OF IMMUNOLOGY

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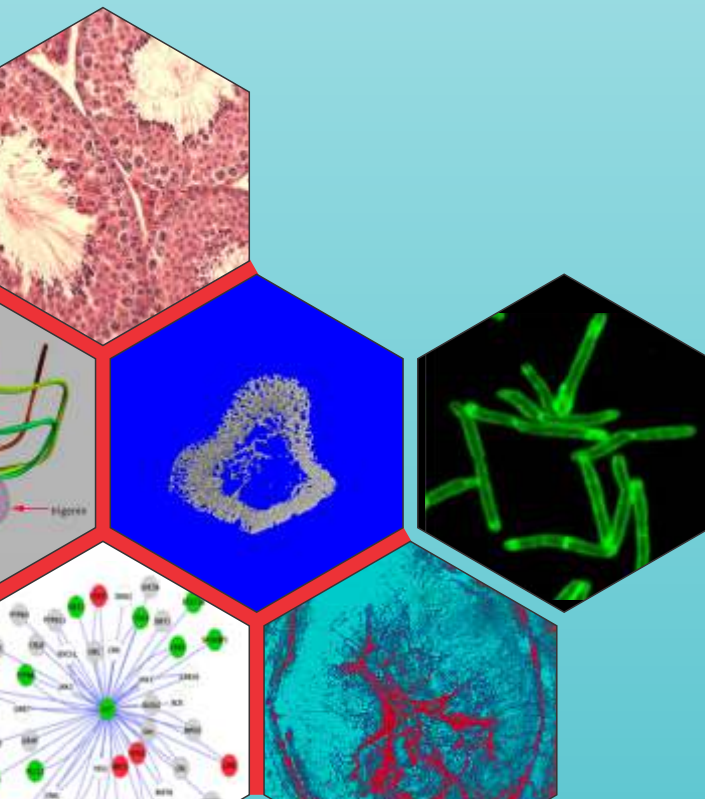
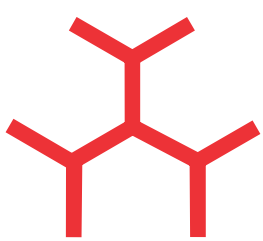


NATIONAL INSTITUTE OF IMMUNOLOGY



ANNUAL REPORT
2018-19

CONTENTS

MANDATE OF THE INSTITUTE

- To undertake, aid, promote, guide and co-ordinate research of high caliber in basic and applied immunology.
- To carry out research for development of new vaccines and immunological reagents for communicable diseases.
- To develop immunological approaches for regulation of male and female fertility.
- To interact with industry for manufacture of vaccines and immunological reagents.
- To organise postgraduate courses, workshops, seminars, symposia and training programmes of a specialized nature in the field of immunology, vaccine development and related areas.
- To organise training programmes for technicians in immunological methods and related techniques.
- To establish affiliation with recognised universities and institutions of higher learning for the purpose of enabling research scholars to register for postgraduate degrees.
- To serve as a national reference centre for immunology and to provide consultancy services to medical and veterinary institutions, public health agencies and industries in the country.
- To provide and promote effective linkages on a continuing basis between various scientific and research agencies/laboratories and other organisations working in the country in the field of immunology, vaccine development and related areas.
- To collaborate with foreign research institutions, laboratories and other international organisations in fields relevant to the objectives mentioned above.

FOREWORD

It is with a sense of immense pride and satisfaction that I present this Annual Report (2018-2019).

The promotion of research in basic and applied immunology forms a central mandate of the National Institute of Immunology; our work strives to provide immunological solutions to the many diseases that are primarily prevalent in India. Using the tools of modern biology, we are pursuing programs which aim to elucidate the factors that promote tumorigenesis and infection, as we also develop new vaccines to fight these conditions. Influences upon the ageing process, as well as signals which precipitate the onset of chronic diseases (such as diabetes and systemic autoimmunity) comprise other interests. Recently, we have refocused our research activities on the following broad areas: (a) Immunology and Vaccines (b) Infectious and Autoimmune Diseases (c) Cancer Biology and Immunotherapy (d) Metabolic Disorders and Chronic Diseases (e) Structural and Computational Biology, and (f) Genetics, Cell and Development Biology. Here, I briefly highlight some of the progress we have made in these areas, and outline the new research programmes we have initiated over the last year.

The characterization of host-pathogen interaction has been a long-standing interest - diseases currently in our ambit in this regard include AIDS, tuberculosis, typhoid, pneumonia, malaria and dengue fever. The determinants of inflammation are also under study.

How HIV-1 and the Dengue virus modulate host cellular machinery for their own advantage is being assessed. It appears that CHIP – E3 ligase is a potent suppressor of HIV-1 replication. The role of de-ubiquitinases and micro RNAs in Dengue infection are being explored.

Mycobacterium indicus pranii (MIP) is being studied for its utility as an alternative, more potent vaccine against tuberculosis (TB). Correlates of protective immune responses induced by MIP have been identified; MIP, when administered intra-nasally, more effectively induces homing of antigen-specific Th1 cells to the lung airways. These findings have important implications for the development of future vaccines against TB.

The histidine biosynthesis pathway, essential for *Mycobacterium tuberculosis* (*M.tb*), is absent in humans; enzymes that comprise this pathway therefore represent ideal targets for the development of new anti-TB drugs. A number of potent imidazole- and triazole- scaffold inhibitors against HisB have been designed; they exhibit significant anti-TB activity *in vitro*, and are non-toxic. An *M.tb* dienoyl CoA reductase required for breakdown of fatty acids has now been characterized. These studies provide deeper understanding of the mechanisms by which *M.tb* survives in lipid-rich niches. Abrogation of ligand binding, or sequestration of the ligand, leads to aberrant localization of PknB, causing cell death; these observation open up new avenues of intervention.

A major research effort at the Institute has been on the development of nanoparticle-based vaccines against *S. pneumoniae*. The immunological evaluation of nano-formulations containing carbohydrates derived from different serotypes of *S. pneumoniae*, as well as of pneumococcal protein-polysaccharides conjugates, is underway. The role of *S. pneumoniae* purine nucleoside phosphorylase in host-pneumococcal interaction is being elucidated; mice infected with mutant strains deficient in *pnp1* or *pnp2* demonstrate significantly lower bacterial load, indicating a requirement for the enzyme for optimal bacterial growth *in vivo*. Immunodominant proteins

from *S. pneumoniae* and are also being assessed for their utility as protein alone-based vaccines.

Cell signaling events in *Plasmodium falciparum* and *Toxoplasma gondii* are being explored. In particular, molecular mechanisms via which kinases PfCDPK1 and PfCDPK7 regulate their target proteins are being elucidated. Molecules of the malaria parasite which affect host cellular processes are being identified, as are synthetic and natural molecules which inhibit parasite growth. A novel synthetic molecule, currently under study, has demonstrated inhibitory activity on multiple parasite stages, a highly desirable property in an anti-malarial drug.

Salmonella typhi causes systemic infection typhoid, while non-typhoidal *Salmonella* serovars, such as *Salmonella enteritidis*, produce gastroenteritis. Immunization of mice with live *Salmonella typhi* was shown to generate antibodies against carbohydrate determinants shared by *Salmonella typhi* and *Salmonella enteritidis*. These antibodies not only clear *Salmonella typhi* but can also provide protection against *Salmonella enteritidis* in a mouse model. Neutrophils play a role in the dissemination of the *leishmania* parasite, and emerging observations indicate a role for autophagy in the process. A Halictin peptide induced significant death of both leishmania promastigote and amastigote forms within host cells; the possibility developing a new drug based on these observations is being explored.

Pro-inflammatory cytokines and pathogen-derived substances trigger the canonical NF-κB pathway, activating RelA/NF-κB heterodimers, and inducing the genes which drive immune responses. In contrast, tissue-morphogenetic cues engage the non-canonical NF-κB arm to activate RelB/NF-κB heterodimers, which mediate the expression of immune-differentiation factors. Combining experimental and mathematical analyses, the functional crosstalk between these NF-κB-activating pathways is being characterized.

Studies on basic immunology have gathered pace.

Follicular helper T (Tfh) cells influence both the magnitude and quality of antibody responses. The functional traits of Tfh cells in sustained immunity, upon vaccination as well as upon recovery from natural infection, are being enumerated; studies encompass human subjects immunized with a Japanese encephalitis vaccine, as well as experimental animals.

The events that influence the development and function of dendritic cells are being elucidated; in particular, studies continue to shed further light on the role of *Irf8* in the generation of $CD8\alpha^+$ dendritic cells. Further analysis would help identify the pathways that control cDC1 development and function; such data could aid in the design of improved vaccination strategies.

Enhancer-promoter interactions in vertebrate genomes are modulated by several factors; CTCF has emerged as a factor of significance. CTCF binding sites (CBS) orchestrate a dynamic organization of chromatin loops in eukaryotic genomes. The mouse TCR locus has 21 CBS; insertion of ectopic CBS was shown to impair the loop organization, dramatically influencing VDJ recombination.

Protein synthesis has been known to be de-regulated in a wide range of diseases, including in cancer and autoimmune disease. Many genetic disorders are now known to be associated with mutations that affect tRNA biogenesis. tRNA modifications which are decreased in effector immune cells have been identified; biochemical implications of such the differences are being assessed.

Studies on the biology and therapeutics of cancer have diversified.

Ablation of the cancer-testis antigen SPAG9 was shown to result in reduced cellular proliferation and colony-forming ability, and in the increased apoptosis of tumor cells. Phase II clinical trials (double-blind, randomized, in collaboration with Cancer Institute (WIA), Chennai) are currently underway to evaluate the efficacy of dendritic cells pulsed with recombinant SPAG9 in patients of Stage IIIB cervical cancer. Of the forty five patients completing the vaccination schedule, thirty have demonstrated a complete response.

Studies aimed at elucidating the mechanisms by which long non-coding RNAs and microRNAs prevent the genomic aberrations that promote oncogenesis

are underway. microRNAs of the miR-16 and miR-34 families have been shown to target cyclin A2, a major cyclin protein.

Secretion of gonadotropin by tumors is associated with chemoresistance and poor patient prognosis. Ovariectomy was shown to enhance tumor volumes when gonadotropin-responsive tumor cells were implanted in βhCG transgenic mice. These finding are of relevance in the study of tumors that arise in post-menopausal women.

Mutations in BLM helicase predispose Bloom Syndrome patients to a wide spectrum of cancers. New work has demonstrated that the presence of BLM lowers the binding of proto-oncogene c-Jun to its cognate targets. Over-expression of c-Jun in absence of BLM leads to enhanced tumor growth. The therapeutic aspects of these findings are being explored.

Cyclin F is the substrate recognition subunit of SCF (Skp1-Cul1-F-box protein) ubiquitin ligase complex. The role of SCF ubiquitin ligase complex in regulating oncogenic pathways is being investigated. Data indicates that RBPJ regulates the expression of *IDH1*, which is mutated to an oncogenic form - $IDH1^{R132H}$ - in cancer. Metabolic stress-induced cyclin F therefore appears to attenuate the oncogenic functions of $IDH1^{R132H}$.

The study of chronic diseases remains a continuing focus, as are the factors that influence life-span and the aging process.

Oxidized hemoglobin was shown to generate heightened levels of lupus-associated cytokines from several cell lineages derived from lupus-prone mice and from PBMCs derived from SLE patients. Accumulating evidence suggests that hemoglobin is a disease-perpetuating autoantigen in systemic autoimmunity.

Human calcitonin is not considered as a viable therapeutic option in osteoporosis because of its tendency to self-associate into amyloid fibrils. A Supramolecular Calcitonin Assembly (SCA-I) was shown to release monomers and demonstrated superior anti-osteoclast activity *in vitro*.

Retinitis pigmentosa is a progressive, irreversible retinal degenerative disease which causes partial or complete blindness. Monocytes have been differentiated into retinal neuron-like cells; transplantation of these cells a murine model resulted in improved depth perception and better exploratory behaviour.

Caenorhabditis elegans and mice have been employed as model systems to understand the molecular basis of aging. Since dietary restriction (DR) is known to increase life span and delay onset of age-related diseases, molecular events (including signalling pathways, and transcription and post-transcriptional responses) that follow DR are being deciphered.

Computational and biophysical approaches continue to shed light on as-yet undiscovered molecular pathways, providing predictions about structure, as well as on enzyme action.

Machine learning-based bioinformatics tools have been developed which predict the final cyclised chemical structures of polyketides and nonribosomal peptides encoded by biosynthetic gene clusters (BGC) in a completely automated fashion, given the genome sequence of the BGC as input. This work represents a valuable resource for the discovery of natural products by genome mining.

Acyl carrier protein (ACP) is an indispensable component of the type II fatty acid biosynthesis pathway. The fatty acid synthase (FAS) enzymes that associate with ACP have a conserved ACP recognition motif near their active sites. Comparison of ACP-FAS structures suggests that the helix II of ACP interacts with its partners in a manner comparable to the ubiquitin interacting motif. Study of the biology of human gunanylate binding proteins continues; unlike in human gunanylate binding protein-1 (hGBP-1), the tetramer of a large GTPase hGBP-2 does not appear to be important for GMP formation. The thermostability of human arginase-I was demonstrated to enhance in the presence of Co^{2+} ions. Since the enzyme is a potential candidate for the treatment of L-arginine auxotrophic cancers, the identification of factors that increase stability is useful from a therapeutics perspective.

Staphylococcus aureus sortase A-catalyzed ligation of recombinant or synthetic LPXTG polypeptides to an aminoglycine derivatized moiety occurs efficiently *in vitro*, and has inspired numerous applications. Cyclic sortases are being designed for enhanced stability, and the substrate specificity of a class E sortase from *Thermofida fusca* is being assessed.The structural and enzymological aspects of glycosomal enzymes in the parasitic protozoa *Leishmania* are being elucidated, with a particular focus on phosphoglycerate kinase and its isoforms. Metabolic glycan engineering has been employed for the inhibition of glycosylation, using synthetic hexosamine analogues. The ability to modulate glycosylation using small molecules has the potential to extend understanding of the pathology of autoimmune disorders, as well as aid in treatment.

Reproductive biology has been a traditional focus; both basic and applied aspects of the field continue to engage our interest.

Contraceptive vaccines for use in the management of wildlife populations were evaluated. Regulatory approvals have been obtained to establish proof-of-concept in female beagle dogs at Palamur Biosciences Private Limited, Secunderabad, India.

Infertility is an emerging global problem, and compromised Sertoli cells (Sc) function could be a contributing factor. Events that govern the regulation of sperm production by Sc are being elucidated, using high throughput genomics and proteomics tools. To aid in this work, transgenic animals with Sc-specific knockdown of specific genes have been generated.

NII overview

NII actively interacts with Institutes in the Delhi-NCR region in areas of shared interest. We have recently signed MOUs with IIT Delhi and INMAS (DRDO) Delhi to initiate collaborative research programmes. We also interact with several local colleges under the Science Setu program. Doctoral Students, Research Fellows, Post-Doctoral Research Associates and highly-trained Technical Personnel are flag-bearers of our research programmes. Our administrative staff members carry out their roles with dexterity and commitment, ensuring the smooth functioning of our research endeavors. I express gratitude towards all members of the NII community for constantly striving to better themselves, for there is no better way serve an Institution and its ideals.

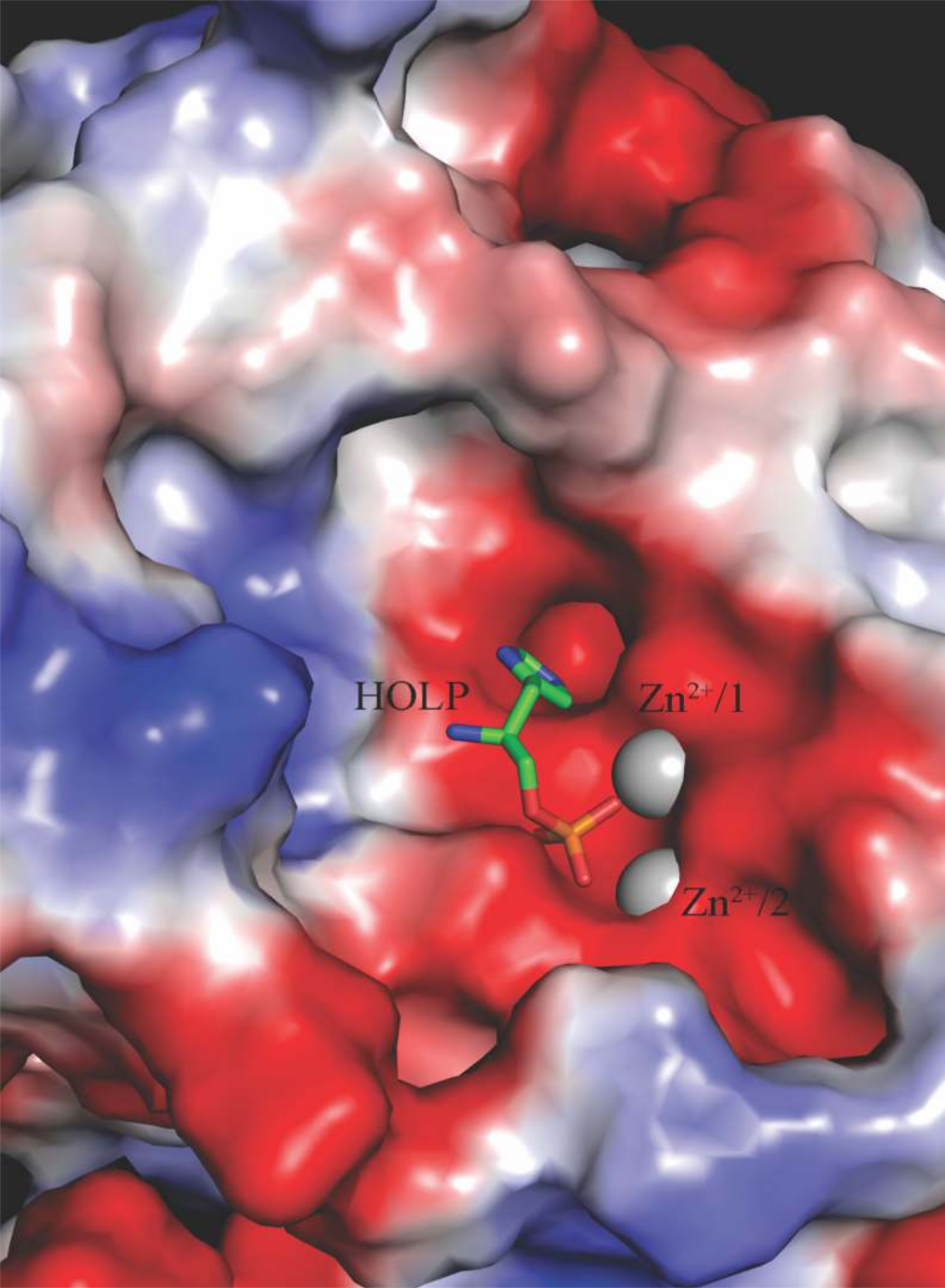
The last few years have seen tremendous advances in the understanding of the immune system; as with any definitive science, quantitative understanding of immunological phenomenon is gaining increasing significance. Emerging information and insights carry with them new opportunities, and it is always our endeavour to develop novel therapeutic modalities in our spheres of work. Towards this end, a Flagship Programme (ImmunoEngineering) is being developed. This Programme will use and create novel tools in the areas of Synthetic Immunology, Nanotechnology, Artificial Immune Systems, High Definition Cellular and Molecular Imaging, Biomaterials and Microfabrication, Therapeutic Delivery System, and Immunophysics. To begin with, the Programme will focus on three areas: (i) Immunotherapy for Cancer (ii) Creation of Cell Therapy Platforms, and (iii) Discovery and Development of Adjuvants from the Natural Products Repository of India. As the Programme evolves, it is expected that it will act as an impetus for the development of novel vaccines, as well as for new therapeutics for autoimmune diseases; we also desire to contribute in the areas of regenerative medicine and implantable chips.

I wish to thank members of our Governing Body and Scientific Advisory Committee for their constant and un-wavering encouragement; their feedback and critical appraisals propel us from strength-to-strength, enabling us to realize our full potential. We are deeply appreciative of the scientific, administrative and financial support we receive from the Department of Biotechnology; we are co-travelers in this exciting journey, so full of promise and opportunity.

Amulya K. Panda
Director

Date: 16th August, 2019

RESEARCH REPORTS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IMMUNITY AND INFECTION

Understanding the role of interferon regulatory factors in cell development and innate immunity

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Dendritic cells (DCs) are composed of multiple subsets that collectively provide early innate immunity leading to subsequent adaptive immunity. Plasmacytoid dendritic cells (pDC), $CD4^+$ DC, $CD8\alpha^+$ DC and $CD4^-CD8^-$ DC are four major subtypes in the murine spleen. These subtypes of DCs express different sets of genes and assume distinct functions. We are interested in understanding the mechanisms of DC subtype development and their specific functions. Members of Interferon regulatory factors (IRFs) play critical role in DC biology. Interferon regulatory factor 4 (*Irf4)* and Interferon regulatory factor 8 (*Irf8)* plays pivotal role in generation of diverse DC subtypes. The development of $CD8\alpha^+$ DC and pDC requires *Irf8*, whereas $CD4^+$ DC subset is dependent on *Irf4*. In a simplified system of DC classification, $CD8\alpha^+$ DC and equivalent population from various tissues is classified as cDC1 whereas $CD4^+$ DC equivalent population is classified as cDC2 subtype. Various studies from knockout mouse model led to identification of critical role for Inhibitor of DNA binding 2 (*Id2*), Basic leucine zipper transcription factor 3 (*Batf3*) and *Irf2*, *Relb* in development of $CD8\alpha^+$ DC and $CD4^+$ DC respectively. Our efforts are mainly focused at understanding the cross talk between IRFs and other transcription factors in defining DC subtype development and understanding contribution of TGF-β signaling in *Irf8* directed DC development.

Understanding the interactions between transcription factors in defining DC subtype development

Irf8, *Batf3* and *Id2* play an essential role in development of cDC1 subtype. We had reported hierarchical relation between transcription factors *Irf8*, *Id2* and *Batf3* that are important for classical $CD8\alpha^+$ DC development. To better understand the mechanism of development of $CD8\alpha^+$ DC; we established yellow fluorescent protein (YFP) based bimolecular fluorescence complementation (BiFC) assay in a living cell to define interaction between IRF8, BATF3, ID2 and known partner PU.1 as a positive control. N-terminal half of YFP was expressed as a fusion protein ahead of ID2, BATF3 and PU.1; later half of YFP was cloned as a fusion protein at C-terminus of IRF8 and its DNA binding domain (DBD) mutant $IRF8^{K79E}$ and a IRF association domain (IAD) mutant $IRF8^{K289E}$. IRF8-YCC and $IRF8^{K79E}$-YCC when co-expressed with YNN-PU.1 led to efficient YFP signals but $IRF8^{K289E}$-YCC failed to show YFP signals with YNN-PU.1. YNN-ID2 did not show YFP signals when co-expressed with IRF8-YCC, whereas YNN-BATF3 had showed interaction with IRF8-YCC and $IRF8^{K79E}$-YCC but not with $IRF8^{K289E}$. Our results from bimolecular fluorescence complementation (Bi-FC) assay suggested that

BATF3 directly interacts with IRF8 in guiding CD8α$^+$DC development; whereas ID2 may aid CD8α$^+$DC differentiation by blocking pDC development. We confirmed that cre-recombinase mediated deletion of *Batf3* in cDC1 BMDC cultures from *Batf3$^{fl/fl}$* mice selectively decreased transcript levels of CD8α$^+$DC subtype, suggesting *Batf3* specific transcription program at developmental stages. For in-depth understanding of the BATF3 and IRF8 interaction in CD8α$^+$ DC development, we prepared the partner interaction dead (BATF3^{H55Q}) and the DNA binding dead mutant of BATF3 (BATF3^{S43D}). BATF3^{S43D} mutant interacts with IRF8 but heterodimer could not bind to the IRF8-BATF3 composite elements thus competing with endogenous BATF3 and resulted in decrease in the CD8α$^+$DC specific transcripts and enhancing that of pDCs. BATF3^{H55Q} mutant did not have any effect on CD8α$^+$ DC specific transcription as it could not compete with endogenous BATF3 for interaction with IRF8. Thus, we confirm that it is not the BATF3-IRF8 interaction but its recruitment to chromatin is essential for the differential transcription towards development of CD8α$^+$ DC subtype. To identify the cDC1 genes that are guided by the IRF8-BATF3 interaction, we performed IRF8 and BATF3 ChIPseq data analysis. IRF8 is expressed mainly in pDC and cDC1 subtype and ChIPseq data analysis revealed 258 binding sites that are common in these two DC subsets while 3586 and 2371 IRF8 binding sites were specific to each DC subset respectively. Similar ChIPseq data analysis for BATF3 suggested 6941 and 837 binding sites in cDC1 and cDC2 subtype respectively with only 4 binding sites being common to both theses DC subtypes. Further analysis of 3586 IRF8 and 6041 BATF3 binding sites specific to cDC1 subtype; 1135 sites showed overlapping peaks for IRF8 and BATF3 suggesting possibility of IRF8-BATF3 composite element at these sites. We sorted CD24$^+$DC subtype equivalent cDC1 subtype population form BMDC cultures and performed RNA seq analysis to identify transcripts specifically regulated by IRF8-BATF3 interaction. Co-relation of co-occupancy of IRF8 and BATF3 with the cDC1 transcriptome suggested that approximately half of IRF8-BATF3 specific peaks bound in intergenic regions. We are further analysing genes that specifically show co-recruitment of IRF8 and BATF3 with respects to their transcription status and specificity of expression with respect to cDC1 subtype. Our analysis will help us to identify the pathways that directly regulate cDC1 development and functions and this information can be employed to develop future strategies to regulate immune responses under physiological conditions.

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Plasmodium proteins involved in virulence and host modulation: Host-parasite interactions in Plasmodium liver stages

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Basic theme is to identify, new parasite molecules that affect the host cellular processes. Such parasite proteins are potential antigens and should be evaluated for their vaccine potential.

1. Role of *Plasmodium berghei* heat shock protein PBANKA_093830 (*Pb* HspJ2) in parasite life cycle:

In parasitophorous vacuole proteome Heat Shock Proteins [HSPs] were abundant (~34%), especially under stress condition, and major HSP are predicted for transport into the host cytosol. HSPs and chaperons are potential antigens for vaccine. PBANKA_093830 is a heat shock protein family member with DNAJ2 domain. In order to know the role of PBANKA_093830 in parasite growth and development we generated a knockout parasite line. A clonal population was obtained. We find knockout parasite grow normally in blood stages but fail to form gametocytes and later stages (Zygote, Ookinete, Oocyst) of the parasite, indicating essentiality for sexual stages development. To reveal the role of *Pb*HspJ2 we compared the transcriptome of *Pb*HspJ2 knockout parasites with wild type. We found 117 transcripts (genes) were down regulated more than four folds in *Pb*HspJ2 knockout. 25% of down regulated transcripts were related to gametocyte development and cell motility.

2. Studies on an immuno-modulatory protein of malaria *(TIP):*

Longitudinal evaluation of CD4+FoxP3+ T-reg cell levels in a human sporozoite challenge study documented that an increased number of these cells was associated with higher parasite loads and a decline in pro-inflammatory cytokines. It appears that malarial parasite somehow modulate the immune response including the modulation of T cells. To know Malaria TIP funtion, we administered four dose of endotoxin free recombinant protein intravenously to mice. On day-2 mice were challenged with blood stage parasites. Protein injected mice showed higher growth of parasites (175± 25%) compared to untreated indicating its role in parasite growth in the host. Using sandwitch ELISA method TIP protein was detected unequivocally in serum of malaria infected mouse up to 1:64 dilution. This indicates TIP protein is released in the blood circulation.

3. Drug discovery:

a) Neem-derived compounds: Seventeen chemical entities were purified from Neem leaves and tested against blood stage malaria parasites. Nine of them showed antimalarial activity. We tested two best inhibitors against liver-stage parasites in the *in-vitro*

culture model and found efficacious in low nano-molar range. We further plan to test these inhibitors in the Mouse model of malaria. *b) Synthetic Hydroxy-ethyl-amine (HEA) derivatives:* Several derivatives of hydroxy-ethyl-amine backbone were tested for blood and liver stage parasite inhibitory potential. A few of them were active in low micromolar range (IC50 < 2.0 uM). Efforts are underway to find derivative with better inhibition potential.

Publications

Original peer-reviewed articles

1. Singh S, Rajendran V, He J, Singh AK, Achieng AO, Vandana, Pant A, Nasamu AS, Pandit M, Singh J, Quadiri A, Gupta N, Poonam, Ghosh PC, Singh BK, Narayanan L, Kempaiah P, Chandra R, Dunn BM, Pandey KC, Goldberg DE, Singh AP, Rathi B. (2018) Fast-acting small molecules targeting malarial aspartyl proteases, plasmepsins, inhibit malaria infection at multiple life stages. **ACS Infect Dis.** 5: 184-198.

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3. Pradhan S, Kalia I, Roy SS, Singh OP, Adak T, Singh AP, Dhar SK. (2019) Molecular Characterization and Expression Profile of an Alternate Proliferating Cell uclear Antigen Homolog PbPCNA2 in Plasmodium berghei. **IUBMB Life.** 71:1293-1301.

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Functional analysis of host and viral genes that affect HIV and Dengue pathogenesis

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Viruses have limited number of genes to combat the host challenge and therefore most of the viral proteins are poly-functional. It is our goal to understand mechanistically how this host-pathogen interaction favouring ultimately the pathogen is achieved. Similarly, Dengue virus is a major public health problem and this virus exploits various cellular machinery with hallmark pathogenic events, especially the varying roles played by small RNAs constitute the major objective. Intra-cellular stability of viral and host proteins is governed my multiple mechanisms, Ubiquitination and De-ubiquitination involving various E3 ligases (including CHIP) being a major degradation pathway will be studied in details.

Infection with HIV-1 invariably results in generation of latent reservoir which can get activated when presented with the stimulus. Although this complex issue is addressed before using variety of models, we have used the serum starvation/deprivation approach to understand mechanistic details. We used latently infected monocytic cell line U1 and T-cells – J1.1. Serum starvation was able to break latency in U1 cells and under similar conditions, the T-cells failed to do so. We asked if cell death pathway and autophagy was involved with activation. These pathways were not involved. We further report that serum starvation up regulated ERK/JNK pathway. Treatment of cells with U0126, an ERK kinase inhibitor, potently inhibited HIV-1 replication. In summary, we show that latently infected monocytic cell line when subjected to serum starvation results in breakdown of latency.

It is becoming increasing clear that CHIP controls the half life of several important cellular proteins like P53. We have preliminary evidence that the stability of the two regulatory proteins (Tat and Vif) are controlled by CHIP. Transient transfetion of CHIP results in potent degradation of these viral proteins. Knock down studies are underway to establish the role of CHIP in virus replication.

We observed a remarkable bi-phasic response – in the early stages of infection the SOCs 3 levels are down regulated but eventually gets up-regulated during the late stages. We also provide evidence that SOCs levels are down-regulated with viral RNA also. We next investigated the effect of SOCs3 on NF-kB signalling. We observed that SOCS3 causes degradation of p65 via proteasomal pathway, thereby inhibiting NFkB signalling. Hence this study reveals a novel role of SOCs3 in the biology of HIV-1 involving Nf-kB signalling and host immune evasion.
Innate immunity relies heavily on various signalling adaptors involving TRAFs. We show that ubiquitination of TRAF 6 is mainly carried out by Vpr that increases NfkB and create pro-inflammatory state. We generated over-expression systems along

with knock-out shRNA constructs for TRAF3 and TRAF6.

We observed that NS5 gene of Dengue virus alone modulates the expression of mir-590 to regulate an important De-ubiquitinase (DUB)–USP42 in human microglia. The importance of this observation with respect to DENV replication is being carried out. Using mimics and anti-mir we were able to establish that mir-590 plays an important role in viral replication.

Publications

Original peer-reviewed articles

1. Ali A, Farooqui SR, Banerjea AC (2019). The host cell ubiquitin ligase protein CHIP is a potent suppressor of HIV-1 replication. **J Biol Chem.** 294:7283-7295.

2. Sood V, Lata S, Ramachandran VG, Banerjea AC (2019) Suppressor of Cytokine signaling 3 (SOCS3) degrades p65 and regulate HIV-1 replication. **Front Microbiol.** doi: 10.3389/fmicb.2019.00114.

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4. Raja R, Lata S, Trivedi S, Banerjea AC (2018) Serum deprivation/starvation leads to reactivation of HIV-1 in latently infected monocytes via activation ERK/JNK pathway. **Sci Rep.** doi: 10.1038/s41598-018-32316-2.

Review/Proceeding

1. Lata S, Mishra R, Banerjea AC (2018) Proteasomal degradation machinery: Favorite target of HIV proteins. **Front Microbiol.** doi: 10.3389/fmicb.2018.02738.

