MOIL

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

ab initio modeling: GAUSSIAN

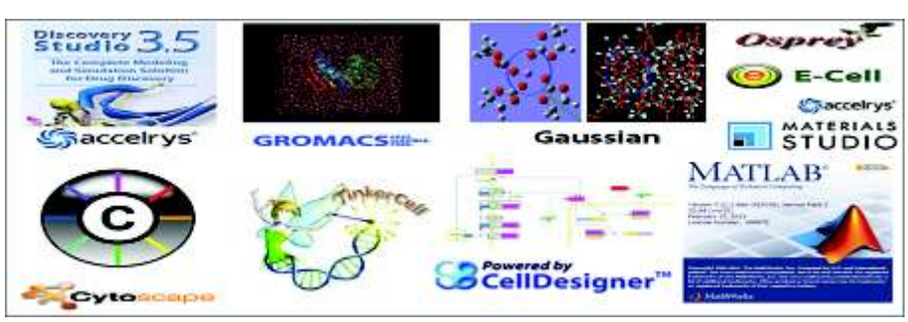
Systems Biology Tools: Virtual Cell, M-cell, Cell Designer, GEPASI, Cytoscape, Osprey, E-Cell, SimBiology

Artificial Intelligence: SVMlight and SNNS

Material Modeling and Simulation: Material Studio 5.5

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

Statistical packages: MATLAB and R



Library

The NCCS library has a collection of books, journals and magazines in frontier areas of biotechnology having relevance to NCCS research activities. The library holds approximately twelve thousand four hundred bound journals, two thousand eight hundred books and two hundred NCCS Ph.D. theses. It subscribes to twenty scientific journals and twenty eight other periodicals in print form. In the development of its collection, the library's priority is to support NCCS research activities and it is expanded in consultation with NCCS scientists. The library's print collection is growing by approximately 1200 volumes per year. The library provides access to seven hundred and thirty four online Journals (including the Methods in Enzymolgoy series) of various publishers, such as Springer, John Wiley, Nature Publishing group, Mary & Libert, Oxford, Elsevier Science Direct, through the 'DeLCON' online journal consortium of DBT. In addition, it also independently subscribes to six online journals.

The library is equipped with Linux-based SLIM21 with RFID Interfaced library software for the library housekeeping operation and Web-OPAC for online searching of library documents. The library has also installed barcode technology for circulation (issue & return) of library documents. The library maintains library information (in Hindi & English) on its webpage, which includes free Online Medical database link, NCCS research publication list, library forms, 'NCCS in the News', Ph.D. theses, a list of NCCS alumni and other Scientific Grants/ Funds/ fellowships-related links. During the period, April 2013 - Mar 2014, the library has created Digital Archive of NCCS Ph.D. theses, 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, and 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 also provides the service of scanning documents for plagiarism using the iThenticate Anti-Plagiarism Software.

Team

Mr. Krupasindhu Behera, *Technical Officer*

Mr. Rameshwar Nema, *Technical Officer*

Computer Section

M.V. Krishnasastry

Moving Towards a 'Paperless Office'

The computer section has put in efforts to introduce the Human Resource Management System, developed by C-DAC, Pune. Currently, the system is being configured and tested, before it can be made available for use by staff members. Complete operationalization of this system will lead to automation of a substantial portion of day-to-day administration.

The computer section has developed a major back-end application using 'model-view-controller' architecture which helps scientists and heads of various departments, to update their profile, publications, seminar notices, other notices, circulars, tenders (both on intranet and main websites) and to track bills etc., internally. Another major utility of this application is to provide a platform for online submission of complaints related to instrumentation and maintenance of electrical, civil and air conditioning, which are likely to arise at the institute. The system enables online filing of complaints, assignment of work, updating of work, review of progress and finally acknowledgement of the work done by the end user, for satisfactory closure of the request/complaint. The system has flawlessly handled over 1500 requests / complaints in 12 months.

The computer section is also developing a New Dynamic Web site which will be launched soon. The new web site will allow scientists to update their profiles and publications, and will enable the administration, stores and purchase and academic sections to post notices, tenders, circulars, etc., directly from their desktop.

Acquisition of New Hardware

A new Wireless Switch (3COM WX2200 with 15 WAP) was configured and installed. Wireless arrangements were made for live online sessions for a symposium organized at NCCS in collaboration with the University of Pune.

Technical

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

Additional licenses of Symantec protection suite SPSS 3.0 were installed and configured for gateway level e-mail antivirus, antispam, desktop and laptop antivirus and network access control.

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 softwares were installed/ updated on user computers at NCCS. These include MS Office 2010, Adobe Suite X, Sigma Plot Suite 12.0 and Reference Manager 12.0. Win. Vista was upgraded to Win7. In addition, the Paypack salary software was installed and configured for the NCCS salary process, which takes care of TDS and EPF deductions.

General Assistance

The computer section made provisions for internet connectivity and other arrangements in the newly inaugurated auditorium, to ensure smooth functioning during the many presentations made by distinguished speakers.

The computer section actively participated in the NCCS Silver Jubilee celebrations by assisting with the preparations and providing full technical support throughout.

NCCS Facilities

a) FACS Core Facility

There are seven instruments in the FACS core facility of the Centre under the supervision of Dr. L.S. Limaye. These are operated on a rotation basis by six dedicated operators.

The technicians at the facility are listed below:

1. Hemangini Shikhare.
2. Pratibha Khot
3. Amit Salunkhe
4. Rupali Jadhav (till Dec.2013)
5. Ashwini Kore
6. Vikas Mallav (operator provided by BD and posted in NCCS under the BD-NCCS STEM CELL COE from Dec.2011)

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

Immunophenotyping & Cell Cycle Analysis:

Equipment	Surface /Intracellular staining	DNA Cell cycle	Total Samples Acquired
FACS Calibur	2428	6183	8611
FACS Canto II (Old)	5924	85	6009
FACS Canto II (New)	10266	-	10266

Sterile Sorting:

EQUIPMENT	SORTING	ACQUISITION **	TOTAL
FACS Aria II SORP	337	2554	2891
FACS Aria III SORP	193	944	1137
FACS Aria III Standard	187	1016	1203

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

BD Pathway 855:

Around six users from NCCS have used this instrument during the period under consideration. Imaging of samples was done for live cell staining (96 well plates)

Team

Suresh Basutkar, *Technical Officer*
Narayan Kadlak, *Technician*
Pramod Surve, *Technician*
Kailash Bhandalkar, *Helper*

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

Samples from users outside NCCS:

Users from institutes like NCL, ARI, IBB, IISER, IRSHA, the Department of Biochemistry University of Pune and Rasayani Biologics Pvt. Ltd. have utilized our FACS facility on a payment basis during the period under consideration. 506 samples were acquired, including those for Surface/ Intracellular staining and DNA cell cycle analysis.

Activities under BD-NCCS COE programme:

1. Calibur training:

Training on Calibur for NCCS students was carried out in batches throughout the year every month by a BD application specialist. 46 students from NCCS received training through this programme, resulting in the instrument being used independently by these students.

2. Canto-II training and examination:

An examination was conducted for the trained students 02 – 03 May 2013 for students trained on Canto II. 19 out of 20 students successfully qualified this exam and started using the instrument independently.This year a fresh batch of 21 students are trained by BD on Canto-II and will appear for the examination on 29-30 April 2014

National Science Day:

On the occasion of the National Science Day celebrations on 26th February 2014, the operators of the FACS facility presented an informative poster entitled 'NCCS Flow Cytometry Core Facility'. BD had also arranged a demonsration of the BD Accuri machine. These displays were visited by a large number of students from colleges, who found the enthusiastic interactions and discussions during this poster session and instruments demonstration very beneficial.

b) Imaging facility

Technical Officers

Ashwini N. Atre

Trupti Kulkarni

The imaging facility has three scanning confocal laser microscopes, which includes Zeiss 510 Meta and Leica TCS SP5 models. All the systems are inverted

microscopes and have a wide range of lasers, such as Blue Diode laser (405nm), Argonlaser (458/477/488/514 nm), He-Ne (543nm, 594nm and 633nm) and DPSS 561nm. The systems can be used for doing FRET, FRAP, 3D imaging and reconstruction, and live imaging, which are required for most cell biology research. The FLUOVIEW FV10i microscope from M/s Olympus has a compact design and does not require a dedicated darkroom.

All three instruments are used by in-house users as well as by users from neighboring organizations.

c) DNA sequencer

NCCS has one ABI 3730 and one ABI 3730xl. A total of 9,400 samples were run on these machines during this financial year.

d) IVIS Imaging System

Dr. Gopal C. Kundu is in charge of this facility.

Technical Staff

Dr. Mahadeo Gorain, Technician

The IVIS imaging system is a central facility of NCCS which is used for bioluminescent and fluorescent imaging of cells or whole small animal under in vitro or in vivo conditions. The IVIS imaging instrument was used by more than 25 researchers from the various laboratories at NCCS as well as by collaborators in other institutes. The research scholars used the bioluminescence and fluorescence imaging with different strains of mice (NOD/SCID/ NUDE/ C57/BalC etc) as well as with tissue culture plates (96 well, 24 well and 12 well etc).

The IVIS imaging system is a high-sensitivity, low noise, in vivo imaging technology platform that enables non-invasive visualization and tracking of cellular and genetic activity within a living organism in real time. The system provides both bioluminescence and fluorescence imaging capability.

The Xenogen IVIS Imaging System is capable of imaging bioluminescence and fluorescence in live 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. For fluorescence imaging, the instrument

can operate in reflectance or trans- illumination mode. Filtered light from a broad-band lamp provides the excitation source in both modes. In the reflectance mode, light is delivered to four reflectors that are located on the ceiling of the imaging chamber.

Standard filter sets of the 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

IVIS Imaging System

Bioluminescent and Fluorescent Imaging:

This system can be used for in-vitro and in-vivo studies based on bioluminescence and fluorescence techniques. The lens system includes user-accessible filter wheels that accept up to 22 filters, including a large set of fluorescence filters and A set of bioluminescent filters to use in spectral imaging studies.

Benefits and Features:

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

(ranging from 560 nm to 660 nm) facilitate 3D diffuse tomographic reconstruction and determination of the depth and location of a bioluminescent reporter.

