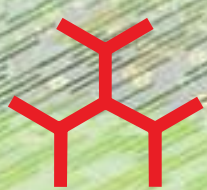
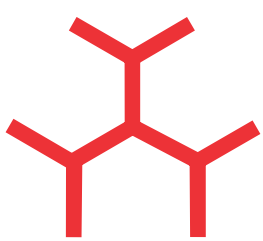
NATIONAL INSTITUTE OF IMMUNOLOGY



ANNUAL REPORT
2019-20

NATIONAL INSTITUTE OF IMMUNOLOGY



ANNUAL REPORT
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MANDATE OF THE INSTITUTE

- To undertake, aid, promote, guide and co-ordinate research of high calibre 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 has been an interesting year, full of exciting discovery. As always, it is a pleasure and privilege to present the Annual Report of the Institute. I am fortunate to be associated with a bunch of energetic and capable colleagues; their commitment to the ideals of scientific rigor and excellence is what drives the Institute forward. Our students are the best and brightest; their dynamism and passion is a critical ingredient in our intellectual pursuits, and of campus life.

While our interests are broad, and encompass large areas of the biological sciences, we lay special emphasis on immunology; we believe this institutional characteristic, carefully and intentionally nurtured by preceding Directors over the years, bestows upon us unique, multi-dimensional strengths. Needless to say, it is a characteristic we feel proud of.

While on-going programmes have kept us busy, the SARS-CoV-2 pandemic has thrown up sudden challenges and unique opportunities. Benefitting from our inherent institutional strength and expertise, several of us have embarked upon the design and evaluation of a protein-based vaccine; initiatives in drug discovery are also underway.

As our understanding of immunology broadens, we increasingly imbibe the tools and techniques of physical and engineering science. In order to fully exploit the potential of such cross-fertilization, it is our endeavour to also adopt the methods of precision analysis and quantitative determination that characterize those disciplines. As we enter into the realm of ImmunoEngineering, these skills will help us achieve our societal goals.

Here is a brief compendium of our research pursuits over the course of the year.

Biophysics and Molecular Design

Computational approaches for biomolecular structure and substrate specificity are being developed, and exploration of their applicability in the identification of novel biosynthetic pathways and regulatory networks continues. Ribosomally-synthesized, post-translationally modified peptides (RiPPs) constitute a large class of natural bacterial products of diverse structure and bioactivity. RiPPMiner, developed earlier, used a machine learning approach to predict RiPP class, leader cleavage sites and cross-linked chemical structures of RiPPs, utilizing only the sequence of the precursor peptide as input. RiPPMiner-Genome, a significantly updated version of RiPPMiner, takes genomic sequences as input and identifies RiPP biosynthetic gene clusters and modifying enzymes, significantly enhancing the accuracy of RiPP chemical structure prediction.

The process of developing tools to understand the structure and function of glycoconjugates in living systems continues. Earlier studies demonstrated the ability of peracetyl *N*-thioglycolyl-D-galactosamine (Ac_5GalNTGc) to inhibit mucin type *O*-glycosylation (MTOG) in Jurkat, K562 and U937 cells. Significantly, Ac_5GalNTGc was shown to inhibit MTOG in murine primary T-cells as well. Inhibition of MTOG on CD43 affected its exclusion from the immunological synapse; consequential effects on immune activation or suppression are being explored.

Investigations into the structure-function relationship of glycolytic enzymes in *Mycobacterium tuberculosis* are underway. Atomic level details of enolase (an essential enzyme for mycobacterial growth and virulence) were deciphered, both in native and substrate-bound form. Structural and biochemical studies are providing insights into mechanism of action; emerging data may assist in the design of specific inhibitors.

The methods of structural biology are being employed to study the behaviour of *Leishmania* phosphoglycerate kinase isoforms. The relevance of information theory in the study of protein folding is being explored. The understanding of the ligand interactions of proteins involved in the fatty acid metabolism of *Leishmania* has advanced. AutoDock was employed to screen NCI small molecule libraries; two inhibitors, effective against promastigotes, axenic amastigotes, as well as intracellular amastigotes, were identified.

Work into understanding the mechanisms of GTP catalysis by GTPases have continued. Along with structural data, these analyses could provide insights into drug design. Chimeric constructs of hGBP-1 and hGBP-2 have helped discern why hGBP-1 induces a relatively lower amount of GMP upon GTP catalysis. Data indicates that, compared with hGBP-1, reduce levels of GMP may be a result of differences in the readjustment of the active site after the first phosphate cleavage of GTP.

Transpeptidase sortases are being investigated for their utility in mediating peptide ligation for the semi-synthesis of proteins. Sortase-mediated semi-synthesis of histones, carrying site-specific acetylation of Lys-5 and Lys-4 in H2B (H2BK5Ac) and H3 (H3K4Ac) respectively, was carried out with a view to define the specificity of histone deacetylases (HDACs). Data indicates that HDAC1 preferentially deacetylates H2BK5Ac.

Cancer Biology and Chronic Diseases

Using *Caenorhabditis elegans* as a model system, signalling events that culminate in altered gene expression during the aging process are being deciphered. Dietary restriction is known to increase life span and delay age-onset diseases; the cascade of molecular events leading to such outcomes is being delineated.

Molecular pathways involved in tumor cell growth, migration and invasion are being delineated, with specific focus on the tumor-associated antigens SPAG9, AKAP4 and HSP70-2. On-going Phase II clinical trials aim to assess the effects of the administration of autologous dendritic cells (primed recombinant SPAG9 protein) in patients of cervical cancer; trials in patients of ovarian cancer are also on the anvil.

Work on extending understanding of the function of tumor suppressor genes has continued. ACLY was identified as a Caspase-10 interacting protein; cleavage of ACLY abrogates its enzyme activity, suppressing the generation of acetyl-CoA, which is critical for lipogenesis and histone acetylation. In an *in vivo* model, Caspase-10 was shown to down-regulate ACLY-promoted tumor metastasis.

Identifying the underlying mechanisms which drive pathogenesis in neurological disorders has proceeded apace. Exposure to low doses of Bisphenol A (the endocrine-disrupting chemical employed in the manufacture of polycarbonate plastics and epoxy resins) caused neuronal degeneration and demyelination, a finding that has implications in multiple sclerosis and neuromyelitis optica. AdipoRon (an adiponectin agonist) was shown to augment brain insulin sensitivity, reduce $A\beta_{42}$ accumulation and improve cognitive function in APP/PS1 mice.

The processes responsible for inhibition of DNA replication during stress are being elucidated; unravelling of such regulatory mechanisms is likely to cause genomic instability. Gene targets of lncRNAs dysregulated in cancer are being identified and their influence on the cell cycle is being established.

Bloom Syndrome and Rothmund-Thomson Syndrome are associated with germline mutations in BLM and RECQL4 helicases, respectively; tumorigenesis is a characteristic of both syndromes. Several substrates of the BLM-interacting E3 ligase FBW7 are proto-oncogenes which have been implicated in human cancers. Previous work has found that BLM promoted the FBW7-dependent degradation of c-Myc, affecting tumor initiation. FBW7a was also found to degrade p53 via the proteasomal pathway, affecting its function during DNA damage.

Cell and Molecular Biology

Signaling pathways which mediate cell death in eukaryotic cells are being elucidated. Stimuli like starvation, oxidative stress and drugs were shown to enhance autophagy in the *Leishmania* parasite; the ability to counteract such stress appears to be dependent on Atg8. An analogue of Halictine-2 (an antimicrobial peptide from the venom of the eusocial honey bee) was shown to demonstrate high anti-

leishmanial activity, raising the possibility of its use as a drug.

Disruption of the regulation between protein homeostasis and energy metabolism occurs in many diseases, though mechanisms remain obscure. The mTORC1 pathway was shown to be involved in determining the levels of resting-state protein synthesis in B cells. Vitamin D was demonstrated to regulate energy metabolism and protein homeostasis in skeletal muscles; in Vitamin D receptor knockout mice, a dysregulation of the mTOR pathway correlated with muscle atrophy.

The DNA binding protein CTCF contributes to chromatin organization. Mice carrying mutations in various CTCF binding sites have been generated in order that cooperation and/or redundancy can be evaluated; initial analysis has revealed altered usage of V segments during V-to-DJ recombination. Work on elucidating the activity of the enhancer Eb (an important regulatory element of the TCRb locus) has also been initiated.

Study of the processes of cellular differentiation and reprogramming continue; besides shedding light on basic biology, such work will assist in the development of novel therapeutics. Amongst other work, early transfer of “reprogrammed monocytes” into mice who have undergone cecal ligation and puncture (an established model of sepsis) was shown to enhance survival.

Contraceptive vaccines are being evaluated for the management of street dog populations. A recombinant protein (comprising a T cell epitope of TT, a fragment of dog ZP3, a T cell epitope of bovine RNase, GnRH, a T cell epitope of *Plasmodium falciparum* CSP, and another copy of GnRH) induced anti-fertility effects when immunized in female beagle dogs.