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Regulation of immune responses under physiological conditions and under conditions of stress

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We have been been trying to understand the mechanistic basis for our finding that a) IgA-high CBA mice are resistant to DSS-colitis while IgA-low B6 mice are susceptible and b) that disease in B6 mice correlates with a less-robust intestinal barrier and c) with higher fecal bacterial loads and poor coating of fecal bacteria with IgA. To determine which of these factors contributed to disease susceptibility, we carried out homologous fecal microbial transplants with purified IgA-coated and IgA-uncoated bacteria and found that only B6 recipients receiving IgA-uncoated B6 bacteria were susceptible to DSS-colitis. Ampicillin-treatment of B6 mice, reported to eliminate a cohort IgA-uncoated bacteria, also decreased disease severity. Genetic experiments with F1 mice where the female was either CBA or B6 revealed that only pups from the former were resistant. Further, in F1xParent backcrosses, resistance segregated with the IgA-high phenotype of the proximal female (and not with barrier genes). Cohousing adults of the two strains did not change susceptibility patterns of either, indicating a role for early maternal effects determined by IgA amounts in breast milk. However, in foster-nursing experiments, B6 pups transferred to CBA females immediately after birth continued to be susceptible as adults while CBA pups foster-nursed on B6 females were resistant, indicating that early maternal effects were not sufficient, and that high IgA made in adult life can compensate for suboptimal IgA received in early life. In confirmation, we also found that providing oral immunoglobulins to adult B6 mice led to amelioration of disease.

In our analysis of the effect of fever temperatures (39C) on T cell responses during priming, we found that fever-range temperatures led to the induction of GATA-3. However, this was independent of IL-4 signaling which is the major pathway of GATA-3 induction. Rather, the opening of the temperature-gated channel TRPV1 led to induction of Notch signaling with downstream effects on GATA-3 and a shift in T cell priming to a non-inflammatory Th2-response. In confirmation, we found that addition of a gamma secretase inhibitor, to prevent Notch processing, reversed the effect of fever temperatures on transcript induction and Th2 modulation.

We have also been looking at the contribution of the non-canonical NF-kappaB (NFkB) pathway is the regulation/maintenance of immune homeostasis through effects on Tregs. We found that Treg development and suppressive function were normal in mice deficient in nfkb2 (p100); however, differentiation into the effector Treg pool was higher in these mice. In bi-parental chimeras of wildtype and nfkb2-null mice, the nfkb2-null genotype was over-represented in the Treg pool, as was a higher ratio of effector:central Tregs. Further, when central

Tregs from the two backgrounds were sorted from the chimeras and then transferred at a 1:1 ratio into congenically-marked wildtype mice, higher proliferative activity, higher Treg numbers, and greater conversion to effector Tregs was seen in the NFkB2-null compartment. In keeping with the inhibitory effect of NFkB2 on Relb, we found higher Relb activity in Tregs generated in vitro from NFkB2-null mice. However, in Relb-null/wildtype chimeras, Treg proportions and effector conversion were equivalent in the two compartments. Our data are in keeping with a very recent report indicating that p100 restrains RelB-mediated Treg activation.

Publication

Original peer-reviewed articles

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2. Balyan R, Gund R, Chawla AS, Khare S, Pradhan SJ, Rane S, Galande S, Durdik JM, George A, Bal V, Rath S (2018) Correlation of Cell-Surface CD8 Levels with Function, Phenotype and Transcriptome of Naive CD8 T Cells. **Immunology.** 156: 384-401.
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Analysis of *Salmonella* - host cell interactions

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Infections with pathogenic *Salmonellae* continue to be a major public health problem particularly in the developing world. The clinical manifestations produced by different *Salmonella* serovars range from localized gastroenteritis to more serious systemic infection depending upon the serovar and the type of host. In humans, *Salmonella enetrica* serovar Typhi (*S*.Typhi) causes systemic infection typhoid while non-typhoidal serovars, *S*.Typhimurium and *S*.Enteritidis, produce only gasteroenteritis. *S*.Typhi does not establish infection in mice while *S*.Typhimurium infection in susceptible strains of mice produces a systemic disease analogous to human typhoid. The reasons for different clinical outcomes produced by these two closely related *Salmonella* serovars and for the host specificity exhibited by them are not completely understood. In our laboratory, we have been studying host-pathogen cross-talk and the regulation of immune responses during infection with these bacteria.

Accessibility of O-antigenic determinants determines antibody-mediated cross-protection with *Salmonella*

Salmonella serovars share a high degree of homology at the genome and the proteome level. However, whether infection or immunization with one serovar provides protection against related serovars has not been investigated in detail. We reported previously that antibodies generated in mice in response to infection with live *S*.Typhi readily cross-react with antigens including LPS from *S*.Typhimurium in ELISA but do not bind live / intact *S*.Typhimurium. T cells from *S*.Typhi - immune mice also responded efficiently to antigens of *S*.Typhimurium *ex vivo*. *S*.Typhi-immunized mice significantly cleared *S*.Typhimurium from the spleen in an intraperitoneal model of infection but did not survive longer than unimmunized mice. To understand the reasons for the inability of anti-*S*.Typhi antibodies to bind live / intact *S*.Typhimurium, we analyzed reactivity of these antibodies with two other closely related *Salmonella* serovars, *S*.Enteritidis and *S*.Paratyphi A. Anti-*S*.Typhi antibodies bound *S*.Enteritidis but not *S*.Paratyphi A, which suggested that the antibodies capable of binding live / intact *Salmonella* were directed against determinants that are present or accessible on *S*.Typhi and *S*.Enteritidis but not on *S*.Typhimurium and *S*.Paratyphi A. Significantly, mice immunized with *S*.Typhi cleared *S*.Enteritidis better than *S*.Typhimurium and survived for a long duration, highlighting a crucial role for antibodies in imparting immunity against *Salmonella*. Further experiments revealed that anti-*S*.Typhi antibodies capable of binding live / intact *S*.Enteritidis were directed against O-9 antigenic determinant of LPS, which *S*.Enteritidis shares with *S*.Typhi. On the other hand, antibodies against O-12 determinant, which *S*.Typhi shares with *S*.Typhimurium, *S*.Enteritidis and *S*.Paratyphi A, though present in the sera of immunized mice, did not bind live / intact *Salmonella* due to surface inaccessibility of this determinant. Similar phenomenon was observed with antibodies generated against *S*.Typhimurium and *S*.Enteritidis. These results suggest that antibodies against one *Salmonella* serovar can render protection against

other *Salmonella* serovars provided shared O-antigenic determinants are accessible on the surface of bacteria. These findings have implications for vaccine development against pathogenic *Salmonella*.

Lysophosphatidylcholine (LPC) activates caspase-1 – mediated inflammatory response by bringing about release of ATP and sensitizing cells to this danger signal

LPC is one of the major lipids present in the plasma with concentrations ranging from 20-200µM. These levels increase following infection or inflammation. Our previous studies suggested that LPC and other serum borne lipids can specifically amplify TLR-activated inflammatory responses from cells. We now show that at higher concentration (reminiscent of inflammation), LPC also activates NLRs and brings about caspase-1 – dependent release of IL-1 from macrophages. This release was almost completely abrogated in presence of ATP hydrolase, apyrase, which indicated a role for ATP in the activation of the inflammasomes with LPC. Analysis of ATP in cell – free supernatants revealed that LPC indeed triggers release of this nucleotide from cells in a time and dose-dependent manner. Inhibition of purinergic signaling during treatment of cells with LPC prevented caspase-1 activation. Taken together, our findings identify a previously unrecognized mechanism by which LPC instigates another danger signal to activate caspase-1 – mediated inflammatory response.

Publication

Original peer-reviewed article

1. Shivcharan S, Yadav J , Qadri A (2018) Host lipid sensing promotes invasion of cells with pathogenic *Salmonella*. **Sci Rep.** doi: 10.1038/s41598-018-33319-9.

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Microbial interface biology and associated host immune response

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We are interested in deciphering the molecular and cellular basis of B-cell response against protein and polysaccharide antigens present on the human bacterial pathogen *Streptococcus pneumoniae* (pneumococcus). Our other research interest is to find out how pneumococci cause disease and what interventions can be made to stop this from happening. The research is focused on the pneumococcal products and strategies that allow the pathogen to avoid being destroyed by the mammalian immune system, and the types of immune response that can circumvent these strategies and products. The main objectives are (a) identification and characterization of virulence factors from *S. pneumoniae*, (b) how these virulence factors interact with the immune system and host cell to alter its cellular and molecular processes, and (c) evaluating the vaccine potential of pneumococcal surface proteins.

Characterization of purine nucleoside phosphorylases from *S. pneumoniae*

Nucleotides play a vital role in biological processes like replication and transcription, and serves as the primary energy currency of the cell. Nucleotides are biosynthesized by *de novo* and salvage pathways. Unlike the *de novo* pathway which utilizes simple precursors, salvage pathway makes use of preexisting cytoplasmic nucleobases and nucleosides or imported from the extracellular milieu via nucleoside/nucleobase transporter(s) to form nucleotides. We aim to characterize a key enzyme of the purine salvage pathway, purine nucleoside phosphorylase (PNP) from *S. pneumoniae* and elucidate its role in host-pneumococcal interaction. PNP catalyzes the cleavage of glycosidic bond of nucleosides in the presence of inorganic phosphate to produce a purine base and sugar moiety.

Bioinformatic analysis revealed that pneumococci codes for two PNPs (i. e. PNP1 and PNP2) in its genome.Our *in vitro* enzyme kinetics analysis showed that recombinant PNP1 (rPNP1) and PNP2 (rPNP2) have overlapping, but not identical, substrate specificities. The catalytic efficiency of rPNP1 for the common substrates guanosine (16 fold), deoxyguanosine (15 fold) and inosine (9 fold) was higher than that of rPNP2. Xanthosine phosphorolysis was catalysed by rPNP1 and not rPNP2. Adenosine was used as a substrate by rPNP2 and not by rPNP1. Mutations in the putative conserved nucleoside-, phosphate-, and ribose-binding residues resulted in complete abrogation of the enzymatic activity of PNP2. We have previously reported similar findings for PNP1. Exogenous supplementation of chemically defined minimal medium (CDM) with some substrates restored the growth of D39Δ*pnp1* and D39Δ*pnp2* to wildtype level. This restoration correlated with the *in vitro* catalytic efficiency of rPNP1 and rPNP2. Complementing D39Δ*pnp1* and D39Δ*pnp2* with the corresponding enzymatically dead PNP did not restored their growth suggesting that enzymatic activity of PNPs are essential for growth of pneumococcus.

Mice infected with mutant strains deficient in *pnp1*, *pnp2* or both genes showed significantly lower bacterial load in blood, lung and spleen compared to

mice infected with wildtype or genetically complemented strains. We did not observe any additive effect due to the absence of *pnp1* and *pnp2* in the double mutant. Mice infected with strains deficient in *pnp1*, *pnp2* or both the genes survived longer than mice infected with wildtype or genetically complemented strains.

Characterization of SPD_1629*, a putative nucleobase transporter*

Bacterial ABC transporters are responsible for the uptake of a wide range of substrates including essential nutrients like sugars and metal ions. The availability of nutrients and/or metal ions may have an impact on the fitness and/or virulence of bacterial pathogens. Uracil isan essential component of CDM for pneumococci. Wildtype *S. pneumoniae* fails to propagate in CDM that lacks uracil. We are interested in deciphering how uracil is taken up by pneumococci. Blocking the transport of uracil or getting the bacterial pathogen to take up a lethal analogue of uracil through a transporter or permease can potentially serve as a strategy against *S. pneumoniae*. We have previously demonstrated that SPD_0739 is the substrate binding protein of an ATP-binding cassette (ABC) transporter that is involved in the import of nucleosides with cytidine, uridine, guanosine and inosine as the preferred substrates. Deoxycytidine, deoxyuridine and thymidine were transported less efficiently, and nucleobases and ribose sugar were not transported via SPD_0739.

The growth of wildtype *S. pneumoniae* was inhibited by 90% in the presence of 200µ M 5-fluorouracil, a lethal analogue of the nucleobase uracil. In rich medium, the growth of wildtype and D39Δ*spd_0739* strain was inhibited to the same extent in the presence of 200µ M 5-fluorouracil. The *in vitro* growth kinetics of D39 and D39Δ*spd_0739* are found to be comparable in the absence of 5-fluorouracil. This led us to conclude that 5-fluorouracil is not taken up through SPD_0739. We hypothesized that uracil is imported in *S. pneumoniae* through a transporter other than SPD_0739. *In silico* analysis revealed the presence of 3 putative nucleobase permeases, namely SPD_0267, SPD_1141 and SPD_1629, in the genome of pneumococcal strain D39. We reasoned, if 5-fluorouracil were to be transported exclusively via one of these 3 putative permeases then that mutant would exhibit a 5-fluorouracil resistant phenotype. Results of experiments involving *in vitro* growth of mutants in the presence of 5' fluorouracil showed that D39Δ*spd_1629* was partially resistance to 5-fluorouracil. This observation suggested that uracil is transported through SPD_1629 though not exclusively. To identify the natural substrate(s) of SPD_1629, a competition assay was performed wherein various pyrimidine nucleobases (cytosine, uracil and thymine), purine nucleobases (guanine, adenine, hypoxanthine and xanthine), a purine nucleoside (inosine), and sugar (ribose) were allowed to compete with 5-fluorouracil. The sensitivity of wildtype D39 strain to 5-fluorouracil is expected to reduce if the added substrate competes for transport via SPD_1629. Our observations suggest that only uracil was able to compete with 5-fluorouracil and partially restore the growth of D39Δ*spd_1629*.

Publication

Original peer-reviewed article

1. Jhelum H, Sori H, Sehgal D (2018) A novel extracellular vesicle-associated endodeoxyribonuclease helps *Streptococcus pneumoniae* evade neutrophil extracellular traps and is required for full virulence. **Sci Rep.** doi: 10.1038/s41598-018-25865-z.

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Studies on immune response from antigen loaded biodegradable polymer particles and protein refolding from inclusion bodies

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The major theme of research is to evaluate polymeric particle based delivery system for improved immunogenicity of different antigens such as Tetanus Toxoid (TT), Hepatitis B surface antigen (HBsAg), viral and carbohydrate (Vi polysaccharide and *S. pneumoniae* polysaccharides) based vaccines. We have also developed a novel scaffold fabrication method in our laboratory that involves the fusion of microparticles in presence of alcohol. Another research activity of the laboratory is the analysis of inclusion body formation and development of mild solubilization processes for improved recovery of bioactive proteins.

The main objective of the project is to improve the immunogenicity of antigens entrapped in biodegradable polymer particles. High-throughput refolding of inclusion body proteins into bioactive form is another objective of the research group. Researches in the following areas are conducted in the laboratory to achieve the objectives:

1. Analysis of immune response from antigen loaded polymer particles and evaluation of adjuvant properties associated with particle formulation. Currently we are focusing on improving the immunogenicity of carbohydrate based antigens using polymeric nanoparticles based delivery system.

2. Evaluation of polymer particle based membrane as scaffold for growth of cells and tissue developments. Currently we are focusing on developing theses polymer membranes as an artificial skin substitute for wound healing.

3. Solubilization and refolding of inclusion body proteins from *Escherichia coli*. This involves analysis of inclusion body formation and understanding of protein aggregation with an aim to recover higher amount of bioactive protein.

A. Formulation and development of nanoparticle based pneumococcal vaccine

A major research effort of the laboratory is to develop nanoparticle based Pneumococcal vaccine. The activities have been in three different areas such as (i) use of carbohydrates from different serotypes of *S. pneumoniae* and immunological evaluation of its nanoformulations, (ii) conjugation of pneumococcal protein with polysaccharides and its immunological evaluation, and (iii) purification and characterization different immunodominant protein from *S. pneumoniae* and its evaluation as protein based vaccine.

To evaluate the immunogenicity of polysaccharide based particulate vaccine, four capsular polysaccharides were selected i.e. serotype 1, 14, 6B and 5. Pneumococcal diseases due to these serotypes are highly prevalent among Indian population. To begin with, Pneumococcal Capsular Polysaccharide type 1 (PCP-1) entrapping polymeric nanoparticles were formulated and immunized in Balb/c mice. Immune response studies showed particle entrapped polysaccharide elicited enhanced IgG response than soluble polysaccharide and elicited memory antibody response from single point immunization. Optimization of polymeric nanoformulation entrapping other serotypes is under progress.

To further improve the immunogenicity of carbohydrate based vaccine, several homologous pneumococcal proteins are under investigation. SP0845 is one such protein which has been identified at NII and has shown promising results in pneumococcal infection model. Conjugate vaccine of PCP1 and SP0845 has been already made and characterized; immunization of this conjugate vaccine is under way. These conjugate vaccines will be evaluated and compared to that of nanoparticles entrapping only polysaccharides. This will provide information whether nanoparticles based polysaccharide vaccine are superior or comparable to that of conjugate vaccine. We are also evaluating nanoparticles based Pneumococcal vaccine entrapping only protein antigens of *S. pneumoniae*. We have selected four proteins such as SP0845, PsaA, Pneumolysin and ABC transporter protein of *S. pneumoniae* for this purpose. These proteins are involved at different stages of infection. Apart from animal immunization and challenge experiments, interaction of nanoparticles with antigen presenting cells such as macrophages & dendritic cells are being carried out to understand the molecular mechanism of antigen presentation using nanoparticles.

B. Fusion of polymeric particle into membrane like structure and its evaluation as scaffold for cell growth and artificial skin substitute

A new method of scaffold fabrication, designed earlier in our laboratory involved the fusion of PDLLA particles in presence of alcohol to produce a porous scaffold that can be used for growth of various types of cell *in vitro* as well as for delivering bio molecules. PDLLA based scaffold being hydrophobic doesn't support efficient cell attachment and proliferation. To reform the problem composite scaffolds were designed for specific cells requirement and tissue regeneration. For skin wound healing, PDLLA-Gelatin composite scaffold was prepared having bi-layered structure mimicking skin dermis and epidermis. The mechanical property as well as biocompatibility of the composite scaffold was found to be comparable with the Integra® that is currently used for surgical treatment of skin wound and burn healing. Primary human fibroblast and keratinocytes were seeded and their growth was monitored *in vitro* by live-dead staining and cell-viability assay kit. Cell infiltration was evaluated and compared with Integra®. For skin regeneration studies, C57BL/6 mouse model was used. Trichrome staining analysis of wound bed area suggested more collagen deposition in comparison to control group as well as Integra® (positive control) group. IHC and IF staining for αSMA was done for monitoring fibroblast activity, Ki67 staining for cell proliferative capability and F4-80 staining for macrophage infiltration. All results suggested that PDLLA-Gelatin composite scaffold supported as well

as promoted cellular adherence, differentiation, and proliferation, mimicking natural extracellular matrix (ECM).This membrane may be an ideal alternative to Integra® as an artificial skin substitute.

C. Solubilization and refolding of inclusion body proteins

Strong chaotropic agents such as high concentrations of urea and guanidine hydrochloride offer good solubility over a wide range of IBs but result in poor recovery of bioactive protein. We have developed a novel mild and versatile solubilization agent that solubilize wide range of IBs with the solubilization efficiency comparable to strong denaturing agents (Indian Patent Application No. 201811017082). Interestingly, the agent has also been observed to improve the refolding efficiency. Out of the 14 different inclusion body proteins tested, the IBs of human growth hormone (hGH), a therapeutic protein, was taken as model IBs for further downstream processing. The overall recovery of bioactive protein from IBs was close to 50%. The versatile agent was found to be protecting the secondary structure and destabilizing the tertiary structure of purified protein. This novel solubilization buffer can be used for recovery of bioactive protein from inclusion body aggregates.

We are planning the best combination of pneumococcal polysaccharide and protein conjugates entrapped in polymer particle as a single dose vaccine. Independently we are also developing polymeric formulation entrapping immunogenic protein of *S. pneumoniae*.

Technology transfer on dry powder alum is being discussed with Indian companies.

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1. Singhvi P, Panda AK (2018) "A method for solubilization of protein aggregates." **Indian Application Number 201811017082; Filed on 7/05/2018.**
2. Jha D, Kumar R, Panda AK (2018) "A Formulation for modulating immune response." **Indian Patent Application Number 201811036025; Filed on 25/09/2018**.
3. Meena J, Panda AK (2018) "A novel conjugate for vaccination against typhoid comprising chemical conjugate of vi polysaccharide and flagellin, a process for producing the same and a composition comprising the conjugate." **European Patent Application Number 17788946.6; Filed on 26/11/2018**.
4. Meena J, Panda AK (2018) "A novel conjugate for vaccination against typhoid comprising chemical conjugate of vi polysaccharide and flagellin, a process for producing the same and a composition comprising the conjugate." **US Patent Application Number 16/096387; Filed on 15/10/2018**.

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Disorders of proliferation: Analysis of novel pathways and targets

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A. Characterization of lupus-associated immune responses

Apoptotic blebs, anti-apoptotic cell-reactive antibodies

Our work has previously documented the disease-perpetuating effects of apoptotic cell-reactive antibodies in lupus. In extension of this work, human apoptotic cell-reactive IgG antibodies were found to induce IL-1β secretion from monocytes and neutrophils isolated from SLE patients; secretion diminished upon incubation with a caspase-1 activation blocker, upon blocking Fc receptors, and upon use of glyburide. Further, Mito-TEMPO, TLR 9 antagonism, as well as Dnase also inhibited secretion. The aberrant humoral recognition of cell death can therefore lead to inflammasome activation.

Apoptotic blebs were generated upon the individual incubation of tumor cells with six different cytotoxic agents. Variations were observed in the abilities of the bleb preparations to specifically enhance levels of cell-surface markers on lupus B cells, as well as to heighten the secretion of the lupus-associated cytokines from splenocyte cultures. Further experimentation aims at attributing these effects to specific bleb constituents.

Hemoglobin

While previous work demonstrated that immunization with hemoglobin (Hb) preferentially accelerates kidney disease in lupus-prone mice, ongoing investigations focus on characterizing differential inflammatory responses to Hb. PBMCs from SLE patients generated heightened levels of lupus-associated cytokines in response to Hb. Fe^{3+} Hb triggered the preferential release of such cytokines from splenocytes derived from lupus-prone mice; a similar bias was observed in the release of IL-8, IL-12 and IL-17A from plasmacytoid dendritic cells. CD8 T cells, CD4 T cells and B cells purified from lupus-prone mice were also more responsive to the inflammatory effects of Fe^{3+} Hb. Infusion of Fe^{3+} Hb into lupus-prone mice induced enhanced release of lupus-associated cytokines, compared with infusion into healthy mice. Hb appears to be preferentially inflammatory and immunogenic in a lupus milieu.

B. Delineation of the role of hCG in tumorigenesis

The secretion of human chorionic gonadotropin (hCG) by tumor cells is associated with poor prognosis in cancer patients. While hCG induced chemoresistance in tumor cells, the incubation of TLR ligands along with hCG resulted in synergistic chemo-protective effects. Apoptotic blebs were isolated upon the individual incubation of tumor cells with six different cytotoxic agents. While the combination of hCG with such blebs (which contain

endogenous TLR ligands) also induced enhanced chemo-protection, the extent of cyto-protective synergy varied, depending on particular apoptotic bleb-drug combinations; the cause and consequences of these findings are under investigation.

The implantation of gonadotropin-responsive tumor cells in βhCG transgenic mice and non-transgenic littermates resulted in higher tumor volumes in the former; only in transgenic mice did ovariectomy cause further increases in tumor incidence and volume. Significantly, estrogen and progesterone demonstrated potent cytotoxic effects on tumor cells *in vitro*, with co-incubation resulting in synergism. Whether steroid supplementation in ovariectomized βhCG transgenic mice can influence tumor growth is being assessed. Given the association between post-menopausal status and the heightened risk of cancers of different lineages, whether circulating hCG (low levels of which have been detected after menopause) can be an aggravating factor is worthy of consideration.

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Original peer-reviewed articles

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Study of immunotherapeutic potential of *Mycobacterium indicus pranii* (MIP) and the underlying mechanisms in animal models of tuberculosis and tumor

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1. To investigate the protective efficacy of MIP immunization in live or killed form, through parenteral route / by aerosol route, against subsequent infection with *M.tb* in animal models and study of underlying mechanisms of protection in MIP immunized animals.
2. To evaluate Immunotherapeutic activity of MIP and its cell wall fractions in mouse syngeneic tumor models and simultaneous study of mechanism of MIP mediated host immune activation.
3. Analysis of the role of macrophages in TB-IRIS development.

A. MIP mediated pulmonary immune response when given by intra nasal route / parenteral route

MIP induces expression of protective chemokines in lung

In order to study biomarkers of MIP mediated immunity, surface expression of certain chemokine receptors on T-cells were analysed. Significantly higher percentage of T-cells in airway lumen expressed CXCR3 post MIP i.n. vaccination. Simultaneous upregulation of cognate ligand(s) for CXCR3 i.e., CXCL11 and also CXCL10 to some extent were observed in mice lung tissue. Effector T-cell subsets with upregulated expression of CXCR3 has been implicated in control of M.tb in previous studies. In systemically immunized mice, these cells remained in lung vasculature and failed to populate lung mucosa. In 'post *M.tb* challenge' studies, increased expression of chemokine ligands CCL19 and CCL21 in draining lymph node was observed in 'MIP i.n.' group. M.tb is known to delay its trafficking by down-regulating expression of CCR7 marker on infected DCs. Increased expression of CCL19 and CCL21 in lung draining lymph nodes in MIP i.n. group suggest efficient migration of infected DCs to draining lymph nodes, resulting in effective T-cell priming and their recruitment to lungs.

Intra-tracheal transfer of MIP primed airway luminal T-cells confer protection in naïve mice

Protective potential of MIP elicited airway luminal Th1 cells was evaluated by adoptively transferring these T-cells isolated from 'MIP i.n.' group into naïve mice, followed by challenge with M.tb. Intra-tracheal transfer of T cells resulted in ~1 log CFU reduction as compared to 'infected control'. Conclusively, our results identified correlates of protective response induced by MIP, against TB, in mouse model. More specifically, the findings suggested that MIP given by nasal route more effectively induced homing of antigen-specific Th1 cells in the lung airways.

B. Role of MIP in modulation of cell death pathways in M.tb infected macrophages

Previously, we had reported that MIP is a potent inducer of autophagy in macrophages. Co-infection

of macrophages with MIP and M.tb resulted in enhanced clearance of M.tb from the infected macrophages. To examine whether MIP induced autophagy is exclusively by active mechanisms or some of its component/s has the ability to induce autophagy. It was observed that MIP lipid fraction has crucial role in autophagy induction. Mycobacterium cell wall consists of various complex lipid molecules and one of the unique molecules is Lipoarabinomannan. MIP LAM was purified and tested for immunostimulatory properties on macrophages and dendritic cells. MIP LAM was able to induce significant amount of autophagy as compared to untreated control or M.tb LAM treated macrophages. This study enhanced our understanding of the mechanisms behind MIP mediated protection in M.tb infection.

C. Analysis of the role of macrophages in TB-IRIS development

TB-IRIS is a major problem in the treatment of HIV and M.tb co-infection. It is widely accepted that a hyperactive immune response is to blame for the pathogenesis of TB-IRIS. Macrophages are the major host cells exploited by M.tb for its growth and multiplication and they are very much likely to play an important role in the activation of hyperactive $CD4^+$ T cell response and the pathogenesis of TB-IRIS. Peritoneal macrophages were isolated from wild type and $TCR\beta^{-/-}$ mice and infected with M.tb. Higher level of pro-inflammatory cytokines and co-stimulatory markers expression in $TCR\beta^{-/-}$ mice macrophages provided evidence of inherent higher activation status of macrophages in mice which lack T cells even in the absence of added T cells. Initial findings of our study showed that somehow antigen presenting cells are differently activated in the absence of T cells which supports our hypothesis.

Publications

Original peer-reviewed articles

1. Saqib M, Khatri R, Singh B, Gupta A, Bhaskar S (2019) Cell wall fraction of *Mycobacterium indicus pranii* shows potential Th1 adjuvant activity. **Int Immunopharmacol.** 70: 408-416.
2. Kumar P, Bhaskar S (2019) Myeloid differentiation primary response protein 88 (MyD88)-deficient dendritic cells exhibit a skewed cytokine response to BCG. **BMC Res Notes.** doi: 10.1186/s13104-019-4086-6.
3. Kumar P, John V, Gupta A, Bhaskar S (2018) Enhanced survival of BCG-stimulated dendritic cells: involvement of anti-apoptotic proteins and NF-kB. **Biol Open.** doi: 10.1242/bio.032045
4. Sharma A, Saqib M, Sheikh J, Chaudhuri T, Bhaskar S, Ehtesham N, Hasnain S (2018) *Mycobacterium indicus pranii* protein MIP_05962 induces Th1 cell mediated immune response in mice. **Int J Med Microbiol.** 308: 1000-1008.

Review/Proceeding

1. Singh B, Bhaskar S (2018) Methods for detection of autophagy in mammalian cells. **Methods in Microbiology.** doi:10.1007/7651_2018_190.

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The NF-kB system and signaling crosstalk

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The NF-kB system modulates immune activations as well as immune maturation processes. The canonical NF-kB pathway transduces signals from pro-inflammatory cytokines, including TNF, to activate the expression of inflammatory and immune response genes. In this pathway, signal induced degradation of IkBs trigger transient nuclear translocation of the RelA:p50heterodimer, whose activity is dynamically controlledby IkB-mediatednegative feedback. On the other hand, the noncanonical NF-kB module promotes partial proteolysis of p100 (encoded by *Nfkb2*) into p52 and liberates a sustained RelB:p52activity in the nucleus. RelB:p52 is activated in response toimmune organogenic cues, such as those signals though lymphotoxin-b receptor, and mediatesthe expression of immune differentiation factors. We are addressing if crosstalk between these NF-kB -activating pathways tunes inflammation.Combining mathematical studies and experimental analyses, we could elucidate that the noncanonical *Nfkb2*pathway contributes to the NF-kB dimer homeostasis, and thereby modulates canonical NF-kB response. We could demonstrate that the noncanonical signal transducer p100/*Nfkb2*provokes colonic inflammation in the experimental mouse model of colitis because of the newly identified NF-kB crosstalk. Our work bear promises for novel therapeutic intervention strategy in human IBD involving noncanonical NF-kB pathway.

Publication
Review/Proceeding

1. Roy P, Sarkar UA, Basak S. (2018) The NF-kB Activating Pathways in Multiple Myeloma. **Biomedicines**. 6: 59. pii: E59. doi: 10.3390/biomedicines6020059.

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Biology of follicular helper T cells in protective immunity

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Understanding the determinants of long-term sustained protective immunity may provide significant lead for the rational design of vaccine. By providing help to B cells, follicular T helper (Tfh) cells are crucial to the generation of potent germinal centers and germinal center derived humoral responses. The biology of Tfh cells in sustained protective immunity is largely uncharacterized. Therefore, the conceptual framework of our research program is to resolve the traits and function of Tfh cells in long-term sustained immunity in context of vaccination or recovery from infection. The global aim is to identify and harness the positive attributes of Tfh cells for rational development of the immunization strategies.

In order to determine the positive traits and function of Tfh cells in establishment of sustained and broadly protective immunity, we utilize the models of vaccination or acute viral infection.

A. Biology of Tfh cells in protective humoral immunity establishment to licensed human vaccine

Here, our investigations are focused in delineating the biology of Tfh cells in the humoral immunity establishment to commonly used SA14-14-2 live attenuated Japanese encephalitis (JE) vaccine. Studies in the framework of this project are aimed at defining the mechanism and function of Tfh cells in the humoral immunity establishment to JE-vaccine. The preliminary data obtained from archives human serum samples was instrumental in designing a strategy for studying Tfh cells in sustained protective immunity in controlled human vaccination. We are in the process of launching the vaccine trial in few months. We are also embarking on the mouse models for detailed mechanistic investigations on Tfh cells and ontogeny of their heterogeneity during vaccine response. We have successfully established an animal model for studying the biology of Tfh cells during the process of protection conferred by JE-vaccine. Accumulated data indicate the crucial role of CD8 T cell subset in Tfh differentiation and antibody responses to JE-vaccine. Further attempts will be focused on demonstrating the implication and the mechanism by which specialized CD8 T cell subset regulates the Tfh differentiation program.

B. Function of Tfh cells in protective immunity to natural infection

In this project, our attempts are focused on providing the insight into the determinants of antibody response to dengue virus. Dengue severity is marked by the presence of low affinity, cross-reactive and poorly neutralizing antibodies. Understanding the biology of Tfh cells during the course of infection may provide the leads for formulating optimal antibody

response in dengue. Our previous analysis suggested a selective induction of circulating Tfh cells in acute phase of dengue virus infection. For systematic investigations, we enrolled dengue patients progressing though all the phases of illness up to approximately 1-year post onset of symptoms. Altogether, in antigen-specific and longitudinal analyses, we demonstrate that the memory $CD4^{+}CXCR5^{+}$ cells co-expressing PD1 and ICOS are circulating counterparts of GC-Tfh cells in dengue. We further demonstrate that this Th1-biased Tfh subset harbors strong signature of B-cell help, triggers plasma cell differentiation and induces low level NS1-IgG in ex vivo assays. Our future efforts will be to define the underlying mechanism of differentiation, transcriptomic landscape and the characteristic help-axis of the identified major Tfh and related subset in dengue.

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Modulation of intracellular trafficking in host cells by various intracellular pathogens

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Salmonella species cause gastroenteritis and enteric fever in infected hosts. SipA is one of the major effectors which induces membrane ruffling and actin rearrangement of host cells. SipA is a bifunctional protein which is cleaved by caspase-3 into two domains. C-terminal of SipA regulates actin polymerization, whereas the function of N-terminal is not clearly established.