- Heated animal shelf (up to 40° C).
- NIST traceable absolute calibrations.
- Class I Laser Product.

e) Central Sterilization Facility

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

Team

Suresh Basutkar, *Technical Officer*
Narayan Kadlak, *Technician*
Pramod Surve, *Technician*
Kailash Bhandalkar, *Helper*

Microbial Culture Collection

Yogesh Shouche
yogesh@nccs.res.in

Participants

Tapan Chakrabarti, *Consultant*
Kamlesh Jangid, *Scientist*
Omprakash Sharma, *Scientist*
Ashish Polkade, *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*
Shrikant Pawar, *Technical Officer*
Hitendra Munot, *Technical Officer*
Dimple Notani, *Technician*
Vishal Thite, *Technician*
Sonia Dhage, *Technician*
Mahesh Sonawane, *Technician*
Madhuri Vankudre, *Technician*
Shalilesh Mantri, *Technician*
Vikram Kamble, *Technician*
Umera Patawekar, *Technician*
Vikas Patil, *Technician*
Sunil Dhar, *Technician*
Ashok Shinde, *Technician*
Vipool Thorat, *Technician*

Background

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

The Microbial Culture Collection (MCC) was started at NCCS and has established complete infrastructure required for microbial growth, long term preservation and identification.

Aims and Objectives

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

Work Achieved

MICROBIAL PROSPECTING PROJECT

Since its establishment, MCC received ~2,00,000 cultures collected from various ecological niches as part of the microbial prospecting project undertaken by DBT in collaboration with nine participating institutes/universities. These safe deposit cultures have been categorized as below, on the basis of their screening for different bio-active compounds:

a) 'Normal' Cultures: All the cultures which are isolated by each institute.
b) 'Three star' Cultures: All normal cultures are then screened by Piramal Life Science Limited (PLSL), Mumbai for four different activities. Three star cultures are the ones which show one or more of these activities.
c) 'Re-fermented' Cultures: All the three star cultures undergo second level of screening (fermentation) to check whether they retain the activity. Re-

fermented cultures are those three star cultures which retain their activity after second fermentation.

d) 'Scale-up' Cultures: Re-fermented cultures that have shown potentially novel molecules/compounds during screening at PLSL and have been selected for large scale fermentation by PLSL are designated as 'Scale-up' cultures. 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 the latter half of 2013, since 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, a major proportion of the normal cultures has undergone first passage and will be completed for the remaining cultures by late 2014.

Cultures dispatched to PLSL

MCC continues to supply cultures to PLSL for scale up activity and other purposes.

Identification of cultures

So far, a total of 7938 pure 'Three Star' cultures have been received at MCC, of which 6973 could be sequenced. 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 trials 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. From a total of 2404 'normal' cultures that have been taken for sequencing, 881 have yielded good quality sequences. The remaining samples are being processed.

A total of 1051 'three star' cultures were processed for their identification by MALDI-TOF mass spectrometry, of which 789 (75%) were identified and 262 (25%) could not identified. Of those identified, 12 cultures were identified as *Candida* and included multiple species viz., *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. palmioleophila* and *C. tropicalis*.

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 B*acillus*, *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 55 bacterial and 5 fungal cultures in form of stabs and slants, respectively. Very recently, 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). Of the 812 cultures received for general deposit, >30% could not be accessioned either due to receipt of contaminated cultures from the depositor or non-authentication by sequence analysis at MCC (Table 6). 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 438 authenticated cultures (346 bacterial and 92 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 12 lyophilized ampoules are stored at 4 ˚C.

IDA/Patent deposits

In addition to the general deposits, MCC also receives deposits for IDA from Indian and overseas institutes. So far, MCC has accessioned 19 bacterial cultures under IDA and seven are in process. 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 are stored at MCC at 4˚C.

Identification services

Since October 2012, MCC has begun 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 434 cultures of bacteria and fungi together (including 47 for paid service) were sequenced at MCC. For bacterial identification, the 16S rRNA gene sequence was used whereas for fungi, the ITS region sequence was used.

Phylogenetic analysis

MCC has received 4 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 the type strain sequences from databases like RDP and EZTaxon are used. The alignment is done with the ClustalX software. Phylogenetic trees are constructed with the 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 is now ready to provide MALDI analysis as a part of the bacterial identification service.

FAME Analysis

MCC started providing FAME analysis as a service in February 2013. Since then, a total of 37 bacterial cultures (17 anaerobic and 20 aerobic bacterial cultures, including one paid for) have been analyzed on the MIDI system. The major anaerobic genera identified included *Bacteroides*, *Camylobacter*, *Tissierella*, *Treponema*, *Peptococcus*, *Clostridium* and *Coprococcus*. Among the aerobes, *Sphingomonas*, *Virgibacillus*, *Bacillus*, *Paenibacillus*, *Psuedomonas*, *Rhodococcus*, and *Rhizobium* were identified as common genera. However, FAME profile of four cultures did not match with MIDI aerobic (RTSBA) or anaerobic (SMOORE6) libraries.

DNA-DNA hybridization and GC (mol %)

DNA-DNA hybridization (DDH) and G+C content (mol%) are important molecular characteristics and also play 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 36 internal MCC cultures. A revised fee structure is proposed for providing these as a service.

ISO Certification

MCC 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 was due in early 2014.

FUTURE PLANS

Additional Services to be offered

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

MALDI-TOF

In addition to identification of cultures by MALDI-TOF, customers also require creation of a MALDI database for which the number of replicates required is large and needs additional consumables along with technical expertise. A revised fee structure for MALDI-TOF typing of single cultures as well as database creation is therefore proposed and being submitted to the Scientific Advisory Committee (SAC) for its approval.

DNA-DNA Hybridization and GC content analysis

MCC is now receiving requests from Indian and overseas customers for DNA-DNA hybridization and GC content analysis. However, the rates need revision before these services are offered as a paid service to its customers in the near future.

DNA isolation from cultures

In the past year, there have been many requests, especially from colleges and universities, to carry out DNA isolation for them. Since there are many universities that cannot perform high quality DNA isolation procedures in their laboratories, MCC would like to offer this service in the near future.

Anaerobic microbes

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

Hazard group 3 microbes

Specifications have been drawn by an expert committee chaired by Dr. D. T. Mourya, Director National Institute of Virology, Pune, for the establishment of Biological Safety Level 3 laboratory to handle Hazard Group 3 organisms. 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 will soon begin to supply these cultures, once the modalities are worked out by the partners involved.

MCC Publications

1. Canion A, Prakash O, Green SJ, Jahnke L, Kuypers MM, Kostka JE. Isolation and physiological characterization of psychrophilic denitrifying bacteria

from permanently cold Arctic fjord sediments (Svalbard, Norway). Environ Microbiol. 2013 May;15(5):1606-18.

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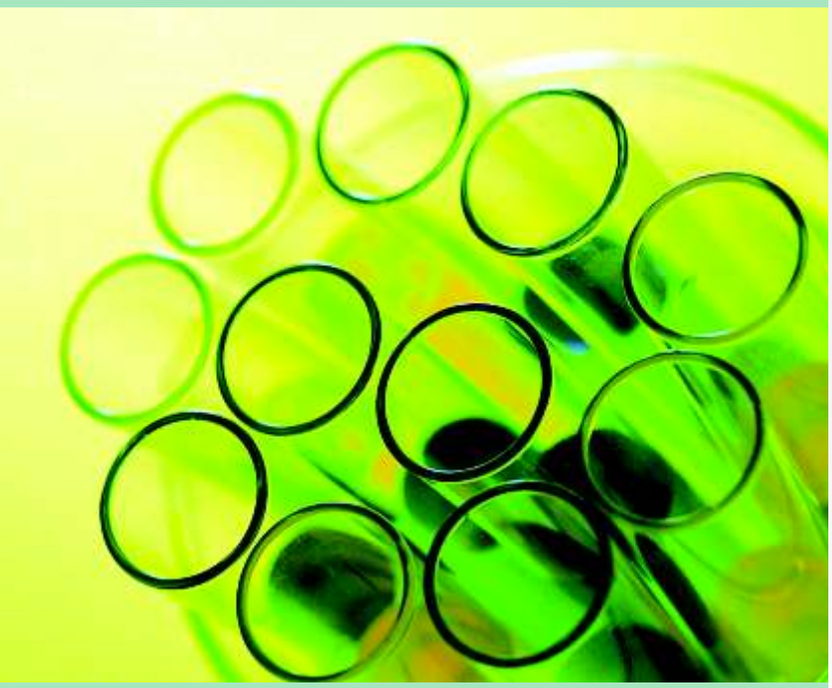
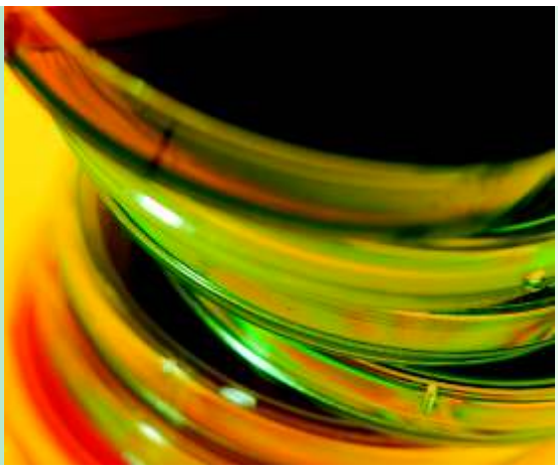


Other Information



Publications / Book Chapters / Patents

Publications & Patents

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Book Chapters / Invited Reviews / Editorials

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5. Milsee Mol and Shailza Singh (2014) "Computational Design of Biological Systems: From Systems to Synthetic Biology" in "Frontiers in Computational Chemistry", Bentham Science Publishers.

Patents (filed / sealed)

Dr. Vaijayanti P. Kale

'The creation of an "Artificial Bone Marrow micro Environment" (ABME) and uses thereof'

Granted:

Japan: 2007-557602 (August 2013);

Korea: 2007-7020266 (July 2013)

ARIPO: AP/P/2007/00416 (Jan 2014)

Dr. Samit Chattopadhyay

'Tumor suppressor Activation polypeptide and uses thereof'. PCT filed September 2010, Indian patent file number: IPR/4.19.20/06083/2006

Granted:

United States (April, 2013): Patent Number # US 8420100 B2

Dr. Sandhya Sitaswad

'Anti-tumor activity of AECHL-I, a novel triterpenoid isolated from Ailanthus excelsa in vitro and in vivo'. (Manish S. Lavhale, Santosh Kumar, Shrihari Mishra, Sandhya Sitasawad). PCT/IN2008/000795.

Granted:

United States: US Patent No. 8,519,163

Japan: Japanese Patent No. 5468611

Germany (June, 2014)

Awards/ Honours/ Memberships/ Extramural Funding

Awards / Honours / Memberships

Sharmila Bapat

- Elected as Office Bearer of the executive Committee of Indian Association of Cancer Research (IACR) 2014-2016.

Samit Chattopadhyay

- Awarded the prestigious J. C. Bose National Fellowship by the Department of Science and Technology, Government of India, in recognition of PI's active outstanding performance and contribution to science, 2013.
- Dr. Chattopadhyay's former Ph.D. student, Dr. Pavithra Lakshminarasimhan Chavali, was awarded the INSA Young Scientist Award.