Using transcriptomics, proteomics and bioinformatics, factors in Sertoli cells that regulate germ cell division and differentiation are being elucidated; such work could shed light on the causes of idiopathic male infertility. A role of the Hippo transducer YAP in regulation of the c-AMP and Notch signaling pathways in functionally mature Sertoli cells has been demonstrated. Data suggests that YAP regulates TLR-2 signaling in Sertoli cells.

Immunology and Vaccines

Use of antigen-entrapped polymer particles to enhance immunogenicity has been a long-standing interest; current studies focus on protein and carbohydrate antigens of *S. pneumoniae* and *S. typhi*. Immunogenicity studies of particle-entrapped SP0845-PCP1 conjugate are in progress. Preliminary evidence suggests that nanoparticle-based immunization enhances the germinal centre reaction.

Infection with closely-related *Salmonella* serovars is associated with distinct clinical outcomes in both mice and humans, the reasons for which are incompletely understood. In a murine model, two observations were of interest. Firstly, MyD88 was required for inflammatory responses to metabolically-active *S.*Typhi, but was largely dispensable for antibody responses. Secondly, *Salmonella* infection transiently suppressed antibody responses to unrelated antigens, a fact that has implications for vaccines employing adjuvants which seek to heighten MyD88-dependent effects.

Exploration of *Streptococcus pneumoniae*-host cell interaction has continued. PCP1 was shown to induce phosphorylation of ERK in RAW264.7 cells, acting via TLR-2. *O*-acetylation on PCP-1 was critical for cell binding, and deacetylation affected the molecule's immunogenicity. In other work, a uracil transporter was demonstrated to be required for pneumococcal fitness and virulence.

The role follicular T helper (Tfh) cells play in protective immunity is being assessed. Upon immunization of mice with the Japanese encephalitis (JE) vaccine, CD8 T cells were required for Tfh differentiation, as well as for the generation of optimal protective antibody responses. In humans immunized with the live attenuated JE-vaccine, the frequency of activated Tfh cells correlated with the magnitude of antibody-forming cells, reiterating their influence on the generation of protective humoral immunity.

Studies on elucidating the development and function of dendritic cells were carried forward. A novel role of RELB as a negative regulator of the type I IFN signaling pathway was described. Expression of RELB in FLT3-ligand derived DCs (which normally express low levels of RELB) reduced expression of CpG-stimulated *Ifna, Ifnb, Il12p40* and *Tnfa* transcripts. Further, RELB-

expressing NIH3T3 cells exhibited lower transcript levels of interferon-stimulated genes upon viral infection.

Systemic autoimmune diseases like SLE (or lupus) occasionally "flare" during pregnancy. In a possible explanation of this association, apoptotic blebs (implicated in lupus onset) were shown to synergize with human chorionic gonadotropin (hCG, a pregnancy-associated hormone) to elicit lupus-associated inflammatory cytokines and autoantibodies specifically in splenocytes cultures from lupus-prone mice. Previous work had indicated that hCG also exhibits pro-tumorigenic activity; emerging observations in transgenic mice suggest that ovarian steroids help ameliorate such effects.

Cross-talk of non-canonical NF-kB signaling pathway with the canonical NF-kB signaling pathway, and with the IRF3-type 1 interferon axis, is being elucidated. The canonical RelA NF-kB pathway was found to exert a pro-viral role during infection, arising from the ability of RelA to suppress cell death. Further, whether non-canonical NF-kB signaling influenced TNF-induced gene expression was assessed, employing microarray analysis on specific NF-kB component-deficient mouse embryonic fibroblasts. Data suggests that the p100 subunit modified transcriptional responses to TNF via both RelA- as well as RelB-dependent mechanisms.

CD4 cytotoxic T cells mediate protective roles in several viral infections. Long-term culture of human T cells under a Th1 polarizing protocol resulted in differentiation into a CD4 cytotoxic T cell-like lineage. Global gene expression and epigenetic analysis is aiding in defining the molecular events driving such differentiation. Further, transcription factors and lncRNAs involved in the generation of CD4 cytotoxic T cells are being elucidated; whether these cells express pathogen-specific features is also being determined.

Infectious Diseases

Learning how viral genes exploit cellular processes to promote viral replication has been a long-standing interest. The Tat protein from HIV subtype B was shown to exhibit significantly higher RNAi-silencing suppressor activity than the Tat protein from HIV subtype C. The E3 ligase CHIP was shown to modulate the stability of Tat, and transfection of CHIP significantly reduced HIV-1 replication. In other work, novel frame-shift mutations in the gene for CCR5 were detected in HIV sero-negative individuals from North India.

Molecules made by the *Plasmodium* parasite which work to manipulate host biochemical pathways in hepatocytes could prove to be good targets for the development of anti-malarial drugs. In this regard, the properties of T-cell immunomodulatory protein (Tip) were investigated. Tip was present in the blood of infected mice and reduced the pro-inflammatory effects of LPS, while enhancing secretion of IL-10 and b; these effects can potentially influence immune responses against the parasite.

Signaling events in apicomplexan parasites, as well as in mammalian neurons, have been enduring interests. Partial knockdown of PfVps15 (a kinase essential for blood stage development of *Plasmodium*) caused a decrease in parasite growth. Data suggests an important role of Vps15 in *Toxoplasma* development as well. Molecular mechanisms that regulate cell cycle-related neuronal apoptosis triggered by Aβ42, and regulated by various miRNAs, are being delineated.

Studies seek to discover how metabolic dysfunction drives pathology in microbial and autoimmune diseases. Diisonitrile lipopetides, absent in planktonic cultures, were identified in biofilm extracts from *M. tuberculosis* (*M.tb*); analysis of the functional properties of these metabolites is in progress. Sequential RNA-seq analysis over the 6-day pigmentation cycle of B16 melanoma cells revealed evidence of metabolic changes, coupled with changes in mitochondrial respiration; potential molecular links between mitochondrial metabolism and pigmentation are being discerned.

Signaling cascades which work to promote survival of *M.tb* are being delineated. Infection of macrophages with *H37Rv* resulted in higher levels of double-stranded breaks in host DNA than did infection with *H37Ra*; damage to host DNA was also observed in *M.tb*-infected mice. Treatment of infected mice with a combination of isoniazid and an ATM inhibitor resulted in a synergistic reduction in bacterial load; reversing pro-survival signals may therefore result in therapeutic benefit.

Mycobacterium indicus pranii (MIP) is being investigated for its utility, both as a potential therapeutic against cancer, and as vaccine against *M.tb*. Immunization with MIP reduced metastasis of B16F10 melanoma cells in mice, possibly due to decreased MMP9 and VEGF levels. Studies also suggest a direct effect of MIP on the progression of the tumor cell cycle. Lipoarabinomannan derived from MIP was shown to induce autophagy in *M.tb*-infected macrophages, enhancing bacterial clearance.

Flagship Programme of NII

NII has initiated a Flagship Programme in ImmunoEngineering. This umbrella project encompasses research on novel adjuvants and vaccines (for infectious diseases and cancer), immunotherapy strategies and artificial antigen presenting cells, scaffolds and drug delivery devices, and on new methods and protocols for regenerative medicine. Several therapeutic modalities involve the adoptive transfer of cells that have been modified *in vitro* using molecular methods, and then grown to high density in bioreactors, in the presence of appropriate cytokines, growth factors and support cells. We therefore aim to also set up an advanced cell culture/fermentation facility to grow human cells/tissues/organs for therapeutic applications.

Acknowledgement

The support of the Department of Biotechnology, in terms of both funds and intellectual inputs, has been an enduring source of strength; the administrative assistance and guidance has allowed the optimal use of our resources.

We look forward to the annual meetings of RAPSAC with great eagerness. The feedback and critique we receive serve to shape the course of our work.

The last few months have been particularly trying, with the on-going pandemic. Members of our administrative staff have stepped up to the challenge, making sure that we are not lacking for supplies and reagents. Members of our technical staff too have responded in an exemplary fashion, keeping critical services going. Support services are helping us tide over the crisis, going above and beyond the call of duty, unmindful of personal inconvenience; staff members at the Primate Research Centre and the Small Animal Facility deserve special mention in this regard.

We shall continue to build on our strengths, and look to the future with great anticipation.