As phagosome maturation depends on the sequential recruitment and removal of different Rabs and SNAREs, we have analyzed the recruitment of different Syntaxins on SCV. Our results have shown that SCV:WT recruit significantly higher amounts of Syntaxin8 at 90 min post infection (*p.i.)*. Subsequently, we have shown that Syntaxin8 specifically interacts with a ~74kDa *Salmonella* effector protein, SipA. SipA is a major effector of Salmonella, which causes gastroenteritis and enteric fever. Caspase-3 cleaves SipA into two domains: the C-terminal domain regulates actin polymerization, whereas the function of the N terminus is unknown. We have shown that the cleaved SipA N terminus binds and recruits host Syntaxin8 (Syn8) to *Salmonella*-containing vacuoles (SCVs). The SipA N terminus contains a SNARE motif with a conserved arginine residue like mammalian R-SNAREs. SipA:R204Q and SipA$^{1–435}$:R204Q do not bind Syn8, demonstrating that SipA mimics a cognate R-SNARE for Syn8. Consequently, *Salmonella* lacking SipA or that express the SipA$^{1–435}$:R204Q SNA RE mutant are unable to recruit Syn8 to SCVs. Finally, we have shown that SipA mimicking an R-SNARE recruits Syn8, Syn13, and Syn7 to the SCV and promotes its fusion with early endosomes to potentially arrest its maturation. Our results reveal that SipA functionally substitutes endogenous SNAREs in order to hijack the host trafficking pathway and promote *Salmonella* survival.Our results provide mechanistic insight into how effector molecules from pathogens manipulate host cellular processes by functionally substituting endogenous SNAREs. It could be possible that pathogens have selected SMs during evolution for easy manipulation of membrane fusion for their benefit. Thus, the disruption of bacterial SNARE-like effector proteins could be a viable target for the development of a therapeutic strategy.

Previously, we have shown that *Leishmania* endocytosed hemoglobin (Hb) via a specific receptor which is a hexokinase located in the flagellar pocket. To identify the Hb-binding domain of the Hb-receptor (HbR), we have cloned and expressed HbR$^{1-416}$, HbR$^{1-126}$, HbR$^{121-276}$ and HbR$^{270-461}$ truncated proteins and determined their ability to bind Hb. We have shown that HbR$^{1-126}$ retained more than 80% Hb binding activity in comparison to full length protein (HbR$^{1-416}$). In order to identify the minimum hemoglobin-binding domain of HbR, the HbR$^{1-260}$have serially truncated from the N-terminus staggered by 20 amino acids. Similarly, we have made twelve truncated proteins of HbR$^{1-160}$ staggered by 20 amino acid from the C-terminus. Hb-binding activity of these truncated proteins have revealed that Hb-

binding domain of HbR span between HbR^{41-80}. Subsequently, we have shown that HbR^{41-80} retained about 90% Hb-binding compared to HbR^{1-416}. Finally, we have synthesized 40 amino acid residues and shown that this peptide specifically binds with Hb. In addition, we have found that HbR^{41-80} peptide completely blocks the Hb uptake in *Leishmania* and thereby inhibits the growth of the parasites. Taken together, our results identified that HbR^{41-80} is the Hb-binding domain of HbR which could be used as a potential therapeutic agent to suppress the growth of *Leishmania*.

Publication

Original peer-reviewed article

1. Singh PK, Kapoor A, Lomash RM, Kumar K, Kamerkar SC, Pucadyil TJ, Mukhopadhyay A (2018) Salmonella SipA mimics a cognate SNARE for host Syntaxin8 to promote fusion with early endosomes. **J Cell Biol**. 217: 4199-4214.

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REPRODUCTION AND DEVELOPMENT

Studies of Sertoli cells and spermatogonial stem cells of the testis and other endocrinology related research

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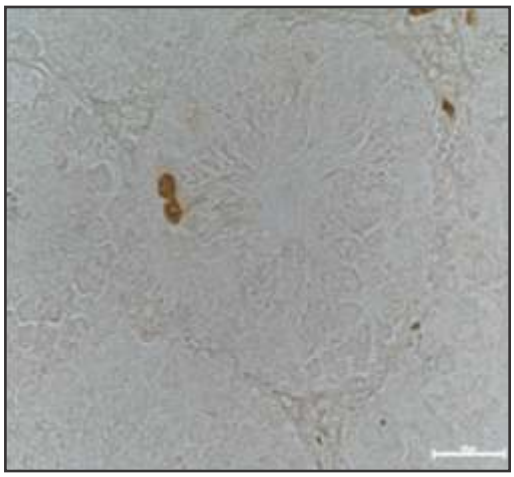
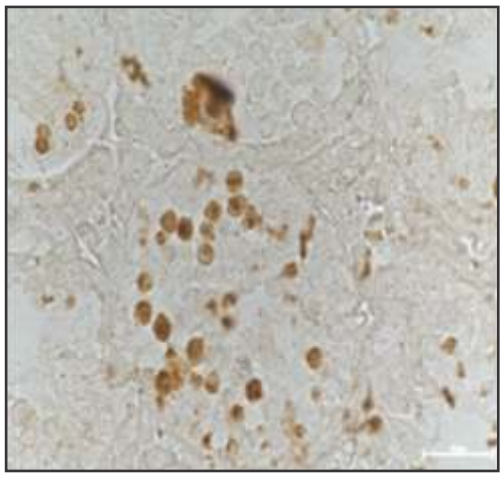
Research in our lab revolves around identification of novel factors which might be involved in development of hormone independent male infertility. Sperm production is a complex process that is regulated by endocrine, paracrine and juxtacrine signals to the germ cells. Within the testes, the somatic Sertoli cells (Sc) play a crucial role in regulating germ cell proliferation and differentiation. These cells respond to endocrine inputs (Follicle Stimulating Hormone and testosterone) to produce factors which in turn, regulate the timely division and differentiation of the germ cells. Any defect in either Sc proliferation and/or impaired maturation would adversely affect spermatogenesis and consequently, lead to infertility or sub-fertility. In such cases, hormone supplementation would fail to initiate sperm production due to an intrinsic defect in the Sc of the affected individual. Therefore, identification of Sertoli cell genes crucial for spermatogenesis can lead to the development of therapeutics for the treatment of "Hormone Independent" cases of Idiopathic male infertility. In this regard, we have previously used a high throughput transcriptomics and proteomics approach to identify novel transcription factors which may be playing a role in Sc function and spermatogenesis (*Mandal et.al, DNA Research, 2017*). Using the high throughput transcriptomics data, we have identified the homoebox gene *Meis1* as an important regulator of Sertoli cell maturation. We have generated transgenic mice with Sc specific knock down of *Meis1*. *Meis1* Knock down (Meis1 KD) transgenic mice had smaller testis as compared to age matched wild type mice. Sc specific *Meis1* knock down led to massive germ cell apoptosis (Figure 1) and disrupted seminiferous tubule architecture. The transgenic mice had significantly lower epididymal sperm counts (as compared to age matched wild type mice) and were found to be sub-fertile. These results highlight the importance of Sc specific factors in regulating spermatogenesis and hence, male fertility.

We are also looking into the role of small RNAs in Sc function.We have identified a number of microRNAs which are differentially expressed in infant and pubertal Sc. Further studies (in-vitro and in-vivo) have been undertaken to decipher the role of these differentially expressed miRNAs in Sc proliferation and functional maturation.



Wild type



Meis1 KD

Fig.1. Detection of apoptotic germ cells in the testes of WT and Meis1 KD mice using TUNEL assay.Our studies on signal transduction in Sertoli cells have revealed a role of Hippo pathway transducer YAP in regulating c-AMP signaling in Sc (Sen Sharma et.al, 2017). We have found that the core hippo pathway components are differentially expressed in infant and pubertal rat Sc. Interestingly, YAP was found to be significantly higher in pubertal Sc, indicating a possible role of this transcriptional co-activator in pubertal Sc function. Follicle Stimulating Hormone was found to regulate *Yap* expression and phosphorylation. Our results suggested differential role Hippo pathway in infant and pubertal Sc. Currently, we are looking into the cross talk between YAP and inflammatory signaling pathways in Sc.

Publications

Original peer-reviewed articles

1. Sen Sharma S, Vats A, Majumdar S (2019) Regulation of Hippo pathway components by FSH in testis. **Reprod Biol.** 19:61-66.
2. Bhattacharya I, Basu S, Pradhan BS, Sarkar H, Nagarajan P, Majumdar SS (2019) Testosterone augments FSH signaling by upregulating the expression and activity of FSH-Receptor in Pubertal Primate Sertoli cells. **Mol Cell Endocrinol.** 482: 70-80.
3. Ganguli N, Ganguli N, Chandra S, Choubey M, Sarkar DP, Majumdar SS (2018) A combinatorial approach for robust transgene delivery and targeted expression in mammary gland for generating biotherapeutics in milk, bypassing germline gene integration. **Appl Microbiol Biotechnol.** 102: 6221-6234.

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Cell death regulation

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The overall theme of the research program is to elucidate the processes that influence cell death programs under varying physiological conditions in diverse model systems.

Regulatory networks driving cell fate decisions are important to investigate in the context of understanding diseases. Broadly, our research programme explores the underlying mechanisms of cell survival and death in diverse intracellular and extracellular conditions. The systems used by us are a lower eukaryotic cell, the protozoan parasite *Leishmania* and the higher eukaryotic mammalian carcinoma cells.

A. Host parasite interaction

The biology of neutrophils has attracted significant attention in recent times because of the realisation of the complex nature of these cells and their capability to carry out a large range of specialized functions. Our studies show a time dependent autophagy increase in neutrophils after *Leishmania* infection. This induction was responsive to block by 3-methyladenine (3-MA), an autophagy inhibitor but sensitive to ULK1/2 inhibition only after 3 h of initial phase. This suggested the prevalence of canonical autophagy during later hours, because ULK1/2 inhibition is able to block only canonical autophagy and not non-canonical autophagy. There was a reduction in macrophage uptake of parasite-exposed human polymorphonuclear cells (PMNs) treated with 3-MA or ULK1/2 inhibitor, suggesting the involvement of both non-canonical and canonical autophagy in neutrophil engulfment by macrophages. The uptake of the PMNs was orchestrated by reduction and diffusion of CD47 or 'do not eat me' signals with no change in phosphatidylserine (PS) or 'eat me signal' expression. The *lpg1*-KO parasites expressing defective lipophosphoglycan (LPG) did not induce autophagy, indicating LPG is necessary for interaction with the neutrophils. In the context of *Leishmania* infection, the data suggest the contribution of both the canonical and non-canonical pathways in initiating infected neutrophil engulfment.

B. Defensive enzymes

The cytosolic tryparedoxin peroxidase (cTXNPx) is reported to be present in the secretome of *Leishmania* but its functional relevance has not been reported so far. Our studies detected the protein on the surface of the parasite as well as a secretory protein in the media. Protein database analysis identified several proteins including Apoptosis Inducing Factor (AIF) being immunopreciptated with the protein. In silico interaction of cTXNPx and AIF emerge to be a strong interaction with polar hydrophobic and hydrogen bond combination.

C. Anti-leishmanial drugs

As a part of work on drug discovery for leishmaniasis, we tested a Halictin peptide made by our

collaborator. The results are very encouraging as this peptide shows significant death of both promastigote and amastigote forms within macrophages. The mechanism appears to be through induction of pores on the membrane which is followed by major biochemical changes within the cell. Most importantly, this peptide increases the efficacy of anti-leishmanial drug antimony. Further applicability as an anti-leishmanial agent is being explored.

Future plans will be to complete our programme on the three above mentioned approaches. The potential anti-leishmanial peptide will be transferred to another collaborator for further studies.

Publication
Original peer-reviewed article

1. Pitale DM, Gendalur NS, Descoteaux A, Shaha C (2019) *Leishmania donovani* induces autophagy in human blood-derived neutrophils**. J Immunol.** 202: 1163-1175.

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Biology of trophoblast cells and immuno-contraception

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Development of contraceptive vaccine

Long term follow-up of the female FVB/J mice immunized with *E. coli*-expressed recombinant $Sp17_C$-$GnRH_2$ using either Squalene-Arlacel A as adjuvant (two injections schedule) or delivered using PLA based micro-particles entrapping the recombinant protein (single injection) showed that the same contraceptive efficacy is maintained up to 150 days after initiating immunization. Regulatory approvals have been obtained to establish proof-of-concept by actively immunizing female beagle dogs, next year, with 3 candidate vaccines targeting dog zona pellucida/GnRH at Palamur Biosciences Private Limited, Secundrabad, India.

Molecular mechanisms associated with migration, invasion and differentiation of the trophoblastic cells

I) Trophoblastic cell migration

HTR-8/SVneo cells under hypoxia (2% O_2) show higher migration and invasion as compared to under normoxia (20% O_2). The migration and invasion under both normoxic and hypoxic conditions is further enhanced after treatment with HGF. Subsequent to treatment with HGF, a significant increase in expression of MMP2 & MMP3 under normoxia and MMP1 & MMP9 under hypoxia was observed. Under hypoxia decrease in TIMP1 expression was also observed. Treatment of HTR-8/SVneo cells with HGF led to an increase in phosphorylation of ERK½ and Akt. Inhibition of MAPK by UO126 and PI3K by LY294002 led to concomitant decrease in the HGF-mediated migration/invasion of HTR-8/SVneo cells. In addition, HGF treatment under hypoxia also led to a significant increase in the expression of hypoxia inducible factor-1α (HIF-1α). Inhibition of HIF-1α by siRNA led to decrease in HGF-mediated migration of HTR-8/SVneo cells under hypoxic conditions (Fig. 1).

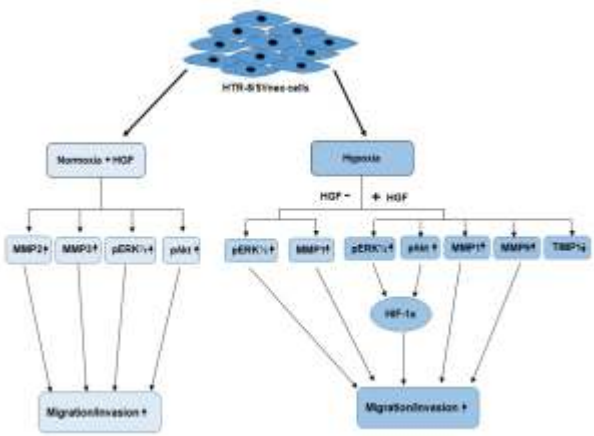

Fig.1. Schematic representation of the role of MMPs and MAPK/PI3K signaling pathways in HTR-8/SVneo cells migration/invasion following treatment with HGF under normoxic and hypoxic conditions. Treatment of HTR-8/SVneo with HGF under normoxia led to activation of ERK½, Akt, MMP2 and MMP3, which increased cell migration/invasion. Under hypoxia alone, increase in expression of MMP1, activation of pERK½ along with increased in migration/invasion was observed. Whereas, HGF treatment under hypoxia increased the expression of MMP1 & MMP9, downregulated TIMP1 and activated both ERK½ and Akt signaling pathways that further increased migration/invasion via recruitment of HIF-1α in the nucleus.

ii) Trophoblastic cell invasion

IFN-g-mediated reduced trophoblast invasion: This year, we showed that Bone Marrow Stromal Cell Antigen 2 (BST2) expression increases after IFN-g treatment and its silencing abrogates the effect of IFN-g on the decreased invasion of HTR-8/SVneo cells. Silencing of BST2 down-regulates the level of IFN-g-mediated expression of E-cadherin. Further, the expression of both BST2 as well as E-cadherin is regulated by STAT1, as its silencing by siRNA down-regulates the IFN-g-mediated increased expression of BST2 and E-cadherin. Additionally, IFN-g activates AKT signaling pathway and its inhibition, abrogates the IFN-g-mediated decrease in trophoblast cells invasion as well as down-regulates the expression of both BST2 and E-cadherin. These results suggest that IFN-g decrease the invasion of HTR-8/SVneo cells by activation of both STAT1 and AKT, which in turn leads to an increased expression of BST2 and E-cadherin (Fig. 2).

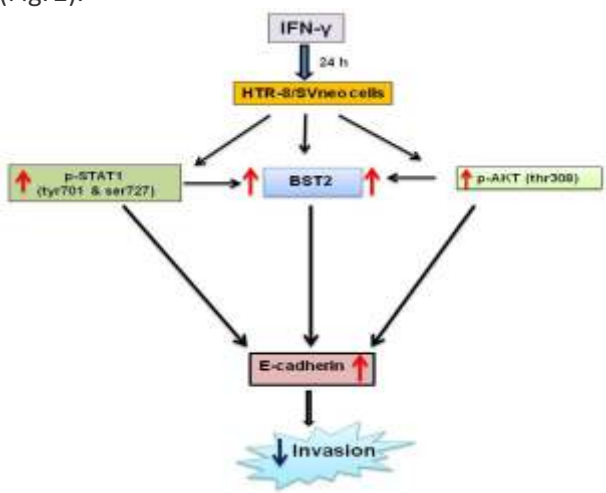

Fig.2. Role of BST2 and E-cadherin in IFN-g-mediated decrease in invasion of HTR-8/SVneo cells. Treatment of HTR-8/SVneo cells with IFN-g up-regulates the expression of BST2 and concomitantly activates JAK/STAT and AKT signaling pathways. The reduction in HTR-8/SVneo cell invasion by IFN-g is brought about by BST2 and E-cadherin through STAT1 and Akt activation. The expression of E-cadherin is regulated by BST2 in addition to STAT1 & AKT signaling pathways.

EGF-mediated increased trophoblast invasion: We confirmed MAPK8 and FAS as targets of miR-92a-1-5p at protein level by Western blotting. Inhibition of MAPK8 by SP600125 and silencing of FAS by siRNA led to reduced EGF-mediated HTR-8/SVneo cells invasion. Further, inhibiting MAPK8 activation significantly reduced EGF-mediated increase in MMP-9/TIMP1 ratio, and MMP-2 & MMP-9 expression. Whereas, silencing of FAS significantly reduced EGF-mediated increase in MMP-2/TIMP1 ratio, and MMP-2 & MMP-9 expression. Thus, the reduced expression of hsa-miR-92a-1-5p leads to loss of its repression control on MAPK8 and FAS when HTR-8/SVneo cells are treated with EGF. The reduction in miR-92a-1-5p levels after EGF treatment is achieved through activated STAT1 and STAT3.

iii) Trophoblastic cell differentiation

Based on miRNA microarray data, the effect of miR-27b-5p on the differentiation of BeWo cells has been studied. Treatment of BeWo cells with forskolin downregulate the expression of miR-27b-5p. Overexpression of miR-27b-5p reduced forskolin-mediated syncytialization of BeWo cells accompanied by a reduction in the secretion of human chorionic gonadotropin (hCG).

Publications

Original peer reviewed articles

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2. Joone CJ, Schulman ML, Fosgate GT, Plagis TA, Crafford JE, Gupta SK, Bertschinger HJ (2019) Antigen-specific $CD4^+$ and $CD8^+$ T-cell responses in PBMC from pony mares immunized with either native or recombinant zona pellucida vaccines. **Theriogenology.** 126:106-113.

3. Mishra NN, Kesharwani A, Agarwal A, Polachira SK, Nair R, Gupta SK (2018) Herbal gel formulation developed for anti-human immunodeficiency virus (HIV)-1 activity also inhibits in vitro HSV-2 infection. **Viruses**. doi:10.3390/v10110580.

4. Chaudhary P, Malhotra SS, Babu GS, Sobti RC, Gupta SK (2019) HGF promotes HTR-8/SVneo cell migration through activation of MAPK/PKA signaling leading to up-regulation of WNT ligands and integrins that target β-catenin. **Mol Cell Biochem.** 435:11-32.

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2. Gupta SK (2018) The human egg's zona pellucida. **Curr Top Dev Biol.** 130:379-411.

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MOLECULAR DESIGN

Structural studies on proteins, dynamics and ligand interactions using NMR

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The theme of our research is to understand the structure, ligand interactions, and dynamics of proteins using NMR.

The primary objective of our study is to structurally and functionally characterize the proteins involved in the fatty acid metabolism, with special emphasis on *Leishmania,* using NMR and other biophysical techniques.

Structure and function of the proteins/enzymes of the type II fatty acid biosynthesis pathway (FAS)

Acyl carrier protein associates and dissociates with innumerable proteins/enzymes of the fatty acid biosynthesis (FAS) pathway in its lifetime by means of an amphipathic helix, also known as the "universal recognition helix. Comparison of the ACP-FAS structures suggest, that the the helix II of ACP interacts with its partners in a manner comparable to the ubiquitin interacting motif (UIM.

Helix II of ACP displays significant similarities to the ubiquitin interaction motif

Thus, $^{1}H^{15}N$ labeled apo-ACP was titrated with increasing concentration of unlabeled ubiquitin.

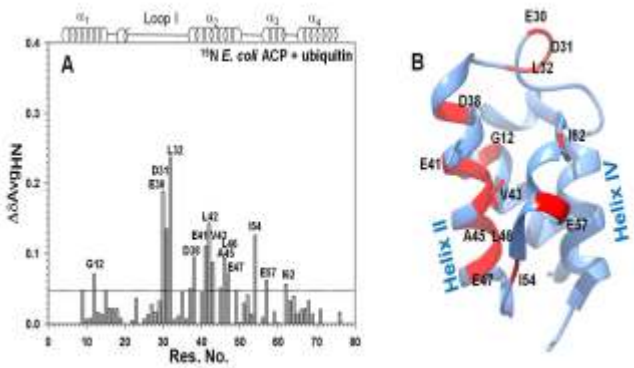

Fig.1. Chemical shift perturbations of *E. coli* ACP backbone upon ubiquitin interaction.

Significant changes in chemical shift were observed for a number of residues present in loop I and helix II of ACP, as illustrated in Figure 1B.

Chemical shift perturbations suggest that ubiquitin binds ACP in a manner analogous to the ubiquitin interacting motif (UIM)

$^{1}H^{15}N$ ubiquitin was titrated with increasing concentration of unlabeled *E. coli* apo-ACP. Figure 2A shows the changes in the average backbone amide chemical shifts of ubiquitin upon titration with *E. coli* ACP. Residues of ubiquitin that also form the 1le 44 interaction surface, display significant chemical shift change (>1SD).

Chemical shift perturbations suggest equivalent binding of ACP and the helix II peptide

$^{1}H^{15}N$ labeled ubiquitin was titrated with a synthetic peptide 'Acetyl-GADSLDTVELVMALEEE-NH2', corresponding to residues 33-49 of *E. coli* ACP. Noticeable chemical shift changes were observed in the amides, as illustrated in Figure 2B.

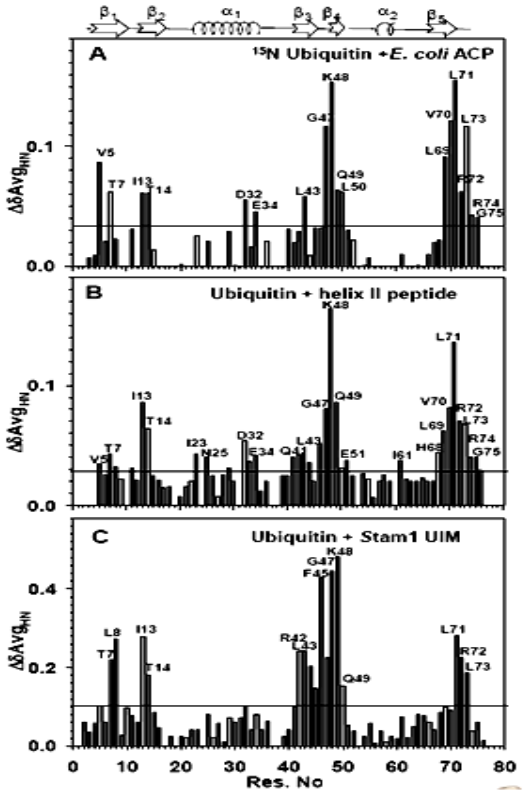

Fig.2. Chemical shift perturbations in the ubiquitin backbone upon titration with *E. coli* ACP, helix II peptide and Stam1 UIM.

E. coli ACP and helix II peptide interact with ubiquitin in SPR

SPR measurements were carried out by immobilizing ubiquitin on the sensor chip, and passing increasing concentrations of *E. coli* ACP over the chip surface. The two proteins interact with a calculated affinity constant (K_D) of 35.3 5 µM. Binding of ubiquitin to the helix II peptide was weaker, and a K_D of 700 ± 50 µM was observed.

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Original peer-reviewed articles

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To develop strategies for making sensors and actuators for biological processes

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A. Transplantion of retinal neuron like cells (RNLCs) in the NOD.SCID-*rd1* mouse model
Transplantation of RNLCs

Retinitis pigmentosa (RP) is a progressive irreversible retinal degenerative disease which causes partial or complete blindness. We have adapted monocytes for further differentiation into retinal neuron like cells (RNLCs) by a two-step approach using appropriate extrinsic factors.

We observed that transplanted mice showed slightly improved depth perception as evident by their capability to step on shallow side more number of times than deep side and a better exploratory behaviour as they were able to locate the opening to the dark chamber easily than the non-transplanted animals. Nevertheless, their aversion to light still remained non-conclusive as they spent almost equal time in both the chambers. They also exhibited a response to head tracking device in scotopic conditions (50 lux) at 0.03 cycles per degree (cpd) and 0.13 cpd. The changes between transplanted and non-transplanted animals at 0.03 cpd were significant.

Molecular level investigation of RP
In RP, the primary causative factor is the genetic mutation which causes loss of photoreceptor. Consequently, the ocular niche environment is compromised and activates a cascade of events. Immune modulation in RP patient's peripheral system indicates their role in retinal degeneration, however not all mutation types may be affected by such a modulation. Hypoxia, since upregulated in almost all patients have a definitive role to play in the pathology and progression of RP. Th2 and Th17 are the responses mostly exhibited during RP owing to healthy T cells stimulated by RP serum.

B. Diagnostic markers in Acute Rheumatic Fever (ARF) and Rheumatic Heart Disease (RHD)

Acute rheumatic fever is caused by Group A Streptococcus pyogenes and post infection squeal turns into Rheumatic heart disease (RHD).

Metalloproteinases (MMPs) are involved in extracellular matrix modeling. We have identified the sets of MMPs in total blood RNA samples from the diseased and healthy subjects. Higher expression of MMP-10 was observed in chronic subjects as compare to healthy. Tissue inhibitors of metalloproteinases (TIMP) are the proteases involve in the regulation of MMPs. TIMP provides a balancing mechanism between excessive

degradation of ECM. There was higher level of all the TIMPs in case of ARF subjects as compared to chronic and healthy subjects. Different collagen intermediates were also quantified in all the subjects. Collagen I and III are the most abundant subtypes of collagen found in the heart. These are the intermediates formed during the synthesis and degradation of collagen.

C. Aerogenic route immunization on non-human primates

Alginate particles in the size range of 2-4 micron were prepared by encapsulating live BCG. Infant Rhesus macaques were immunized with either BCG vaccine intradermally (n=2) or BCG encapsulated alginate particle (BEAP) via tracheal intubation (n=2). Around one log reduction in *Mtb* H37Rv CFUs was observed in immunized animals and reduction was additional 0.5 log in animals immunized with BEAP.

Publications

Original peer reviewed articles

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3. Bhattacharjee J, Das B, Mishra A, Sahay P, Upadhyay P (2017) Monocytes isolated by positive and negative magnetic sorting techniques show different molecular characteristics and immunophenotypic behaviour. **F1000Res**. doi:10.12688/ f1000research. 12802.3.

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Protease-catalyzed splicing of peptide bond

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Sortase enzymes are membrane-anchored transpeptidases found in Gram-positive bacteria and are responsible for covalent anchoring of surface proteins to the peptidoglycan. The prototype sortase A (SaSrtA) from *Staphylococcus aureus*, recognizes a LPXTG sequence motif in substrate proteins, cleaves the scissile T-G peptide bond and transfers protein-LPXT to the terminal glycine of the peptidoglycan pentaglycine branch leading to covalent anchoring of proteins to the cell wall. SaSrtA-catalyzed ligation of recombinant or synthetic LPXTG polypeptide to an aminoglycine derivatized moiety occurs efficiently *in vitro* and has inspired numerous applications. Sortases with newer substrate specificity, enhanced catalytic efficiency and stability are required for expanded applications.

Studies on structure, dynamics and function of sortases

Cyclic sortases: A cyclic sortase (cSrt1) was assembled through the mediation of isopeptide-linked fragment complementing system present in CnaB2 domain of fibronectin binding protein from *Streptococcus pyogenes.* The CnaB2 domain can be split into a 13-residue peptide (SpyTag) and a larger 83-residue (SpyCatcher) complementary fragment. The SpyTag and SpyCatcher encounter generates CnaB2 domain by spontaneous formation of a Lys-Asp isopeptide bond. We designed a construct with SpyTag and SpyCatcher sequences placed at the Srt1 termini for recombinant expression of the cyclic protein in *E coli*.

The conditions for expression and assembly of cSrt1 in *E coli* was optimized. We also generated a linear version of Srt1 by mutating Asp7 to Ala. This mutation disrupts the Lys31-Asp7 isopeptide bond and eliminates the SpyTag-SpyCatcher mediated cyclization of Srt1. Furthermore, we applied the same design and engineering principles, and generated a cyclic (cSrt2), and a linear form of another sortase. Interestingly, a construct containing Srt2 sequence interspersed with flexible linkers and SpyTag/SpyCatcher sequence, behaved in much the same way as Srt1 when expressed in *E coli.* The studies on physico-chemical, catalytic and stability aspects of the above cyclic and linear forms of sortases are in progress.

Structure-function analysis of TfSrtE: We had previously reported the expression, purification and characterization of a class E sortase (TfSrtE) from *Thermobifida fusca.* TfSrtE transferred both LPXTG and LAXTG peptides to Gly-nucleophiles with almost equal efficiency. We further probed the Ala/Pro specificity and found that Pro homologs/analogs, endowed with the ability to generate a 'kink' conformation, were well tolerated at the second position of the pentapeptide recognition motif. This is reminiscent of our earlier studies on substrate specificity of *S. avermitilis* sortase E (SavSrtE). However, TfSrtE also displayed propensity to form a gamut of transpeptidation product utilizing - or ε-amino group of the YALPNTGK peptide substrate under limiting concentration of the GGGKY

nucleophilic substrate. Such products were eliminated when acetyl-YALPNTGA was used as a substrate.

Sortase-mediated protein labeling and conjugation

We applied sortase-mediated peptide ligation for preparing chemically defined acetylated histones. There are 18 human deacetylases (HDAC) that erase acetyl groups from histones and other acetylated proteins. The eraser of acetylation at a particular site in histones is largely unknown.

H2BK5Ac and H3K4Ac were prepared by sortase-mediated ligation of appropriate complementary fragments generated by chemical synthesis, proteolysis or recombinant expression. Eraser assays were carried out in the presence of various HDACs to delineate the specific eraser of K5 and K4 acetylation in H2 and H3 respectively. Preliminary results emanating from deacetylation assays carried out using HEK293 cells transfected with the plasmids expressing Human HDACs revealed the identity of erasers specificity of H2BK5Ac and H3K4Ac.

Publication
Original peer-reviewed article

1. Pawale VS, Yadav P ,Roy RP (2018) Facile one-step assembly of *bona fide* SUMO conjugates by chemoenzymatic ligation. **Chem Bio Chem**. 19: 1137-1141.

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Therapeutic interventions in chronic diseases

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A. α-lipoic acid has protective effect against bisphenol A-induced neurobehavioral toxicity:

Bisphenol A (BPA), a well-known xenoestrogen, is ubiquitously utilized in manufacturing of polycarbonated plastics. Convincing evidence suggests that BPA induces neurotoxicity and certain behavioral deficits. We studied the neuromodulatory effect of α-Lipoic acid (ALA) against neurotoxicity of BPA *in vitro* in C8-D1A mouse astrocyte cell line and *in vivo* in C57BL/6J male mice. *In vitro* ALA (100 µM) protected cells from BPA (30 µM)-induced ROS generation, cell death and increased activity of GFAP. *In vivo* ALA (50 mg/kg) increased the neurospecific acetylcholinesterase activity and decreased the monoamine oxidase activity altered by BPA exposure (10 mg/kg, *per os* x 30 days). Our results suggest that ALA has a promising role in modulating BPA-induced neurotoxicity in C8-D1A mouse astrocyte cells as well as neurochemical and neurobehavioral deficits in C57BL/6J male mice and its antioxidant and free radical scavenging activities may in part be responsible for such an effect.