Jomon Joseph

- Member: Indian Society of Cell Biology
- Member: Society for Biological Chemistry, India

Vaijayanti Kale

- Member: 'Ethical Research Initiatives'
- Appointed as a member of ICMR Expert Committee on Stem Cell Research and Therapy
- Appointed as a member of IC-SCRT of AFMC, Pune

Girdhari Lal

- Early Career Faculty Travel Award (2013) by American Association of Immunologists (AAI), USA.
- DBT-CTEP travel support to present paper in American Association of Immunologists 2013 meeting at Honolulu, Hawaii, USA.

Nibedita Lenka

- Chairman, Institutional Ethical Committee, OCT Therapies & Research Pvt. Ltd. Mumbai. Life Member, Indian Academy of Neuroscience.

Lalita Limaye

- Annual member of International Society of experimental Haematology
- Life member of: Indian society of cell biology; Biotechnology society of India; Indian women scientists association; Indian association of Microbiologists of India

Debashis Mitra

- Fellow, Indian National Science Academy, January 2014

Srikanth Rapole

- Member, American society for mass spectrometry (ASMS)

Padma Shastry

- Ms Reecha Shah, (Research Assistant, ICMR project) was awarded the 5th Becton Dickinson-The Cytometry Society (TCS) Award (2013) for recognition of excellence in flow cytometry in India.

Anjali Shiras

- Travel Award for participation at International Society Stem Cell Research Meeting (ISSCR); Boston, USA; 2013.

Shailza Singh

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

Mohan Wani

- Academic Editor, PLOS ONE journal, 2013-14

Praveen Rahi (Scientist at MCC)

- Selected for the Young Scientist Award in Agriculture Microbiology, by the Association of Microbiologists of India (November, 2013)

Extramural Funding

Manoj Kumar Bhat

- 'Relationship between obesity and cancer, its ramifications in cancer progression and chemotherapy'. 2013-2016 [DST-SERB (DST No. SR/S0/HS-0136/2012, India)]

Samit Chattopadhyay

- ? 'ATM dependent DNA damage repair by tumor suppressor SMAR1'. 2012-2015 (The Council of Scientific & Industrial Research, India: No SC/CSIR/37(1572)/12-13/EMR-II).
- 'Regulation of T cell Development and Differentiation by Nuclear Matrix Protein SMAR1: Its Implications in Immune Responses'. 2012- 2015 (DBT, India: BT/PR14746/BRB/10/899/2010).
- 'Regulation of CD44 splicing by tumor suppressor SMAR1: Implications in cancer metastasis'. 2012- 2015. (DBT, India: BT / PR3624 / MED / 30 / 662 / 2011).

Radha Chauhan

- 'Reconstitution and structural studies on Nup93Nup205 complex of the nuclear pore complex'. 2013 - 2016. (DST-SERB, India)

Jomon Joseph

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

Vaijayanti Kale

- 'The role and mechanism of Free Radical Scavengers and / or Cell Death Cascade regulators in mitigating the Diabetes Mellitus-induced Endothelial Progenitor Cell (EPC) Dysfunction'. 2012-2015 (DBT, India).
- 'A study to determine the safety and efficacy of extra-cellular matrix (ECM) embedded bone marrow-derived endothelial progenitor cells (EPCs) in treatment of impaired wound healing'. 2011-2014 (BRNS, India).

Gopal Kundu

- 'Peptide nanoparticle mediated drug/siRNA delivery to tumor vasculature that suppresses tumor growth and angiogenesis in breast and prostate cancers'. 2011-2014. (Department of Biotechnology, Government of India).

- 'Therapeutic application of targeted shRNA libraries in treatment of breast and prostate cancers'. 2012-2015. (Department of Biotechnology, Government of India).

Girdhari Lal

- 'Cellular and molecular mechanism of CD4 T cell and endothelial cell interaction to control inflammation and autoimmunity'. 2012-2015. (Department of Biotechnology, Govt.of India: BT / PR4610 / MED / 30 / 720 / 2012).
- 'Role of chemokine and its receptors in the pathogenesis and regulation of autoimmunity'. 2011-2016. (Department of Biotechnology, Govt.of India: BT/RLF/Re-entry/41/2010).
- 'CCR6 as therapeutic target to control inflammation and autoimmunity'. 2011-2014. (Department of Biotechnology, Govt. of India: BT/03/IYBA/2010).

Nibedita Lenka

- 2012-2015. Indo-Australia Biotechnology Fund (Round 6).

Lalita Limaye

- 'Generation of mesenchymal stem cells from human umbilical cord tissues, their characterization and differentiation to neural cells'. 2012-2015 (BRNS, Mumbai, India).
- '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, India).
- '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, Govt. of India).

Shekhar Mande

- 'SysTB: A network program for resolving the intracellular dynamics of host-pathogen interactions in TB infection' - PI in a project involving 11 PI's and 4 co-PI's. 2012-17 (DBT, Govt. of India).
- 'Discovery of bioactive natural products from microbes especially actinomycetes in niche biotopes in Manipur' - Co-PI in a project involving 4 collaborators. 2011-14. (DBT, Govt. of India).

- 'Virtual Centre of Excellence on multidisciplinary approaches aimed at interventions against *Mycobacterium tuberculosis*' - Co-PI in a project involving 15 collaborators. 2008-13. (DBT, Govt. of India).
- 'DBT Centre of Excellence for Microbial Biology' - Co-PI in a project involving 6 collaborators. 2008-13. (DBT, Govt. of India).
- '*Mycobacterium tuberculosis*: Bioinformatic and structural strategies towards treatment' - Indian PI in a New Indigo Era-Net grant with three European collaborators. 2010-13. (DBT, Govt. of India).

Debashis Mitra

- 'Structure based discovery of novel antiviral molecules with potential to inhibit drug resistant viruses' - Co-PI in a project involving 3 collaborators. 2010-2014. (DBT, Govt. of India).
- 'Identification of novel cellular targets and new lead molecules to inhibit HIV-1 infection'. 2012-2015. (DBT-Tata Innovation Fellowship grant).

Srikanth Rapole

- 'An attractive and promising strategy for early cancer diagnosis through the assembly of the human cancer volatome'. 2013-2016 (DBT New INDIGO, India).
- 'Metabolomic profiling for identification of novel potential biomarkers in breast cancer using mass spectrometry and bioinformatics'. 2013-2016 (DBT RGYI Grant, India).
- 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'. 2013-2013 (DBT Basic Science, India).

Arvind Sahu

- 'Studies of species specificity in poxviral complement regulators'. 2011-2014 (Department of Biotechnology, Govt. of India).
- 'Soluble mediators of the immune system against *Aspergillus fumigatus*'. 2014-2016 (Department of Science and Technology and Indo-French Centre for the Promotion of Advanced Research).

Vasudevan Seshadri

- 'Role of protein disulfide isomerase in glucose stimulated insulin biosynthesis'. 2011-2014. (DBT, Govt. of India).

Padma Shastry

- 'Role of Prostate Apoptosis Response-4 (Par-4) and its interactive proteins in chemo resistance/ sensitivity in gliomas using multi-cellular spheroids (MCS) as model'. 2011-2014. (ICMR, India).

Anjali Shiras

- 'Identification of biomarkers for diagnosis and prognostication by Next Gen sequencing of oligo-dendroglial tumor exome'. 2011 - 2014. (DBT, Govt. of India).
- 'A Novel strategy for re-programming of somatic cells to induced pluripotent stem (iPS) cells by a single Non-coding RNA - Ginir for applications in regenerative medicine'. 2014 – 2015. (DST- UKIERI, India-UK).

Yogesh Shouche

- 'Establishment of Microbial Culture Collection and Biological Research Centre'. 2009-2013. (DBT, Govt. of India).
- 'Screening of bio-molecules from bacterial diversity isolated from various niches of Western Ghats'. 2011-2013. (DBT, Govt. of India).
- 'Small intestinal and whole gut met genome in patients with celiac disease, their first degree relatives and controls'. 2011-2013. (DBT, Govt. of India).
- 'The origins and process of microbata development in different geographic areas: creating new nutritional tools for microbiota modulation'. 2012-2015. (DBT, Govt. of India).
- 'Tracking the shift in gut microbiome from healthy to diabetic state: an omic approach'. 2015-2016. (Unilever)

Shailza Singh

- 'Systems and Synthetic Biology for *Leishmania*'. 2013-2016. (Department of Science and Technology, Govt. of India).
- 'Systems Biology of *L.major*: Therapeutic Implications'. 2012-2015. (Department of Biotechnology, Govt. of India).
- 'Drug Target Identification in *L.major* and *S.mansoni* through Biochemical Network Modeling'. 2012-2015. (Department of Biotechnology, Govt. of India).

Sandhya Sitasawad

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

Deepa Subramanyam

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

Mohan Wani

- 'Studies on understanding the role of IL-3 in regulation of human osteoclasts and osteoblasts differentiation'. 2013-2014, (DBT, Govt. of India).

Conferences / Seminars / Symposia / Workshops / Meetings

Participation by Faculty

Sharmila Bapat

- 'Ovarian cancer stem cells and transformation-associated pathways' (Invited Talk), "4th International Conference on Stem Cells and Cancer (ICSCC-2013): Proliferation, Differentiation and Apoptosis", 19-22 October 2013, Haffkine Institute, Parel, Mumbai, India.
- 'Tumor Heterogeneity in Ovarian Cancer' (Invited Talk) at one-day meeting during visit of Prof. Harold Varmus, Director, NIH, USA on 21st January, 2014 to National Centre for Biological Sciences, Bangalore, India.
- 'Tumor Heterogeneity' (Invited Talk), Half-Day Symposium on Computational Aspects in Cancer Biology, 11th February, 2014, NCCS, Pune, India
- 'Deconstruction of a tumor' (Invited Talk), 33rd Convention of the Indian Association of Cancer Research (IACR), 13-15 February, 2014, at Rajiv Gandhi Centre for Biotechnology, Trivandrum, India
- 'Emerging concepts in molecular stratification of high-grade Serous Ovarian cancer (HGSC)' (Invited Talk), "Trends in Cancer Research" symposium for medical students, 21-23 March, 2014, at Krishna Institute of Medical Sciences, Karad, India.

Radha Chauhan

- 'Structure and functional basis of nuclear pore complex assembly and functions' (invited talk), Indo-UK meeting on Complementary Approaches in Structural Biology, 27-29 January, 2014, IISER Pune, India

Sanjeev Galande

- International workshop on "Unraveling the developmental regulatory network in early animals, Evangelische Akademie Tutzing, Germany, 23-25 Spetember 2013.
- Workshop on NGS analysis, IISER Pune, February 23-27, 2014.