Amulya K. Panda

Date: 31st August, 2020 Director

RESEARCH REPORTS

IMMUNITY AND INFECTION

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

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Dendritic cells (DCs) are collection of heterogeneous population of antigen presenting cells that initiate innate immunity, activate co-stimulatory signals, produce high levels of effector cytokines and define an adaptive immune outcome. Plasmacytoid DCs (pDC), $CD8\alpha^+$ cDC1, $CD4^+$ and $CD4^-CD8^-$(double negative, DN) cDC2 are major DC subtypes represented in mouse spleen and their equivalent populations can be found in other tissue sites. Extensive research using specific gene knock out mice models have identified molecules playing pivotal roles in DC differentiation yet the mechanistic details of DC diversification from a common dendritic cell precursor population remains poorly understood. Type I Interferon (IFN) signaling plays a critical role in DC biology and is shown to control the homeostasis, apoptosis, migration and functions of DCs. Type I IFN signaling synergistically regulates FLT-3 L directed development of pDCs from common lymphoid progenitors and blocking IFN signaling hinders pDC development from this progenitor population. *Irf4*, *Irf2* and *Relb* are essential for the development of cDC2 subtype. Abrogation of cDC2 development due to *Irf2* deficiency could be rescued in $Irf2^{-/-}Ifnar1^{-/-}$ mice suggesting a negative regulation of cDC2 development by hyper type I IFN signalling. We analyzed effect of RelB expression on type I IFN signaling in DCs.

Studies on DC development and functions have been facilitated by two *in vitro* culture systems guided by FLT3-L (FL-DC) and GM-CSF (GM-DC). GM-DC culture resembles $CD11b^+$cDC2 subtype and was our preferred model to study role of RelB in cDC2 biology. RelB, an NF-kB family transcription factor enters the nucleus as a RelB:p52 heterodimer and regulates target gene promoters. Nuclear translocation of RelB:p52 heterodimer is blocked by cell permeable peptide inhibitor SN52. We observed a modest increase in IFN stimulated genes (ISG) levels upon treatment with SN52 as against mutant form SN52M; suggesting that RelB might play important role in DC biology by suppressing type I IFN signaling. Among the different DC subsets, pDCs are intricately linked to production of type I IFN through IFN feedback loop. Hence, to further study the effect of RelB on IFN signaling at molecular level, we expressed *Relb* in on-going FL-DC culture which supports development of pDC and other cDC subtypes. *Relb* expression in FL-DC cultures led to reduction in the pDC population and further, we also noticed defect in induction of CpG-stimulated *Ifna, Ifnb, Il12p40* and *Tnfa* transcripts. Though, RelB is expressed at negligible levels in FL-DCs and we reported for the first time, an induction of RelB in FL-DC cultures upon TLR stimulation as early as 2 hours post CpG stimulation, increasing thereafter. Noticeably, increase in RelB levels coincided with the decline in type I IFN transcript levels and concomitantly *Relb* expressing FL-DCs demonstrated an impaired induction of ISGs

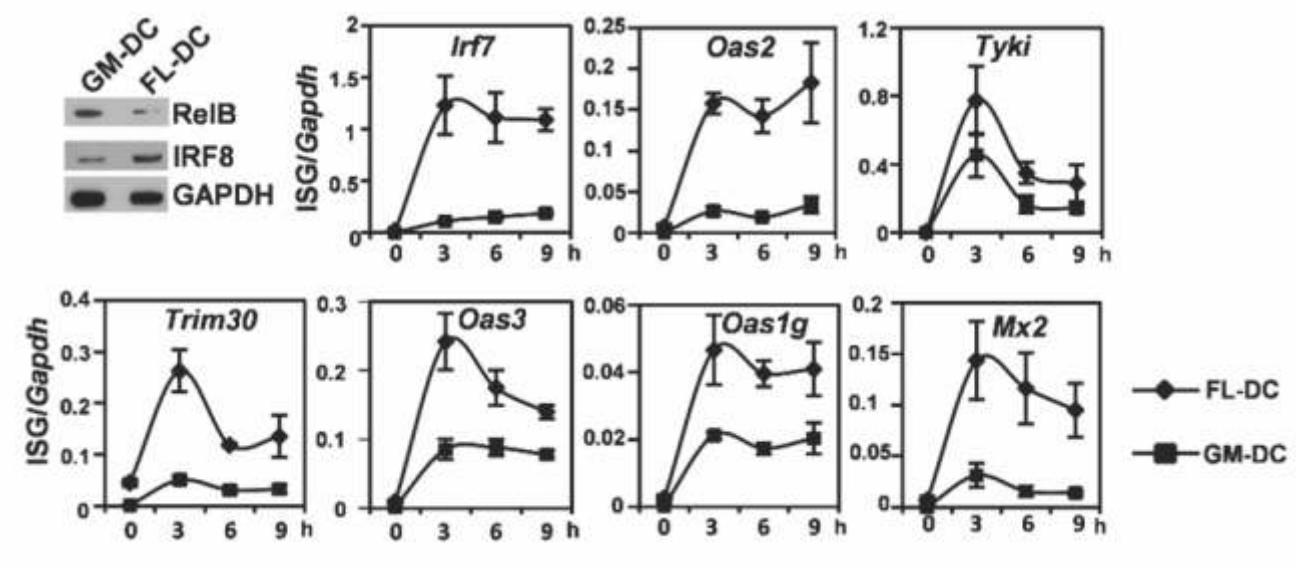

Fig.1: FL-DC and GM-DC cultures express differential levels of RelB (protein levels were examined on DC harvested on day 8 without IFNα2 treatment). GMDC culture derived cDC2 are inherently defective in type I IFN signaling. FL-DCs and GM-DCs were harvested on day 8, stimulated with IFNα2 (500 U/ml) and ISG induction was analyzed at different time points. X-axis represents time in hours (h) post IFNα2 stimulation.

both at steady state levels as well as under TLR stimulated state.

GM-DCs express high levels of RelB and exhibit a lower induction of ISGs upon IFN stimulation with respect to FL-DCs which express negligible levels of RELB (Fig. 1). This difference in ISG expression could be an effect of differential expression of *Relb* and differences in DC subtypes in two culture systems. Hence, to confirm our observation independent of limitations of DC cultures, we analyzed the RelB expressing NIH3T3 fibroblasts which exhibited suppression in type I IFN signaling and displayed lower induction of ISG transcripts in comparison to control population. Our observation is also in agreement with previously reported global gene expression analysis study from *Relb*$^{+/+}$ and *Relb*$^{-/-}$ mice GM-DC cultures. We noticed that RelB could also inhibit the activation of *Ifnb* promoter by IPS1, further confirming our observations. To understand the mechanism of suppression of type I IFN signaling, we analyzed the level of different members of the NF-κB signaling pathway in *Relb* expressing NIH3T3 fibroblasts. Although, p65 and p50 were expressed at comparable levels, strikingly that of IkBα, an inhibitor of canonical NF-kB signaling showed selective increase in protein levels. Further, we demonstrated that effects of RelB could be mediated through IkBα as expressing a phosphorylation defective i.e. stabilized mutant, IkBαM also displayed suppression of type I IFN signaling.

Suppression of type I IFN signaling by RelB could down regulate cellular antiviral responses by interfering with type I IFN signaling and indeed, RelB expressing NIH3T3 cells exhibited lower ISG transcript levels upon New Castle disease Virus (NDV) infection. This compromised induction in ISG levels was also translated into higher NDV transcripts suggesting an increased viral replication in RelB expressing cells (Fig. 2). Consistent with our finding that IkBαM lowered type I IFN induced ISGs and hypothesis suggesting that observed effects of RelB may be mediated by increased IkBα levels; IkBαM expressing NIH3T3 fibroblasts exhibited dampened antiviral responses by lowering ISG levels. Hence, our study shows novel role of RelB in modulation of host antiviral machinery and notably, the first report suggesting a connection between NF-kB signaling to type I IFN signaling.