B. Development and Characterization of Supramolecular Calcitonin Assembly and Assessment of its Interactions with the Bone Remodeling Process:

Osteoporosis is the most common metabolic bone disease, which poses an immense socio-economic burden on the society. In estrogen deficit condition osteoclasts become more viable and active which leads to accelerated bone loss. Over activity of osteoclasts can be regulated by inhibitory action of calcitonin. Therefore calcitonin is considered as a one of the promising therapy for postmenopausal osteoporosis. Despite of in-depth understanding of bone biology, till date the therapeutic use of drugs to treat osteoporosis is lacking due to adverse side effects and inadequate long-term compliance including complexity of dosing. Calcitonin derived from salmon is one among such therapies which is approved by FDA for use in the treatment of postmenopausal osteoporosis. Unfortunately, human calcitonin was never employed as frontline treatment for osteoporosis because of its lower potency and higher propensity to aggregate whilst being less immunogenic compared to salmon calcitonin. *Hence, a*ggregation and formation of amyloids remain the major challenge in the field of peptide therapeutics. One innovative way to

overcome this problem is to develop macro-molecular cluster of monomers that can release biologically active monomers in a sustained manner. Herein we have advanced the calcitonin therapy by regulating the aggregation of human calcitonin and developed conglomerate of monomers termed Supramolecular Calcitonin Assembly (SCA-I) and biophysically characterised. SCA-I releases biologically active calcitonin monomers in a sustained manner for a period of at least three weeks. SCA upon single injection in OVX rats releases biologically active monomers in a sustained manner for 3 weeks and significantly improves bone quality as observed increase in bone formation parameters and decrease in bone resoption parameters.

This was further substantiated by histomorphometric analysis where increased numbers of osteoblasts were seen at endosteal surface. This study demonstrates that SCA-I is more effective compared to the human calcitonin monomers on osteoclasts and has site-specific effect on bone in a model of post-menopausal osteoporosis. This approach opens up an innovative way to use and study the function of human calcitonin.

Publications

Original peer-reviewed articles

1. Manglani K, Vijayan V, Pathak C, Khandelwal M, Singh P, Chellappa S, Yadav VK, Surolia A, Gupta S (2019). Development and characterization of supramolecular calcitonin assembly and assessment of its interactions with the bone remodelling process. **Bone.** 122:123-135.
2. Khan J, Salhotra S, Ahmad S, Sharma S, Abdi S, Banerjee BD, Parvez S, Gupta S, Raisuddin S (2018). The protective effect of α-lipoic acid against bisphenol A-induced neurobehavioral toxicity. **Neurochem Int**. 118:166-175.

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1. Vijayan V, Gupta S (2018) How homocysteine modulates the function of osteoblasts and osteocytes. **In: Non-proteinogenic Amino Acids**. doi:10.5772/ intechopen.76398.
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Patents

1. Vijayan V, Gupta S (2018) "Peptide complex with immunomodulatory and anti-inflammatory function." **Indian Patent Application Number 201811016307; Filed on 30/04/2018**.
2. Vijayan V, Siddique IA, Gupta S (2018) "Carboxylatedosteocalcin for treatment of amyloidosis or diseases associated with abnormal protein folding." **Indian Application Number 201811016306; Filed on 30/04/2018**.

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Molecular mechanism of enzymatic reactions and enzyme-ligand interactions

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Tetramer of hGBP-2 is catalytically incompetent for GMP formation

To understand whether the lower GMP formation in hGBP-2 is due to its inability to form a tetramer, analytical gel-filtration measurements were performed in the absence and presence of GDP. AlF_4. With GDP. AlF_4, the protein eluted as a mixture of monomer, dimer and tetramer with tetramer being the predominant form, indicating that unlike hGBP-1, hGBP-2 did not tetramerize completely after the first phosphate cleavage of GTP. The inability to produce an increased GMP suggests that the hGBP-2 tetramer is either catalytically less efficient or not important for the second phosphate cleavage compared with that of hGBP-1.

Biochemical and biophysical studies on *H. pylori* ADC

H. pylori ADC is a 616 amino acids long protein. The recombinant protein was found to be catalytically active. The pH dependent activity assays displays a bell-shaped curve with an optimum activity at pH ~8.5, suggesting that at least two ionisable residues at the active-site are involved in catalysis. The K_m and k_{cat} were determined to be 3.4 mM and 55.2 min^{-1}, respectively. The CD studies showed that the enzyme is primarily helical. Using CD measurements, the T_m value was estimated to be 65.8±0.2°C, suggesting that the protein is thermostable. To investigate the pH dependent activity further, a detailed steady-state kinetics was performed at various pH. The S-shaped pH dependent curve of k_{cat} gave the value of pK_a 7.2 ± 0.1. This may correspond to the ionisation of either a His or Cys residue. *H. pylori* ADC exhibits highest sequence identity with the homologue of *C. jejuni* (~61%). Based on the crystal structure of *C. jejuni* ADC, a Cys residue is conserved at the active site of *H. pylori* ADC. Thus, the above pK_a value may correspond to the ionisation of a Cys residue. To verify this, Cys487Ala mutant was prepared, which shows 5% of wild type activity. The T_m value of this mutant suggests that Cys487 has a marginal role in the stability. All these results indicate that Cys487 is a catalytic residue.

Exploring the metal ions-induced stability and function of a bimetallic human arginase-I, a therapeutically important enzyme

To investigate whether the variation in the retention of catalytic activity between the Co^{2+}- and Mn^{2+}-reconstituted hArg-I observed in the human serum is due to the difference in their secondary structure, CD measurements were performed in the far-UV region. The CD values for both the holo proteins were more than the apo, suggesting that the protein exhibits higher secondary structural content in the presence of the metal ions. A marginal increase in the CD value for the Co^{2+}-protein was observed as compared with its Mn^{2+}-counterpart. The CD spectra analyzed by Chen method provides an estimate of the helical content 28, 36 and 41% for the apo, Mn^{2+}- and Co^{2+}-

proteins, respectively. These data suggest that the helical content of the protein is increased in the presence of either of these two metal ions, but more increase was observed with the Co^{2+} ions.

To further investigate the importance of the metal ions in structural stability, heat-induced denaturation studies of the holo and apo proteins were performed. The T_m value for the Co^{2+}- and Mn^{2+}-proteins was determined to be 83±2.0 and 81±2.0°C, respectively, while that for the apo enzyme was 69±3.0 °C. These results suggest that hArg-I is a thermostable protein. A comparison of the T_m values further indicates that the holo proteins are significantly more stable than the apo. Additionally, the data suggest that the Co^{2+}-protein is slightly more stable than that of the Mn^{2+}-protein.

Publications

Original peer-reviewed articles

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2. Sadarangani V, Rahman S, Sau AK (2018) Metal ions-induced stability and function of bimetallic human arginase-I, a therapeutically important enzyme. **Biochim Biophys Acta.** 1866:1153–1164.

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Mechanistic studies of *Mycobacterium tuberculosis* proteins

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We have been majorly pursuing two projects involving structural and functional aspects of proteins from *Mycobacterium tuberculosis (Mtb)*, the organism that causes tuberculosis (TB) in humans. The first one focuses on understanding the 3D structures and the biochemical properties of enzymes of histidine (His) biosynthesis pathway to derive the molecular mechanisms underlying their actions and to design novel enzyme specific anti-TB small molecule compounds through a structure-guided approach. In the second project, we focus on elucidating the roles of *Mtb* membrane associated proteases in host factors modulation.

Structural and biochemical analysis of HisN (Rv3137)

We have carried out comprehensive structural and biochemical characterization of HisN (histidinol phosphate phosphatase (HolPase)) that catalyses the dephosphorylation of L-histidinol phosphate (HOLP) to yield L-histidinol in the His pathway. In order to derive mechanistic insights into the function of *Mtb* HolPase, we determined its X-ray structure at 1.95 Å resolution . Analysis of the 3D structure showed that the polypeptide chain folds as two distinct structural domains- named here as the N-terminal (1-136 aa) and the C-terminal (152-260 aa) domains. The tertiary structure comprises of 10 helices (seven α-type and three 3_{10}-type), 12 β-stands and 21 loops (Fig. 1A). The N-terminal domain folds into a mixed six-stranded β-sheet underneath of which lie two long α-helices (Fig. 1A). On the other hand, the C-terminal domain folds as a globular shape structure comprising of a mixed five-stranded β-sheet sandwiched between six helices. Both domains are connected by a 15-residue long loop. The biological functional unit of *Mtb* HolPase is a homodimer as observed both in the solution and in the crystal structure. The crystal asymmetric unit of the native crystal possesses two copies of such a dimer. The monomers of the biological unit are assembled in an inverted manner and are related to each other by a 2-fold rotational symmetry perpendicular to the plane of the paper (Fig. 1B). The dimer interface buries a surface area of about 1817 $Å^2$, approximately 16% of the total accessible surface area of a monomer. The dimer is stabilized largely by hydrogen bonds, salt bridges and Van der Waals interactions. (Fig. 1C). Analysis of the electrostatic surface potential of the residues lining the interface clearly shows that charge and shape complementarities contribute in the formation of a stable dimer assembly (Fig. 1D). Neither intra-subunit nor inter-subunits disulphide bridges are present in HisN.

In addition we, elucidated the complex structure of the enzyme bound with the substrate HOLP. Mainly, based on the enzyme-substrate cocrystal structure and kinetic data, we propose a reaction mechanism underlying the action of Rv3137 (Fig. 1E. 1F and 1G). The enzyme possesses a co-catalytic Zn^{2+} motif and catalyzes the hydrolysis of HOLP to form HOL and

phosphate. The reaction is initiated by the activation of a water molecule in the active site pocket by $Zn^{2+}/1$ leading to the ionization of the water molecule (Fig. 1E). In the second step, the hydroxyl ion generated in the process initiates a nucleophilic attack on the scissile bond between P and O of HOLP (Fig. 1F). This is followed by electron delocalization and donation of proton to the leaving HOL, eventually leading to the dephosphorylation of the substrate. The source of the general acid that ultimately donates a proton to the leaving group alcohol of the product might be one of the interacting Aspartates, most likely Asp^{213} (Fig. 1F). Following the leaving of HOL group, two Zn^{2+} move from sites '1 and 2' (ready state) to '1 and 3'('relaxed' state) holding the free phosphate in proximity and L3 loses its stability (Fig. 1G). In the second project, we have determined the 3D structure of the periplasmic portion of *Mtb* High-temperature requirement A1 (mHtrA) protein.

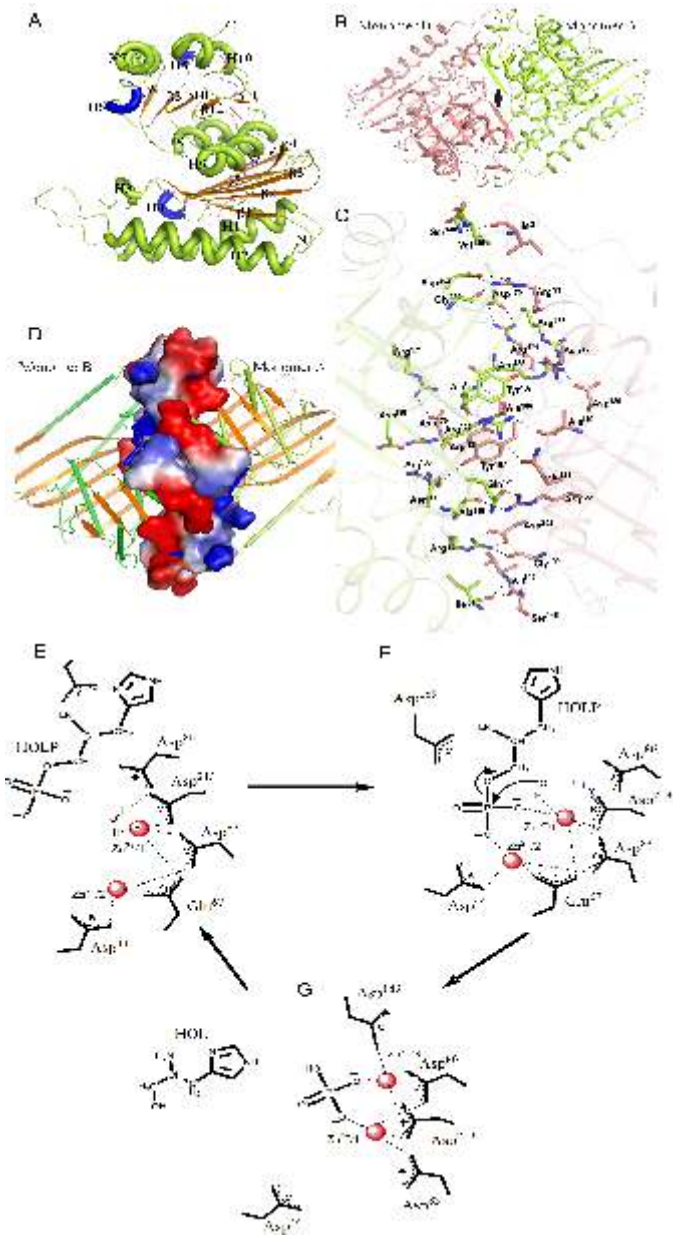

Fig.1. Presentation of the 3D structure, mode of dimerization of native Mtb HolPase and elucidation of dephosphorylation mechanism. A. The 3D structure of Mtb HolPase is depicted in cartoon representation. Helices (α-type, green and 3_{10}-type, blue) and β- strands are numbered. **B.** Cartoon representation of a biological dimer showing the inverted mode arrangement of the monomers. The line of symmetry passes between the two monomers and is represented as a black ellipse. The monomers A and B are represented in limon and salmon colours, respectively. **C.** Hydrogen bonding interactions between the two monomers are shown by the dotted lines. **D.** Electrostatic potential surface map at the dimer interface. **E.** Water molecule is positioned appropriately and is activated by $Zn^{2+}/1$. **F.** The hydroxyl ion attacks the phosphate moiety of the substrate HOLP and the product HOL is released. **G.** Zn^{2+} ions move to positions '1 and 3' from '1 and 2.' Free phosphate is held closely to the two Zn^{2+} ions.

Publications

Original peer reviewed articles

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2. Dwivedy A, Jha B, Singh KH, Ahmad M, Ashraf A, Kumar D Biswal BK (2018) Serendipitous crystallization and structure determination of bacterioferritin from Achromobacter. **Acta Crystallogr F Struct Biol Commun**. 74: 558-566.

3. Kumari M, Pal RK, Mishra AK, Tripathi S, Biswal BK, Srivastava KK, Arora A (2018) Structural and functional characterization of the transcriptional regulator Rv3488 of *Mycobacterium tuberculosis* H37Rv. **Biochem J**. 475: 3393-3416.

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Molecular modelling of proteins and protein-ligand complexes using knowledge-based approaches and all atom simulations

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The specific objective of the various projects are (A) *In silico* identification of secondary metabolites by genome mining (B) *In silico* analysis of Phosphorylation and Protein-Protein interactions networks in *P. falciparum*

A. Machine learning based approach for predicting cyclization patterns of polykedites and non-ribosomal peptides

We have developed a machine learning based bioinformatics tools which can predict final cyclised chemical structure of polyketides (PK) and nonribosomal peptides (NRP) in a completely automated fashion given the genome sequence as input. Major challenge in developing such methods has been deciphering correct cyclization pattern of a linear polyketide or nonribosomal peptide from among large number of combinatorial possibilities (**Fig.1**).

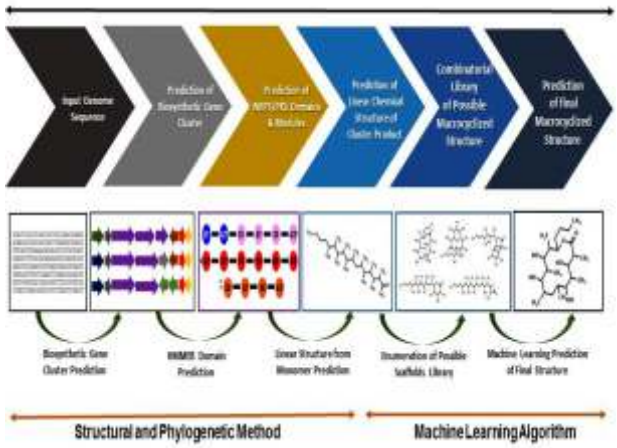

Fig.1. Flowchart depicting various steps for predicting chemical structure of secondary metabolites by genome mining.

In order to develop the machine learning approach, we compiled a manually curated dataset of 850 natural products from PK/NRP families, which consisted of approximately 350 linear polyketides and nonribosomal peptides and 520 PK/NRP molecules which form macrocyclic structures. For every molecule all possible linear as well as macrocyclic structures were enumerated based on known macrocyclization reactions for PKs and NRPs. All these structures were labelled as positive or negative depending on whether they corresponds to a naturally occurring linear or cyclic PK or not. The various chemical structures in both positive and negative data sets were converted into feature vectors using Morgan circular finger prints based on atom types, chemical connectivity and neighbouring bonded atoms. The data was divided into training and test sets using leave-one-out as well as n-fold cross validation approaches. Using the training set, random forest classifiers were trained to discriminate between positive and negative data sets and prediction accuracy of the classifier was benchmarked on the test set using different

statistical measures. Our results indicate that even though complete gene to metabolite prediction can be carried out with fairly high accuracy, error in prediction of linear structure is affecting prediction accuracy of final secondary metabolite.

B. Analysis of Phosphorylation network and Protein-Protein interactions in *P. falciparum*

We have deciphered peptide binding mode in PfCDPK1 by carrying out several microsecond scale MD simulations. Explicit solvent MD simulations for 1 µs duration were carried out on the crystal structure (PDB ID:3Q5I) of ligand free PfCDPK, the structure of PfCDPK1 in complex with its known substrate peptide KRLSVSA and KRLSSEE. Our long time scale MD simulations revealed a distinctly different binding mode for peptide recognition by PfCDPK1 compared to the binding pose observed in peptide bound crystal structure of mouse PKA. Interestingly, while the substrate peptide showed flexibility in the binding site, interactions between calmodulin domain and kinase domain remained conserved throughout the 1µs simulation. Analysis of crucial interactions between these two domains have provided us key insights for design of small molecule inhibitors for inhibiting this interaction.

In addition, we have also attempted to identify novel PPI inhibitiors for various types of PPIs in plasmodium. We have carried out docking studies for Atg8:Atg3 PPI complex based on the crystal structure (PDB ID:4EOY) of AIM bound Atg8. Currently work is in progress to carry out MD simulations for Atg8 in complex with selected inhibitors to identify high affinity binders based on MM-PB/SA approach.

Publications

Original peer-reviewed articles

1. Kaur H, Sain N, Mohanty D, Salunke DM (2018) Deciphering evolution of immune recognition in antibodies. **BMC Struct Biol.** doi: 10.1186/s12900-018-0096-1.
2. Kaur P, Rausch M, Malakar B, Watson U, Damle NP, Chawla Y, Srinivasan S, Sharma K, Schneider T, Jhingan GD, Saini D, Mohanty D, Grein F, Nandicoori VK (2019) LipidII interaction with specific residues of *Mycobacterium tuberculosis* PknB extracytoplasmic domain governs its optimal activation. **Nat Commun.** doi: 10.1038/s41467-019-09223-9.

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Chemical Glycobiology: Glycoform modulation, carbohydrate-based drug design and glycomics

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Glycosylation forms a major, diverse, complex, and dynamic post-translational modification (PTM) of proteins. Similar to nucleotides and amino acids employed, respectively, for biosynthesis of genes and proteins, glycosylation employs various monosaccharide building blocks for biosynthesis of glycans. Our laboratory strives to develop tools for engineering, interception, and inhibition of glycosylation.

A. Modulation of glycosylation of cluster of differentiation (CD) antigens.

Mucin type *O*-glycosylation (MTOG) is characterized by the biosynthesis of GalNAc- -Ser/Thr (Tn-antigen) which is further elaborated to Thomson-Friedenreich (TF) antigen, sialyl-Tn, sialyl-TF, di-sialyl-TF, Lewis, and blood group antigens. The efficacy and ability of GalNAc analogues for metabolic glycan engineering (MGE) depends on several factors, including expression and activity of various glycosyl transferases. In order to investigate cell-line dependent effects of MGE, U937 (human histiocytic lymphoma; both C1GalT and C2GnT are active), K562 (human chronic myeloid leukemia; C2GnT defective), and Jurkat (human T-lymphoma; C1GalT defective) were chosen as they predominantly express core-2 glycans, core-1 glycans, and Tn-antigens, respectively. Using a panel of peracetylated GalNAc analogues, namely, *N*-thioglycolyl-D-galactosamine (Tg), *N*-glycolyl-D-galactosamine (Gc), and GalNAc (Ac) along with dimethyl sulfoxide (D, vehicle) as a control we investigated the effects on MTOG.

Estimation of cell surface thiols (CST) revealed that K562 and U937 cells showed, respectively, 3.5-fold and 7.5-fold increase in CST levels, upon incubation with Tg, but not in controls. U937 cells showed decrease in *Maackia amurensis* lectin-II and increase in *Vicia villosa* agglutinin epitopes suggesting hyposialylation and inhibition of MTOG induced by Tg. By contrast only milder effects were observed for K562 cells. U937 cells treated with Tg displayed homotypic cell clumps indicative of enhanced cell-cell interactions due to loss of sialic acids. CD43, which is estimated to carry 80-90 mucin-type *O*-glycans, was chosen to investigate changes to MTOG. We employed a panel of anti-CD43 antibodies, *viz*., 1G10 and L60 (both recognize neuraminidase-sensitive epitopes), L10 (recognizes neuraminidase-resistant epitopes), and *C*-term (recognizes the polypeptide regardless of glycosylation) (Fig.1). In U937 cells, both 1G10 and L60 epitopes were reduced upon treatment with Tg, but not with controls D, Ac, or Gc, indicating loss of sialoglycans on CD43. CD43-L10 epitopes, which were not detectable in controls, appeared upon treatment with Tg. *C*-term blots showed significant shifts in relative apparent molecular weight. These results suggested that U937 cells process Tg efficiently

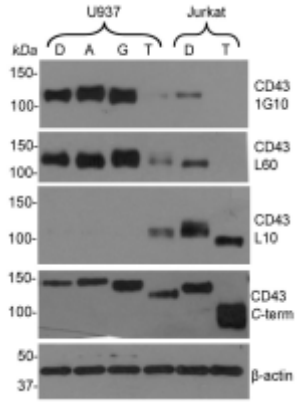

Fig.1. Modulation of CD43 glycoforms induced by GalNAc analgues. Western blots of total lysates of U937 cells incubated with GalNAc analogues (100μM, 48 h) probed with multiple anti-CD43 antibodies, viz., clones 1G10 and L60 (both neuraminidase sensitive), clone L60 (neuraminidase resistant), and *C-term* (which recognizes the CD43 polypeptide). β-actin blots are shown as loading controls. Total lysates of Jurkat cells incubated with GalNAc analogues were included as positive controls. Blots shown are representative of at least two biological replicates. D,DMSO, A, Ac_4GalNAc, G, Ac_5GalNGc and T, Ac_5GalTGc.

resulting in higher CST levels, lower MAL-II and higher VVA epitopes, and significant inhibition of MTOG on CD43.

B. Modulation of glycans of the central nervous system (CNS) across the blood-brain barrier (BBB).

Using the carbohydrate-neuroactive hybrid (CNH) strategy developed in our laboratory we have shown that synthetic ManNAc analogues were able to reach CNS across BBB when conjugated to carrier molecules such as vitamins and fatty acids. We investigated the effect of retinoic acid differentiation on SH-SY5Y (human neuroblastoma) cells *in vitro* and applied MGE to enrich and identify differentially expressed *N*-azidoacetyl-D-neuraminic acid-carrying sialoglycoproteins. Mass spectrometry and proteomics studies identified 131 candidate proteins, including integrins, CD276, and ICAM1, that were uniquely found in SH-SY5Y cells upon treatment with both retinoic acid and peracetyl *N*-azidoacetyl-D-mannosamine.

Publications

Original peer-reviewed article

1. Dwivedi V, Saini P, Tasneem A, Agarwal K, Sampathkumar SG (2018) Differential inhibition of mucin-type *O*-glycosylation (MTOG) induced by peracetyl *N*-thioglycolyl-D-galactosamine (Ac_5GalNTGc) in myeloid cells. **Biochem Biophys Res Commun.** 506: 60-65.

Review/Proceeding

1. Goswami S, Parashar S, Dwivedi V, Shajahan A, Sampathkumar SG (2018) Chemical and biological methods for probing the structure and functions of polysialic acids**. Emerg Top Life Sci.** 2**:** 363-376.

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Role of carbohydrates in modulating the structure and function of glycopeptides

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The project is aimed for understanding the role of carbohydrate domains in modulating the structure and function of glycopeptides by involving different model systems such as antimicrobial and thrombin-inhibitory peptides.

Objectives

1. Synthesis and structural characterization of glycosylated amino acids
2. Structure-function analysis of the synthetic glycoconjugates

Glycosylation has been known to effect the backbone conformation of peptides profoundly. Thus, to investigate any local conformational changes in indW9(β-Glc)TP7K occurring around the site of glycosylation, high resolution 2DNMR studies were carried out in membrane mimicking environment of zwitterionic DPC at 37^{o}C and pH 4.7 and a comparison was made with indolicidin. For confirming the involvement of sugar in the attained conformation of indW9(β-Glc)TP7K, NMR studies of non-glycosylated peptide, indW9TP7K were also performed. The proton resonance assignments of the major peaks were performed by adopting standard procedure of Wuthrich. The peaks in acquired spectra were well dispersed and resolved. The TOCSY spectra acquired for both the peptides was used to assign the respective spin systems. The sequential connections were established between the identified spin systems using NOESY spectra by connecting H^{α}_{i}-H^{N}_{i+1} cross peaks. The sequence of glycopeptide indW9(β-Glc)TP7K, differs from indolicidin as it has a glycosylated threonine at 9thposition instead of tryptophan and lysine in place of proline at 7th position. Thus, there were difference in the chemical shift values of the amide and alpha protons around the substitution site due to obvious chemical differences.

To have a comparative structural overview, the representative conformations of the peptides indW9(β-Glc)TP7K, indW9TP7K and indolicidin (PDB:1G89) were overlayed and no significant differences were observed among the three peptides. The presence of sugar known to introduce β turns or rigidity in the backbone did not lead to any significant observable conformational change in indW9(β-Glc)TP7K backbone compared to indolicidin structure reported earlier. Absence of significant changes in structure upon glycosylation (as observed by NMR studies) probably accounts for the possibly retained functions and mode of action of the peptide.

Continuing the studies for understanding the role of glycosylation in modulating the structure and function, the model system of helical antibacterial peptide, HAL-2; GKWMSLLKHILK-NH_2), has been undertaken. Halictines are among the shortest linear cationic α-helical antimicrobial peptides found in nature. HAL-2, GKWMSLLKHILK-NH_2(GKWMS, has one *O*-glycosylation site at Ser^{5} position. The preferred choice for the saccharide part was β-glucose as this sugar is involved in many important biological phenomena and secondly the critical building block, Fmoc-Ser-(Ac_4-β-D-Glc)-OH is

convenient to synthesize. The critical building block is required for synthesizing the glycosylated peptide. Mostly in proteins/peptides, Thr and Ser positions are the potential *O*-glycosylation sites. Thus, HAL-2 analogs containing β-Glc-Ser and β-Glc-Thr as well as their non-glycosylated counter parts were synthesized for comparative study of the effect of glycosylation on structure and function. It was observed that GKWMS exhibited lower level of toxicity with comparison to that of GKWMT. Interestingly, the glycosylated versions of these peptides i.e. GKWM(Glc)S and GKWM(Glc)T displayed opposite results to their non-glycosylated forms wherein peptide analog with glucosylated Thr [GKWM(Glc)T] showed more % cell viability of macrophages and lesser haemolytic activity against rat erythrocytes in comparison to glucosylated Ser [GKWM(Glc)S] containing peptide. MIC studies revealed that Thr substitution increased the antibacterial activity of the peptide irrespective of whether it is glycosylated or non-glycosylated. Killing kinetics was found to be similar for all the four peptides: GKWMS, GKWMT,GKWM(Glc)S,GKWM(Glc)T. Presence of Ser or Thr did not influence the secondary structure of the peptide in different environments and glycosylation of these peptides kept the α-helical structure of the peptides intact in 10mM SDS and 50% TFE.

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Delineating immune metabolism interaction in disease pathogenesis of tuberculosis and vitiligo

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The thematic focus of our laboratory is to elucidate the complex interplay between metabolic reprogramming and immunity in the context of pathogenic disease Tuberculosis and autoimmune skin disorder Vitiligo. Our endeavor is to define how metabolic dysfunction drives disease pathogenesis. We anticipate that these studies wouldidentify novel therapeutic targets and strategies that will tackle the underlying causes, rather than just the symptoms.

Objectives

A. Mycobacterial lipid metabolism and its implications in TB disease pathogenesis

Tuberculosis (TB) continues to be among the leading causes of infectious disease mortality in India. The primary causative organism, *Mycobacterium tuberculosis* (Mtb), is believed to survive in hypoxic necrotic tissue by assimilating energy from host-derived lipids.These processes require a variety of hydrolases and lipases that release fatty acyl chains. Fatty acids are then broken down to acetyl coenzyme A (CoA) by the evolutionary conserved β-oxidation machinery consisting of five core catalytic functions. In our previous study, we showed that degradation of unsaturated fatty acids by mycobacteria require auxiliary a novel *cis-trans* enoyl CoA isomerase. However, this complex was unable to breakdown fatty acids with *cis* unsaturation at even carbon. We have now characterized Mtbdienoyl CoA reductasethat is required forbreakdown of these specialized fatty acids. Our studies thus provide understanding of the mechanism by which Mtb survive in these lipid-rich niches.

B. Deciphering mechanisms underlying melanogenesis and depigmenting disorder Vitiligo

Vitiligo is an acquired pigmentary disorder characterized by areas of depigmented skin resulting from loss of epidermal melanocytes. The cause of the destruction of epidermal melanocytes is complex and not yet fully understood. The Unfolded Protein Response (UPR) is a homeostatic endoplasmic reticulum (ER)-nuclear crosstalk mechanism operative in mammalian cells. Secretory cells with higher protein folding load on the ER-Golgi axis are heavily dependent on UPR for their adaptation and functioning and survival. In our laboratory, we are interested to identify the mechanisms that link triggering factors with the disease progression. We set out to study UPR during melanogenesis and examined whether apart from the classical pathway any other explicit UPR

response is required during pigmentation. This study was performed by usingB16 melanoma cells-based pigmentation oscillator model previously reported from our laboratory. Analysis of three classical UPR pathways revealed a two-fold increase in Xbp1 splicing during early initiating phase of pigmentation on day 3 which returned back to normal levels by day 4. We also observed ATF4 levels going up during pigmenting phase on day 4 and day 5 but activation of ATF6 was completely absent. Based on transcriptome studies, we investigated into other ER-resident proteins. Our analysis identified regulation of Creb3l2 to be coincident with melanogenesis programming at both mRNA and protein level. We demonstrated that the activated CREB3L2 N-terminus localizes to the nucleus by performing nuclear fractionation experiments on Day 5 cells as well by employing tagged CREB3L2 with mCherry at its N-terminus and EGFP at its C-terminus. We are presently understanding the regulatory role of CREB3L2 on melanogenesis and whether these effects are mediated by the central transcription factor Microphthalmia inducible transcription factor (MITF).

Publication

Original peer-reviewed article

1. Bothra A, Arumugam P, Panchal V, Menon D, Srivastava S, Shankaran D, Nandy A, Jaisinghani N, Singh A, Gokhale RS, Gandotra S, Rao V (2018) Phospholipid homeostasis, membrane tenacity and survival of Mtb in lipid rich conditions is determined by MmpL11 function. **Sci Rep**. doi:10.1038/s41598-018-26710-z.

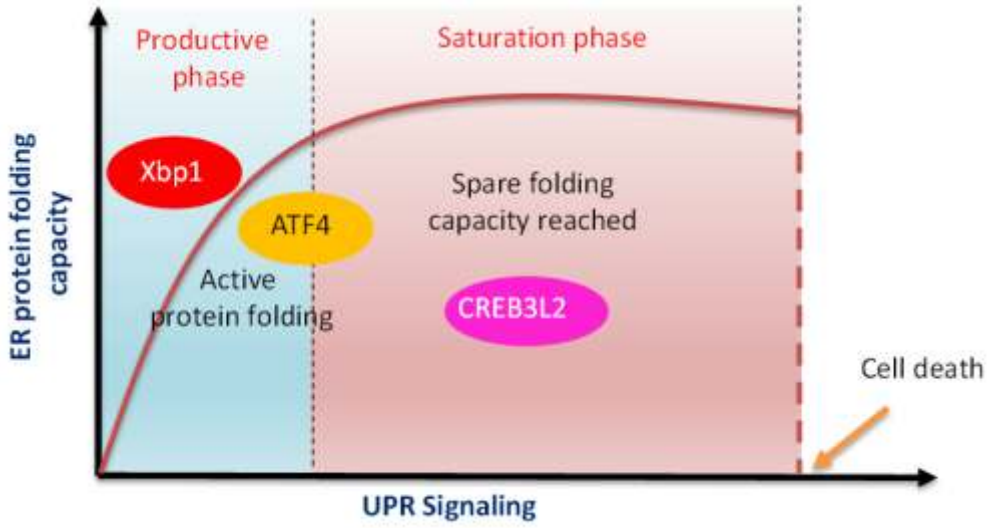

Fig. Kinetics of unfolded protein response during melanogenesis

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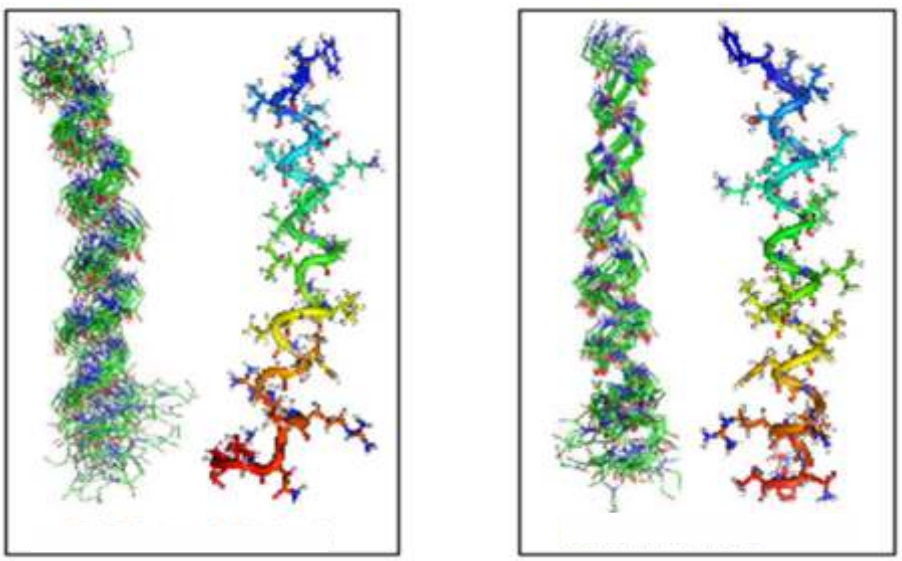
Biophysical and biochemical characterization of *Leishmania mexicana* phosphoglycerate kinase: An enzyme in the glycolytic pathway of parasitic protozoa

Vidya Raghunathan

Trypanosomatida cause deadly diseases in humans. Of the various biochemical pathways in trypanosomatida, glycolysis, has received special attention because of being sequestered in peroxisome like organelles critical for the survival of the parasites.