Jomon Joseph

- 'Role of Nup358 in microRNA pathway', Frontiers in Modern Biology meet, June 15-16, 2013. Department of Biochemistry, Indian Institute of Science, Bangalore, India.
- 'Regulation of miRNA function by a nucleoporin', International Conference on Electron Microscopy and XXXIV Annual Meeting of the Electron Microscope Society of India (EMSI), July 3-5, 2013. Hyatt Regency, Kolkata, India.
- 'Non-traditional roles of nucleoporins: Nup358 regulates cell polarity', XXXVII All India Cell Biology Conference on Cell Dynamics and Cell Fate, December 22-24, 2013. J.N. Tata Auditorium, IISc, Bangalore, India
- 'A novel role for Nup358 in miRNA pathway', 7th Discussion Meet of the RNA Group. March 6-8, 2014. Indian Institute of Chemical Biology, Kolkata, India

Vaijayanti Kale

- 'Modulating stem cell functions in vitro', National Conference on Frontiers in Biotechnology and Bioinformatics (NCFIBB2014) on "Macromolecular Interactions in Biology", 30th - 31st January 2014, D.Y. Patil University, Belapur, Navi Mumbai, India (invited talk).

Girdhari Lal

- 'Role of follicular helper (Tfh) and follicular regulatory (Tfr) CD4 T cell in tolerance' (Invited talk), 4th International conference on stem cells and cancer (ICSCC 2013), Haffkins Institute, Mumbai, India on 19-22nd October, 2013.
- 'From microarray technology to epigenomics', (Invited Lecture Series), Academic Staff College, Sambalpur University, Orissa, August 16-18th, 2013.
- 'History of T cells from laboratory to clinic: Cytokine and autoimmunity' (Invited talk), Department of Life Science, Sambalpur University, Orissa, 17th August, 2013.
- 'Tumor microenvironment induces selective migration of specific subsets of natural killer (NK) cells and inhibits their cytolytic function' (Lal G, Paul S, Singh AK), American Association of Immunologists conference (IMMUNOLOGY 2013), 3-7th May, 2013, at Honolulu, Hawaii, USA (J Immunol 2013 190:53.20).
- 'IL-21R+ marginal zone precursor (MZP) B cells in the germinal center induce IL-10 dependent allogeneic tolerance' (Lal G, Nakayama Y, Sethi A, Singh AK, Burrell BE, Kulkarni N, Brinkman CC, Iwami D, Bromberg JS), American Transplant Congress held at Seattle, USA, 22nd May, 2013. (Presentation in basic science plenary session).

Nibedita Lenka

- Workshop on "Patient needs and Stem Cell Research", Co-organized by the Centre for Bionetworking, Univ. of Sussex, UK and Sochara, India, St. John's Research Institute St. John's Medical College campus, Koramangala, Bangalore, India, October 5, 2013 (Invited Member).
- 'Unraveling the Cell fate decision Machinery using Embryonic Stem Cell System' (Invited Speaker). National Training Programme on Advances in Stem Cell Therapy in Livestock and Pets and its Business Potential, 2013. Indian Veterinary Research Institute, Izatnagar, UP, India.
- 'Delineation and functional characterization of midbrain precursors during neural differentiation from Embryonic Stem cells'. International Conference on Neurosciences (Brain Plasticiy and Neurological Disorders), 2013, Ravenshaw University, Cuttack, Odisha (Invited Speaker & Session Chair).
- 'Neurogenesis: Default or instructive?' 6th World Congress on Preventive and Regenerative Medicine. 2013, KIIT University, Bhubaneswar, Odisha (Invited Speaker).
- 'Stem Cells -The Biology and Implications'. Department of Biotechnology, Utkal University, Bhubaneswar, 2013 (Guest Lecture).
- 'Permissive and Instructive cues underlying Early Neurogenesis. Pune Biologists Meet, 2014, NCCS, Pune (Invited Speaker).
- 'Exploration of therapeutic efficacy of Embryonic Stem Cell derived Dopaminergic neurons using hemi-parkinsonian Animal models'. National seminar on Stem Cell Biology and the Era of Regenerative Medicine, 2014, AIIMS, Raipur (Invited Speaker).

Lalita Limaye

- 'Efficient expansion of umbilical cord blood derived CD 34+ cells in a co culture with mesenchymal stem cells in the presence of apoptotic inhibitors' (Darshana Kadekar,V.P.Kale and L.S.Limaye), International Society of Stem Cell Research (ISSCR) 11th Annual meeting, June 12-15, 2013 , Boston, USA.
- 'Cryopreservation, ex vivo expansion & differentiation of hematopoietic & mesenchymal stem cells from umbilical cord blood' (Invited talk), National Seminar on Biotechnology for Sustainable Growth and Development, C.K.Thakur College, Panvel, India 3rd January, 2014.

Shekhar Mande

- 'Redox proteins of *Mycobacterium tuberculosis*', Indus-II synchrotron, Indore, April 2013.
- 'Networks in Biology', CSIR Foundation day talk, Indian Institute of Chemical Biology, Kolkata, September 2013.

- 'Netowrks in Biology', MC Nath memorial oration, Department of Biochemistry, RSTM Nagpur University, Nagpur, October 2013.
- 'MTBSS: *Mycobacterium tuberculosis*, Bioinformatics and Structural strategies towards treatment', EU-INDIA STI days, Paris, France, October 2013.
- 'Very high resolution crystal structure of NrdH protein', International conference on interdisciplinary areas with chemical sciences, Chandigarh, October 2013.
- 'Structural understanding of thiol-basd redox homeostasis in *Mycobacterium tuberculosis*', Shri GB Joshi memorial oration, Pune, November 2013.
- 'Very high resolution crystal structure of *Mycobacterium tuberculosis* NrdH'. Indian Crystallographic Association, New Delhi, November 2013.
- 'Interdisciplinary sciences in modern biology', INSPIRE camp, Nagpur, December 2013.
- 'Redox processes in *Mycobacterium tuberculosis*', National symposium on innovations in TB diagnostics, drug targets and biomarkers, Wardha, January 2014.
- 'Very high resolution structure of *M. tuberculosis* NrdH', Indo-UK meeting on Proteins: structure, function and dynamics, Pune, January 2014.
- 'Very high resolution structure of *M. tuberculosis* NrdH, Molecular architecture, dynamics and assembly in living systems', Indian Biophysical Society, Kolkata, February 2014.
- 'Interdisciplinary sciences in modern biology', Bhopal, February 2014.
- History of crystallography', Indian Institute of Science, Bangalore, February 2014.
- 'History of crystallography', Indian Crystallographic Association, IISER, Mohali, March 2014.
- 'Historical Developments in the Understanding of Protein Structure and Function'. Talk for Science Academies' Summer Research Fellows, NCCS, Pune, 3rd July, 2013.

Debashis Mitra

- 'Host factors in HIV-1 pathogenesis and novel strategies targeting the virus, Debashis Mitra. 79th Annual Meeting of Indian Academy of Sciences, 8th – 10th November 2013, Chandigarh, India.
- Indian National Science Academy (INSA) Anniversary Meeting, 16th - 28th December 2013, Lucknow, India

B. Ramanamurthy

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

Srikanth Rapole

- 'An integrated proteomics and metabolomics approaches for the discovery of potential biomarkers in breast cancer' (Invited talk), Indo-French seminar on recent trends in proteomics, organized by CEFIPRA & IISc, Bangalore, April 6-8, 2013.
- 'Proteomic analysis using mass spectrometry' (Invited talk), National workshop on genomics, proteomics and bioinformatics (GPB-2013), Annamalai University, Chidambaram, July 19-21, 2013.
- 'Proteomics Applications' (Invited talk), One day lecture series on proteomics and applications, Aravind medical research foundation (AMRF), Madurai, August 3, 2013.
- 'An attractive and promising strategy for early cancer diagnosis through the assembly of the human cancer volatome' (Invited talk), EU-INDIA STI Cooperation days focus on health, organized by New Indigo, October 11-12, 2013, Paris, France.
- 'Proteomics data analysis and statistical validation' (Invited talk), Workshop on proteomics, biomarkers, and diagnostic, Regional Center for Biotechnology (RCB), New Delhi, October 21-25, 2013.
- 'Identification of novel potential biomarkers for breast cancer using mass spectrometry based proteomic and metabolomic approaches' (Invited talk), Human cancer volatomics workshop organized, Rostock medical center, Rostock, January 29-31, 2014.
- 'Identification of novel potential biomarkers for breast cancer using integrated proteomic and metabolomic approaches' (Invited talk), Recent trends in biology, Pune University, March 28-29, 2014.
- Human cancer volatomics workshop organized by Rostock medical center, January 29-31, 2014 in Rostock, Germany.

Arvind Sahu

- 'Complement's plea for entry into the antiviral defense club' (Invited talk), Department of Microbiology and Cell Biology, IISc, Bangalore, April 7, 2014.
- 'Virally encoded complement regulators as tools to understand human complement regulation' (Invited talk), Regional Centre for Biotechnology (RCB), Gurgoan, April 16, 2014.

- 'Complement inhibitors: a resurgent concept in anti-inflammatory therapeutics' (Invited talk)', B.V. Patel PERD Centre, Ahmedabad, April 21, 2014.
- 'Complement's plea for entry into the antiviral defense club' (Invited talk), Department of Biochemistry, AIIMS, New Delhi, April 28, 2014.
- 'Rational designing of C3-convertase inhibitors', 22nd Molecular Immunology Forum, Bose Institute, Kolkata. January 9-11, 2014.

Padma Shastry

- 'Contrasting effects of fungal lectin, *Rhizoctonia bataticola* lectin (RBL) in human leukemia cells and normal CD3 and CD34 positive cells' (Invited talk), Glycobiology of cancer; Lectins as tools and targets (GCLTT), Karnatak University, Dharwad, India.,7th to 9th November 2013.
- 'Role of prostate apoptosis response-4 (Par-4) in human glioma stem cells during drug-induced apoptosis' (Invited talk), 33rd Annual Convention of Indian Association for Cancer Research, Kochi,India, 13th to 15th February 2014.

Anjali Shiras

- 'Glioma stem cell - endothelial cell niche interactions define malignant progression in Glioblastoma' (Anjali Shiras, Aman Sharma and Dattatraya Mujumdar), International Society for Stem Cell research (ISSCR), 12th - 15th June, 2013, Boston, USA.
- 'Role of long non - coding RNAs - Ginir and Giniras in Cell growth, Regeneration and Homeostasis' (Invited talk), Institute of Bioinformatics and Applied Biotechnology; Bangalore, India, September 2013.
- 'Role of long non-coding RNAs - Ginir and Giniras in Cell growth and Stemness' (invited talk), KIIT University; Bhubaneswar, India, November 2013.
- 'Long non-coding RNA Ginir promotes cellular transformation, tumorigenicity and metastasis of Mouse Fibroblasts' (Invited Talk), RNA biology Meeting; Kolkata, India, March 2014.
- 'Elucidating Mechanisms of Cancer Stem cell – Niche Interactions in Glioma' (Invited talk), 2nd International Conference on Angiogenesis: Theragnostics in Cancer and Cardiovascular Diseases; KIIT; Bhubaneswar, India, February 2014.