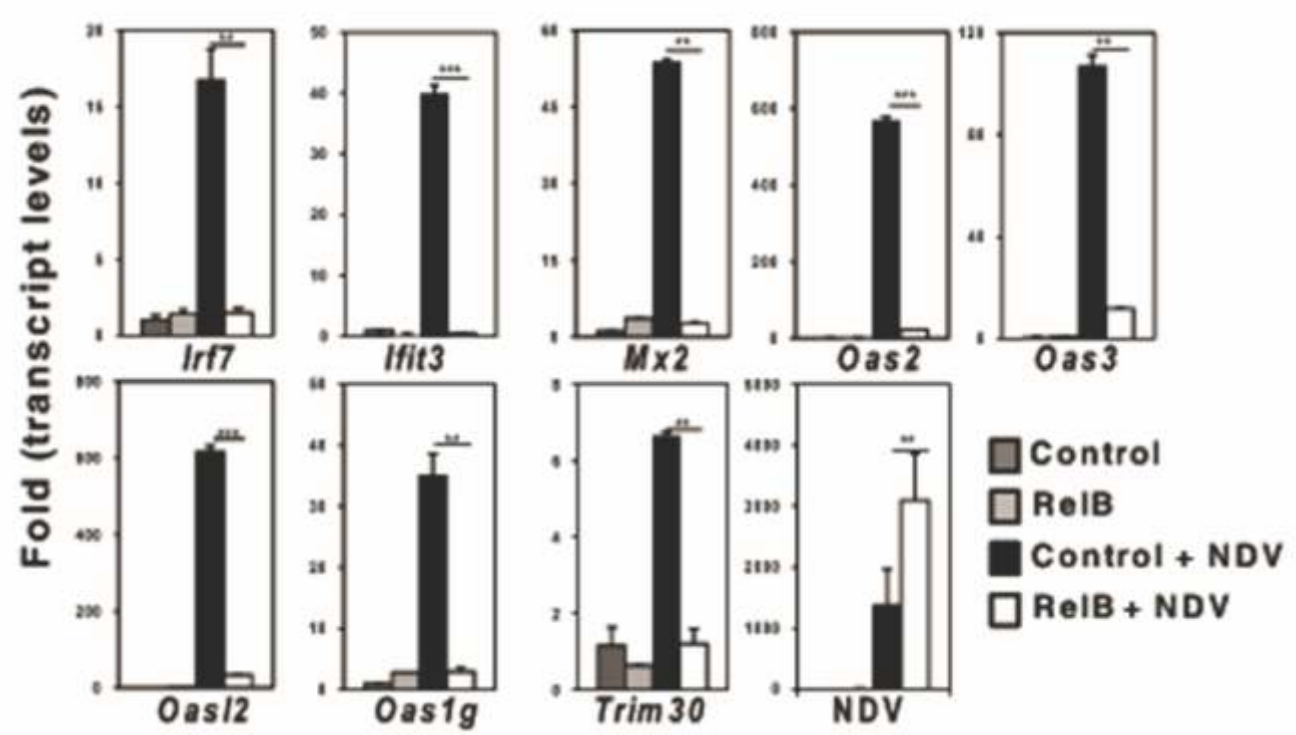

Fig. 2: RelB mediated suppression of type I IFN signaling leads to higher viral load in NDV infected cells. Control and *Relb*-expressing NIH3T3 cells were infected with NDV (LaSota strain) for one hour at MOI of 5 and qPCR analysis performed 24 h post viral infection showed a noticeable suppression of ISGs with higher viral load of NDV in RelB expressing population as compared to the Control cells.

Hyper type I IFN signaling was reported to abrogate cDC2 development. In collaboration with Dr. Ranjeny Thomas at Queensland University, Australia; we developed *Relb*$^{-/-}$*Ifnar1*$^{-/-}$ double knock out mouse strain and demonstrated that cDC2 development was rescued in *Relb*$^{-/-}$ mice in *Ifnar1* null background indicating that the hyper type I IFN signaling in *Relb*$^{-/-}$ mice was responsible for the abrogation of cDC2 development. Type I IFN signaling plays a critical role in DC development and functions. Hyper type I IFN signaling is deleterious to development of cDC2 subtype. Overall, our study projects a novel role of RelB as a negative regulator of the type I IFN signaling pathway deciphering the unexplored mechanism of regulation of cDC2 development by RelB. We demonstrate that RelB expression dampens antiviral responses by lowering ISG levels.

Publication

Original peer-reviewed article

1. Saha I, Jaiswal H, Mishra R, Nel HJ, Schreuder J, Kaushik M, Singh Chauhan K, Singh Rawat B, Thomas R, Naik S, Kumar H, Tailor P (2020) RelB suppresses type I interferon signaling in dendritic cells. **Cell Immunol.** doi: 10.1016/j.cellimm.2020.104043.

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

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Theme of research

Plasmodium species introduce effector molecules into hepatocyte cytosol to manipulate host metabolic and /or signaling pathways for its own benefit. 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 or possibly as drug targets.

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

Previously we reported that *Pb*HspJ2 is important for malaria gametocyte development. To find the interacting partner of *Pb*HspJ62 we performed GST-pull down assays with parasite lysate. Eluted proteins Mass-spectrometry analysis was done to identify the interacting partners. We further report that *Pb*HspJ2 interacts with ApiAP2 transcription factor found in malaria parasites. ApiAP2 is known to play role in gametocyte development. In the *Pb*HspJ2 knockout parasite transcriptome a majority of the downregulated genes were related with gametocyte develoment. When we analyzed promoter region (~1000 bp) of differentially expressed genes (DEG) in *Pb*HspJ62 knockout parasites, we found a conserved motif (TCT ACA) in 50 genes, out of total 214 DEG, which is the binding motif for ApiAP2 transcription factor. These 50 genes are related with gametocyte development. Overall results indicate that *Pb*HspJ62 protein is essential for the correct functioning of ApiAP2 transcription factor at the specific commitment step of gametocyte formation.

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. We found that malaria immunomodulatory protein (TIP) binds to macrophages and dampen the proinflammatory immune response. TIP also upregulates the mediators of anti-inflammatory immune response. RAW cells stimulated with LPS generate inflammatory response and upregulate IL1β, IL6, IL12p40, IFNγ, TNFα. Purified endotoxins free PbTip in combination with LPS reduced the levels of above-mentioned cytokines significantly and also increased the level of TGF-β, after stimulation. Decrease in levels of proinflammatory cytokines and increase in level of anti-inflammatory

cytokine such as IL10 and TGF-β might be helping the parasite to decrease immune responses against the parasite in the host. Anti-inflammatory milieu may also affect the Th1-Th2 balance as it is known in the case of nemotode infections.

3. Drug discovery

Artemisinin (antimalarial drug) is water insoluble. We have made a liposome nanoparticles based formulation in aqueous buffer which is as active as the artemisinin dissolved in ethanol. Box-Behnken design was used for formulation design and optimisation of liposome nanoparticles. Artemisnin-Liposome formulations were synthesised using conventional thin film hydration followed by bath sonication and mechanical extrusion. The electron microscopy images of the artemisinin loaded liposomes revealed the spherical structures of ART-LIPO. The observed entrapment efficiency of 81.68 % indicated that most of the drug was encapsulated into the liposomes.

Publications

Original peer-reviewed articles

1. Quadiri A, Kalia I, Kashif M**,** Singh AP (2020) Identification and characterization of protective CD8+ T epitopes in a malaria vaccine candidate SLTRiP. **Immun Inflamm Dis.** 8: 50-61.

2. Kumar B, Singla D, Kashif M, Sharma R, Dixit R, Singh AP, Saxena AK, Abid M, Pandey KC (2019) Metacaspase-3 of plasmodium falciparum: An atypical trypsin-like serine protease. **Int J Biol Macromol.** 138: 309-320.

3. Garg S, Shivappagowdar A, Hada RS, Ayana R, Bathula C, Sen S, Kalia I, Pati S, Singh AP, Singh S (2020) Supression of perforin-like protein pores inhibit plasmodium multistage-growth, transmission and erythrocyte senescence. **Front Cell Infect Microbiol.** doi: 10.3389/fcimb.2020.00121.

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

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Theme of research

Viral (HIV and Dengue) Pathogenesis is very complex involving multiple viral and host factors. Viruses have limited number of genes and most of the viral proteins are therefore poly-functional. Viruses exploit the host cellular machinery for their advantage and is our goal to understand mechanistic details of host-pathogen interactions. This understanding is necessary to generate specific anti-viral approaches.

Objectives

How different viral structural and regulatory genes of HIV-1 and Dengue exploit the cellular machinery to promote its own replication is the major focus of our work. Besides studying the ploly-functional nature of viral proteins, it is important to study factors that govern the intracellular stability of viral proteins. How a combination of specific miRNAs and cellular ubiquitin machinery which involves De-ubiquitinases (DUBs) also, influence viral outcomes constitute an important part of our ongoing studies.

HIV-1 Tat subtype-specific modulation of RNAi-silencing suppressor activity and cell death

HIV-1 subtype B is predominant in US and UK but it is genetic subtype C that is predominant in India. Tat is a known RNAi-silencing suppressor and known to induce cell death. HIV-1 Tat C has some remarkable signature changes in many of its domains when compared with subtype B Tat. We, therefore wanted to carry out RNAi silencing of suppressor (RSS) activity of natural variants of HIV-1 Tat (derived from Indian isolates) along with the standard prototype subtype B Tat derived from pNL4-3. A very sensitive mammalian RNAi-silencing suppressor assay system was developed in our lab earlier. Experiments showed that subtype B Tat exhibited significantly more RSS activity when compared to Tat-C and some of the natural variants. We subjected all Tat variants to cellular ubiquitination and once again significant differences in the extent of ubiquitination was observed. These differential activities may help us understand HIV-1 genetic subtype specific differences with respect to pathogenesis.

HIV-1 subtype B and C interactome study

We plan to study and compare the cellular interacting partners of HIV-1 subtype B and C proteins. Interactom e analysis of HIV-1 proteins was done by Mass Spectrometry once. It will be repeated two more times to get a list of proteins interacting with HIV-1 proteins of both subtypes. This will be helpful in understanding the mechanism of HIV-1 pathogenesis and common interacting partners of proteins of both subtypes can be explored to develop new anti-HIV-1 targets.