Leishmania PGK isoforms has some distinct structural features, as PGKB and PGKC differ primarily in the presence of a long extension at the C-terminus of PGKC. Drug development efforts can be targeted, either at the glycosome itself or at the enzymes present within them for which, targeting unique structural features is critical.

We are interested to use X-ray crystallography and related structural biological methods to study the PGK isoforms in *Leishmania* spp. Phosphoglycerate kinase (PGK) from *Leishmania spp*. which, exists in the cytoplasmic PGKB and glycosomal PGKC isoforms shows differences in their biochemical properties. Computational analysis predicted the likelihood of a transmembrane helix only in the glycosomal isoform PGKC, of approximate length 20 residues in the 62-residue extension,ending at, arginine residues R471 and R472. From experimental studies using circular dichroism and NMR with deuterated sodium dodecyl sulfate, we find that the transmembrane helix spans residues 448 ± 2 -476 **(Fig.1).**



NMR Structure in Methanol **NMR Structure in SDS**

Fig.1.

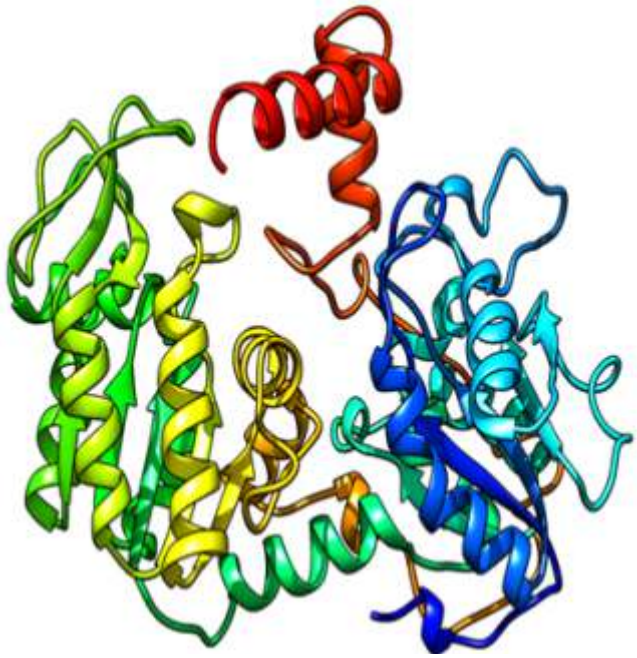


Fig.2.

Further, we sought to establish the three dimensional structure of PGKC_*Lmexicana* by homology modelling and biochemical data. We have a final theoretical 3-dimensional model of *L. mexicana* PGKC (residues 1-479) that enables visualization of the *GXXXG* motif in the enzyme fold (Figure 2). While supporting our biochemical data, the docking interactions reveal new aspects of the tertiary fold of PGKC. The helix which corresponds to TMS (Fig. 1) is a discontinuous helix in the model shown in Figure 2. Furthermore *GXXXG* motif such as is present in our case may have function in stabilizing the protein conformation given the sequence context such as the presence of neighboring β-branched residues. The hydrophobic patch that is formed by ^{462}LLIGIFIG^{469} may represent the localized epistatic interactions controlling the evolvability of PGKC. The perturbation leading to evolution of PGKC may be the drastic change in the environment of the protein, in this case the encapsulation in the glycosome. In the well-studied case of HIV-1 protease, epistatic interaction in mutation covariance cause high evolvability and drug resistance of the protease. The knowledge in this field is still scant and the exact role of epistatic pair-wise interactions or modularity on evolvability of function and robustness of a protein is not fully understood. We hypothesize a switch type of mechanism for the 63-mer between insertion into the membrane with the rest of PGKC in an open conformation *to* a closed conformation of PGKC_*Lmexicana* where the 63-mer stabilizes this conformation. Further research in this direction is likely to give very important results.

Publication

Original peer-reviewed article

1. Srinivasan S, Aggarwal S, Raghunathan V (2018) Theoretical structural model of the glycolytic enzyme Phosphoglycerate Kinase C from Leishmania Mexicana mexicana: Transition from a continuous to discontinuous helix at the C-terminus and the role of GXXXG motif. **Allergy Drugs & Clin Immunol**. 2: 44-55.

GENE REGULATION

Cellular and molecular biology of human cancer

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Ovarian cancer is the most lethal malignancies among women. There is an urgent need to develop novel therapeutic treatment for better cancer management. SPAG9 is a novel tumor associated antigen, expressed in variety of malignancies. However, its role in ovarian cancer remains unexplored. We have investigated role of SPAG9 in ovarian cancer cells employing shRNA approach.

SPAG9 is expressed in ovarian cancer cells

SPAG9 gene and protein expression was examined in three ovarian cancer cells (Figure 1a, 1b). Subsequently, FACS analysis revealed surface localization of SPAG9 protein in A10 and SKOV-3 cells (Fig. 1c). In addition, we also found cytoplasmic localization of SPAG9 in ovarian cancer cells. SPAG9 co-localization was also observed with endoplasmic reticulum, golgi bodies and mitochondria (Fig. 1d). However, SPAG9 did not co-localize with nuclear envelope (Fig. 1d).

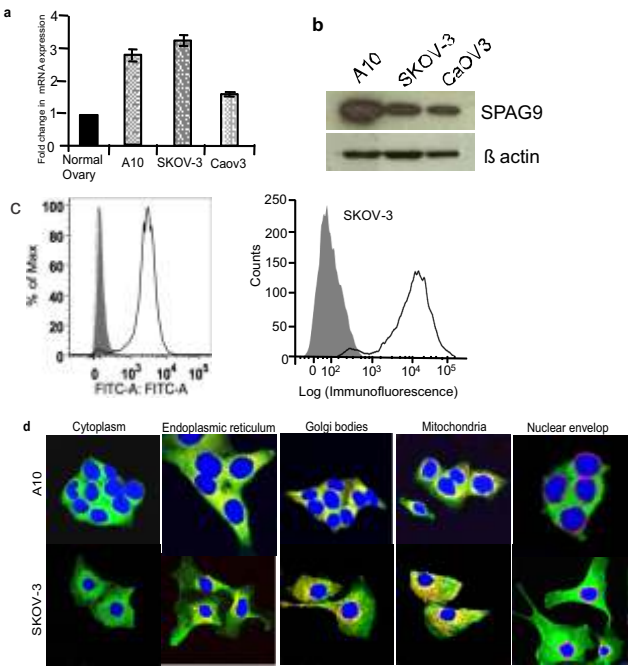

Fig.1. SPAG9 gene protein expression and surface localization in different ovarian cancer cells.

Knockdown of SPAG9 results in reduced cellular proliferation, colony forming ability and enhances cytotoxicity of paclitaxel

Effects of SPAG9 ablation on various malignant properties of ovarian cancer cells were investigated in A10 and SKOV-3 cells. Western blot analysis validated reduced SPAG9 protein expression in A10 and SKOV-3 cells (Fig. 2a). Significant reduction in cellular proliferation (P<0.001) was observed in SPAG9 ablated ovarian cancer cells (Fig. 2b). Further, paclitaxel treatment on A10 and SKOV-3 cells resulted in significant reduction in cellular proliferation (P<0.001; Fig. 2b) as compared to NC shRNA treated A10 and SKOV-3 cells. We further examined the combined effect of paclitaxel and SPAG9 shRNA on cellular proliferation in A10 and SKOV-3 cells which revealed enhanced chemotherapeutic sensitivity with reduced cellular proliferation (Fig. 2b). Further, effect of SPAG9 knockdown on cell viability was assessed by MTT assay in A10 and SKOV-3 cells, which showed significant decrease in cell viability (P<0.001) after SPAG9 ablation in A10 and SKOV-3 cells compared to NC shRNA treated cells (Fig 2c). Down-regulation of SPAG9 in ovarian cancer cells also inhibited the viability with enhanced cytotoxic effect of chemotherapeutic agent paclitaxel. As expected, paclitaxel treatment resulted in significant (P<0.001) reduction in cellular viability in A10 and SKOV-3 cells as compared to NC shRNA alone (Fig. 2c). IC50 of the paclitaxel was 1.4 nM in A-10 and 3.2 nM in SKOV-3 ovarian cancer cells respectively. Combination treatment with paclitaxel and shRNA1 or shRNA2 resulted in significant reduction in cellular viability and enhanced cell death in A10 (P<0.001) and SKOV-3 (P<0.001) cells as compared to untreated cells (Fig. 2c). Combination index (CI) values of interactions between SPAG9 shRNA and paclitaxel were found to be <1 indicating synergistic effect in ovarian cancer cells. Colony-forming ability of ovarian cancer cells revealed a significant reduction in number of colonies formed in SPAG9 depleted cells as compared to NC shRNA treated cells (P<0.001; Fig. 2d). SPAG9 ablated cells showed S phase growth arrest after PI staining at 48h in A10 and SKOV-3 cells respectively (Fig. 2e).

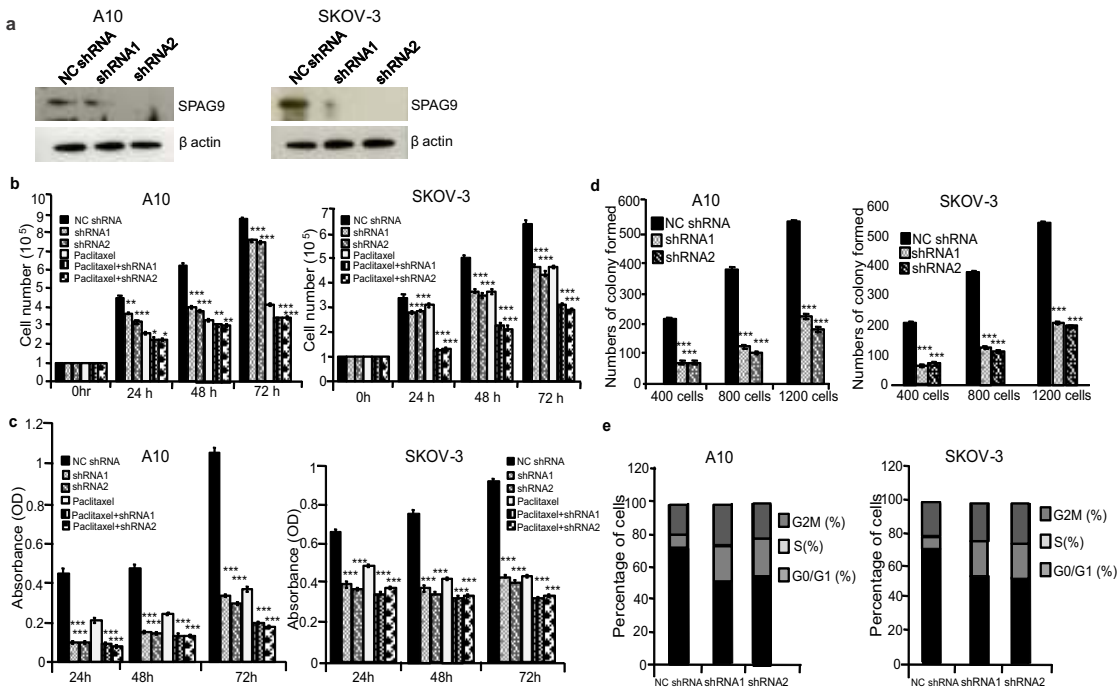

Fig.2. Ablation of SPAG9 results in reduced growth and colony forming ability. (a) SPAG9 protein expression in SPAG9 ablated A10 and SKOV-3 cells as compared to NC shRNA cells. β actin was used as endogenous loading control.(b) Histogram shows significant reduction in cellular proliferation post 24 h, 48 h and 72 h treatment with SPAG9 ablation or paclitaxel alone or combination of paclitaxel with SPAG9 knockdown as compared to NC shRNA transfected A10 and SKOV-3 cells. (c) Cellular viability assay in A10 and SKOV-3 cells. Histogram depicting significant growth reduction post 24h, 48h and 72h treatment in A10 and SKOV-3 cells after SPAG9 knockdown. (d) Histogram shows significant inhibition in colony forming ability in shRNA1 and shRNA2 treated cells compare to NC shRNA treated cells (e) Histogram shows S phase growth arrest in SPAG9 ablated cells at 48h in A10 and SKOV-3 cells respectively. Experiments were performed three independent times in triplicates. Data are represented as mean ± standard error of the mean (* P<0.05; ** P<0.01; *** P<0.001).

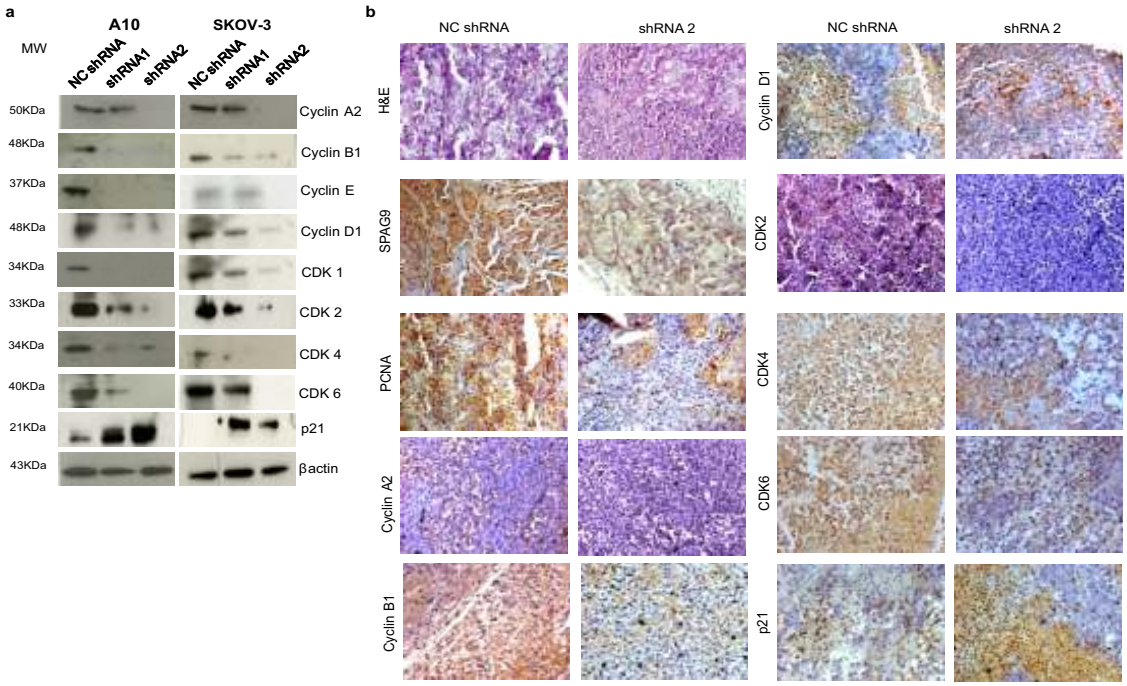

Fig.3. Ablation of SPAG9 alters expression of cell cycle molecules involved in cellular growth and proliferation in vitro and in vivo. (a) Western blot data reveals down regulation of various cell cycle related proteins and up regulation of p21 in SPAG9 ablated A10 and SKOV-3 cells. (b) Representative images of IHC analysis show reduced immunoreactivity of SPAG9 and cell cycle related proteins and increased immunoreactivity of p21 in SPAG9 shRNA2 treated tumor as compared to NC shRNA treated tumor sections. H&E staining shows cytostructure of the tumor cells.

SPAG9 knockdown arrests cellular proliferation and reduced tumor growth *in vivo* mouse xenograft model

Western blot analysis showed a significant decrease in the expression of cell cycle related proteins and upregulation of p21 in both ovarian cancer cells (A10 and SKOV-3) transfected with SPAG9 shRNA as compared to NC shRNA treatment (Fig. 3a). To validate in vitro effect of SPAG9 ablation on cellular proliferation and growth, in vivo A10 ovarian cancer xenograft mice model experiments were carried out which revealed significant reduction in tumor size and volume in SPAG9 shRNA2 treated tumor as compared to NC shRNA treated group ($P<0.001$). Immuno-histochemical analysis of tumor sections showed significant reduction ($P<0.0001$) in SPAG9 expression in SPAG9 shRNA2 treated group as compared to NC shRNA treated group (Fig.3b). We also observed decreased expression of cell cycle molecules and increased immunoreactivity of p21 in SPAG9 shRNA2 treated tumors (Fig. 3b), supporting *in vitro* observations of effect of ablation of SPAG9 on ovarian cellular growth.

In summary our study suggested that SPAG9 might be playing role in ovarian cancer growth. Gene silencing approach revealed that various oncogenic properties of ovarian cancer cells including cell cycle regulation, apoptosis and EMT were down regulated in SPAG9 depleted cells. Also, SPAG9 depletion in ovarian cancer cells revealed reduced tumor growth *in vivo*. Thus, SPAG9 may be a potential novel therapeutic target in ovarian cancer and studies have been initiated in preclinical mouse model system.

Human clinical trials in cervical cancer patients stage IIIb

Clinical experience with DC vaccine at Cancer Institute (WIA) Adyar, Chennai

Based on the Phase 1 study, the Department of Science and Technology, Government of India provided funds to establish a Cancer Immunotherapy Centre, in the Department of Molecular Oncology, Cancer Institute [WIA]. The Centre was established and a Phase 2 double blind clinical trial to assess the efficacy of Dendritic cells primed with either patient's own tumour lysate or using rSPAG9 protein in stage IIIB cervical cancer has been initiated and is recruiting patients. [DCGI Clearance obtained by their letter dated 03 March 2015; CTRI/2016/12/007530].

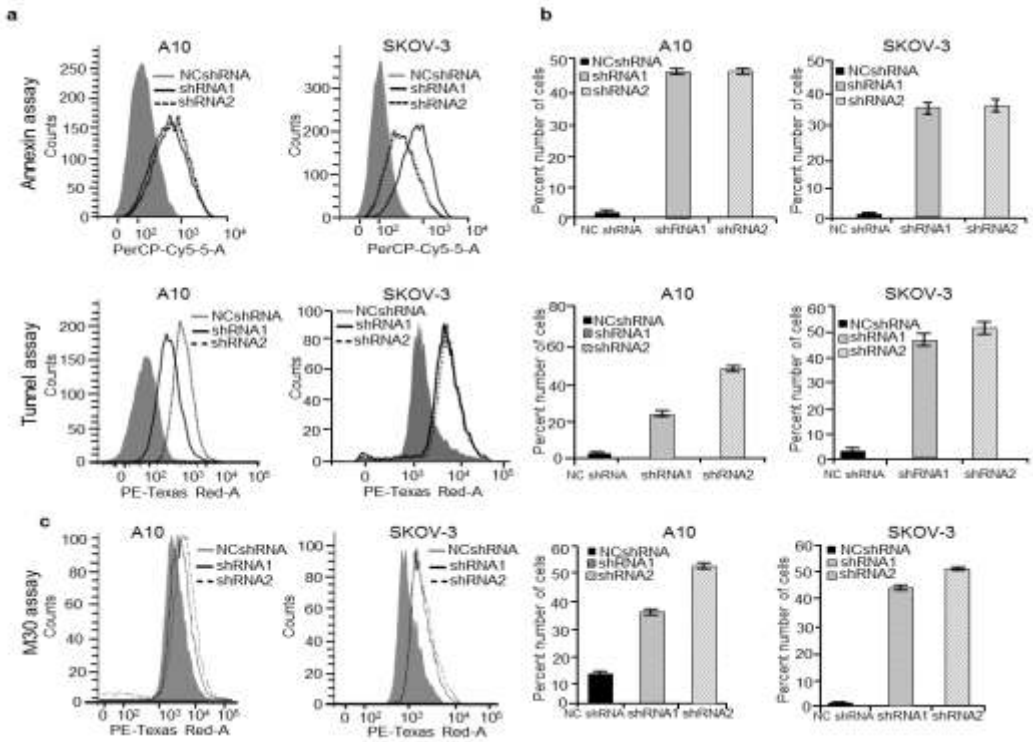

Fig.4. Knockdown of SPAG9 causes DNA damage and initiates apoptosis. (a) Flow cytometric analysis demonstrates the effect of SPAG9 ablation on onset of apoptosis in A10 and SKOV-3 cells by annexin V (phosphotidyl serine expression), TUNEL assay (DNA damage) and M30 assay (caspase activation). (b) Histogram depicts percentage of A10 and SKOV-3 cells with increased phosphotidyl serine expression, increased DNA damage and enhanced caspase activation in SPAG9 ablated cells as compared to NCshRNA cells. Experiments were performed three independent times in triplicates. Data are represented as mean ± standard error of the mean (* P<0.05; ** P<0.01; *** P<0.001).

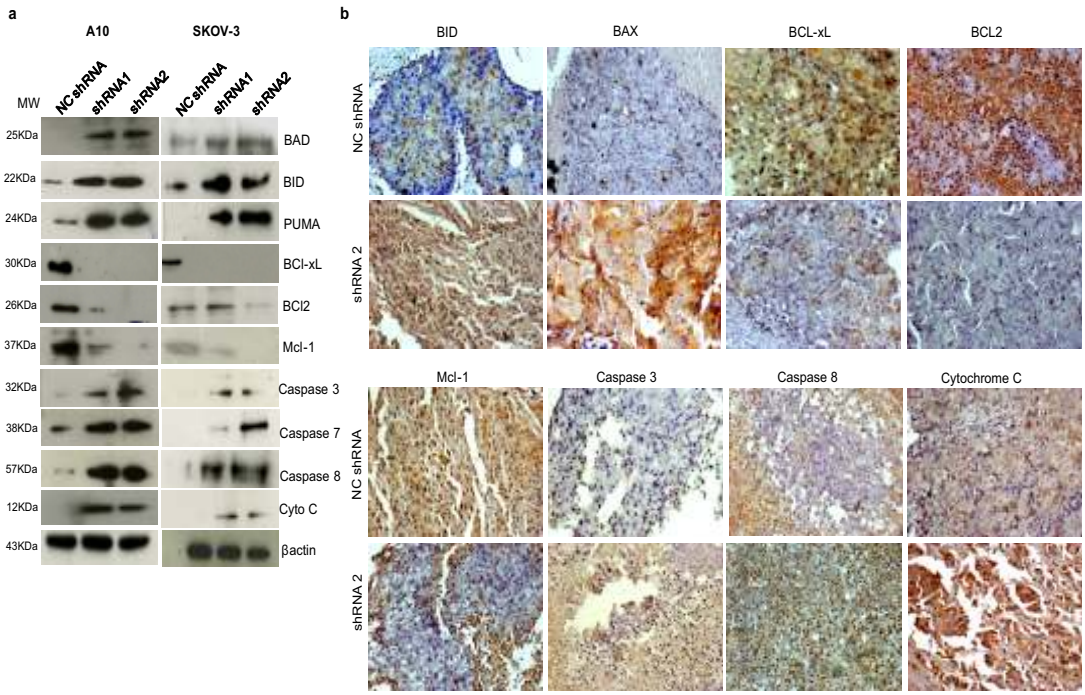

Fig.5. Ablation of SPAG9 protein alters molecules involved in apoptosis process. (a) Western blot shows down regulation of anti-apoptotic molecules and up regulation of pro-apoptotic molecules in SPAG9 ablated A10 and SKOV-3 cells (b) Representative IHC images with increased immunoreactivity of pro-apoptotic molecules and reduced expression of anti- apoptotic molecules in SPAG9 shRNA2 treated mice xenograft as compared to NC shRNA treated mice.

Study design of the ongoing Phase 2 trial in stage IIIB cervical cancer- Cancer Institute, Adyar, Chennai

The study is a phase II, randomized, double blind, three arm trial. Stage III-B cervical cancer patients with HPV positivity and SPAG9 positivity will be included in the study and randomized to one of the three arms:

- Arm 1: Conventional concurrent chemo-radiotherapy with placebo (saline given intradermally)
- Arm 2: Conventional concurrent chemo-radiotherapy with DCs primed with patient's own whole tumour cell lysate given intradermally
- Arm 3: Conventional concurrent chemo-radiotherapy with DCs primed with recombinant SPAG9 protein given intradermally

A total of 54 patients were to be included in this Phase 2 trial wherein 40 patients have been recruited since Jan 2017 for the trial. The randomization was done as block randomization of 18 per block, 3 blocks in total. Till date, 31 patients have finished all ten doses and 30 have had their re-evaluation done employing PET-CT Scan. No DC Vaccine related toxicity has been seen observed except for pain at the site of intradermal injection.

The specific aims for research include the following various aspects of cancer management and focus on following areas of cancer research with team of scientists and clinicians:

1. Early Detection and Diagnosis: [NCI-AIIMS, NII, Cancer Institute Chennai]. The goal is to advance medical research and improve patient outcomes by discovering biomarkers (indicators) for multiple types of cancer. The application of biomarkers to cancer is leading the way because of the unique association of genomic changes in cancer cells with the disease process. The early detection of cancer is crucial for its ultimate control and prevention. Large scale validation of SPAG9 will be carried out in NCI-AIIMS and Cancer Institute, Chennai for ovarian, breast and prostate cancer. Our aim is to use SPAG9 molecules for detecting cancers when they are at their earliest stages, even in the premalignant state. This will mean that current or future treatment strategies will have a higher probability of truly curing the disease.

Publication

Original peer-reviewed article

1. Jagadish N, Fatima R, Sharma A, Devi S, Suri V, Kumar V, Suri A (2018) Sperm associated antigen 9 (SPAG9) a promising therapeutic target of ovarian carcinoma. **Tumour Biol**. doi:10.1177/1010428318773652.

Patent

1. Suri A (2018) "Novel cancer associated antibodies and their use in cancer diagnosis." **Indian Patent Number 295616; Granted on 09/04/2018**.

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Deciphering the role of cell signalling in *Mycobacterium tuberculosis* biology

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The emergence of increasingly drug resistant *Mycobacterium tuberculosis (Mtb)* strains has become a crucial public health concern. In order to effectively treat tuberculosis, it is imperative to find newer targets, which are important for the *in-vivo* bacterial survival and persistence. Phosphorylation based signaling cascades modulated by Eukaryotic like Serine/Threonine Protein Kinases and phosphatase in *Mtb,* transduce extracellular stimuli to a cellular response ensuing pathogen's growth, persistence and pathogenesis. There are 11 eukaryotic-like STPKs in *Mtb*, and we have worked towards analyzing the functional roles of the phosphorylation events mediated by these kinases. Investigating the signaling events in the pathogen contributes significantly towards understanding the biological events that are coupled to them anifestation of the disease.

Protein phosphorylation has come forth as a preeminent circuitry regulating a vast number of physiological processes in the bacterial kingdom. A particular class of receptor-type serine-threonine kinase called PASTA (Penicillin binding proteins And Serine Threonine Associated) kinase is widespread across gram-positive firmicutes and actinomycetes and is known for its functions associated with bacterial cell growth. These protein kinases have an intracellular kinase domain, which shows sequence and structural homology to the eukaryotic serine/threonine kinases, and an extracytoplasmic (Ec) domain made up of varying number of PASTA domains. PASTA kinases are usually required by bacteria under stress conditions like nutrient starvation, antibiotic stress, biofilm formation etc., and are non-essential for their vegetative growth. However, in the pathogenic bacterium *Mtb* the PASTA kinase PknB ($PknB_{Mtb}$) is an essential gene and is proposed to be one of the master regulators of serine/threonine phosphorylation-mediated signaling.

The essential nature of PknB in mycobacteria stems from its ability to influence the activity of a large repertoire of substrates involved in cell wall synthesis, cell growth, cellular metabolism, transcription and translation. Over-expression or depletion of PknB impacts cellular morphology and survival of *Mtb*, which suggests that the expression and activity of this kinase must be critically fine-tuned inside the bacterium. The dynamicity of $PknB_{Mtb}$ regulation implies that the receptor kinase actively monitors its environment and responds accordingly, in an effort to provide survival advantage. PknB is believed to respond to environmental signals through PASTA domain interactions with the specific ligand, identified to be non-crosslinked peptidoglycan (PG) fragments called muropeptides. In line with this, purified PASTA domains of $PknB_{Mtb}$ interact and bind with a synthetic muropeptidecontaining isoglutamine (iGln) and meso-diaminopimelic acid (mDAP) residues at the second and third position of the stem peptide *in vitro*.

The prevailing hypothesis suggests that the interaction of the extracytoplasmic domain with the ligand results in the dimerization of intracellular kinase domain, which is required for the activation of the kinase through activation loop phosphorylations. In consonance with this we have previously reported that the extracytoplasmic PASTA domains are indispensable for the function of PknB and survival of *Mtb*. To date, the hypothesis with respect to $PknB_{Mtb}$ activation has not been tested *in vivo*. We set out to answer the following questions: (i) What are the ligand binding residues in the extracytoplasmic domain? (ii) What is the impact of abrogating ligand binding on the localization and activation of PknB? (iii) What are the physiological ligands that interact with PASTA domains? (iv) What is the impact of abrogating ligand binding on the phosphorylation of target substrates of PknB? Here we report the results of our efforts to elucidate the mechanism of regulation of PknB activity.

mDAP and iGln interacting residues influence the survival

With the help of *in silico* molecular simulations we identified potential ligand-binding residues in the linker regions between PASTA3 and 4 domains. Data suggested that Ser_{556} and Lys_{557} (SK) interact with the carboxy terminal region of the iGln and Asn_{559} and Gln_{560} (NQ) interact with the mDAP residues in the muropeptide ligand. We employed previously described *Mtb* conditional mutant of *pknB* (*RvΔB*), wherein the native locus has been modified to bring its expression under pristinamycin inducible promoter, which allows the bacterium to grow efficiently in the presence of pristinamycin but not in its absence. To assess the impact of all four putative ligand interacting residues, we generated a PknB tetra mutant (PknB-GM)in pNit-3F, which could be induced with isovaleronitrile. The *RvΔB* strain was transformed with pNit-3F-PknB and pNit-3F-PknB-GM and the expression of both proteins was confirmed. While the wild type could successfully rescue the phenotype both *in vitro* and *ex vivo*, the tetra mutant failed to do so. Thus, putative iGln and mDAP interacting residues in the PASTA domain of PknB seem to be necessary for its functionality. Further experiments suggested that both iGln and mDAP interacting residues are individually critical for PknB functionality, especially during the *ex vivo* infection scenario where even marginal perturbations in ligand binding are not endured.

Mutations in the PknB-Ec abrogate its binding to LipidII

PG synthesis at the poles and septum region involves two stages. In the first stage, which happens in the cytoplasm, LipidII is synthesized from UDP-GlcNAc precursor by sequential action of multiple Mur family enzymes. LipidII is composed of N-acetylglucosamine (NAG)-N-acetylmuramic acid (NAM)-pentapeptide (stem peptide) connected to the membrane anchored decaprenyl phosphate through a pyrophosphate link. The nature of the lipid moiety, the amino acids in the stem peptides and modification in the NAG and NAM sugars vary from species to species. We examined the interaction between the PknB-Ec and lysine- or mDAP-containing LipidII molecules. The results showed that the extracytoplasmic domain of $PknB_{Mtb}$ binds specifically with mDAP-containing LipidII and the binding is abrogated upon mutating ligand-interacting residues.

Abrogation of ligand binding perturbs localization of PknB

Nascent PG biosynthesis takes place at the poles and mid cell (septum) regions and hence the precursors such as LipidII and muropeptides are anticipated to be concentrated at these niches. Interestingly, PknB also localizes to both polar and mid-cell regions and the extracytoplasmic PASTA domains govern the localization. Thus the rational supposition would be that the localization of PknB is probably dictated by the interaction between PASTA domain and PG precursors. We examined the localization of GFP-PknB and GFP-PknB-GM mutants in *M. smegmatispknB* conditional mutant (*mc²ΔB*). The results suggest that LipidII is likely to be the major intracellular ligand of PknB, and importantly, ligand binding is essential for the appropriate localization.