Yogesh Shouche

- 'Whole Genome Sequencing of human gut isolates: Insights into their metabolic potentials' (Invited Talk), Indo-German Workshop on Diagnostics

of Translational Genome Sequencing in Clinical and Public Health Microbiology 19-21 March, Chennai.

- 'Insights into Metabolic Potentials of Gut Microbes by Whole Genome Sequencing' (Invited Talk), 7th Asian Conference on Lactic Acid Bacteria (ACLAB7) 6-8th September, New Delhi.
- 'Man and Microbes: Human microbiome studies in Indian context' (Invited Talk), International Symposium on Frontier Discoveries and Innovations in Microbiology and its Interdisciplinary Relevance November 17-20, 2013, Rohatak.
- 'Human Microbiome Indian perspective' (Invited Talk), Metagenomics: Role of next generation sequencing and bioinformatics 15-24, October, 2013, Anand.
- 'DNA barcoding as a tool for the accurate identification of insects' (Invited Talk), 7th Conference on Medical Arthropodology 12-13 December Tezpur.
- 'Probiotics, Microbiome and Gut Function - Transforming Health and Well-Being', 15th - 16th February, 2014, Eros Hotel, New Delhi.

Shailza Singh

- 'Comparative Modeling, Molecular Dynamics Simulations and its Application in Drug Design' (Invited Talk), Raipur Medical College under the aegis of CHIPS, Govt. of Chhatisgarh, 13-15th March 2014.
- 'Systems Biology unraveling Negative Autoregulatory Synthetic Gene Circuit in Leishmania: Structure of Transcription Gene Networks' (Invited Talk), 17th ADNAT Convention, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, February 23-25, 2014.
- 'Identification of novel Inositol phosphoryl ceramide synthase (IPCS) inhibitors in *Leishmania* by in silico screening: Structural basis for its interaction' (Invited Talk), National Conference on Recent Trends in Structural Biology, Jamia Millia Islamia, New Delhi, 18th December 2013.
- 'Numerical Simulation of Stochastic Gene Circuits in Infectious Disease Leishmania: Role of HPC' (Invited Talk), CDAC, 12th September 2013.
- 'Regulatory Dynamics of Network Robustness and Orthogonality in Tristable Circuit of *Leishmania*: A Systems Biology Perspective' (Invited Talk on), International Conference on Biotechnology and Bioinformatics, organized by Dr. D.Y. Patil Vidyapeeth and The Biotech Research Society, Hotel Le Meridien, Pune, 25th November 2013.

Sandhya Sitaswad

- 13th Annual Meeting of the Society for Free Radical Research-India (SFRR-India) and conference SFRR-INDIA-14, January 27-30, 2014, Lonavala, India.

- 2nd International Conference on Angiogenesis, February 2-4, 2014, KIIT, Bhubaneshwar, India.

Deepa Subramanyam

- 'Exploring the role of endocytosis in early mammalian development' (invited talk); Mumbai / Annual Meeting of the Indian Society of Developmental Biologists; Mumbai, India, December 2013.

Mohan Wani

- Guha Research Conference (GRC), Araku-Vizag, Andra Pradesh, December 6-10, 2013.
- 'Immunomodulatory role of IL-3 in regulation of pathological bone loss', Dept. of Microbiology, April 6, 2013.
- 'Veterinary stem cells and its applications in degenerative diseases of canines' at College of Veterinary Science, Shirval, Pune, October 5, 2013.
- 'Laboratory animal models for biomedical research', Institute of Genomics and Integrated Biology, New Delhi, November, 2013.
- 'Development of experimental laboratory animals of human diseases' at Dept. of Zoology, University of Pune, February 25, 2014.
- 'Regulation of bone remodeling by IL-3' at Dr. H. S. Gour Central University, Sagar, Madhya Pradesh, March 24, 2014.

Participation by Students / Project Staff in Workshops, Conferences, etc.

- Aditi Singh: 'Nup358 interacts with Dishevelled and aPKC to regulate neuronal polarity' (poster presentation), "Hippocampus: From Synapses to Behaviour" workshop, December 1-3, 2013, IISER, Pune, India.

- Aftab Alam: 17th Transcription Assembly Meeting JNCASR, Bangalore. March 17-18, 2014.

- Ajitanuj Rattan: Role of complement in Influenza virus Pathogenesis, 3rd Molecular Virology meeting Organized by National Institute of Virology, Pune, Jan 10-11, 2013.

- Akshada Gajbhiye: 'Comprehensive proteomic analysis of breast cancer using high-throughput complementary proteomic approaches towards early markers in disease diagnosis and prognosis' (poster presentation),

International symposium on medical proteomics and 5th annual meeting of Proteomics Society of India, 28-30 November, 2013, IISc, Bangalore. (She won: (a) the **Best Poster Award** & the **1st prize in the science sketch award**).

- Aman Sharma: 'Perivascular niche interactions of glioma stem cells - endothelial cells promote angiogenesis response in Glioblastoma' (Aman Sharma, Dattatraya Mujumdar and Anjali Shiras), Gordon Research Conference, 4 - 9th August, 2013, Rhode Island, USA.

- Ankita Chouksey: 'Structural and functional study of Nup93 with Nup205 and reconstitution of the complex in-vitro' (Ankita Chouksey and Radha Chauhan). National Seminar on crystallography 43A, 28-30 March, 2014, IISER Mohali, India.

- Ashwani Kumar: 'Structural studies on Formate Hydrogen Lyase complex' (Ashwani Kumar, C. M. Santosh Kumar, Swastik Phulera and Shekhar C. Mande), National Seminar on Crystallography-43A, 28-30 March, 2014, Mohali, India.

- Ashwani Kumar: Practical apsects of membrane crystallography, 9-12 December, 2013. inStem, Bangalore, India.

- Balkrishna Chaube: 'A mechanistic insight into the regulation of metabolic reprogramming and energy homeostasis in tumor microenvironment' (Balkrishna Chaube, Parmanand Malvi, Benoit Viollet and Manoj Kumar Bhat), 5th International Conference on Translational Cancer Research, 6th-9th, February, 2014, New Delhi, India.

- Balkrishna Chaube: 'Mitochondrial respiratory Complex I activity regulate reprogrammed metabolism and tumorigenesis via elevating NADH/NAD+ ratio' (Balkrishna Chaube, Parmanand Malvi, Shivendra Vikram Singh, Naoshad Mohhamad and Manoj Kumar Bhat), 38th Mahabaleshwer Seminar: Mitochondria, Metabolism and Energetic, 27th - 30th January, 2014, Mahabaleshwer, India.

- Balkrishna Chaube: 'Understanding the regulation of energy metabolism of cancer to restrain cell growth, proliferation and survival' (Balkrishna Chaube, Parmanand Malvi, Shivendra Vikram Singh, Naoshad Mohammad and Manoj Kumar Bhat), 1st Indian Cancer Congress, 21st- 24th, November 2013, New Delhi, India.

- Deepak Khuperkar and Indrasen Magre: Workshop on "3D Live Cell Imaging with Light Sheet Fluorescence Microscopy" October 15-18, 2013. IISER, Pune, India

- Deepak Khuperkar: 'Inter-cellular transport of Ran GTPase' (poster presentation), Frontiers in Modern Biology-2013 meet, June 15-16, 2013. Department of Biochemistry, IISc, Bangalore, India.

- Hina Ojha: International Workshop on Structural Biology & its Application to Parasitic Diseases, Organized jointly by National Centre for Cell Science and University of Pune, 3-7 March, 2014.

- Jeetendra Kumar: 'Dendritic cell based cancer immunotherapy: Exogenous addition of Arachidonic acid to the culture media as a novel strategy to generate dendritic cells with enhanced functionality' (Jeetendra Kumar, Rupali Gurav, Vaijayanti Kale and Lalita Limaye) - Poster presentation, IX DAE-BRNS LIFE SCIENCES SYMPOSIUM (LSS-2013) on Current Advances in Immuno Biology and Cancer 28-30th November, 2013,Mumbai, India.

- Jeetendra Kumar: 'Targeting Human umbilical cord blood derived Dendritic Cells as an allogenic source for cancer immunotherapy' (Jeetendra Kumar, Vaijayanti Kale and Lalita Limaye) - Poster presentation, International Conference on Recent Advances in Cancer Prevention and Therapeutics. 19-20th November, 2013 at the School of Life Sciences, Central University of Gujarat, Gandhinagar, India.

- Khushman Taunk: Workshop and hands-on training on volatile breath analysis, organized by Rostock medical center, January 29 to Feb 26, 2014, Rostock, Germany.

- Neeraja Kulkarni: Poster presentation, 'Chemokine receptor signaling affects the differentiation & function of CD4 Tcells' (Kulkarni N, Sethi A, Singh A K, Shaikh S I, Sharma M, Boppana R & Lal G), NCCS mini-symposium on cell biology, 8th May, 2013, NCCS, Pune.

- Mamata Khirade: Workshop on microarray data analysis using R and bioconductor packages; 16 - 18 Jan, 2013; Trivendram, Kerala, India.

- Manas Sahoo, Swati Gaikwad and Santosh Kumar Yadav participated in "Frontiers at Modern Biology-2013" meet, June 15-16, 2013. Department of Biochemistry, Indian Institute of Science, Bangalore, India.

Meghal Desai receiving the prize for oral presentation and certificate of excellence.

- Meghal Desai : 'Computational Synthetic Prediction of post-deletional metabolic optimality in Leishmania: Steady state mathematical model analysis' (Meghal Desai and Shailza Singh), Accelerating Biology: The Next Wave', 18-20th Feb, 2014, organised by CDAC, YASAHDA, Pune. (Meghal Desai was awarded the **3rd prize for oral presentation and certificate of excellence** by Mr. Raj Shah, Chairman & CEO, Capital Technology and Information Services,Washington,DC).

- Nandaraj Taye: 'Wnt 3a activation inhibits tumor suppressor protein SMAR1 and in turn positively regulates Cyclin D1' (oral presentation), 17th Transcription Assembly Meeting, JNCASR, Bnagalore, March 17-18, 2014.

- Naoshad Mohammad: 'Cholesterol depletion by methyl-beta-cyclodextrin augments tamoxifen induced cell death by enhancing its uptake in melanoma' (Naoshad Mohammad, Parmanand Malvi, Avtar Singh Meena, Shivendra Vikram Singh, Balkrishna Chaube, Garikapati Vannuruswamy, Mahesh J Kulkarni and Manoj Kumar Bhat), 5th International Congress on translational Cancer Research, 6th- 9th, February, 2014, New Delhi, India.