Genetic polymorphisms in the HIV-1 co-receptor – CCR5 from North India

Chemokine receptor type 5 (CCR5) serves as a co-receptor for Human Immunodeficiency Virus (HIV) to enter human CD4 +ve T cells, langerhan cells and macrophages. In the absence of CCR5, HIV fails to successfully initiate infection. Our lab was the first in India to report a CCR5-delta 32 individual and then we studied the inheritance of this remarkable mutation in that family (Gene 207, 141-147, 1998; & J Human Virology, 1, 187-192, 1998; covered by all National News Papers of India and also covered in Nature Medicine). Various natural mutations of the CCR5 gene which influences the rate of AIDS progression has since been described. Genetic characterization of the CCR5 gene in individuals from North India revealed several natural point mutations in HIV-1 seronegative and seropositive individuals. Interestingly, we identified novel frame-shift mutations in the CCR5 gene in HIV seronegative individuals, as well as the earlier reported CCR5Δ32 mutation. Additionally, we observed a number of mutations present only in HIV seropositive individuals. This is the first report to describe the genetic variations of CCR5 in individuals from North India.

Role of CHIP (carboxy terminus Hsp70 interacting protein) –E3 ligase in controlling intracellular stability of HIV-1 Tat

It is becoming increasing clear that CHIP controls the half life of several important cellular proteins like P53. HIV-1 Tat is the major protein responsible for increasing transcription of all the HIV-1 genes and is made very early during the infection. It is critically involved with breaking the HIV-1 latency. Hence, it is important to study the various cellular factors responsible for governing the intracellular stability of the Tat protein. Since E3 ubiquitin ligases are involved in proteasomal degradation of several proteins, we wanted to explore if CHIP was involved in modulating the stability of HIV-1 Tat protein. Co-transfection of Tat and CHIP expressing plasmid DNA resulted in decreasing the levels of Tat protein in a dose-dependent manner. A CHIP mutant lacking the U-box domain was unable to degrade the Tat protein. Furthermore CHIP promoted ubiquitination of Tat by wild-type as well as lysine-linked-K48 ubiquitin. CHIP Transfection resulted in significant reduction of HIV-1 replication. CHIP-knock down studies also established that CHIP is a potent suppressor of HIV-1 replication.

Studies aimed at studying factors governing stability of HIV-1 Vif protein

Vif protein plays an important role in neutralizing a cellular restriction factor APOBEC3G. This step is critically important in maintaining the genomic integrity of the virus. We wanted to explore how the intracellular stability of this protein is governed. Our preliminary results suggest that just like HIV-1 Tat protein, CHIP is also responsible for its degradation and stabilization of APOBEC3G. CHIP knock down studies are planned to address the specificity issues.

Role of Exosomes in Dengue infection

The major aims of this project involve investigation of bystander effects of DENV infection and role of released exosomes in intercellular communication between Dengue infected and uninfected cells. Micro-vesicles also known as extracellular vesicles are known to package biological molecules (mRNA, miRNA, Transcription factors etc) and currently known for horizontal transfer. A preliminary study of micro-RNA profiling was carried out and indeed specific miRNAs were identified that were uniquely present in Dengue infected cells which also included few unique De-ubiquitinases (DUBs). The importance of this observation with respect to replication and pathogenesis will be studied in future.

Dengue virus nonstructural protein-1(NS1) impairs TGF-β signaling via recruiting Smurf2

TGF-β signaling is tightly regulated to ensure cellular functions. Modulation of the TGF-β pathway has been well documented for different viruses. In order to establish the role of dengue virus non structural proteins in TGF-β signaling, we investigate the involvement of NS1, a multifunctional viral protein that interacts with several other host proteins. Our preliminary results suggested that dengue virus NS1

protein interferes the TGF-β signaling. NS1 was also able to interact with SMAD transcription factors (Smad2, Smad3 and Smad4) and degrade these proteins by recruiting E3 ligase SMURF2. We observed Notch3 down regulation upon infection and this will be explored in context with platelet reduction.

Intracellular stability of HV-1 Vif protein involving natural variants from North India) will be explored in details especially in context of APOBEC3G biology. Intracellular P53, p21 protein stability by HIV-1 Nef will be explored. Role of NS1 dengue gene in influencing several key cellular functions (perturbation in cellular miRNAs and DUBs) will be explored.

Publications

Original peer-reviewed articles

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Analysis of *Salmonella* - host cell interactions

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Theme of research and objectives

Pathogenic *Salmonella* serovars continue to be a major public health problem particularly in the developing world. The clinical manifestations produced by these pathogens 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 self-limiting gasteroenteritis in normal humans and non-typhoidal invasive *Salmonella* (iNTS) disease in immunocompromised subjects. iNTS is a significant health issue in sub-saharan Africa and other AIDS affected countries. *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 typhoidal and non-typhoidal *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 regulation of immune responses during infection with these bacteria.

MyD88-dependent inflammatory responses are largely dispensable for antibody response to *Salmonella*

Infection with pathogenic microorganisms activates inflammatory and innate immune responses that include production of cytokines, chemokines and antimicrobial peptides. These responses are generated as a result of sensing of pathogen - derived molecules by innate immune receptors including TLRs and NLRs. These responses not only play a critical role in bringing about clearance of the pathogen but also participate in shaping the adaptive immune response. We investigated the role of these early responses in generation of antibodies against *Salmonella* by immunizing WT mice and mice lacking the TLR adaptor, MyD88, with this pathogen. The results showed that the production of inflammatory cytokines including CXCL8, IL-6, IFN-g in response to infection with *S*.Typhi required metabolically active bacteria as the cytokine response was poorly elicited with antibiotic- treated *S*. Typhi. Further, this early inflammatory response was largely dependent on signals generated through MyD88 adaptor as mice lacking this adaptor showed highly reduced cytokine production. The induction of inflammatory response with live *S*.Typhi in WT mice was associated with splenomegaly and significant changes in splenic cellularity. However, these changes did not affect antibody response to *Salmonella* as WT and MyD88 deficient mice showed comparable antibodies against antigens of *Salmonella*. The only noticeable difference was that MyD88 deficient mice showed increased IgG_1 kind of antibodies in particular against *S*.Typhi LPS, very likely due to reduced TLR-driven IFN-g in this strain. Importantly, immunization with antibiotic-treated *S*. Typhi resulted in significantly muted antibody response to antigens of this pathogen which suggests that viability of bacteria might have a considerable influence on immune response to *Salmonella*. Our results indicate that non-MyD88 sensors including TRIF might play an important role in regulating anti-*Salmonella* antibody response.

Infection of mice with *Salmonella* produces transient suppression of antibody response to non-*Salmonella* protein antigens

The results described above suggested that the changes in splenic cellularity brought about by MyD88-dependent inflammatory responses may not significantly influence antibody response to *S*.Typhi, and this antibody response may be largely extrafollicular and not dependent on fully established germinal center. Considering the importance of germinal center formation in antibody response in general, we asked if the changes in cellularity brought about by this pathogen might affect immune response to non-*Salmonella* antigens. Mice were infected with *S*.Typhi and immunized with ovalbumin or tetanus toxoid on the day when changes in splenic cellularity brought about by this infection were at peak. Compared to uninfected mice, infected mice showed significantly reduced antibody response against these two antigens. This reduction was, however, not seen in response to immunization with Vi capsular polysaccharide. These results suggest that *Salmonella*-induced early inflammatory and innate immune responses, which are critical for bacterial clearance, might dampen antibody response to non-*Salmonella* protein antigens. These findings have implications for understanding immunity during co-infections.

Publications

Original peer-reviewed articles

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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.

Immunomodulatory and antigenic properties of capsular polysaccharide from *S. pneumoniae* serotype 1

Circulating capsular polysaccharide in the range of 0.1 to 100 µg/ml has been detected in patients suffering from bacterial pneumonia and these concentrations appear to be stable for at least 10 to 15 days. Here, we sort to analyze the immunological consequences of the interaction of pneumococcal capsular polysaccharide from serotype 1 (PCP1) with host immune cells.

Antibodies directed against the capsular polysaccharide have been shown to confer protection against *S. pneumoniae* infections. PCP1 is a component of the pneumococcal polysaccharide and glycoconjugate vaccines. Chemically, PCP1 is a linear polymer of trisaccharide repeating units, consisting of D-galactopyranosyluronic acid and 2-acetamido-4-amino-2, 4, 6-trideoxy-D-galactopyranosyl residues. The native PCP1 contains a non-stoichiometric amount of *O*-acetyl groups.

Previously, we had shown that binding of PCP1 to the surface of immune cells led to proinflammatory cytokine production which was not cell line or cytokine restricted. PCP1 failed to induce TNF-a production from RAW264.7 cells when pre-incubated with a TLR2 blocking antibody. The surface binding of PCP1 was abrogated in the presence of TLR2 blocking antibody. Recent data from the lab showed that PCP1 failed to bind TLR2 deficient RAW264.7 cells and induce TNF-a production.