PknB-GM is hyper-phosphorylated in the activation loop

If the prevailing hypothesis with respect to the activation of PknB is accurate, abrogation of ligand binding should result in decreased phosphorylation of the activation loop residues. To determine the extent of activation loop and juxtamembrane phosphorylations, we resorted to isobaric TMT labeling. Intriguingly, contrary to the current belief, we observed hyperphosphorylation of both activation loop and juxtamembrane domain in the 3F-PknB-GM mutant compared with 3F-PknB. While the role of activation loop phosphorylation is known, the role of juxtamembrane phosphorylation needs further investigation. The data suggests that abrogation of ligand binding results in hyper phosphorylation of PknB, strongly suggesting that the ligand binding plays a regulatory role.

PknB-GM hyperphosphorylates specific & non-specific targets

The analysis of the activation loop phosphorylations in PknB suggested that PknB-GM is hyperphosphorylated, which led us to infer it to be a hyperactive kinase. In line with this prediction, complementation with the PknB-GM mutant resulted in the remarkable hyperphosphorylation of cellular proteins. To confirm that mutant is indeed hyper-phosphorylated in the activation loop, we probed with phosphospecific antibodies. Consistent with mass spectrometry data, we observed ~1.5 fold increase in the normalized activation loop phosphorylation in the ligand binding mutant PknB-

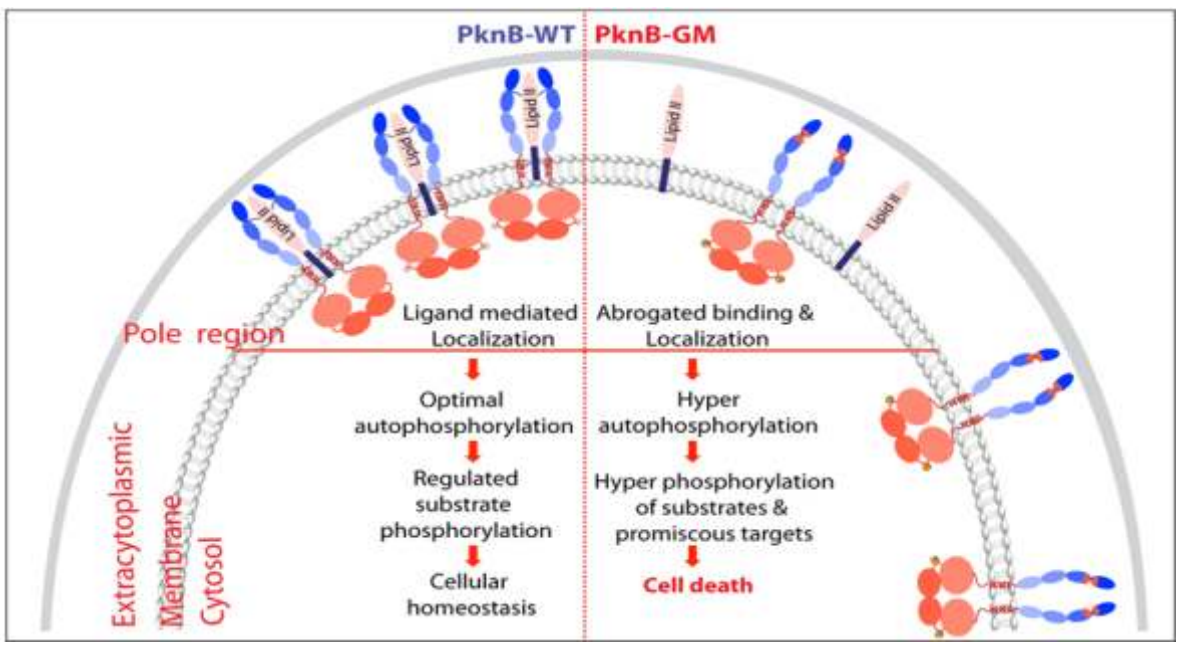

Fig.1. Model depicting PknB regulation wherein LipidII interacts with specific region of PASTA 3-4 linker region of PknB and defines its localization to polar/septal niches and regulates the activity to optimal levels and hence maintains cellular homeostasis and cell survival.

GM. *In vitro* kinase assays with immunoprecipitated 3F-PknB and 3F-PknB-GM using GarA showed that PknB-GM has higher activity compared with the PknB, which could be a combinatorial effect of higher phosphorylation of loop as well as the juxtamembrane residues. Finally, to validate the data we performed parallel reaction monitoring (PRM) to quantitate the TatA-T60 phosphopeptide. Quantitation of TatA-T60 phosphopeptide with respect to the corresponding heavy peptide using PRM evidently demonstrated ~2 fold increase in its levels in *RvΔB::B-GM* sample compared with *RvΔB* &*RvΔB::B* samples.

Collectively, these data demonstrate that the abrogation of ligand binding perturbs the normal regulatory circuits of PknB, resulting in aberrant localization, hyperactivation of the kinase, and indiscriminate target-specific and promiscuous phosphorylation events, leading to eventual cell death (Figure 1).

Publications

Original peer-reviewed articles

1. Kaur P, Rausch M, Malakar B, Watson U, Damle NP, Chawla Y, Srinivasan S, Sharma K, Schneider T, Jhingan GD, Saini D, Mohanty D, Grein F, Nandicoori VK (2019) LipidII interaction with specific residues of Mycobacterium tuberculosis PknB extracytoplasmic domain governs its optimal activation. **Nat Commun**. doi: 10.1038/s41467-019-09223-9.
2. Yadav K, Yavvari PS, Pal S, Kumar S, Mishra D, Gupta S, Mitra M, Soni V, Khare N, Sharma P, Srikanth CV, Kapil A, Singh A, Nandicoori VK, Bajaj A (2019) Oral delivery of cholic acid-derived amphiphiles helps in combating salmonella-mediated gut infection and inflammation. **Bioconjug Chem**. 30: 721-732.
3. Joshi AC, Kaur P, Nair RK, Lele DS, Nandicoori VK, Gopal B (2019) Selectivity among anti sigma factors by Mycobacterium tuberculosis ClpX influences intracellular levels of extracytoplasmic functions sigma factors. **J Bacteriol**. doi:10.1128/JB.00748-18.
4. Misra R, Menon D, Arora G, Virmani R, Gaur M, Naz S, Jaisinghani N, Bhaduri A, Bothra A, Maji A, Singhal A, Karwal P, Hentschker C, Becher D, Rao V, Nandicoori VK, Gandotra S, Singh Y (2019) Tuning the Mycobacterium tuberculosis alternative sigma factor SigF through the multidomain regulator Rv1364c and osmosensory kinase, protein kinase D. **J Bacteriol**. doi:10.1128/JB.00725-18.
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Review/Proceeding

1. Khan MZ, Kaur P, Nandicoori VK (2018) Targeting the messengers: Serine/threonine protein kinases as potential targets for anti-mycobacterial drug development. **IUBMB Life**. 70: 889-904.

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Elucidating the molecular mechanisms of aging and innate immunity using *Caenorhabditis elegans* as a model system

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Our lab uses a combination of genetics, molecular biology and genomics in *Caenorhabditis elegans* to understand signalling events that lead to alterations in gene expression during aging.

A. Deciphering the coordinate regulation of genes downstream of the Insulin/IGF-1-like (IIS) pathway

The IIS pathway regulates germline development, dauer formation, stress tolerance as well as longevity. We identified a cyclin-dependent kinase (CDK) in a reverse genetic screen. Interestingly, we found that the *cdk* knockdown led to increased nuclear localization of the FOXO transcription factor (TF) DAF-16. We found that *cdk* knockdown arrested the germline of *daf-2(e1370)* worms at pachytene; however, the arrest was relieved in *daf-2(e1370);daf-16(mgdf50)*.

B. Involvement of novel kinases in DR

To understand why *flr-4(n2259)* mutant increases life span in a food-type-dependent manner, we performed RNA-seq analysis. We found that genes involved in xenobiotic biotransformation is upregulated specifically in the mutant grown on HT115, but not in OP50. The phosphorylation of PMK-1, the p38 ortholog, was found to be higher when the *flr-4(n2259)* worms were fed HT115. Consequently, the expression of a xenobiotic gene reporter was specifically upregulated in the mutant grown on HT115, in a p38 MAPK-dependent manner.

C. Role of the Endoplasmic reticulum (ER) in DR-mediated longevity

We found that the levels of ERAD genes are upregulated early in development and is maintained at a high level during adulthood in *eat-2* mutant worms. We also found that the expression of the ERAD genes are dependent on the FOXA transcription factor PHA-4. Mechanistically, we found that the transient UPR response during DR is caused by depletion in glucose resulting in lower levels of glycosylated ER proteins.

To study conservation in mammals, we used a cellular model of Huntington's disease. Cells overexpressing PolyQ150 were subjected to a transient pretreatment with Tunicamycin. We found that the treatment decreased PolyQ150 aggregation. Using cycloheximide chase assay, we showed that the PolyQ150 proteins are degraded faster in cells preconditioned with transient dose of Tunicamycin.

D. Using mice models of diabetes to study the efficacy of Rifampicin on reducing hyperglycemia-related complications

We tested two delivery routes to assess the effect of Rifampicin on type I and type II diabetes models in mice. First, in the Type 1 model, intra-peritoneal route (IP) ororal gavage did not have any effect on blood glucose and HbA1c.We concluded that Rifampicin may not be able to counter insulin-dependent diabetes. On the other hand, we found that IP of Rifampicin significantly decreased HbA1c levels in db/db mice. The treated mice had lower body weight and improved glucose homeostasis, as determined by oral glucose and insulin tolerance tests.

Role of C. elegans p53 ortholog cep-1 in DR-mediated longevity

We used multiple DR paradigms in *C. elegans* to determine the requirement of *cep-1.* In two different non-genetic modes of DR, *cep-1* was dispensable for the increased longevity. However, in another genetic model of DR, the *eat-2* mutants, *cep-1* was required for DR-induced life span extension. In order to identify the pathways that *cep-1* genetically interact with, we knocked down the transcription factor using RNAi in different signalling pathway mutants. We found that *cep-1* is partially required for TOR pathway mutants to increase life span. On the other hand, germline-induced life span is completely dependent on *cep-1*. Interestingly, *cep-1* was found to regulate autophagy during DR, but that was independent of the TOR pathway.

Publications

Original peer-reviewed articles

1. Kumar N, Mukhopadhyay A (2019) Using ChIP-based approaches to characterize FOXO recruitment to its target promoters. **Methods Mol Biol**. 1890: 115-130.
2. Verma S, Jagtap U, Goyala A, Mukhopadhyay A (2018) A novel gene-diet pair modulates C. elegans aging. **PLoS Genet**. doi:10.1371/journal.pgen. 1007608.

Patent

1. Mukhopadhyay A, Chakraborty K, Matai L (2018) "A method of mimicking benefits of dietary restriction by transiently upregulating ER stress response." **Indian Patent Application Number 201811047507; Filed on 14/12/2018.**

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Epigenetic regulation of the eukaryotic genome : Role of CTCF in organizing chromatin

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Appropriate organization of chromatin at various levels is essential to accomplish spatial and temporal regulation of nuclear processes vital for metazoan development and cellular functions. This is governed by specific interactions of myriad cis-acting elements and trans-acting factors. Although the mechanisms underlying these interactions are not completely understood, CTCF has emerged as an important contributor to chromatin organization. The multifunctional nature of CTCF and the consequent diversity in the functional outcomes of CTCF binding is extremely intriguing. To decipher the nature of chromatin domains organized by this multifunctional protein, we are investigating the role of CTCF in regulation of murine TCRb locus whose transcription and RAG mediated VDJ recombination is exquisitely well regulated during development. Besides the appropriate enhancer-promoter interactions, higher order chromatin reorganization is necessary to bring gene segments (V, D and J) in spatial proximity prior to RAG mediated VDJ recombination. Since CTCF is an important global factor contributing to long range interactions of chromatin, it is of interest to decipher the chromatin structure and organization of the wild type and genetically manipulated Antigen Receptor loci to understand various aspects of CTCF based chromatin organization that may influence the interactions of regulatory elements important for transcription and VDJ recombination.

TCRb has 21 CTCF binding sites (CBS). Chromosome Conformation Capture (3C-qPCR) analysis has previously revealed thymocyte specific interactions amongst CBS suggesting their importance for maintaining the integrity of RC as well as facilitating "locus contraction" that precedes V-to-DJ recombination in Double Negative (DN) thymocytes. Further, ectopic CTCF binding within RC reduced all intrachromosomal interactions at TCRb locus by about 50%.

It was particularly intriguing that endogenous CBS of TCRb locus facilitate locus contraction and V-RC proximity yet ectopic CBS impaired the usage of upstream V segments. The intrachromosomal interactions detected by 3C-qPCR also suggested that various CBS of TCRb locus and the ectopic CBS differed in their function and recapitulated the functional diversity of CTCF observed in the global context. The diversity can be due to location with respect to other regulatory elements and/or specific molecular features associated with each CBS. Analysis of the various CBS using Fluorescence Polarization assay indicated that almost all the CBS of TCRb locus had high affinity to bind CTCF *in vitro*. We also examined the ability of the endogenous CBS to organize an enhancer blocking insulator in a cell culture system. Each of the CBS tested along with their flanking regions (about 200bp on either side), appeared to act as an efficient enhancer blocking insulator in this *in vitro* assay.

It will be useful to determine other aspects which distinguish ectopic CBS from the CBS endogenous to TCRb. Additionally, using ablation of specific CBS, we plan to test the contributions of individual CTCF binding sites of TCRb locus to various regulatory aspects including organization of barrier and/or enhancer blocking insulator near RC, establishment and stabilization of RC and locus contraction. We have initiated CRISPR-Cas9 based ablation of specific CBS of the TCRb locus to dissect the functional requirement of specific CBS for chromatin organization, transcription and VDJ recombination.

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Role of cell signaling in eukaryotic development

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We are interested in dissection of signaling and trafficking mechanisms that operate in diverse cell types. Here is a brief description of our recent studies:

I. Dissection of intracellular signaling and trafficking cascades that operate in *Plasmodium falciparum* and *Toxoplasma gondii*.

A. Role and eegulation of key kinase substrates by phosphorylation

Previously, we used a quantitative phosphoproteomics based approach to identify targets of kinases that are modulated by second messengers like calcium and phosphoinostitides in *P. falciparum*, which include PfCDPK1 and PfCDPK7. In addition, we have also performed similar studies on *Toxoplasma* homologue of CDPK7, TgCDPK7. In order to carry forward our quest to establish parasite signaling networks, it is important to validate substrate of these kinases and elucidate the role of phosphorylation in their function. Following is a summary of some of the results related to these studies: Quantitative phosphoproteomics studies revealed that PfCDPK1 may target diverse classes of proteins including proteins involved in invasion. Three major classes of proteins that were affected: cAMP signaling module, low (RAMA, RAP1) and High Molecular Weight Complex of rhoptries (RhopH3) and the inner membrane complex (IMC) proteins.

We demonstrated that the phosphorylation of PfPKA-R at S149 is critical for PfPKA activity, which in turn plays an important role in host RBC invasion. We identified some rhoptry proteins like RAMA and RhopH3 to be differentially phosphorylated in phosphoproteomics studies. Recent studies have indicated that RhopH3 is critical for host RBC invasion. Therefore, it was interesting to investigate if its phosphorylation at S804, which was less phosphorylated in PfCDPK1 depleted parasites, plays a role in this process. We used a CRISPR-CAS9 based approach to generate parasites in which S804 was mutated to A. While there was no discernable change in intraerythrocytic development, there was a significant decrease in the ability of S804A mutant parasites to invade host RBCs. Clearly, these data indicated that the phosphorylation of RhopH3 at S804 by PfCDPK1 is critical for invasion.

B. Dissection of the role of CDPK7 in *T. gondii* and *P. falciparum*

We have extended our interest in PIP-mediated signaling and trafficking by understanding the function of Calcium Dependent Protein Kinase 7 (CDPK7) in both *Plasmodium falciparum* and *Toxoplasma gondii.* We performed further analysis of

TgCDPK7 inducible knock down (TgCDPK7-iKD) and PfCDPK7-KO lines. Comparative phosphoproteomics of Pf/TgCDPK7 depleted parasites indicated that several proteins involved in phospholipid (PL) biogenesis and vesicular trafficking to be targeted by CDPK7. TgCDPK7 was found to regulate vesicular localization of TgRab11a by mediating its phosphorylation at S207. Importantly, TgRab11a phosphorylation was critical for parasite division and trafficking of key proteins.

II. Molecular mechanisms that regulate Cell Cycle Related Neuronal Apoptosis (CRNA)

The cell cycle machinery of neurons has to be suppressed to promote differentiation and for the maintenance of their terminally differentiated state. The reactivation of the cell cycle in response to neurotoxic insults leads to neuronal cell death and some cell cycle related proteins contribute to this process. As reported in the previous years, we had elucidated a novel pathway that promotes CRNA by amyloid peptide $A\beta_{42}$. This pathway involves aberrant activation of the MEK-ERK pathway and degradation of TAp73. We had reported that E3 ubiquitin ligase Itch to be involved in TAp73 degradation as depletion of Itch prevented TAp73 ubiquitnation in response to $A\beta_{42}$, which also prevented CRNA. We have now made efforts to understand the underlying mechanisms. Itch undergoes hyperphosphorylation in response to $A\beta_{42}$ which occurs due to aberrant activation of the JNK pathway. As a result of this, its self-ubiquitination is also enhanced and these events facilitate its interaction significantly with TAp73 in $A\beta_{42}$ treated cortical neurons or neurons from an animal model for Alzheimer's Disease (TgAD). Point mutants of the autophosphorylation and self-ubiquitination site were able to prevent TAp73 degradation and reverse CRNA. These studies provide novel insights into how Itch may be regulated in conditions that mimic neurodegeneration, which contributes to CRNA.

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Determining the signaling and repair pathways that are altered in human cancer

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Project A. Determining the role of BLM helicase in maintaining nuclear genome integrity

BLM suppresses the expression of c-Jun targets

c-Jun is a potent transcriptional activator of genes which positively regulate the carcinogenesis process. Since BLM enhances the Fbw7a-mediated degradation of c-Jun, we wanted to determine whether and how its presence affected the binding of c-Jun to its transcriptional targets. c-Jun ChIP-seq analysis revealed that the binding of c-Jun to 2584 gene promoters was significantly diminished within proximal promoters (-500 to +200 bp) of transcription start sites (TSS) in the presence of BLM. Multiple biological processes which directly or indirectly affect the neoplastic transformation process were upregulated in absence of BLM, as indicated by GO analysis. For eleven c-Jun targets, known to have effects in the neoplastic transformation and DNA repair processes, reduced c-Jun promoter occupancy was observed in cells with BLM. Further, in almost all tested cases expression of the c-Jun target genes were repressed in cells expressing BLM. Several of these c-Jun target genes also had increased protein levels in absence of BLM following IR.

BLM diminishes c-Jun mediated neoplastic transformation

Based on the above results we wanted to determine whether regulation of c-Jun levels by BLM led to a decrease in the capability of cells to undergo neoplastic transformation and thereby carcinogenesis. Cells which lack BLM but stably overexpress c-Jun had maximum clonogenic potential (i.e. the survival fraction is highest) and enhanced capability of anchorage independent growth. Consequently this effect was reflected in vivo during nude mice xenograft assays using the same cohort of stable isogenic lines. Overexpression of c-Jun in absence of BLM led to maximum growth of tumours. There was greater proliferation and angiogenesis in these cells, as evident from higher levels of both PCNA and CD31.

Project B. Determining the role of RECQL4 helicase in maintaining mitochondrial homeostasis

RECQL4 and PolgA levels are decreased by MITOL

In multiple organisms it is known that the robustness of the UPS system is dependent on the aging process. To determine whether mitochondrial UPS system is also altered during the aging we wanted to

determine whether the transcript and protein levels of RECQL4 and PolgA were altered in the brain and liver cells of young and aged mice. While the transcript levels of RECQL4 and PolgA were not statistically altered, the levels of both these proteins were decreased in the mitochondrial extracts isolated from the brain and liver cells of the aged mice. This decrease was due to greater ubiquitylation of RECQL4 and PolgA in the aged brain and liver mitochondrial extracts, indicating that these two proteins were undergoing increased turnover.

To determine the E3 ligase(s) involved in the turnover of RECQL4 and PolgA, overexpression of five known mitochondrial E3 ligases (MITOL, PARKIN, MULAN, RNF185 and KEAP1) were carried out and the levels of endogenous RECQL4 and PolgA determined. Only MITOL decreased the levels of RECQL4 and PolgA in multiple experimental system in which the levels of MITOL were decreased. Hence the half-life of both endogenous RECQL4 and PolgA were increased upon depletion of MITOL as revealed by cycloheximide chase experiment. Reciprocal immunoprecipitations with overexpressed proteins also indicated that MITOL interacted with both RECQL4 and PolgA and consequently colocalized in the mitochondrial nucleoids.

MITOL ubiquitylates RECQL4 and PolgA at specific residues via K6-linkage

Since the levels of RECQL4 and PolgA are regulated by MITOL, we next wanted to determine whether these two proteins were ubiquitylated by the E3 ligase. Indeed *in vitro* ubiquitylation indicated that MITOL WT but not MITOL CD ubiquitylated both RECQL4 and PolgA. This effect was also recapitulated *in vivo.* Next, we wanted to know the linkage via which MITOL ubiquitylated RECQL4 and PolgA. Usage of R- and O- ubiquitin mutants indicated that both RECQL4 and PolgA were ubiquitylated by MITOL via K6-linkage. Further we wanted to determine the sites on RECQL4 and PolgA which were ubiquitylated by MITOL. Using a combination of two independent ubiquitylation site prediction algorithms (UbPred and UbiPred) and a database (PhosphoSitePlus) a number of lysines were predicted on both RECQL4 and PolgA which could potentially be ubiquitylated by MITOL. *In vitro* and *in vivo* ubiquitylation of RECQL4 indicated that out of the predicted four lysine residues, two of them were targeted by MITOL for ubiquitylation. *In vitro* and *in vivo* ubiquitylation with lysine to alanine mutants of PolgA indicated that only one of the residues was ubiquitylated by MITOL.

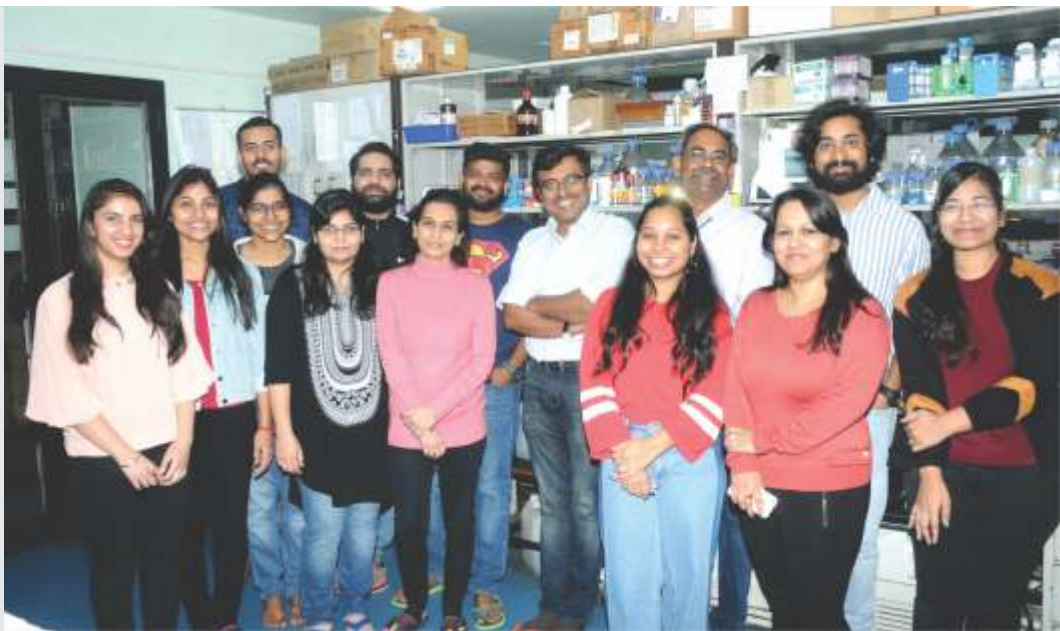
Publication

Original peer-reviewed article

1. Priyadarshini R, Hussain M, Attri P, Kaur E, Tripathi V, Priya S, Dhapola P, Saha D, Madhavan V, Chowdhury S, Sengupta S (2018). BLM potentiates c-Jun degradation and alters its function as an oncogenic transcription factor. **Cell Rep**. 24: 947-961.

Patent

1. Sengupta S (2018) "BLM mediated reactivation of Fbw7α mutant in cancer." **Indian Patent Application Number 201811022241; Filed on 14/06 2018**.



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Understanding the regulation of DNA replication

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Our laboratory is working towards understanding the mechanisms by which microRNAs and checkpoint proteins stall the cell cycle, thereby preventing genomic instability and cancer.

Objectives

We are investigating the cellular response to aberrations in replication complexes. The objective is to identify yet unknown checkpoint pathways that monitor the replication apparatus. We are evaluating the role of microRNA in regulating the DNA replication machinery as the cell progresses from one phase to the next. We are trying to understand the role of replication proteins in centrosomal stability. Summing up, we are attempting to unravel the protective regulatory control of mammalian cells, failure of which is likely to cause genomic instability.

Role of long non-coding RNAs in carcinogenesis

To identify iflncRNAs epigenetically inactivate the tumor suppressor genes in cancer, we analyzed the mRNA and lncRNA expression data of lung adenocarcinoma patients along with their adjacent normal tissue. We analyzed if lncRNAs regulate proximal protein-coding genes in cis: using this approach we have identified 55 possible regulatory interactions between lncRNA and protein coding genes where they lie within 20 kb of each other. Utilizing a high-throughput, genome-wide screen for miRNAs and mRNAs that are differentially regulated in osteosarcoma (OS) cell lines, we have observed that miR-449a and miR-424, belonging to the miR-34 and miR-16 families, respectively, target the major S/G2 phase cyclin, cyclin A2 (CCNA2) in a bipartite manner. We have identified a miRNA mega-cluster coding approximately 40 miRNAs which gets induced after oxidative stress. Out of the 40miRNAs of this cluster 18 miRNAs have significant effect on cell proliferation, DNA replication and G1 to S transition and are predicted to target multiple cell cycle genes. We have observed that miR-1* from this cluster directly targets CCND1, a major cyclin regulating G1-S transition (Figure 1). Over expression of this miRNA blocks the cell cycle in S-phase and decreases the proliferation, invasion and migration of U2OS cells, demonstrating its tumor-suppressive functions**.**

GINS4 is required for centrosome integrity during mitosis

We have shown that depletion of Sld5 results in chromosome congression, multipolarity and reduced centrosome proteins localization at spindle poles. We now show that replication factor Sld5 is essential for dynein-dependent transport of proteins to centriolarsatellites which serve as reservoirs for centrosomal proteins. These events culminate in poor recruitment of centrosomal maturation factor, PLK1, leading to poor maturation of centrosome in G2 phase. Thus, replication factor Sld5 is essential for

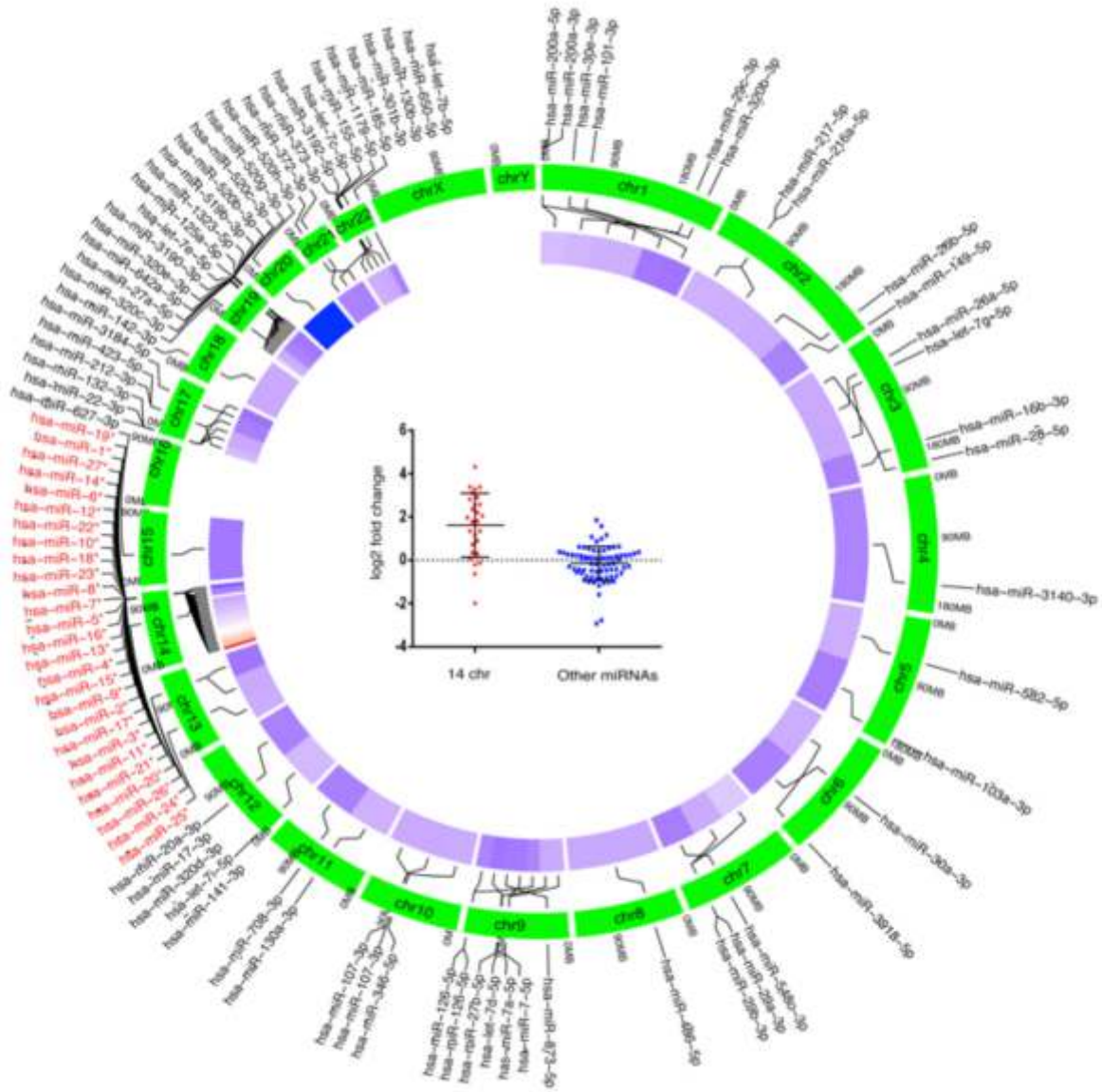

Fig.1. Circos plot to depict expression of miRNAs in whole genome after oxidative stress. Green circular structure represents the whole genome with each bar representing each chromosome. Location of the miRNAs in genome is shown by the line pointing at the location in the chromosome. Inner most circular bar is showing the expression levels of each miRNA after oxidative stress. Blue, white and red bar color representdownregulated, unchanged and upregulatedmiRNAs, respectively. Locus at the chromosomes 14 is highlighted in light red, showing that the cluster gets upregulated after oxidative stress. The inset dot plot depicts that miRNAs from this cluster at chromosome 14 are significantly upregulated in comparison to the miRNAs tested from the rest of the genome.

dynein mediated transport of centrosomal protein to the centriolar satellite for maturation of centrosome during the G2 phase (Figure 2).

Publication

Original peer-reviewed article

1. Shekhar R, Priyanka P, Kumar P, Ghosh T, Khan MM, Nagarajan P, Saxena S (2019) The microRNAs miR-449a and miR-424 suppress osteosarcoma by targeting cyclin A2 expression. **J Biol Chem**. 294: 4381-4400.

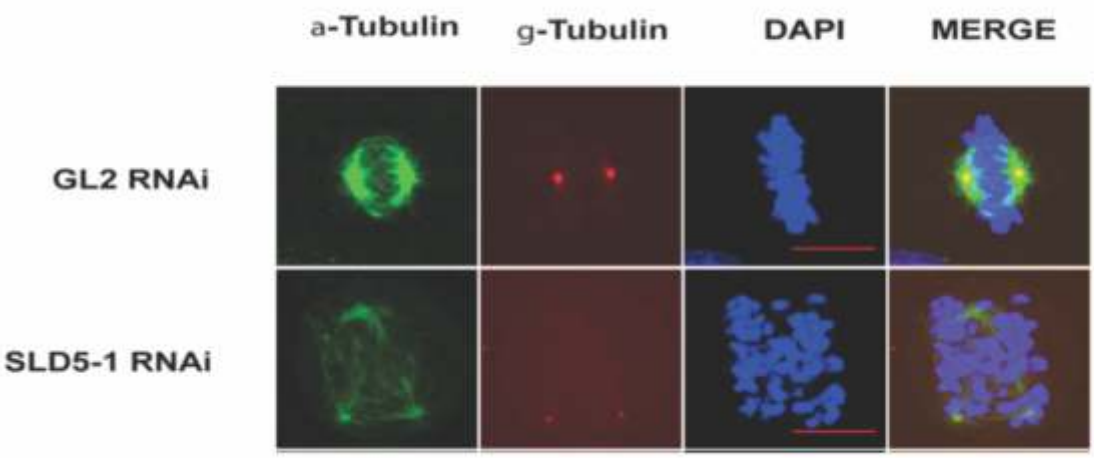

Fig. 2. Depletion of Sld5 at centrosomes leads to spindle pole defects. HeLa cells transfected with control GL2 or SLD5 siRNA were costained for α-tubulin (green) and γ-tubulin (red) and with DAPI for DNA (blue). GL2 siRNA-transfected cells displayed normal spindle pole formation; however, Sld5-depleted cells displayed asymmetric mitotic spindles, chromosome congression failure, and multipolar spindles and decreased γ-tubulin staining at centrosomes.