- Naoshad Mohammad: 'Depletion of membrane cholesterol by methyl β-cyclodextrin enhances the therapeutic effectiveness of tamoxifen in melanoma' (Naoshad Mohammad, Parmanand Malvi, Avtar Singh Meena, Shivendra Vikram Singh, Balkrishna Chaube and Manoj Kumar Bhat), Ist Indian Cancer Congress, 21st - 24th, November 2013, New Delhi, India. (He was awarded the **2nd prize for poster presentation under the 'Skin Cancer'** track).

- Kulkarni N, Sethi A, Singh AK, Shaikh SI, Sharma M, Boppana R and Lal G (2013) Chemokine receptor signaling affects the differentiation and function of CD4 T cells. In NCCS mini-symposium on cell biology, 18th May, 2013, NCCS, Pune. (Poster presentation).

- Nidhi Chaudhary: 'Regulation of DNA damage repair by nuclear matrix protein SMAR1' (poster presentation), EMBO meeting, Athens, Greece. October 7-11, 2013.

- Parmanand Malvi: 'Caloric restriction in ob/ob mice improves chemotherapeutic response in melanoma' (Parmanand Malvi, Balkrishna Chaube, Vimal Pandey, Naoshad Mohammad, Shivendra Vikram Singh and

Manoj Kumar Bhat), 5th International Conference on Translational Cancer Research, 6th - 9th, February, 2014, New Delhi, India.

- Parmanand Malvi: 'Controlling obesity reduces rapid progression of melanoma: In vivo studies in high-fat diet exposed syngenic mouse model' (Parmanand Malvi, Balkrishna Chaube, Vimal Pandey, Maleppillil Vavachan Vijayakumar and Manoj Kumar Bhat), 1st Indian Cancer Congress, 21st - 24th, November, 2013, New Delhi, India.

- Parmanand Malvi: 'Diet-induced obesity impairs chemotherapeutic outcome in melanoma' (Parmanand Malvi, Balkrishna Chaube, Vimal Pandey, Naoshad Mohammad, Shivendra Vikram Singh and Manoj Kumar Bhat), 33rd Annual Convention of Indian Association for Cancer Research (IACR), 13-15 February, 2014, Kollam, Kerala, India.

- Parmanand Malvi: 'High fat diet-induced obesity helps in achieving peak bone mass in young rats' (Parmanand Malvi, Vikrant Piprode, Balkrishna Chaube, Satish T. Pote, Monika Mittal, Naibedya Chattopadhyay, Mohan R. Wani and Manoj Kumar Bhat), 12th International Congress on Obesity, 17th - 20th, March, 2014, Kuala Lumpur, Malaysia. (He was awarded the **International Travel Award** by Science and Engineering Research Board, DST, Govt. of India).

- Pravin Dewangan: 'Structural and functional studies on Nup62 of the nuclear pore complex' (Pravin dewangan, Ankita Chouksey & Radha Chauhan), National Seminar on crystallography 43A, 28-30 March, 2014, IISER Mohali, India.

- Priyanka Chaudhary: 'Regulation of HIV-1 gene expression and replication by Heat Shock Proteins' (Priyanka Chaudhary, Pratima Rawat, Vince Guerriero and Debashis Mitra), Cell Symposia – What Will It Take To Achieve An Aids Free World?, 3 - 5th November 2013, San Francisco, California, USA.

- Pruthvi Raj Bejugam: 'Thermodynamics of the DNA dependent RNA polymerase III as putative ribozyme in Leishmania : Insight into its Simulations' (P.R.Bejugam and S.Singh) - Poster presentation, Accelerating Biology: The Next Wave', 18 - 20th Feb 2014, organised by CDAC, YASAHDA, Pune.

- Saurav Pal: 'Tumor microenvironment induces phynotypic and functional changes in NK cell to promote tumor growth' (Paul S and Lal G), 4th International Conference on Stem Cells and Cancer (ICSCC 2013), Haffkins Institute, Mumbai, India, 19 - 22nd October. (**Best oral presentation award**).

- Shivendra Vikram Singh: 'Mutant p53 status and its relevance with cancer chemotherapy' (Shivendra Vikram Singh, Avtar Singh Meena and Manoj Kumar Bhat), 5th ICTCR cancer conference, 6-9, February, 2014, New Delhi, India.

- Shivendra Vikram Singh: 'Proteasomal inhibition sensitizes cervical cancer cells to Mitomycin C induced bystander killing' (Shivendra Vikram Singh, Amrendra Kumar Ajay and Manoj Kumar Bhat), NCRI Cancer Conference, 3rd - 6th November, 2013, London, UK. (He was awarded the **Company of Biologist Travel Award** by the National Cancer Research Institute).

- Srinadh Choppara: 'APC/C complex functions as a negative regulator of tumor suppressor FBXO31', Poster competition organized by IISER-Pune on the occasion of National Science Day, 28 Feb, 2014 (He won the **Best poster award**).

- Shrirang Inamdar: Parkinson's Disease Education Programme, (Understanding Parkinson's Disease: From Clinics to Basics), Dept. of Neurophysiology and Neurology, NIMHANS, Bangalore, India, March 5-7, 2014.

- Shruti Joshi: Poster presentation at XXXVII All India Cell Biology Conference (AICBC) on Cell Dynamics and Cell Fate, organized by Institute for Stem Cell Biology & Regenerative Medicine, Bangalore, India from 22-24 December, 2013.

- Shruti Joshi: **Best Poster Award** at National Research Scholars Meet (NRSM) in Life Sciences-2013 held at Advanced Centre for Treatment Research and Education in Cancer, Navi Mumbai, Indiaon December 19-20, 2013.

- Shweta Singh: 'Requirement of species-specific factors by human stem cells cultured on murine stromal cells expressing constitutively active AKT signaling', First International and Third National Conference on Biotechnology, Bioinformatics and Bioengineering, 28-29 June, 2013, Tirupati, India (Organized by Society for Applied Biotechnology).

- Sonali Shinde : 'Gene Regulatory Network Modeling in Schistosomiasis: An Insight into the catalytic site for active site directed drug design' (Sonali Shinde and Shailza Singh) - Poster presentation. 25th November 2013, International Conference on Biotechnology and Bioinformatics, Hotel Le Meridan.

- Sonar S : 'Inflammation in peripheral tissues loosens the blood-brain barrier (BBB) and induces T cell infiltration into neuronal tissues' (Sonar S and Lal G), "NCCS mini-symposium on cell biology", 18th May, 2013, NCCS, Pune. (**Best oral presentation award**).

- Suchismita Panda: 'Role of Ginir in Cell Proliferation in mouse fibroblasts' (Suchismita Panda, Varsha Shepal and Anjali Shiras), Symposium on Advances in Non - Coding Genomics, 13 - 14th September, 2013. Institute of Bioinformatics and Applied Biotechnology, Bangalore, India.

- Sudarshan Shetty and Sandeep Walunjkar: "Probiotics, Microbiome and Gut Function - Transforming Health and Well-Being", the 15th - 16th February, 2014, Eros Hotel, New Delhi.

- Suprita Ghode: 'Identifying the role of Neuropilin-1 in the regulation of HSC fate', First International and Third National Conference on Biotechnology, Bioinformatics and Bioengineering, 28 - 29 June, 2013, Tirupati India (Organized by Society for Applied Biotechnology).

- Surbhi Chouhan: 'DKK4 regulates glucose induced canonical wnt signaling pathway in hepatocellular carcinoma' (Surbhi Chouhan and Manoj Kumar Bhat), 33rd Annual Convocation of Indian Association of cancer research, 13th - 15th, February, Kollam, Kerela, India, 2014.

- Surbhi Chouhan: 'Hyperglycemia activates wnt β-catenin signalling in hepatocellar carcinoma' (Surbhi chouhan and Manoj Kumar Bhat), First Indian Cancer Congress (ICC), November 21st - 24th, 2013, New Delhi, India. (She was awarded the **best poster of conference award** and the **1st prize in poster presentation under 'Gastrointestinal Cancer'** track).

- Swapnil Kamble: Discrete molecular classes of ovarian cancer suggestive of unique mechanisms of transformation and metastases. Kamble SC,Gardi NL, Deshpande TU, Budhe SR, Bapat SA. IACR,2014, 7-9 Feb, 2014, Trivandram, Kerala, India.

- Tushar More: 'Metabolic profiling using NMR and LC-MS based approaches towards detection of early disease markers of breast cancer in diagnosis and prognosis' (poster presentation), Indo-US symposium on mass spectrometry based metabolomics in disease biology, 23-24 January, 2014, RGCB, Thiruvananthapuram, India.

- Vadreenath Tripathy: SERB School of Neuroscience, VII Edition, University of Hyderabad, Hyderabad, India, Dec. 9-21, 2013.

- Vineetha Mandlik : 'Biochemical network Modeling of IPCS in L. major: Detecting systems cues for therapeutic intervention' (Vineetha Mandlik and Shailza Singh) - Oral Presentation, DBT JRF Regional Meet, November 21-22, 2013, ICT Mumbai.

Symposia, Workshops & Other Events Organized by NCCS

International Workshop on Structural Biology and Its Application to Parasitic Diseases

(organized in association with the Dept. of Biotechnology, S.P. Pune University)

3-7 Mar, 2014

Speakers: Prof. Wim Hol (Univ.of Washington, USA), Prof. Paul Michels (Université Catholique de Louvain, Belgium), Dr. Shekhar Mande (NCCS, Pune), Prof. Jayanta Pal (S.P. Pune Univ) & Dr. Milind Patole (NCCS, Pune).

Prof. Paul Michels
delivering a lecture

Participants at the workshop

Hands-on session being
conducted by Dr. Payel Ghosh

Computational Aspects in Cancer Biology

Half-day meeting of clinicians, cancer biologists & computational biologists, which included talks by Prof. M. Vidyasagar, Dr. Sharmila Bapat & Dr. Gopal Kundu.

(Organizing Committee: Dr. Sharmila Bapat, Dr. Gopal Kundu)

11th February, 2014

'Predicting clinical parameters in endometrial and lung cancer'
- Prof. M. Vidyasagar, FRS

'Tumor heterogeneity'
- Dr. Sharmila Bapat

'Current strategies & future directions of breast cancer research'
- Dr. Gopal Kundu

International Conference on Angiogenesis Research, Theragnostics in Cancer and Cardiovascular Diseases (co-organized with KIIT University, Bhubaneshwar)

(NCCS faculty member in the organizing Committee: Dr. G. C. Kundu)

2-4 Feb, 2014

Nobel laureate, Prof. Ferid Murad and other dignitaries at the inauguration of the conference.