We tested whether intracellular signaling events were triggered in RAW264.7 cells as a result of surface binding of PCP1. Our data indicated that PCP1 induced phosphorylation of ERK but did not trigger phosphorylation of p38 and JNK in RAW264.7

cells. The role of MAPK-ERK pathway in the proinflammatory activity of PCP1 was confirmed using an ERK specific inhibitor PD98059. Pre-incubation of RAW264.7 cells with increasing concentration of the inhibitor resulted in a dose dependent reduction in TNF-α production from PCP1 treated cells.

Next, we wanted to assess the impact of *O*-deacetylation of PCP1 on its antigenic properties. PCP1 was treated with alkali to remove *O*-acetyl groups. In contrast to PCP1, alkali-treated PCP1 failed to induce RAW264.7 cells to produce TNF-α. We observed that alkali treated PCP1 was unable to bind RAW264.7 cells as assessed by flow cytometry. To check whether *O*-deacetylation affected anti-PCP1 antibody responses, PCP1 or alkali-treated PCP1 were injected intraperitoneally in BALB/c mice and antigen-specific serum antibody levels were assessed by ELISA using PCP1 as the capture antigen. Mice immunized with alkali treated PCP1 elicited lower anti-PCP1 antibody response as compared to PCP1 suggesting that a significant proportion of anti-PCP1 antibody response is directed against the alkali-labile side chain like acetyl group, we observed some antibody response against PCP1 'backbone' as well. To determine whether antibodies directed against alkali-sensitive group(s) of PCP1 such as *O*-acetyls have any role in the ability of PCP1 to confer protection, BALB/c mice were immunized intraperitoneally with either alkali-treated PCP1 or PCP1 and 7 days later, the mice were challenged with virulent pneumococcal strain ATCC 6301. The data showed that 11 out of 12 (92%) mice survived in the PCP1 immunized group while 6 out of 12 (50%, with a median survival time of 4.25 days) mice survived in the alkali-treated PCP1 immunized group. None of the 12 mice in the group given saline survived. This experiment suggested that alkali treatment of PCP1 resulted in the loss of group(s) that served as an important target for generating protective antibodies in PCP1 immunized mice and conferring immune protection. Our data suggests that loss of alkali-labile group(s) of PCP1 compromises its ability to induce protective antibodies. Care should be taken to ensure that non-saccharide moieties like acetyl groups are not lost during chemical coupling of PCP1 to the carrier protein during the manufacture of pneumococcal glycoconjugate vaccine.

SPD_1629 is a uracil transporter, and is required for pneumococcal fitness and virulence

Uracil is an essential component of chemically defined minimal medium used for growing pneumococci. *S. pneumoniae* fails to replicate in chemically defined minimal medium devoid of uracil. We are interested in identifying and characterizing the pneumococcal transporter responsible for uptake of uracil. Blocking the transport of uracil or getting the bacterial pathogen to take up a lethal analogue of uracil can potentially serve as an intervention strategy against *S. pneumoniae*.

Previously, we have shown that pneumococcal growth was inhibited by 5-fluorouracil. Bioinformatic analysis of the pneumococcal genome revealed the presence of 3 putative permeases (SPD_0267, SPD_1141 and SPD_1629) that may be involved in the transport of nucleobases. We observed that D39D*spd_0267* and D39D*spd_1141*, like the wild type D39 strain were sensitive to 5-fluorouracil toxicity. D39D*spd_1629* was however partially resistant to 5-fluorouracil suggesting that uracil is transported through SPD_1629. Of the various purine and pyrimidine nucleobases tested in a competition experiment only uracil was able to compete out 5-fluorouracil and partially restore the growth of D39D*spd_1629*.

We generated a pneumococcal mutant deficient in SPD_0267, SPD_1141 and SPD_1629. The growth profile of D39D*spd_1629* and the triple mutant D39D*spd_0267*D*spd_1141*D*spd_1629* in the presence of 5-fluorouracil was comparable thus discounting the involvement of SPD_0267 and SPD_1141 in the uptake of uracil. Unlike wildtype strain, D39D*spd_1629* was unable to take up ^{3}H-uracil thereby confirming that SPD_1629 was a uracil transporter. Mice challenge experiment showed that all mice infected intraperitoneally with D39D*spd_1629* survived whereas mice infected with D39 succumb to infection within 48 h. Similar results were obtained when bacteria were given intranasally. We estimated the bacterial load in

blood, lungs and spleen of mice following intraperitoneal challenge. The bacterial load in mice infected with D39Δ*spd_1629* was significantly lower than that of mice given the wild type strain. This suggested that pneumococci deficient in SPD_1629 is severely compromised in virulence. To test the ability of D39Δ*spd_1629* mutant to compete with its wild type counterpart we determined the competitive index by co-infecting mice with both the strains. We found that with time there was a decrease in the competitive index suggesting that SPD_1629 plays a crucial role in pneumococcal fitness and virulence.

Publications

Original peer-reviewed articles

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Patent

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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 research objective of the laboratory is to improve the immunogenicity of antigens entrapped in biodegradable polymer particles. In recent years, our focus has been on improving the immunogenicity of carbohydrate antigen using polymeric nanoparticles. Polymeric nanoparticles are used to develop scaffold for tissue engineering applications. 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.

(A) Immunogenicity of polymeric nanoparticle entrapped pneumococcal antigens

Polymeric particles entrapping protein/ carbohydrate antigens are being routinely used in the laboratory to improve their immunogenicity. The 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 (1, 14, 6B and 5) of *S. pneumoniae* and immunological evaluation of its nanoformulations, (ii) conjugation of pneumococcal protein (SP0845) with polysaccharides and its immunological evaluation, and (iii) purification and characterization different immunodominat protein (PsaA, SP9845, ABC transporter protein and penumolysin) from *S. pneumoniae* and its evaluation as protein based vaccine.

SP0845 protein was purified to homogeneity and conjugated to PCP1 and 14 using CDAP conjugation method. The conjugates were purified and characterized. Briefly, pneumococcal polysaccharide was activated using organic cyanylating reagent 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP). The prepared conjugate was purified using

gel filtration chromatography and was further characterized using proton NMR. Protein and polysaccharide content of purified conjugate was estimated using BCA assay and anthrone assay respectively. Immunization studies with PCP1-SP0845 conjugate and nanoparticle entrapping the conjugate are in progress.

Despite the polysaccharide and glycoconjugate polymer particle based pneumococcal vaccines, several conserved pneumococcal surface proteins are under investigation as candidate vaccines. Proteins such as pneumococcal surface protein A (PsaA), lipoprotein SP0845, an ABC transporter protein and penumolysin are being formulated using polymer particle to improve their immunogenicity. The objective is to evaluate mixture of pneumococcal protein to elicit comparable protective antibody response against *S. pneumoniae*. This may lead to development of a protein based pneumococcal vaccine.

(B) Fusion of polymeric particle into membrane like structure and its evaluation as scaffold for growth of different cell types

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. Using this basic scaffold fabrication method we have used polymers such as chitosan, PCL, eudragit, gelatin along with PLA to fabricate scaffold having diverse mechanical strength and surface properties. Fusion of PDLLA-Eudragit nanoparticles with methanol lead to the formation of a transparent membrane. These scaffold have show good potential for the growth of corneal epithelial cells. These polymeric membranes have similar cell growth promoting ability that of amniotic membrane.

PDLLA-Gelatin scaffold fabrication was optimized for skin healing studies. FGF-2 entrapped PDLLA-Gelatin membrane and dummy PDLLA-Gelatin scaffold group showed more number of myofibroblasts and collagen deposition as compared to control and Integraâ group because of larger surface area available for cell attachment. Presence of macrophages in both PDLLA-Gelatin membrane treated mice groups were comparable to that of Integraâ group. Formation of new blood vessel was observed in FGF-2 entrapped PDLLA-Gelatin membrane group. Regenerated skin wound bed appeared healthy as no fluid deposition was observed in all the groups. The PDLA gelatin based scaffold was comparable to that of Integra membrane available for commercial use.

PDLA based scaffold were redesigned for bone tissue engineering. Microparticles based porous scaffold enhance the overall surface area for cell-matrix interaction and also increased the compressive strength. Further, these scaffolds were functionalized with citric acid derived carbon nanodots via EDC/sulfo-NHS. Carbon nanodots are reported to increase electrical and mechanical properties of the scaffold. Results showed that the functionalized scaffolds enhance the osteogenic differentiation of mesenchymal stem cells in comparison to the blank scaffold as carbon dots provide stimulus to the MSCs that promotes differentiation. Currently, the mechanical, electrical and anti-microbial properties of carbon nanodots functionalized polymeric scaffolds are being evaluated in mice model for bone tissue engineering applications.

(C) Solubilization and refolding of inclusion body proteins

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.