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The role of tumor suppressors in stress response

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The focus of the lab is to understand the function and regulation of tumor suppressors. Here, we report the work carried out on two such proteins viz. cyclin F and caspase 10. Cyclin F is the substrate recognition subunit of SCF (Skp1-Cul1-F-box protein) ubiquitin ligase complex. We plan to investigate the role of SCF ubiquitin ligase complex in regulating oncogenic pathways. Caspase10 belongs to cysteine-aspartate protease family of initiator caspases and comes under the category of an unexplored caspase in terms of substrate specificities. Due to the redundant nature of its role in apoptosis, the mechanism of caspase-10-mediated tumor suppression is poorly understood.

Previously, we had identified RBPJ (Recombination signal binding protein for immunoglobulin kappa J region) as a novel cyclin F-interacting protein. RBPJ is an effector of the Notch signalling pathway. Aberrant notch signalling has been implicated in several cancers. Thus, we decided to investigate effect of Cyclin F onnotch/RBPJ oncogenic functions. Our results indicate that cyclin F binds to RBPJ, but upon metabolic stress, the interaction was lost due to downregulation of RBPJ. Moreover, metabolic stress-induced cyclin F promotes polyubiquitylation of RBPJ, resulting in its proteasomal degradation. To determine the effect of RBPJ downregulation on cellular metabolic processes, we examined the transcript levels of genes involved in energy metabolism. We observed that *IDH1*(Isocitrate dehydrogenase 1) expression could be regulated by RBPJ. IDH1 has been observed to be mutated in various cancers. The mutant enzyme produces high levels of the metabolite 2-hydroxyglutarate (2-HG) which brings about epigenetic alterations that promote tumorigenesis. To explore the physiological significance of cyclin F-mediated regulation of $IDH1^{R132H}$we plan to investigate the effect of cyclin F on the malignant phenotype of $IDH1^{R132H}$ viz. invasiveness, migration potential and tumorigenicity.

We performed a proteomic screen to identify caspase 10 interacting proteins. The interaction screen revealed that caspase 10 interacts with many metabolic enzymes. We observed that ACLY (ATP citrate lyase) was the only enzyme cleaved under glucose starvation conditions. ACLY generates acetyl CoA which is critical for both lipogenesis and protein acetylation, including histones. To corroboratea direct role for caspase-10 in ACLY cleavage, we examined ACLY status upon metabolic stress in the presence of caspase-10 inhibitor. We observed that there was no cleavage of ACLY upon caspase-10 inhibitor treatment. Next, to identify the putative aspartate residue in ACLY which is targeted by caspase-10, we analyzed the protein sequence of ACLY *in silico* for caspase-10 consensus sites. Four aspartate residues (D79, D144, D318 and D1026) were predicted to be targeted by caspase-10. To identify which of these aspartate residues is targeted by caspase-10, we performed site-directed mutagenesis. Mutation of ACLY at D1026 abrogated the caspase-10-mediated cleavage. These results indicated that ACLY could be cleaved at D1026 upon metabolic stress. Altogether, our results demonstrate that ACLY is a caspase-10 substrate and

is cleaved at D1026 residue upon metabolic stress. We further observed a significant decline in acetyl-CoA levels under metabolic stress conditions at extended time points. However, we did not observe any significant change in acetyl-CoA levels in the absence of caspase-10 over the time course of metabolic stress. Taken together, these results indicated that caspase-10 modulates ACLY-mediated maintenance ofacetyl-CoA levels. Further the effect of caspase-10-mediated cleavage of ACLY on lipogenesis and global histone acetylation would be investigated.

Publication

Original peer-reviewed article

1. Deshmukh RS, Sharma S, Das S (2018) Cyclin F-dependent degradation of RBPJ inhibits $IDH1^{R132H}$-mediated tumorigenesis**. Cancer Res**. 78: 6386-6398.

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Role of non-coding RNA mediated gene regulation in human development and disease

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Protein synthesis has been known to be deregulated in a wide range of diseases including cancer and autoimmune disorders. tRNAs are short non-coding RNAs that are used as adapters for protein synthesis. Their function is to match the codons with cognate amino acids. In recent years, many genetic disorders have been identified to be associated with mutations that affect tRNA biogenesis pathways. Our aim is to understand the basic principles that connect tRNA modifications with physiology.

Major projects underway in our laboratory are:

A. Translation regulation in immune cells.

To systematically address the regulation of protein synthesis during immune cell activation, we analyzed the translatome (riboseq) of resting B and T cells and compared it with the transcriptome (RNAseq).

Among the 2355 genes we detected in riboseq, translation of 202 genes was significantly high in B cells while that of 160 genes was high in T cells. Gene set enrichment analysis (GSEA) of the ribosome profiling data had shown that, apart from T cell receptor and TCR signaling genes, the major sets that were enriched in T cells were those involved in cell-cell adhesion and the regulation of cell-cell adhesion. On the other hand, the predominant gene sets that were enriched in B cells included genes involved in antigen presentation, endocytosis, phagocytosis, clathrin-coated vesicles, endoplasmic reticulum, Golgi and unfolded protein response, all processes essential for antigen presentation by B cells upon activation.

A comparison of RNAseq and riboseq data in B and T cells had shown that in both cell types most of the transcripts show good correlation between RNAseq and riboseq reads, except for ribosomal protein coding genes and histone coding genes. This decrease in the translation efficiency for ribosomal protein coding genes could be due to decreased mTOR activity in resting cells.

B. Regulation of translation by pathogens.

Translation regulation by *Mycobacterium tuberculosis.*

To identify the extent of regulation of gene expression at the level of protein synthesis, we analyzed the translatome and transcriptome of *M. Tb* under oxidative stress for 1 hour. The very short time period was chosen because translation regulation is known to occur rapidly upon stress induction.

The analysis of riboseq results had shown that genes associated with specific pathways are upregulated in the translatome upon stress induction. The major pathways we found are the thioredoxin system, the

rubredoxin system, both of which are known to be required to alleviate the oxidative damage. Apart from these, we also find the mycolic acid biosynthesis pathway genes, DNA repair associated genes, cholesterol and fatty acid degradation associated genes are upregulated within an hour of oxidative stress.

While a large number of genes that are upregulated in translatome are upregulated in transcriptome as well, several genes upregulated in riboseq are not upregulated in the transcriptome, indicating a role for translation regulation. This data indicates that a combined transcriptional and translational regulation determines the early response of *M. Tb* response to oxidative stress.

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ANCILLARY RESEARCH

Production of transgenic and other animal models for biomedical research

Subeer S. Majumdar

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Theme of the research is to generate transgenic animals for using them as a system for the study of functional genomics and mammalian development and other animal models for use in biomedical research.

Objectives

i. To develop new easier techniques for making transgenic animals. To develop transgenic animal models using genes relevant to human health and diseases.
ii. To study biology of spermatogonial stem cells and to use germ cell transplantation technique for restoration of fertility following chemotherapy.
iii. To provide services of making various transgenic animals to various laboratories of the nation. To extend collaborative help in using specific animal models (transgenic or non-transgenic) for biomedical research.

A. Isolation, Culture, *in-vitro* Transfection of spermatogonial stem cells and germ cell transplantation for restoration of fertility following chemotherapy

Fresh and cryo preserved SSC are routinely being isolated and cultured in the lab in an attempt to improve and enhance the rate of SSC division *in-vitro* to obtain sufficient large population of SSC for autologous transplantation and colonization efficiency of stem cells. Since, SSC are very hard to transfect, attempts are being carried out to transfect and electroporate SSC *in-vitro* using various parameters and transfection techniques. Germ cell depleted mice are routinely being generated by direct testicular injection of busulfan for transplantation experiments.

B. Generation of various transgenic mice for other investigators

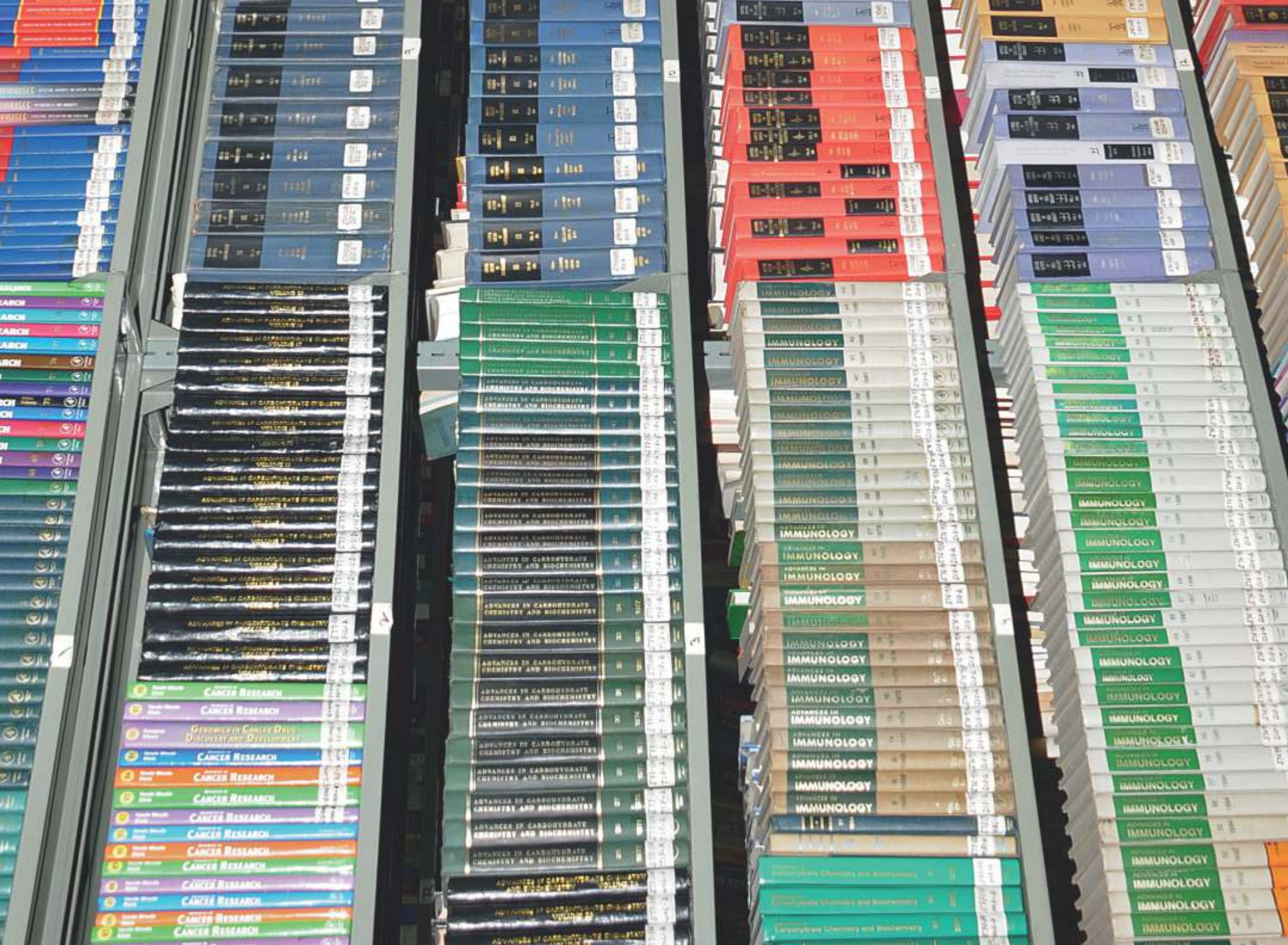
This transgenic service is routinely being provided by NII to various laboratories of the country. Collaborative work for making various transgenic animals for other investigators is undertaken as and when the constructs are provided. Fore-founder animals are given to P.I.'s for generating transgenic lines to address their respective scientific goals.

We have generated tissue specific transgenic mouse model expressing K8 wild type and phosphor dead mutants(K8S73A/K8S431A) for Dr. Milind Vaidya (ACTREC, Mumbai). All the three constructs used for generation of transgenic mice showed successful integration and protein level expression by techniques at molecular and genomic level, confirming a successful generation of tissue specific

transgenic mouse model. The transgenic mice have ascertained a positive impact of K8 phosphorylation on neoplastic transformation of skin-SCC.The manuscript for the same result has been communicated for publication.

We have generated transgenic mice for Dr. Deepak Modi (NIRRH, Mumbai) which carries HOXA10 shRNA.HOXA10 is transcription factor required in uterine biogenesis, embryo implantation and its levels are low in uteri of infertile women. To understand the mechanisms by which HOXA10 regulates uterine functions, a transgenic mice was developed that overexpressed an shRNA against HOXA10. The transgenic mice is being bred further for its colony expansion. Analysis and characterization work is still on.

We have also generated positive human BLM (hBLM) transgenic mice for Dr. Sagar Sengupta, NII, which will express Flag-tagged hBLM (Flag-hBLM) in the B-cell lineage. Mice are being analyzed and studied by P.I. for their functions.


ADVANCES IN CARBOHYDRATE CHEMISTRY AND BIOCHEMISTRY
ADVANCES IN CANCER RESEARCH
ADVANCES IN IMMUNOLOGY

PUBLICATIONS AND PATENTS

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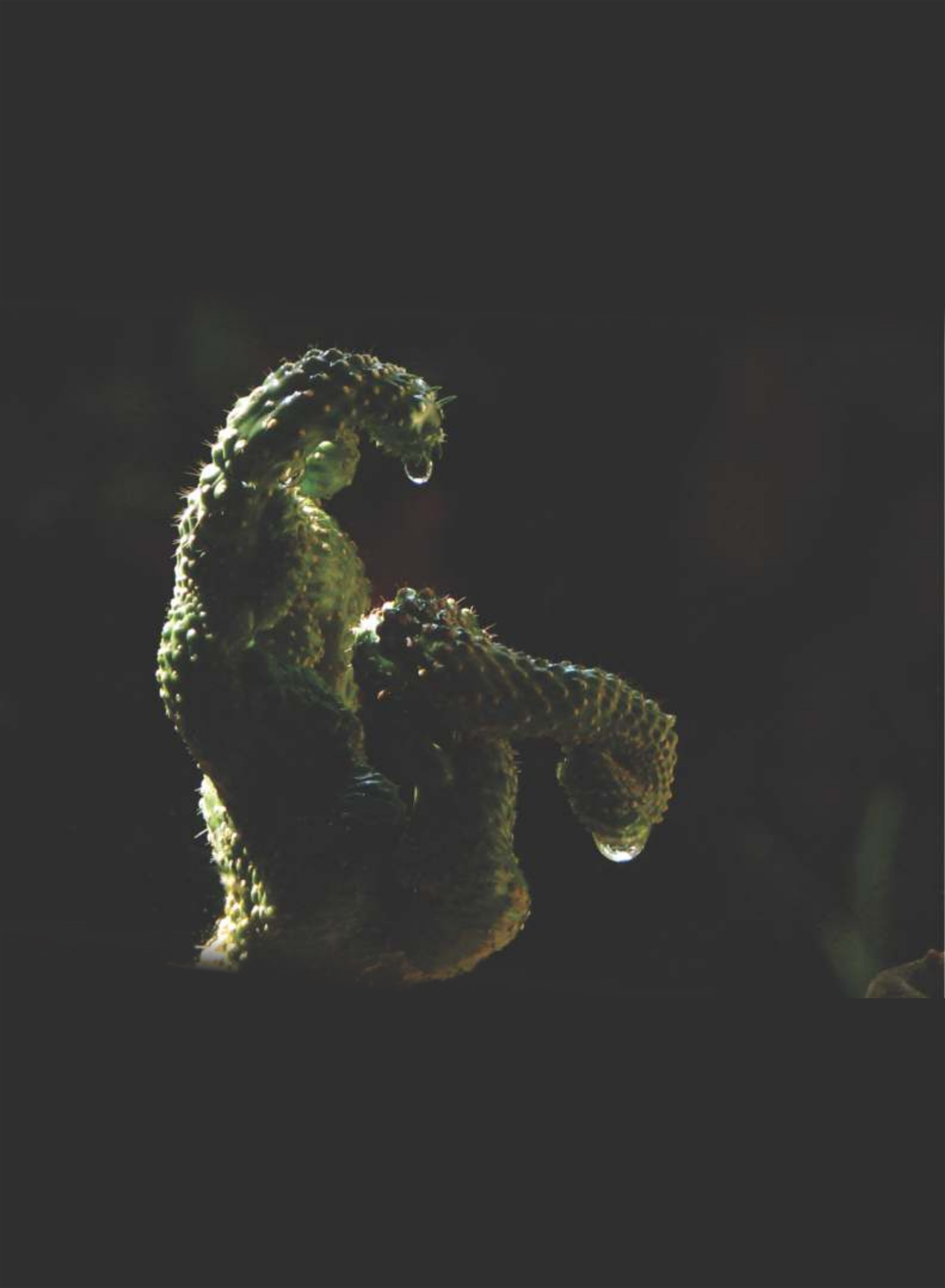


AWARDS AND DISTINCTIONS

Akhil C Banerjea was presented the Lifetime Achievement Award – For promoting HIV and AIDS research in India - at the International Conference "Combat-HIV 2019" at Hyderabad.

Bhavya Jha, a Ph.D student, was awarded the International Union of Crystallography, Young Scientist award in December 2018.

Chandrima Shaha received the Shanti Swarup Bhatnagar Medal of INSA (2019).

Nirmala Jagadish received the Labhsetwar Award (2019).

Sagar Sengupta was conferred the JC Bose Fellowship (2018).

Sanjeev Das was elected Fellow of the National Academy of Sciences, India (2018)

Soumen Basak received the National Bioscience Award for Career Development in recognition of his research in the area of immunology and cell signaling. He was also selected as a member of the Molecular Immunology Forum, India.

Subeer S Majumdar was conferred the JC Bose Fellowship (2018).

Vinay Nandicoori was elected Fellow of the Indian National Science Academy (2019).

Ph.D DEGREES AWARDED TO NII SCHOLARS

Annexure-III

Thirty two scholars of the Institute were awarded the degree of Doctor of Philosophy by Jawaharlal Nehru University on the completion of their work. The details are as follows:

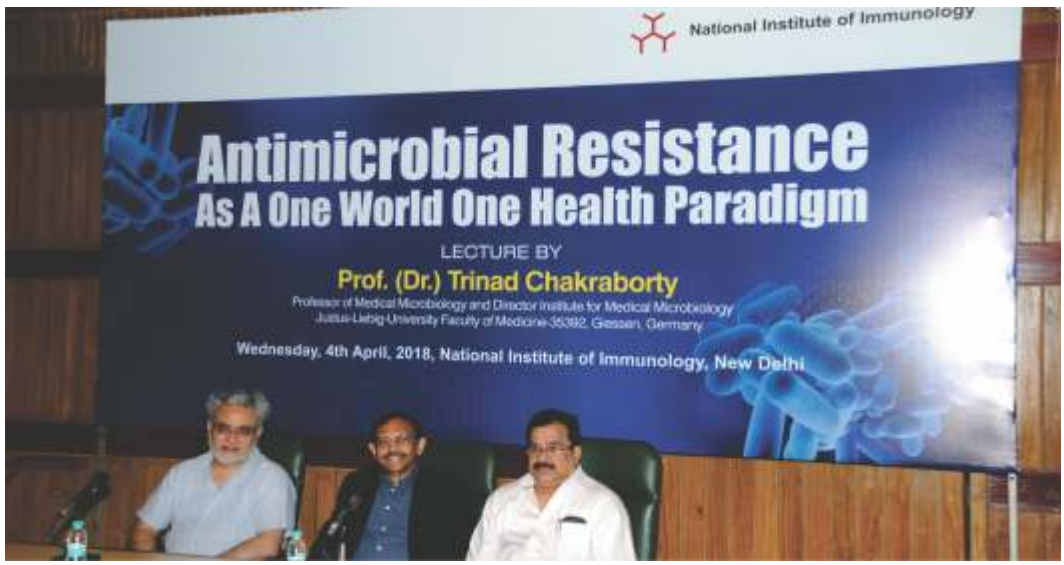
S. No.	Student's Name	Topic of Research	Guide
1	**Ms. Neetu Sain**	Multi-scale analysis of interaction networks involving peptide recognition modules	Dr. Debasisa Mohanty
2	**Ms. Rajni Kumari**	Understanding the role of caspase-10 in cellular metabolism	Dr. Sanjeev Das
3	**Mr. Vikash Kumar**	Studies on role of a-kinase anchor protein-4 (AKAP4) in ovarian carcinogenesis	Dr. Rahul Pal / Dr. Anil K. Suri
4	**Mr. Tapas Mukherjee**	Crosstalk between homeostatic LTβR signal and dynamical TNF signal in regulating NF-κB responses	Dr. Soumen Basak
5	**Ms. Ankita Malik**	Regulatory mechanisms associated with epidermal growth factor-mediated invasion of HTR-8/SV neo trophoblastic cells	Dr. Rahul Pal / Dr. Satish K. Gupta
6	**Mr. Jitender Yadav**	Understanding immune activities and their modulation during infection with pathogenic *Salmonella*	Dr. Ayub Qadri
7	**Mr. Ibrar Ahmed Siddique**	Mechanistic studies on novel anti-Alzheimer's compounds and the role of GTRAP3-18/JWA in autophagy	Dr. Sarika Gupta
8	**Mr. Ashish Kumar**	Sestrin2 as stress regulator: implications in autophagy and apoptosis	Dr. Apurba Kumar Sau / Dr. Chandrima Shaha
9	**Ms. Anshu Sharma**	Exploring the immunoprophylactic potential of mesenchymal stem cells for Type 1 diabetes	Dr. Kanwaljeet Kaur / Dr. Rajni Rani
10	**Ms. Preeti Jain**	Delineating FtsQ mediated regulation of cell division process in *Mycobacterium tuberculosis*	Dr. Vinay K. Nandicoori
11	**Ms. Sudeepa Rajan**	Understanding the structure-function relationships of interferon inducible human guanylate binding protein-1 and -2	Dr. Apurba K. Sau
12	**Mr. Abhisek Dwivedy**	Structural, functional and biochemical studies of histidinol dehydrogenase from *Mycobacterium tuberculosis*	Dr. Bichitra K. Biswal
13	**Ms. Neelam Oswal**	Identification and characterisation of innate and adaptive immune components in barrier associated pathological situations.	Dr. Anna George/ Dr. Vineeta Bal
14	**Ms. Ritu Shekhar**	MicroRNAs regulating cell proliferation during oncogenic transformations	Dr. Sandeep Saxena
15	**Ms. Sneh Lata Gupta**	Analysis of marginal zone B cell and B-1 B cell frequencies and function in inbred and mutant mice strains	Dr. Anna George

S. No.	Student's Name	Topic of Research	Guide
16	**Ms. Utpreksha Vaish**	Investigation of molecular mechanisms involved in vitiligo aetiopathogenesis	Dr. Sangeeta Bhaskar / Dr. Rajni Rani
17	**Ms. Hina Jhelum**	Identification and functional characterization of a nuclease from *Streptococcus pneumonia*	Dr. Devinder Sehgal
18	**Mr. Robin Kumar**	Immunogenicity of serotype independent particulate antigen formulation for pneumococcal disease	Dr. Amulya K. Panda
19	**Ms. Rohini Dwivedi**	Investigating the effect of sugars on antimicrobial peptides of cathelicidin family	Dr. Kanwaljeet Kaur
20	**Ms. Priyanka Bansal**	Role of signaling and trafficking pathways in the development of Apicomplexan parasites	Dr. Pushkar Sharma
21	**Ms. Preeti Sahay**	Development of acute liver failure (ALF) model in rats to evaluate the therapeutic potential of Neohep cells	Dr. P.K. Upadhyay
22	**Mr. Kamal Kumar**	Role of GRASP homologue in the secretory pathway of *Leishmania*	Dr. Amitabha Mukhopahyay
23	**Mr. Nikunj Harilal Raninga**	Human guanylate binding proteins: Revealing mechanism of GTP hydrolysis and structure-function relationship	Dr. Apurba K. Sau
24	**Ms. Prabhjot Kaur**	Investigating the mechanism of PknB mediated signaling in *Mycobacterium tuberculosis*	Dr. Vinay K. Nandicoori
25	**Ms. Sonia**	Role of caspase-1 in host-pathogen cross-talk during infection with *Salmonella*	Dr. Ayub Qadri
26	**Ms. Atika Dhar**	Studies of context-specific roles for pleiotropic signal transduction pathways in differentiation and function of cellular lineages in the immune system	Dr. Anna George / Dr. Satyajit Rath
27	**Ms. Prajna Tripathi**	Role of ClpB in stress management in *Mycobacterium tuberculosis*	Dr. Vinay K. Nandicoori / Dr. J.K. Batra
28	**Mr. Souvik Sen Sharma**	A study of Hippo signaling in testicular Sertoli cells	Dr. Rahul Pal / Dr. Subeer S. Majumdar
29	**Ms. Parna Kanodia**	Characterization and comparison of human immunocytome from adult siblings and unrelated healthy volunteers	Dr. Anna George / Dr.Vineeta Bal
30	**Ms. Ananya Gupta**	Analysis of anti-tuberculosis immune response induced in lungs by *Mycobacterium indicus pranii,* given via intranasal route and delineate the mechanism behind protection	Dr. Sangeeta Bhaskar
31	**Ms. Ruhi Sandeep Deshmukh**	Understanding the role of cyclin F in cancer metabolism	Dr. Sanjeev Das
32	**Ms. Roseleen Ekka**	Role of key protein kinases and signaling in development of malaria parasite	Dr. Pushkar Sharma

LECTURES AND SEMINARS

Public Lecture

Prof. Trinad Chakraborty, Professor of Medical Microbiology and Director, Institute for Medical Microbiology, Justus-Liebig-University, Faculty of Medicine Giessen, Germany delivered a lecture on "**Antimicrobial Resistance: As A One-World One-Health Paradigm**" on 4th April, 2018.



Dr. Rahul Pal (left) and Dr. Amulya K. Panda (right) with Prof. Trinad Chakraborty

Foundation Day Lecture

The 32nd Foundation Day of NII was celebrated on 3rd October, 2018. **Prof Purnendu Ghosh,** Honorary Professor and Executive Director of the Birla Institute of Scientific Research, Jaipur delivered a lecture on **"Engineering Of Life"**.

Dr. Amulya K. Panda (right) honoring Prof. Purnendu Ghosh after his lecture

Special Lecture

Prof. Rafi Ahmed, Emory Vaccine Centre, Atlanta, USA, was invited to deliver a lecture on "**T Cell Exhaustion And PD-1 Immunotherapy"** on 14th November, 2018.

Prof. Rafi Ahmed (left) planting a tree at the Institute along with Dr. Nimesh Gupta and Dr. Amulya K. Panda

Open Day

NII hosted an **"Open Day" as a pre-event of the 4th India International Science Festival (IISF)** which was held on 5th-8th October, 2018 at the Indira Gandhi Pratishthan, Lucknow. Laboratories visits, interactive lectures and poster presentations were organized.

Dr. Amulya K. Panda and Lt. Col. (Dr.) D.K. Vashist (Senior Manager) with students on the occasion of the Open Day

SEMINARS

S No.	Topic	Speaker	Date
1	RAD51 paralogs: Unravelling the new roles in genome stability and tumor suppression	**Dr. Ganesh Nagaraju** Dept. of Biochemistry Indian Institute of Science, Bangalore	11th April 2018
2	Making an impact: Publishing with BMC Biology and BMC	**Dr. Mirna Kvajo** BMC Biology Springer Nature 1, New York Plaza, New York, USA	21st May 2018
3	Tumor microenvironment regulates Mantle cell lymphoma survival	**Dr. Lalit Sehgal** Lymphoma/Myeloma Group UT MD Anderson Cancer Center Houston, USA	24th May 2018
4	Simulating biology stimulates hypotheses	**Dr. Chetan Gadgil** Chemical Engineering Division CSIR-National Chemical Laboratory, Pune	7th June 2018
5	Human cytotoxic CD4+ T lymphocytes and viral infections	**Dr. Veena S. Patil** Vaccine Discovery Division La Jolla Institute for Allergy and Immunology, La Jolla, USA	11th June 2018
6	Bioentrepreneurship: Opportunities and options	**Ms Shreya Malik** Biotech Consortium India Limited, New Delhi, & **Ms. Aditi Kumar** SIDBI Innovation and Incubation Center, IIT-Kanpur	23rd July 2018
7	New insights into DNA binding by NF-κB	**Dr. Gourisankar Ghosh** Department of Chemistry and Biochemistry University of California San Diego, USA	6th August 2018
8	Organisation of the Golgi apparatus determines it's glycan output	**Dr. S. Parashuraman** Institute of Protein Biochemistry National Research Council of Italy, Naples, Italy	28th August 2018
9	Drug discovery: NME translation from synthesis to clinical use drug	**Dr. V.P. Kamboj,** Chairman, BCIL New Delhi & Former Director CDRI, Lucknow	28th August 2018
10	Learning and memory in the eukaryotic cell: Evidence from computational modeling of the MAPK pathway	**Prof. Sitabhra Sinha** Institute of Mathematical Science Chennai	14th September 2018
11	A conserved apicomplexan signaling platform governs natural and induced egress in *Toxoplasma gondii*	**Prof. Dominique Soldati-Favre** Department of Microbiology and Molecular Medicine, University of Geneva, Switzerland	17th September 2018

S No.	Topic	Speaker	Date
12	Modulation of an autoimmune disorder by the activation of innate immunity	**Dr. Umesh S Deshmukh** Oklahoma Medical Research Foundation, Oklahoma City, USA	19th September, 2018
13	Immunological tolerance and regulatory T-cells	**Dr. Dipayan Rudra** Academy of Immunology and Microbiology (AIM)/ POSTECH Campus Institute for Basic Science (IBS) South Korea	31st October 2018
14	Poised epigenome	**Dr. Vinesh Vinayachandran** John Hopkins Bloomberg School of Public Health John Hopkins University Baltimore, USA	1st November 2018
15	Role of SNARE-mediated exocytosis and eutophagy in pathogenesis of pancreatitis	**Dr. Shubhankar Dolai** Department of Medicine University of Toronto	12th November 2018
16	Nanomedicine for autoimmune diseases and cancer	**Dr. Santi Swarup Singha,** Microbiology, Immunology & Infectious Diseases University of Calgary Calgary, Canada	13th November 2018
17	Understanding B cell memory-one cell at a time	**Prof. Michael McHeyzer-Williams** Department of Immunology and Microbiology, Scripps Research, California Campus, California, USA	20th November 2018
18	Molecular biomarkers of colorectal cancer: Translational opportunities	**Dr. Upender Manne** Department of Pathology University of Alabama at Birmingham Birmingham, USA	6th December 2018
19	Sestrins – the guardians of well-being	**Dr. Andrei Budanov** School of Biochemistry and Immunology Trinity Biomedical Science Institute, Trinity College, Dublin, Ireland	13th December 2018
20	The coming of age of *de novo* protein design	**Prof. David Baker** Department of Biochemistry University of Washington Molecular Engineering and Sciences	24th December 2018
21	Metabolic enzyme NNMT regulates the first critical PRC2 activity in embryonic lineage	**Prof. Hannele Ruohola-Baker** Institute for Stem Cell & Regenerative Medicine University of Washington Seattle, USA	24th December, 2018
22	Using Zika virus to target glioblastoma stem cells	**Dr. Milan G. Chheda** Department of Medicine Division of Oncology Washington University Medical School, St.Louis, USA	2nd January 2019

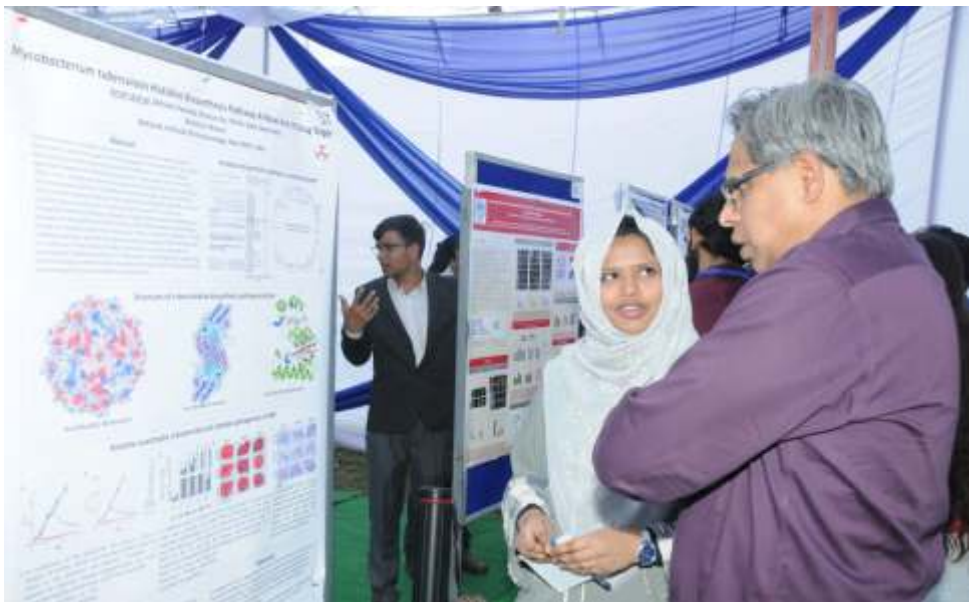
S No.	Topic	Speaker	Date
23	*M. tuberculosis* immune evasion strategies	**Dr. Jennifer Philips** Division of Infectious Diseases Washington University School of Medicine, St. Louis, USA	3rd January 2019
24	Data integration in cancer biology and why it is important to find clinically relevant signatures	**Dr. Binay Panda** Ganit Labs Foundation Bangalore	8th January 2019
25	Targeting BRCA2 deficiency with a WRN-specific helicase inhibitor	**Dr. Arindam Datta** Laboratory of Molecular Gerontology National Institute on Aging National Institutes of Health Bethesda, USA	9th January 2019
26	Molecular mechanisms that regulate gene expression and genomic stability	**Dr. Jayasha Shandilya** Department of Biological Sciences University at Buffalo Buffalo, USA	21st January 2019
27	DNA damage response intertwines with tissue homeostasis in *D. melanogaster*	**Prof. B.J. Rao** Indian Institute of Science Education and Research, Tirupati Andhra Pradesh	29th January 2019
28	Role of malaria parasite cell surface circumsporozoite protein (CSP) in enhancement of cellular flexibility and motility: Is it a lubricant?	**Dr. Rama Koti Ainavarapu** Department of Chemical Sciences TIFR, Mumbai Maharashtra	29th January 2019
29	Role of host molecular pathways in HIV-1 pathogenesis	**Dr. Sneh Lata** National Institute of Immunology New Delhi	1st February 2019
30	Bacterial protein translation regulation by BipA and LepA (EF4) under stress conditions	**Dr. Veerendra Kumar** Institute of Molecular and Cell Biology Singapore	4th February 2019
31	To cleave or not to cleave, that's not the question: Mechanisms of regulated proteolysis in *Streptococci*	**Prof. Indranil Biswas** Department of Microbiology, Molecular Genetics and Immunology University of Kansas Medical Centre Kansas City, KS, USA	4th February 2019
32	The molecular “kiss of death”, finding the enemy within: How host cells recognize and respond to a microbial pathogen hidden in a vacuole?	**Dr. Arun Kumar Haldar** Division of Biochemistry CSIR- Central Drug Research Institute, Lucknow, Uttar Pradesh	11th February 2019
33	Malaria parasite plasmepsins: Not just old degradative enzymes	**Prof. Daniel Goldberg** Washington University School of Medicine, St. Louis, USA	18th February 2019

S No.	Topic	Speaker	Date
34	Uncovering the functional cross-talk between host genetics and microbes in the pathogenesis of related inflammatory disorders	**Dr. Garima Juyal** JNU, New Delhi	19th February 2019
35	Chromosome replication proteins: At the crossroads of centriole duplication cycle and Rb-E2F pathway	**Dr. Manzar Hossain** Cold Spring Harbor Laboratory Cold Spring Harbor, USA	20th February 2019
36	Development of nanoparticle drug therapies against tuberculosis using Zebrafish as a screening system	**Professor Gareth Griffiths** Dept. of Biosciences University of Oslo, Blindern, Oslo, Norway	25th February 2019
37	Phosphorylation-induced conformational change regulates p19INK4d during the human cell cycle	**Dr. Amit Kumar Singh** Francis Crick Institute and King's College, London, UK	11th March 2019
38	Autoimmunity to extracellular DNA	**Dr. Boris Reizis** Department of Pathology, New York University School of Medicine, New York, USA	13th March 2019
39	Role of reduced protein translation in neurodegenerative conditions	**Dr. Somashish Ghosh Dastidar** Center for Neurodegeneration & Neurotherapeutics Duke University, USA	19th March 2019
40	Dissecting the immunopathogenesis of *Mycobacterium abscessus* infection in zebrafish embryos	**Prof. Kremer Laurent** IRIM Montpellier, France	20th March 2019
41	Stem cell-niche interactions regulate hematopoiesis and innate immune response in Drosophila	**Dr. Rohan J. Khadilkar** Life Sciences Institute, Faculty of Medicine, University of British Columbia, Vancouver, Canada	22nd March 2019

CONFERENCES/SYMPOSIA/WORKSHOPS

National Science Day

National Science Day was celebrated on 28th February, 2019. An NCR-Cluster Young Emerging Scientist Symposium was organized on the occasion. Ph.D. students and Research Scholars celebrated the day with great fervour. The day constitutes a major science festival at NII, during which young researchers present posters describing their research.