Pune Biologists' Meet (Focus of the 1st Meeting : '**Cell Trafficking**')

31st Jan, 2014

Symposium on 'Proteins: Structure, Function and Dynamics'

(organized in association with IISER-Pune & NCL, Pune)

30th Jan, 20144

EMBO Global Exchange Lecture Series by Prof. Titia Sixma (*Head, Div. of Biochemistry, Netherlands Cancer Institute*)

(A series of lectures organized by NCCS in Pune & Hyderabad, in association with IISER-Pune, CDFD and CCMB)

16-20 Dec, 2013

Prof. Titia Sixma delivering a talk at NCCS under the aegis of the 'EMBO Global Exchange Lecture Series'.

3rd Meeting of DBT-Ramalingaswamy Fellows

(organized In Pune on behalf of DBT, Govt. of India)

12-14 Sep, 2013

Opening remarks by
Dr. Meenakhi Munshi (Joint Director, DBT)

Presentation being made by
a DBT-Ramalingaswamy Fellow

Discussions between DBT-Ramalingaswamy
Fellows and mentors

Students' Mini-symposium on Cell Biology (18th May, 2013)

A symposium was organized by the research scholars of NCCS, for students from all research institutions in Pune and the S. P. Pune University.

Invited Speakers (& judges for the oral & poster presentations):

Prof. Tapas Kundu (JNCASR, Bangalore), Dr. Roop Mallik (TIFR, Mumbai) & Prof. Shubha Tole (TIFR, Mumbai).

Student who won the prize for 'Best Oral Presentation':

Sandip Sonar (NCCS, Pune)

Students who won the prize for 'Best Poster Presentations':

1st Prize: Abdul Khalique (NCCS, Pune)

2nd Prize: Chaitanya Mungi (IISER-Pune)

Oral presentations

Poster presentations

Prize distribution

Ph. D. Degrees Awarded to NCCS Research Students

(01.04.2013 – 31.03.2014)

No.	Research Scholar	Thesis Title	Month & Year of Award	Research Guide
1	Pankaj Verma	Phylogeny of prokaryotes from estuarine and costal sediments of India	June 2013	Dr. Ramesh Bhonde (co-guide: Dr. Yogesh Shouche)
2	Remya Raja	Studies on role of osteopontin in regulation of angiogenesis and breast tumor growth in response to hypoxia	June 2013	Dr. Gopal Kundu
3	Avtar Singh Meena	Functional significance of wild-type /mutant p53 and molecular mechanisms underlying chemotherapeutic drugs susceptibility/resistance	July 2013	Dr. Manoj Kumar Bhat
4	Pankhuri Vyas	Role of the nucleoporin Nup358 in PAR complex mediated polarization during neuronal differentiation	July 2013	Dr. Jomon Joseph
5	Vishal S. Parekh	Isolation, characterization and differentiation of human islet progenitor cells from non-endocrine sources	July 2013	Dr. Anand Hardikar
6	Rajkumar Singh Kalra	Protein Profiling and Identification of Transformation Associated Pathways in Epithelial Ovarian Cancer	September 2013	Dr. Sharmila Bapat
7	Sarang N Satoor	Understanding the role of Multigenerational under-nutrition in metabolic disorders	October 2013	Dr. Anand Hardikar
8	Pabitra Kumar Sahoo	A novel role for disheveled in translational regulation of cytoplasmic mRNAs	October 2013	Dr. Jomon Joseph
9	Malik Johid Reza	Studies on molecular basis of complement regulation in Herpesvirus saimiri complement control protein homolog (HVS-CCPH)	October 2013	Dr. Arvind Sahu
10	Shardul Kulkarni	Glucose induced translation regulation of insulin mediated by 5'utr binding proteins	November 2013	Dr. Vasudevan Seshadri
11	Nidhi Chaudhary	Regulation of DNA damage repair by nuclear matrix protein SMAR1	January 2014	Dr. Samit Chattopadyay
12	Dhiraj Prakash Dhotre	Comparative genome analysis of mosquito vector	January 2014	Dr. Yogesh Shouche
13	Nachiket P. Marathe	Studies on prevalence and transfer of antibiotic resistance genes in the environments containing high levels of antibiotics	February 2014	Dr. Yogesh Shouche
14	Girish Jayant Kulkarni	Molecular characterization of *Ochrobactrum* spp. isolated from human gastric biopsies	March 2014	Dr.Yogesh Shouche
15	Prashant Kumar Pandey	Microbial ecology of gastrointestinal tract of human infants	March 2014	Dr.Yogesh Shouche
16	Navjot Kaur	Molecular and Functional Studies on a Regulatory RNA involved in cell proliferation and programming	March 2014	Dr. Anjali Shiras

Commemorating the NCCS Silver Jubilee

25th Foundation Day

The Silver Jubilee Foundation Day was celebrated on the 26th of August, 2013, with much enthusiasm by the entire NCCS family.

Many activities were organized on this occasion, with the major events listed below:

Inauguration of the NCCS auditorium by
Prof. P. Balaram (Former Director, IISc., Bangaluru)

Silver Jubilee Foundation Day Address by Prof. P. Balaram
'Chemical Diversity in Biology'

Directors' Address

Dr. U.V. Wagh
Founder Director

Dr. G.C. Mishra
Former Director

Dr. S.C. Mande
Director

Felicitation of NCCS employees who have completed 20 years of service

- by Prof. W.N. Gade (Vice Chancellor, S.P. Pune University)






Cultural programme

(organized and presented by the staff and students of NCCS)




Silver Jubilee Orations

'Brain development & the emergence of behavior'

Prof. Vijayraghavan, FRS

Secretary, Department of Biotechnology

14th June, 2013

'Eradicating Cancer in Our Lifetime: Novel Approaches and New Horizons'

Prof. Ananda Chakrabarty

Distinguished University Professor, University of Illinois at Chicago, USA

29th July, 2013

'Regulation of NF-kB and Ral pathways by atypical Ras proteins'

Prof. Sankar Ghosh

Silverstein and Hutt Family Professor and Chairman, Department of Microbiology & Immunology, Columbia University, New York, USA

29th August, 2013

'The complement system: complex formations and conformational changes'

Prof. Piet Gros

Director, Bijvoet Center, Utrecht University, The Netherlands

18th Dec, 2013

'The Use of Recent Advances in Electron Microscopy to Study Ribosomes at High Resolution'

Dr. Venkatraman Ramakrishnan FRS

6th January 2014

'Machine Learning Methods in Computational Cancer Biology'

Prof. M. Vidyasagar FRS

Cecil & Ida Green Chair in Systems Biology Science, University of Texas at Dallas, USA

12th February, 2014

Talks by Other Invitees

Dr. Manvendra Singh
University of Pennsylvania, USA
'Role of Semaphorin 3D in Cardiovascular development and disease'
3rd April, 2013

Faculty members of the Department of Health Sciences
Curtin University, Australia
23rd April, 2013

Dr. Natalia Markova
Drug Discovery Group, GE Healthcare, Sweden
'Discussions on trouble shooting of ITC200 which Includes assay development mostly on biological samples'
13th May 2013

Dr. Naresh Chandra Bal
Ohio State University, USA
'Role of two muscle Ca2+-handling proteins in health and disease'
11th June, 2013

Prof. Madhav Gadgil
Chairman, Western Ghats Ecology Expert Panel & Emeritus Scientist, NCCS &
Dr. S. Narendra Prasad
Former Sr. Principal Scientist, Salim Ali Centre for Ornithology & Natural History, Coimbatore
'A decision support system for sustainable development of the Western Ghats (Use of modern spatial technology tools)'
2nd July, 2013

Prof. Madhav Gadgil

Dr. S. Narendra Prasad

Dr. Sachin Deshmukh
Mind/Brain Institute, Johns Hopkins University, Baltimore
'Objects, space, and memory: how the hippocampal cognitive map comes together'
1st July, 2013

Dr. Vasanta Subramanian

University of Bath, United Kingdom

'Role of Primary cilia and hedgehog signalling in cellular reprogramming, brain stem cells, patterning and cerebellar ataxia'

18th July, 2013

Dr. Deepshikha Chakravortty

Associate Professor

Department of Microbiology and Cell Biology, IISc, Bengaluru.

'Bacteria and Their Fascinating Machinery'

Jointly organized by the Association of Microbiologists of India (AMI) Pune Unit & MCC

23rd July 2013

Dr. Gautam Soni

Kavli Institute of NanoScience, Dept. of Bionanoscience, TU Delft, The Netherlands

"From Gene Sequencing to Gene Silencing'

25th July, 2013

Dr. Radhika Subramanian

The Rockefeller University, New York, USA

'Marking and measuring dynamic cellular structures'

27th August, 2013

Dr. Sri Devi Narasimhan

Scientific Editor, Cell

'Behind the Scenes at Cell Press'

15th October 2013

Dr. Jonas Cicenas
Swiss Institute of Bioinformatics, Geneva, Switzerland
'"Omics" research in cancer'
23rd October, 2013

Ms. Girija Natu
Information officer, DAAD-India Information Center, Pune
'Opportunities for research, collaborations & funding in Germany'
28th October, 2013

Prof. Anuradha Ray
Dept. of Medicine, Univ. of Pittsburgh, USA
'The Rise and Fall of Tregs Impacting Asthma Pathogenesis'
17th December, 2013

Prof. Titia Sixma
Head, Div. of Biochemistry, Netherlands Cancer Institute
'Protein-Protein Interactions Regulate Ubiquitin Conjugation in the DNA Damage Response' (EMBO Global Exchange Lecture Series)
19th December, 2013

Prof. Usha Menon
Head, Gynaecological Cancer Research Centre, University College London, UK
'The evolution of invasive epithelial ovarian cancer - insights from UKCTOCS'
24th December, 2013

Prof. Shyni Varghese:
Dept. of Bioengineering, Univ. of California, San Diego, USA
'Unraveling the impact of extracellular matrix in tissue regeneration and disease'
31st January, 2014

Dr. Emilie Marcus
CEO, Cell Press & Editor, Cell
'Publishing & the editorial process'
5th of February, 2014

Dr. Mahendra Rao
Director, NIH Center for Regenerative Medicine, USA
'Stem Cells for Screening and Therapy'
17th February, 2014

Dr. Arindam Basu
University of Pennsylvania, Philadelphia.
'Bringing Polycombs to DNA: Did we figure it out?'
11th March, 2014

Dr. Kirti Sharma
Max Planck Institute of Biochemistry, Munich
'Quantitative mass spectrometry for drug discovery and signal transduction research'
18th March, 2014

Dr. Prasad Purohit
State University of New York, USA
'Molecular events and kinetics of nicotinic acetylcholine receptor channel activation'
26th March, 2014

Public Outreach

1) 'Science and Non-science'

Talk by Nobel laureate, Dr. Venkatraman Ramakrishnan, organized at NCCS for college & school students (Jan, 2014)

2) National Science Day (Feb, 2014)

Popular science talks & displays for NCCS students and staff, as well as students of colleges in Pune

'Who can do science: How students, farmers, housewives & even illiterates can do science'
- Prof. Milind Watve
(IISER-Pune)

'Engineering 3D microenvironment for cellular programming'
- Dr. Prakriti Tayalia
(IIT- Mumbai)

'Wonderful world of life versus deadly cancer'
- Dr. Samit Chattopadhyay
(NCCS, Pune)

Display of instruments

Display of posters

3) 'Radioscope'

NCCS organized talks, discussions & interviews for 'Radioscope', a national science magazine broadcast by All India Radio, New Delhi, to educate the general public about diverse topics in science. These were organized in association with scientists from neighbouring institutions.