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2. Singh A, Panda AK. A process for fabrication of transparent polymeric scaffold for tissue engineering applications. (Indian Patent Application No. 201911036223 filed on 9th September, 2019)

3. Meena J, Panda AK. A highly efficient polymer particulate vaccine formulation entrapping admixture of alum and antigen. (Indian Patent Application No. 201911036242 filed on 9th September, 2019)

4. Chakkumkal A, Panda AK. Vaccine composition capable of inducing memory antibody response from single point immunization. (US Patent Application No. 14/122,923 granted on 30th July, 2019)

5. Anish CK, Khan N, Sehgal D, Panda AK. Design and development of nanoparticles based carbohydrate vaccine against Streptoc-occus pneumonia (Indian Patent Application No. 332055 granted on 14th February, 2020)

Disorders of proliferation: Analysis of novel pathways and targets

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A. Characterization of lupus-associated immune responses

Human chorionic gonadotropin (hCG) and lupus

Our previous work demonstrated that, on splenocytes derived from lupus-prone mice, hCG (a hormone associated with pregnancy) synergized with certain TLR ligands in the secretion of lupus-associated inflammatory cytokines and autoantibodies (*Front Endocrinol. DOI: 10.3389/ fendo.2018.00742, 2018*). Ongoing investigations focus on potential immunological and inflammatory synergy between hCG and apoptotic blebs, since such blebs are a source of endogenous TLR ligands of relevance to lupus. Different apoptotic bleb preparations themselves varied in their capacity to induce cytokine and autoantibody secretion from splenocytes derived from lupus-prone mice. The addition of hCG along with particular bleb preparations led to synergistic enhancement of several inflammatory readouts, as well as of autoantibodies. These observations have potential clinical relevance, given the association of pregnancy with flares of lupus disease.

Hemoglobin (Hb) and lupus

An earlier report from our lab indicated that Hb induces deleterious inflammatory and immunological effects in lupus-prone mice (*Front Immunol. DOI: 10.3389/fimmu.2017.00732, 2017*). In current work, ferric Hb triggered preferential release of lupus-associated cytokines from splenocytes, B cells, T cells and plasmacytoid dendritic cells isolated from lupus-prone mice. PBMCs derived from SLE patients secreted enhanced levels of inflammatory cytokines in response to Hb; RNA-seq analysis revealed the up-modulation of mRNAs for *IL-1*, *Oncostatin M*, *CXCL1*, *CXCL5*, *IL-2RA* and *SOCS3*, all molecules implicated in lupus pathogenesis. Whether haptoglobin, which negates other inflammatory effects of Hb, can also reduce the Hb-driven generation of these molecules, is under study.

Taking a lead from previous data, Hb-self antigen interaction was assessed in pull-down assays; several lupus-associated autoantigens were identified. The combination of ferric Hb + apoptotic blebs (which contain packaged autoantigens) led to the enhanced secretion of lupus-associated cytokines and autoantibodies when incubated with splenocytes derived from lupus-prone mice. Infusion of ferric Hb into lupus-prone mice also induced the secretion of such cytokines and autoantibodies, and enhanced the onset of glomerulosclerosis. Accumulating evidence therefore suggests that Hb plays a significant role in lupus pathogenesis.

B. Delineation of the role of hCG in tumorigenesis

Employing C57BL/$6^{-/-}$ × $FVB^{\beta hCG/-}$ F1 mice, previous work described the effects of the transgenic expression of hCG on implanted Lewis Lung Carcinoma (LLC1) murine lung tumor cells. Of particular interest was the fact that ovariectomy resulted in higher tumor incidence, a decreased lag phase, and higher tumor volumes (*Oncotarget 9: 34670-34680, 2018*). On-going RNA-seq analysis is shedding light on the processes which promote enhanced tumor growth post-ovariectomy. Progesterone and estrogen caused a decrease in the viability of LLC1 cells *in vitro*. In ovariectomized hCG transgenic mice implanted with LLC1 cells, supplementation with progesterone reduced tumor volumes to those in non-ovariectomized transgenic mice. That ovarian steroids reduce the pro-tumorigenic effects of a gonadotropin is interesting, particularly in light of the fact that the incidence of some cancers in women increases post-menopause, coinciding with enhanced circulating levels of hCG.

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Reviews

1. Sachdeva R, Singh P, Bose A, Kalha B, Sarkar M, Pal R (2019) Anticancer immunotherapy: Prospects and challenges. In: **Unravelling cancer signaling pathways: A multidisciplinary approach** (Eds. Bose K and Chaudhari P, Springer Nature, Singapore) pp 189-228.

2. Bose A, Sharma H, Pal R (2019) Review of the "Annual Review of Immunology, Volume 35, 2017". **Curr Sci.** 16:1021-1024.

3. Kalha B, Sachdeva R, Bose A, Sarkar M, Sharma H, Malik M, Pal R (2020) Review of the "Annual Review of Immunology, Volume 36, 2018". **Curr Sci.** 118:480-485.

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

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Objectives

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 fraction' 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. 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 which is due to hyperactive immune response. Macrophages are the major host cells exploited by M.tb for its growth and are very much likely to play an important role in the activation of hyperactive $CD4^+$ T cell response. How $CD4^+$ T lymphocyte deficiency dysregulate the function of M.tb-infected macrophages remains largely unexplored. Studies done till now in the project have shown that macrophages of T cell deficient TCRβ−/− mice have higher activation status even without addition of CD4 T cells. As addition of CD4 T cells further stimulate the secretion of proinflammatory cytokines which raises the possibility that communication between CD4 T cells and macrophages could be a key factor driving the excessive inflammation during mycobacterial IRIS. Confocal microscopic analysis for the co-localization of GFP-M.tb in lysosomes suggest that TCRβ−/− mice macrophages have higher phagosome maturation and lysosome fusion activity.

Transfer of CD4 T cells into TCRβ−/− mice result in fatal wasting disease

To examine the outcome of T-cell reconstitution in TCRβ−/− mice during chronic mycobacterial infection, enriched labeled CD4 T cells were transplanted into TCRβ−/− mice. Recipient mice underwent rapid wasting, losing about 20-30 % of their initial body weight between days 7 and 14 after transfer. All the TCRβ−/− mice succumbed in about two week time after CD4 T cells transplantation. In contrast, infected C57Bl/6 WT recipient mice displayed no significant weight loss and mortality. M.tb load in lungs of TCRβ−/− and wild type mice was determined 30 days after M.tb infection. As compared to wild type mice, TCRβ−/−mice showed about one log higher bacterial load. This study describes a mice model for IRIS disease that recapitulates the fundamental immunological scenario of M.tb associated IRIS. Adoptive transfer of

purified CD4 T cells to TCRβ−/−mice infected with M.tb, led to inflammatory disease. Findings of our study show that somehow macrophages are differently activated in the absence of T cells which supports our hypothesis.

B. To delineate the mechanism of MIP mediated tumor reduction: Direct and Indirect effect of MIP on cancer cells

Earlier studies from our lab on very aggressive and poorly immunogenic melanoma (B16F10) tumor model provide evidence of immunotherapeutic potential of MIP for cancer. MIP therapy altered the immunosuppressive tumor milieu to immunologically active one, which contributed to tumor volume reduction. To analyse whether MIP also has direct effect on cancer cells, its uptake by cancer cells was checked. Significant uptake of MIP by B16F10 cancer cells was observed. Moreover, there was decrease in the number of cancer cells, when co-cultured with MIP *in vitro* at different multiplicity of infection. Multiplication status of the cancer cells after MIP treatment was analysed. CFSE assay showed that there was time dependent decrease in the number of cancer cells with increasing MOI of MIP. Based on the above findings, effect of MIP on cell cycle was studied. Initial studies of cell cycle analysis showed that MIP affects the cancer cell cycle progression. These results suggest that direct influence of MIP on the tumor cell cycle progression could be one of the important mechanism by which MIP therapy reduces the tumor volume.

Publications

Original peer-reviewed articles

1. Gupta A, Saqib M, Singh B, Pal L, Nishikanta A, Bhaskar S (2019) *Mycobacterium indicus pranii* induced memory T-Cells in lung airways are sentinels for improved protection against *Mtb* infection. **Front Immunol.** doi: 10.3389/fimmu.2019.02359.

2. Singh B, Saqib M, Chakraborty A, Bhaskar S (2019) Lipoarabinomannan from *Mycobacterium indicus pranii* shows immunostimulatory activity and induces autophagy in macrophages. **PLOS One** doi: 10.1371/journal.pone.0224239.

3. Kumar P, Das G, Bhaskar S (2019) *Mycobacterium indicus pranii* therapy induces tumor regression in MyD88 and TLR2 dependent manner. **BMC Res Notes** doi: 10.1186/s13104-019-4679-0.

Review

1. Bhaskar S, Singh B (2019) Immunotherapeutic potential of *Mycobacteriumindicus pranii* against tuberculosis. In: ***Mycobacterium tuberculosis*: Molecular infection biology, pathogenesis, diagnostics and new interventions** (Eds. Hasnain S, Ehtesham N, Grover S, Springer, Singapore) pp 407-417.