A Ph.D. scholar discussing her work on National Science Day

Science Setu

The "Science Setu" Programme was launched on 26th March, 2015, aimed to enhance connectivity between NII scientists and undergraduate students in the Delhi region. A "Memoranda of Understanding" (MoU) has been executed with 16 undergraduate colleges of Delhi University and with Manav Rachna International University, Faridabad. Students from these colleges are invited to undergo summer and winter internships. Each NII scientist is assigned a partner college, where she/he interacts with students and college Faculty; interations take the form of lectures, lab exercises or short-term projects. Students from partner colleges also visit NII laboratories to interact with Faculty in a lab environment. The following activities were carried out in the year 2018-19 under the auspices of this Program:

1. NII provided opportunities to 19 students for short-term training in different laboratories of the Institute.
2. The Institute signed an "MoU" with Deen Dayal Upadhyaya College (Delhi University).
3. Twenty five students of B.Sc (Hons) from Institute of Home Economics (Delhi University), visited different laboratories of the Institute on 5th March, 2019.

4th International Yoga Day

On the occasion of 4th International Yoga Day on 21st June, 2018, **Dr. Guru Deo**, Assistant Professor (Morarji Desai Institute of Yoga) delivered a lecture on "**Concept of Stress and Yoga**". **Mr. Rahul K. Srivastava** demonstrated various asanas; scientists, students, administrative staff participated in the one-hour long yoga exercises.

Dr. Amulya K. Panda and Ms. Chandresh Bhagatani (Administrative Officer, left), with Mr. Rahul K. Srivastava and Dr. Guru Deo at the inauguration of the workshop on Yoga.

Mr. Srivastava, apart from conducting the exercises, also spoke about the health benefits of each asana. The Instructors described how yoga can contribute to stress-free living.

Workshop On Self Defence

A workshop on **Self Defence** was organized on 27th November, 2018. Self-defence skills that can be applied when faced with situations of potential personal peril in today's world were demonstrated.

Demonstration of skills at the self defense workshop

Republic Day Celebrations

On the occasion of the **70th Republic Day**, NII organized a marathon and a football match on 26th January 2019 in the Institute. Enthusiastic participation characterized the event.

Staff participating in a marathon run organized on Republic Day

Indian Institute Of Technology (Delhi) - NII Memorandum Of Understanding

Indian Institute of Technology, Delhi and NII signed an MoU on 13th March, 2019 to develop technologies for quick and accurate diagnosis of life-threatening diseases.

Prof V. Ramgopal Rao (Director, IITD), Dr. Sunil Khare (IITD), Dr. Bodh Raj Mehta (IITD),
Dr. Gulshan Wadhwa (DBT) with Dr. Amulya K. Panda
Dr. Debasisa Mohanty and Lt. Col. (Dr.) D.K. Vashist.

Farewell To Ph.d Students

A function was held to bid farewell to Ph.D students of 2013 Batch. The event was marked by the planting of a tree.

Ph.D. students of the 2013 Batch planting a tree alongside some scientists


NEC
NEC

Small Animal Facility

Primate Research Centre

INFRASTRUCTURE

Equipment

While most of the routine equipment is available in various laboratories of NII, some high-end instruments facilities are shared by various research groups and their collaborators. The equipment in these facilities includes: Mass Spectrometer, NMR Spectrometer, Confocal Microscopes, Atomic Force Microscope, Scanning and Transmission Electron Microscopes, High Throughput DNA, sequencer flow cytometers, Dual wavelength X-ray Generator, X-Ray Crystallography, Flow Cytometers, Whole Body Imager, CD Spectrosc0py, Surface Plasmon Resonance System and Amino Acid Sequencer.

BSL-III Facility

There are three Biosafety Level III facilities at NII, one each for handling *Mycobacterium tuberculosis, Streptococcus pneumonia* and HIV.

Small Animal Facility

The Small Animal Facility (SAF) of the Institute is devoted to ensure the humane care and breeding of experimental animals used in research and to provide defined strains of mouse and rats to the scientific community of the institute. At present, the SAF holds 104 mouse strains - 87 mutant strains, 16 inbred strains, 1 out bred strain. In addition, 6 rat strains 1 stock each of rabbits and guinea pigs are also housed.

The propagation of all defined strains is done on a three - tiered system i.e., the Foundation Stock (FS), Pedigreed Expansion Stock (PES) and Production Stock (PS). Genetically-modified mouse strains are bred using either of three strategies:

1. Homozygous mutants (-/-) x Homozygous mutants (-/-)
2. Heterozygous mutants (-/+) x Homozygous mutants (-/-)
3. Heterozygous mutants (-/+) x Heterozygous mutants (-/+)

Defined breeding protocols as well as careful management and husbandry procedures are followed to ensure the purity of each strain of mice. To maximize genetic purity and uniformity of mice, inbred strains are propagated and replaced periodically in such a manner that minimizes the genetic drift and inbreeding depression. Random samples from a few breeders of Foundation, Expansion and Production stock are monitored with the help of a few microsatellite markers to ensure genetic purity. The facility also gets support from various principal investigators in the genotyping of transgenic and knockout mice strains to confirm the genetic purity based on presence or absence of specific genes of interest.

The health monitoring program includes regular screening (using ELISA and PCR) of pathogens including, Mouse Hepatitis virus, Mouse Parvovirus, Mouse Norovirus Pnemonia virus, Mycoplasma and Sendai virus. Bacterial pathogens such as *Pseudomonas aeroginosa, Streptobacillus moniliformis, Bordetella, Bronchiseptica, Citrobacter rodentitium, Pasteurella pneumotropica, Staphylococci* and *E. coli* are screened using culture, as well as by biochemical methods and PCR. Random faecal samples are screened for the presence of endoparasites by the sedimentation method for the presence of *syphacia* and *aspicularis* species. Also, periodic FACS analyses are also carried on immunodeficient mice to assess leakiness.

Health quality procedures are implemented to prevent the transmission of infection between cages; these include careful handling of animals, washing of cages and bottles using an automated washer, autoclaving of cages, use of sterilized corn cob bedding, and use of acidified autoclaved drinking water. The breeding and experimental colonies are maintained in a barrier system using individual ventilated cages of international standards. Necessary action, based on clinical signs, is taken by the veterinarian concerning the necropsy/autopsy of the infected animals. Preventive and recommended schedule of medication is strictly followed to prevent the infection.

Primate Research Centre

The National Institute of Immunology has a separate Primate Research Centre (PRC). Macaques are bred and maintained for generation of in-house animals of known age for approved basic, pre-clinical and toxicological research.

Under the breeding program, group mating is carried out in large open pens under semi-natural conditions, where food and water is provided *ad libitum*. Infants are weaned at six to twelve months (depending on the season), after which they are transferred to open enclosures/semi-natural housing for over-all growth and better development of bones, muscles and coordination. Monkeys are housed in independent cages around puberty. To prevent cross-cage contamination or infection, strict hygienic procedures are adopted as routine practice. Regular health monitoring program for primates to detect Tuberculosis, Simian herpes and Simian hepatitis virus are carried out using ELISA and PPD. Animals are inspected daily and sick animals are isolated and treated after necessary investigations. Primates are fed with recommended standardised pellet feed. In addition, bread, soaked Bengal gram, vegetables and/or fruits are also given daily. Regular supplementations with Vitamins and Calcium are administered. The staff at PRC undergo preventative health check-ups yearly. Surgeries, medication and treatment are performed/administered by a registered veterinarian. Technical staff have the required expertise for surgery, immunization, bleeding, biopsy, electro-ejaculation and fertility studies. A research laboratory is situated at the Centre to cater to the need of researchers.

Clearance of the research proposals by CPCSEA (after primary clearance from the Institutional Animal Ethics Committee) is necessary for conducting research on primates at this Centre. Macaques have been used in research related to infectious diseases, reproduction, endocrinology, immunology and contraception. Staff at the PRC ensure that all procedures are pain-free and involve minimum stress to the animal. Experimental animals are provided with special feed, whenever needed. A constant effort is made to keep the animals in a comfortable and stress free environment, as per the available guidelines. There are seventeen open enclosures with swings and shelters, which are used for rehabilitation, and to enable socializing. Weather permitting, attempts are made to monkeys in groups in open enclosures.

Director's Secretariat

Senior Manager's Office

Administration Department

Finance & Accounts Department

Stores & Purchase Department

Engineering Department

Library and Documentation Services

Academic Cell

Instrument Workshop Department

Computer Centre

Central Instrumentation Facility

SUPPORTING UNITS

Establishment, Personnel And General Administration Services

The Division continued to provide key support services. Its aim is to ensure optimal utilization of human and administrative resources. Effective administrative support aided in effective policy implementation. Handling service matters and staff recruitment, promoting career development, arranging foreign visit of scientists for training/ conferences/bilateral exchange visits, coordinating staff welfare activities and post-retirement dispensation, preparing and submitting periodic reports to the Ministry, and preparing responses to parliament questions from some of the Division's other critical functions. To bolster the capabilities and enhance productivity, the Institute conducts periodical training for its Administrative and Technical Staff; in-house training, imparted by experienced professionals, as well as sponsorship of Staff to recognized training Institutes, are undertaken.

The Administration organized a Blood Donation Camp

Financial And Accounting Services

The Division has been responsible for preparation of the annual budget, management of the utilization of funds, receipt and disbursement of payments, internal auditing, getting accounts audited by statutory and CAG auditors, sending reports to funding agencies, recovery and remittance of TDS from salary and contractors, filling institutional income tax return, obtaining required exemptions of the Income Tax Department, maintaining bank accounts, management of trust for CPF, Gratuity Fund, and recovery and remittance of subscriptions of NPS.

Stores And Purchase Department

The Stores and Purchase Department deals with procurement of chemicals, consumables, glassware, plastic ware, equipment, and other essential research items from across the globe. Essentially, the Department acts as a critical lifeline, permitting research to proceed unhindered. Purchase Committees (comprising three or more Scientists, the Finance and Accounts Officer, the Stores and Purchase Officer and occasionally an external expert who possess specialized knowledge about a particular item) assist the Department in its task, helping to evaluate products in terms of quality and price.

Maintenance of records of each activity, tracking movement of important import documents, following-up on orders, issue of purchase orders, ensuring that procurements are as per the covenants of tender conditions, constitute a major portion of the Department's activities. Close rapport with the other Departments ensures that bottlenecks are anticipated problems quickly mitigated.

Engineering, Maintenance And Instrumentation Services

The Engineering Department has been entrusted with engineering activities, including maintenance, services and capital works. It endeavours to provide efficient services using modern technologies. The major activities undertaken during the reporting year are listed here.

I) Installation, testing and commissioning of rooftop grid-sharing system for solar electricity. ii) Setting up of new laboratories and offices. iii) Installation of LED lighting fixtures and retrofitting of LED lamps in existing fixtures. iv) Servicing/repair of DG sets. v) Installation, testing and commissioning of CCTV cameras. vi) Servicing/repair of air circuit breakers of the maintain LT supply system. vii) Replacement of AHU cooling coils. viii) Creation of covered parking for official vehicles. ix) SITC of air washer. x) Replacement of PVC fills including repair works for cooling towers. xi) SITC/replacement of SAC at various laboratories. xii) Reactivation of drinking water treatment system. xiii) Reactivation of sewage treatment plant filters. xiv) Repair work at the Experimental Animal Facility. xv) Installation of floodlights on the playground.

The Department is currently working on the following projects:

I) Setting up of new laboratories and offices. ii) Installation of LED lighting fixtures and retrofitting of LED lamps in existing fixtures. iii) Refurbishment of APU with allied work at the Experimental Animal Facility. iv) Installation of rain harvesting system. v) Replacement of AHU cooling coils. iv) SITC of 2 MVA transformer and associated work. v) Up-gradation work in SAF and wild mice facility. vi) Up-gradation of kitchens at staff quarters at Dwarka. vii) Repairing of existing internal roads. viii) Miscellaneous civil work in staff quarters. ix) Creation of porta cabin for security personnel. x) Plastering and weather proofing of exterior painting on terraces on the main building. xii) Fabrication of working table top with multi-level shelves for various labs. xiii) Miscellaneous work at the Central Instrument Facility. xiv) Cleaning of sewer line and inspection of manholes. xv) Fixation of stainless steel 3D signage on the main building and residential blocks. xvi) SITC of roof top grid connected system for solar electricity. xvii) SITC of air washer for SAF. xviii) Replacement of defective relays. xix) Servicing/ repairing of DG sets.

Library And Documentation Services

The Library and Documentation Department is a service-oriented supportive unit working as a Knowledge Management Centre. It provides information support to the scientific staff of the Institute using both archival and contemporary digital resources.

The Library has a rich collection of books and journals. NII is the member Institute of the DeLCON Consortium Project of Department of Biotechnology. The Library carries out the purchasing of print journals and books, and is responsible for the subscription to online journals and books. It also helps process publication charges once an article has been accepted for publication. All housekeeping activities are computerized and are updated regularly using the Web-Online Public Access Catalogue (Web-OPAC) database.

The Library is involved with compiling, designing and printing the Parliamentary and Scientific Annual Reports of the Institute in Hindi and English. Every month, the Library prepares pictorial research publications and bibliometrics reports. The Library webpage is continuously updated to include information on recent subscriptions, procurement and publications.

The Library has a searchable Institutional Digital Repository. It contains full texts of research publications of the Institute from the year 2008. The Library conducts an induction programme for new-comers every year, which includes workshops on plagiarism and the use of Turnitin software, and the use of Scopus.

Institutional book binding and photocopying work is carried out at the Library. A Hindi Library with a sizable collection of books and magazines has been set up to popularize the official language.

The Library helped organize the Foundation Day Celebrations and the National Science Day Lecture.

7 Reference Books have been added to the Library, and 92 Hindi Books have been procured. A total of 28 Journals, 8 Current Protocols and 11 Annual Reviews were subscribed too.

The Library has taken an initiative to set up Twitter, Blog, and Facebook accounts of the Institute. Updates of notable events are regularly uploaded on these accounts.

Academic And Training Department

The activities of the Academic and Training Department encompass three major spheres: Students affairs, training for external candidates, and in-House training. The Academic Department has been involved in the conduct of Ph.D. Admissions, Pre-Ph.D. Registration Courses, Doctoral Committee meetings, Academic Committee meetings, and disbursement of Fellowship to scholars. Scientists qualified to receive the following Fellowships can apply to work under Faculty members on projects of mutual interest: Indian Institute of Science Bangalore (DBT-RA), ICMR (SRF/RA), DST-SERB (NPDF) DST-Inspire Faculty, DST (WOS), CSIR (SRA/RA), DHR-Young Scientist, Ramalingaswami entry Fellowship. The Institute also imparts six month training to post-graduate students sponsored by the Indian Academy of Science Bangalore. Under-graduate students from different colleges also visit the Institute under the Science Setu programme. The Department also arranges training courses for scientific, technical and administrative officials of the Institute.

Vigilance Cell

The Institute has a Vigilance Cell headed by a Scientist nominated as part-time Chief Vigilance Officer (CVO) by the Central Vigilance Commission (CVC). The CVO and the support staff perform activities related to vigilance as adjunct duties in addition to their primary responsibilities. The Cell has followed various instructions issued by the CVC from time to time to ensure effective implement-ation of measures for strengthening vigilance and anti-corruption work. Emphasis is laid on preventive vigilance since such vigilance, if properly conceived and executed, aids in plugging weak and vulnerable areas. The National Institute of Immunology has been reviewing existing procedures to identify corruption-prone areas, making policies more transparent to avoid ambiguity and streamlining procedures to achieve a working environment free of corruption. Staff members employed in areas prone to corruption is rotated periodically. Sizeable purchases of chemicals, consumables and instruments are handled through various Purchase Committees, thus eliminating the possibility of occurrences detrimental to quality and price of purchases. Such institutional committees are periodically reconstituted. The Cell has been rendering periodical reports on vigilance activities to the administrative machinery and CVC.

"Vigilance Awareness Week" was observed in the Institute from October 29, 2018 to November 03, 2018. A banner announcing the observance of the Vigilance Awareness Week was put up at the entrance of the Institute. Placards bearing slogans against corruption were displayed in the Institute. A pledge to fight corruption was taken by the NII community on October 29, 2018. As essay writing competition was organized on October 31, 2018 on the theme "Eradicate Corruption – Build a New India". Shri T P. Sharma, Under Secretary at the CVC, delivered a lecture on November 01, 2018 on Vigilance Awareness.

Computer Centre

The Computer Centre has been providing informa-tion technology-related support, which involves managing switches and Wi-Fi controllers in a 850 node LAN, system administration of multiple LINUX based E-mail and Web servers, backup services for mail/web servers, management of UTM devices for network security and integration of internet bandwidth from multiple ISPs. The Computer Center staff facilitates day-to-day troubleshooting, maintenance and anti-virus support of about 830 PCs and other peripheral devices. In addition, the Computer Center also provides specialized services like management of HPC clusters, management of floating licenses for access to bioinformatics software over LAN, IT support for developing in-house software for Pay Roll, and maintenance of the employee database.

NOTABLE ACTIVITIES

Academic courses, training programmes and interaction with other academic institutes

The Institute imparts long-term residential training leading to a Ph.D. Degree of the Jawaharlal Nehru University, New Delhi. Every year, 30-35 scholars are admitted to this Programme on a competitive basis, following a written examination and interviews; entrants are chosen from amongst a large number of applicants from all over the country.

The Ph.D. Programme was launched in the Academic Year 1986-87. In the year 2018-19, a total number of students admitted by the Institute was 22. So far, a total of 446 students have been awarded a Ph.D. degree, including 32 in Academic Year 2018-19.

In addition, the Institute hosts Summer Research Fellows, drawn from various Universities/ Institutions, providing facilities and mentorship. The Institute also accepts students who wish to carry out research projects in fulfilment of the requirements of a Masters programme.

Publications

Seventy four research papers were published this year by scientists and scholars; sixty four were peer-reviewed research papers, while ten were reviews/proceedings. The complete details of these papers are listed in Annexure-I.

Patents and Technology Transfer

The Institute has a policy of protecting the intellectual property rights on inventions made in its laboratories. Early research leads are evaluated for commercial viability and patentability. The Institute files applications first in India and when necessary, at patent offices in other countries. During the year under report, nine patents have been filed and two granted.

Lectures/seminars by visiting scientists/ guest investigators

The Institute continued to receive visiting scientists and guest investigators from all over the world; forty one scientific seminars were organized. These seminars saw enthusiastic participation from scholars and scientists from other Institutions as well. The complete details of these seminars are listed in Annexure-II.

Anti-Terrorism day

"Anti-Terrorism Day" was observed by all employees on 21st May, 2018. at 11:00 AM. The following pledge was undertaken: "We, the people of India, having abiding faith in our country's tradition of non-violence and tolerance, hereby solemnly affirm to oppose with our strength, all forms of terrorism and violence. We pledge to uphold and promote peace, social harmony and understanding among all fellow human beings and fight the forces of disruption threatening human lives and values".

Sadhbhavna Diwas

With the an aim to promote national integration and communal harmony among peoples of all religions, languages and regions, "Sadhbhavna Diwas" was observed on the birth anniversary of late Shri Rajiv Gandhi on 18th August, 2018. The following pledge was undertaken: "I take this solemn pledge that I will work for the emotional oneness and harmony of all the people of India regardless of caste, region, religion or language. I further pledge that I shall resolve all differences among us through dialogue and constitutional means without resorting to violence".

Martyrs' Day

"Martyrs' Day" was observed on 30th January, 2019 in the memory of those who gave their lives in the struggle for India's freedom. A two-minute silence was observed at 11.00 AM.

Rashtriya Ekta Diwas (National Unity Day)

"Rashtriya Ekta Diwas" was observed on the birth anniversary of Late Sh. Sardar Vallabhbhai Patel on 31st October, 2018. At 11:00 AM, the following pledge was undertaken: "I solemnly pledge that I dedicate myself to preserve the unity, integrity and security of the nation and also strive hard to spread this message among my fellow countrymen. I take this pledge in the spirit of unification of my country which was made possible by the vision and actions of Sardar Vallabhbhai Patel. I also solemnly resolve to make my own contribution to ensure internal security of my country."

Independence day

Independence Day was celebrated on 15th August, 2018. The event was marked by a message from the Director, followed by singing of the National Anthem by the students and children of the Staff of the Institute.

Representations of scheduled castes, scheduled tribes, other backward classes and economically weaker sections

The Institute complies with reservation orders as per directives of Government of India, while making appointments, to ensure representation of scheduled castes, scheduled tribes, other backward classes and economically weaker sections (EWS) as per the prescribed percentage.

Representation of persons with benchmark disabilities

The Institute follows reservation orders for Persons with benchmark disabilities as per Government of India directives issued from time to time to ensure representation of persons with benchmark disabilities as per the prescribed percentage.

Implementation of Official Language Policy

The official Language policy of the Government of India is followed by the Institute in letter and spirit:

1. To promote the use of Hindi as a language in official work, a Hindi Pakhwara (Hindi Fortnight) was celebrated at the Institute with great zeal from 1st-14th Sept, 2018. Various competitions, such as Hindi Sulekh (Hindi Writing), Hindi Shrutlek (Hindi Dictation), Hindi Samanya Gyaan (General Knowledge Competition), Hindi Vaad-Vivad (Hindi Debate), Hindi Nibandh (Hindi Essay), and Hindi Kavita Pathan (Hindi Poetry Recitation) were organized. A large number of Faculty Members, Staff Members, and Students participated. Hindi Diwas (Hindi Day) was celebrated on 14th Sept, 2018 at the culmination of the Hindi Pakhwara.

2. In furtherance of efforts to increase the use of Hindi, the Institute organized quarterly Hindi workshops/ lectures during the year.

3. The Institute has implemented the Government of India incentive scheme for originally writing notes and drafts in Hindi. An incentive scheme for encouraging and creating interest amongst Scientific and Technical Staff Members of NII for writing articles and research papers in Hindi was also implemented.

4. The Institute has published the 2st edition of in-house Hindi magazine “JAIPRATIRAKSHA DARPAN” in December 2018; the 3nd edition would be published shortly.

5. The Institute achieved the 95% target for implementation of Hindi in official work. Sincere effort is on-going to achieve the 100% target outlined by the Department of Official Language, Ministry of Home Affairs, Government of India.

"Hindi Kavi Sammelan” on the occaision of Hindi Diwas

RTI ANNUAL RETURN INFORMATION SYSTEM (2018-2019)
ANNUAL RETURN FORM

Ministry /Department /Organization: Department of Bio-Technology (National Institute of Immunology), New Delhi-110067

Year 2018-2019 (upto March 2019)
Insert Mode (New Return)

		Progress in 2018-19			
	Opening Balance as on 01/04/2016	**Received during the year (including cases transferred to other Public Authority)**	**No. of cases transferred to other Public Authority**	**Decisions where request/appeals rejects/appeals rejected**	**Decision where requests/appeals accepted**
Request	274	35	0	0	35
First Appeal	1	0	0	0	0

No. of Cases where disciplinary action taken against any Officer	0

No. of CAPIOs designated	**No. of CPIO designated**	**No. of AAs designated**
	1	1

No. of times various provisions were invoked while rejecting request													
Relevant section of RTI Act 2005													
Section 8 (1)										Sections			
a	b	c	d	e	f	g	h	i	j	9	11	24	Others
0	0	0	0	0	0	0	0	0	0	0	0	0	0

Amount of Charges Collected (in Rs.)		
Registration Fee Amount	**Additional Fee & Any other charges**	**Penalties Amount**
Rs. 250	984	0

Last date of Uploading the Pro-active Disclosures on the website of PA	(Format 08/08 /2019)

Name of the person who is entering/updating data	Dr. Sarika Gupta

RTI ANNUAL RETURN INFORMATION SYSTEM (2018-2019)

NATIONAL INSTITUTE OF IMMUNOLOGY
NEW DELHI

Report on Monthly Disposal of Cases
2018-2019

Year	Month	Opening Balance	Receipt	Disposal	Closing Balance	Cumulative Disposal
2018	April	274	2	0	276	273
2018	May	276	3	3	279	276
2018	June	279	1	2	280	278
2018	July	280	6	1	286	279
2018	August	286	4	5	290	284
2018	September	290	2	4	292	288
2018	October	292	0	2	292	290
2018	November	292	3	1	295	291
2018	December	295	1	3	296	294
2019	January	296	2	0	298	294
2019	February	298	4	5	302	299
2019	March	302	2	4	303	302

ORGANIZATION

NII Society

Prof. G. Padmanaban
President, NII Society
INSA Senior Scientist & Senior Science
Innovation Advisor BIRAC
Department of Biochemistry
Indian Institute of Science
Bangalore

Dr. Renu Swarup
Chairperson, GB, NII &
Secretary, Department of Biotechnology
Ministry of Science & Technology
New Delhi

Sh. B. Anand
Additional Secretary & Financial Adviser
Ministry of Science & Technology
Department of Biotechnology
New Delhi

Sh. Chandra Prakash Goyal
Joint Secretary (Admin)
Department of Biotechnology
Ministry of Science & Technology
New Delhi

Dr. Suchita Ninawe
Adviser/Scientist-'G',
Department of Biotechnology
Ministry of Science & Technology, New Delhi

Dr. S. Venkatesh
Director General
Directorate General of Health Services
Ministry of Health & Family Welfare, New Delhi

Prof. Balram Bhargava
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Director General, Indian Council of Medical Research, New Delhi

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Prof. Pierre - André Cazenave inaugurating the Wild Animal Facility in the presence of Prof. G.P. Talwar (Founder Director) on October 9, 1991.

NII Collaborations

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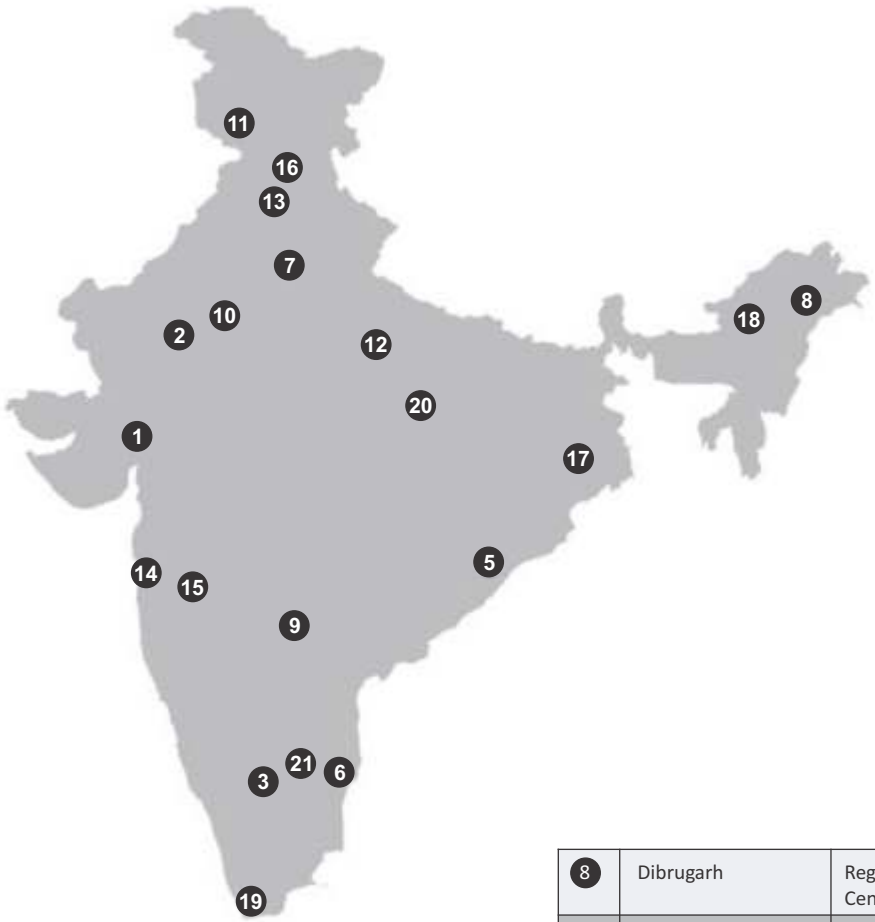

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4	Bhopal	NIREH, IISER (Bhopal)
5	Bhubaneswar	ILS
6	Chennai	Cancer Institute (WIA), TRPVB
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7	NIH Bethesda
8	Northeastern University

9	Imperial College
10	Universite De Rennes
11	University of Geneva
12	University of Pretoria
13	Kumamoto University
14	WEHI
15	University of Queensland

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