12th April, 2013

- **Topics Featured:** 'Stem cell biology'; 'The human microbiome', 'The centenary of X-ray diffraction'; 'SKA-the world's largest radio telescope'.
- **Partcipants:** Dr. Ishwar Chandra (NCRA-TIFR), Prof. Yashwant Gupta (NCRA-TIFR), Dr. Sharmila Mande (TCS Innovation Labs), Dr. Shekhar Mande (NCCS), Dr. Jyoti Rao (NCCS), Dr. Anjali Shiras (NCCS), Dr. Yogesh Shouche (NCCS) & Dr. Deepa Subramanyam (NCCS).

14th Mar, 2014

- **Topics Featured:** 'Scientific Temper'; 'Man's Evolutionary Future'; High-Performance/Super Computers; Science news.
- **Partcipants:** Prof. Madhav Gadgil (NCCS), Dr. Shekhar Mande (NCCS), Prof. Rajat Moona (C-DAC), Prof. Sunil Mukhi (IISER-Pune) & Dr. Jyoti Rao (NCCS).

4) News paper article

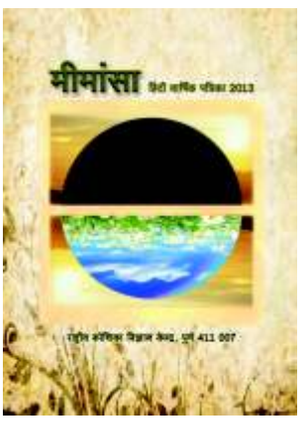
- 'स्फटिकशास्त्राची शतकी वाटचाल' ("Crystallography's Centennial March")
  Article by Dr. Shekhar Mande in the Marathi newspaper, Loksatta (26th January, 2014).

5) Online science magazine article

- "How to tame your virus?"
  This article written by Ph.D. student, Ms. Surya Shrivastava, was published by 'The Briefing' (3rd November, 2013

Other Happenings at NCCS

Launch of the NCCS Annual Hindi Magazine, 'Meemansa' (16th Sep, 2013)



Prize distribution for the Sports Events held at NCCS (15th Aug, 2013)

Prize distribution by:Sensai Yogesh Dhadve (Maharashtra Judo Association)

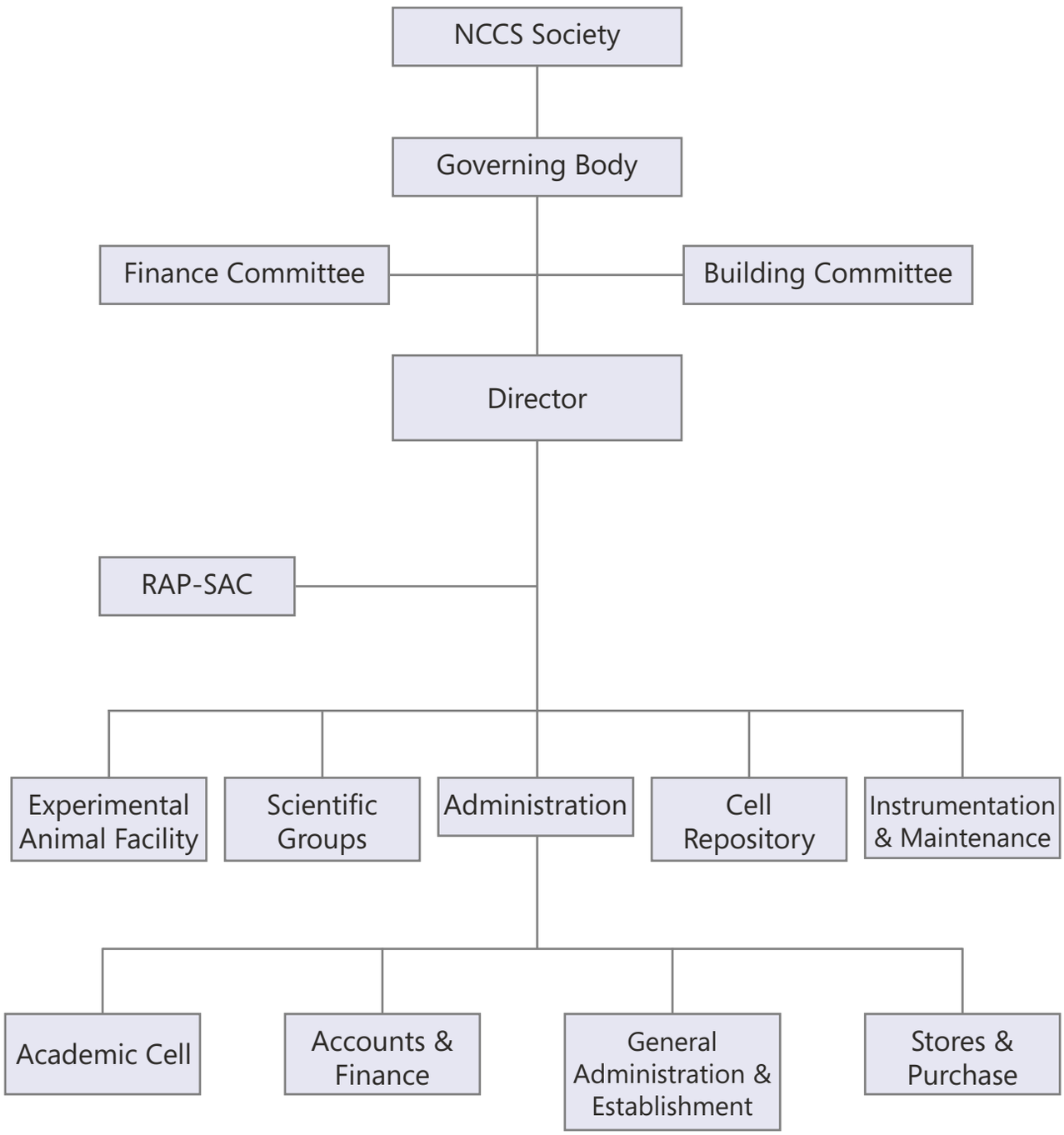
Blood Donation Drive (14th Mar, 2014)

Co-ordinated and organized by Mr. Satish Pote (Technical Officer, Lab 1)

Award given by the Poona Serological Inst., in appreciation of NCCS volunteers who donated blood.

NCCS Organization

NCCS Organization



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Administration

The NCCS Administration consists of the following sections: General Administration & Establishment, Civil Maintenance, Accounts & Finance, and Stores & Purchase. The centre also has an Instrumentation & Maintenance unit. All these sections provide support services to the main scientific activities of the centre.

The NCCS staff strength as on 31st March, 2014, is given below:

Scientists	:	34
Administrative Staff	:	40
Technical Staff	:	69
Total	:	143

Reservation Policy

NCCS follows the Government of India orders on reservation matters. For direct recruitments, we follow respective rosters, 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. The Centre also follows the Government of India reservation policy for physically handicapped candidates.

Right To Information Act 2005

As per the requirement of the RTI Act 2005, NCCS has nominated Shri. V.S. Shinde, Officer 'B' (Administration) as the CPIO and Dr. (Mrs.) V.P. Kale, Scientist 'F', has been nominated as the First Appellate Authority.

Vigilance Matters

Dr. Lalita Limaye, Scientist 'F', has been nominated Chief Vigilance Officer with effect from 24/01/2013 of the centre. Vigilance reports are sent regularly to the nodal ministry, i.e. the Department of Biotechnology, Government of India, New Delhi.

Shri S. P. Singh, Additional Superintendent of Police, CBI, Pune, gave a lecture on 'Vigilance' on 1st Nov, 2013, on the occasion of vigilance week (28th Oct – 2nd Nov, 2013).

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.

Disciplinary Matters

The centre follows CCS (Conduct) rules 1964, CCS (CCA) rules 1965 and NCCS bye-laws for monitoring disciplinary matters at the centre.

Implementation Of Official Language

The Director, NCCS, strongly supports the use of the Official Language in official work, and other related activities carried out at the Institute. 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 this Centre are discussed.

Most of the forms and formats for official documents that are used regularly have been made bilingual. The Institute's website has also been made bilingual.

In the year 2013-2014, the first issue of the annual Hindi magazine of NCCS, 'Meemansa', was launched during the 'Hindi week' celebrations. This was done at the hands of Prof. Rajat Moona (DG, C-DAC), the former Directors of NCCS, Dr. U. V. Wagh and Dr. G. C. Mishra, and Dr. Shekhar C. Mande, Director, NCCS. This magazine includes articles, photographs and other material written and submitted by staff and students of NCCS. It will be published and released every year during the Hindi Week.

Launch of the annual Hindi magazine of NCCS, 'Meemansa'.
Left to right: Dr. S.C. Mande, Dr. G.C. Mishra, Prof. Rajat Moona & Dr. U.V. Wagh.

The 'Hindi week' celebrations also included Hindi essay and 'Shabdavali' ('Paribhashik Shabda') competitions, where the winners were awarded cash prizes and certificates. Prof. Padmaja Ghorpade, Head of the Hindi Department at the Sir Parshurambhau College, Pune, was invited as the examiner for the competitions. Dr. Damodar Khadse, Chairman of the Hindi Sahitya Academy, Govt. of Maharashtra, and Member of the Hindi Advisory Committee, Govt. Of India, was invited as the Chief Guest for the Hindi Day Function held on 20th September, 2013.

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

National Centre for Cell Science
An autonomous institution aided by the Department of Biotechnology, Govt. of India

Contents & Editing : National Centre for Cell Science
Photographs courtesy : Jeetendra Kumar, Phalguni Rath, Sunil Kachare, Mandar Rasane, Ashwini Atre
Layout and Printing : United Multicolour Printers Pvt. Ltd., 264/4, Shaniwar Peth, Pune 411 030
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