The NF-kB signaling system in human health and disease

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Microbial substances activate the canonical RelA NF-kB pathway as well as the IRF3 signaling axis. While RelA induces the expression of pro-inflammatory cytokines and chemokines, IRF3, either alone or in collaboration with RelA, activatesanti-viral genes encoding type-1 interferons. In addition, the tissue-morphogenetic lymphotoxin-b receptor (LTbR) induces the RelB NF-kB activity selectively *via* the noncanonical NF-kB pathway. A variety of biochemical mechanisms link these pathways within an integrated immune signaling network. We are addressing if such an interlinked network provides for signaling crosstalk and if such crosstalk has relevance for human health and diseases. Combining mathematical and experimental analyses, we could elucidate that the constituents of the noncanonical pathway contribute to the dynamical control of canonical NF-kB signaling and instructs the canonical NF-kB gene response. Both canonical NF-kB and IRF3 pathways have been implicated in pathological inflammation. We are currently investigating if noncanonical NF-kB signaling exacerbates aberrant intestinal inflammation associated with colitis involving dendritic cell autonomous signaling crosstalk. We are also examining the plausible role of noncanonical NF-kB signaling in anti-viral immunity. Our work bear promises for novel therapeutic intervention strategies targeting cross-regulatory signaling mechanisms.

Publications
Original peer-reviewed articles

1. Kar M, Khan NA, Panwar A, Bais SS, Basak S, Goel R, Sopory S, and Medigeshi GR. (2019) Zinc chelation specifically inhibits early stages of dengue virus replication by activation of NF-kB and induction of antiviral response in epithelial cell. **Front Immunol.** doi: 10.3389/fimmu.2019.02347.

2. Dhar A, Chawla M, Chattopadhyay S, Oswal N, Umar D, Gupta S, Bal V, Rath S, George A, Arimbasseri GA, Basak S (2019) Role of NF-kappaB2-p100 in regulatory T cell homeostasis and activation. **Sci Rep**. doi: 10.1038/s41598-019-50454-z.

3. Dhawan S, Ghosh S, Ravinder R, Bais SS, Basak S, Krishnan ANM, Agarwal M, Banerjee M and Haridas V (2019) Redox sensitive self-assembling dipeptide for sustained intracellular drug delivery. **Bioconjug Chem.** 30: 2458-2468.

4. Ahad A, Stevanin M, Smita S, Mishra GP, Gupta D, Waszak SM, Sarkar UA, Basak S, Gupta B, Acha-Orbea H, Raghav SK (2019) NCoR1: putting the brakes on the dendritic cell immune tolerance. **iScience** 19: 996-1011.

5. Bais SS, Ratra Y, Khan NA, Pandey R, Kushawaha PK, Tomar S, Medigeshi G, Singh A, Basak S (2019) Chandipura virus utilizes the pro-survival function of RelA NF-kB for its propagation. **J Virol.** doi: 10.1128/JVI.00081-19.

6. Chatterjee B, Roy P, Sarkar UA, Zhao M, Ratra Y, Singh A, Chawla M, De S, Gomes J, Sen S, Basak S (2019) Immune differentiation regulator p100 tunes NF-κB responses to TNF. **Front Immunol**. doi: 10.3389/ fimmu. 2019.00997.

Biology of follicular T helper cells in protective immunity

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Understanding the determinants of long-term sustained protective immunity may provide significant lead for rational design of vaccines against complex pathogens. By providing help to B cells, follicular T helper (Tfh) cells are indispensable for the generation of potent germinal centers (GCs) and GC-derived protective humoral responses. The biology of Tfh cells in sustained protective immunity is largely uncharacterized. Therefore, the conceptual framework of our 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.

A. Biology of Tfh cells in long-lasting protective immunity

Here, we are studying the characteristics and clonotypes diversity of human Tfh cells in sustained immunity established in response to license human SA14-14-2 live attenuated Japanese encephalitis (JE) vaccine. To identify the ideal Tfh traits in long-term protection we are comparing the responses in vaccine responders with the successfully recovered JE-patients. For longitudinal analysis in single-dose immunization with SA14-14-2 vaccine, a cohort was established in Assam. About 130 individuals with no existing flavivirus-IgG or -IgM antibodies were enrolled in the study. Around 63% of the individuals responded to the vaccine, though with varying extent. The comprehensive analysis of circulating Tfh cells suggests a robust expansion in activated Tfh cells (Fig.1A). The significant correlation of the frequency of activated Tfh cells with the magnitude of ASCs confirms robust development of germinal center reaction in response to SA14-14-2 vaccine (Fig. 1B). To characterize Tfh cells in JEV infection we have established the longitudinal cohort of JE patients in Dibrugarh, Assam. The extensive phenotyping suggest that the Tfh cells in patients with successful recovery displayed distinct phenotype as compared to the Tfh cells in vaccine responders. The work is now progressing to define the Tfh-basis of this observation in vaccination versus infection.

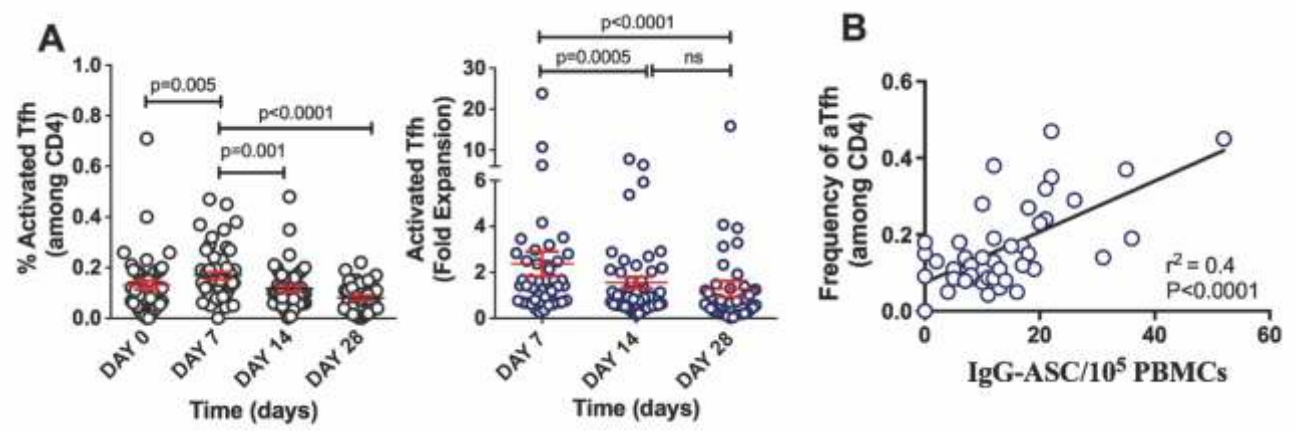

Fig. 1. Characterization of Tfh and Antibody Secreting cells (ASCs) in response to single dose SA14-14-2 vaccine. (A, *left panel*) Frequency of activated Tfh cells at different time points, (A, *right panel*) Fold expansion of activated Tfh cells at Day 7, 14 and 28 over Tfh cells pre-immunization. (B) Correlation of activated Tfh with ASCs. Statistical analysis: (A) Two Way ANOVA (B) Linear regression.

We have also established the JEV challenge and immunization models to delineate the mechanism of protective immunity establishment by historical SA14-14-2 vaccine. Our recent work highlights the crucial role of CD8 T cells in optimal differentiation of Tfh cells and in the establishment of protective antibody responses to the SA14-14-2 vaccine.

B. Function of Tfh cells in humoral immunity establishment to dengue virus

In this program, our attempts are focused on providing the insight into the determinants of antibody response to dengue virus. Understanding the biology of Tfh cells during the course of infection may provide the leads for formulating optimal antibody response in dengue. Here, we are exploring the biology of Tfh-cell and related subsets in various outcomes of dengue virus infection. Moreover, in multicenter program, we are studying the dengue virus specific Tfh-traits and Tfh-epitope composition enriched in Indian population. Our current report from the cohort of 185 dengue patients provides an insight into the cellular basis of antibody responses in dengue and describes a peripheral T helper subset that is capable of driving plasma cell differentiation, superior to the bonafide Tfh cells. Targeting this unique $CXCR5^{-}$ DP Th-subset might limit the exaggerated plasma cell differentiation and the antibody-induced immunopathology in Dengue.

T cell memory in infectious diseases in humans

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The existence of MHC class II-restricted CD4+ helper T cells with cytotoxic potential (CD4-CTLs) has been reported in humans with several viral infections (DENV, HIV, CMV, EBV). The magnitude of CD4-CTL response has been associated with the protection from several viral infections. Vaccinations against many viruses have shown to elicit CD4-CTL response (eg. YFV, polio virus vaccine). Despite their role in protective immune response, our understanding of the biology of CD4-CTLs in humans is limited. Compared to other T_H subsets, the molecular and epigenetic mechanisms that drive the differentiation, maintenance and function of human CD4-CTLs are poorly understood, mainly because of the lack of precise definition of the nature of this subset in humans. CD4-CTLs have long been considered to be short-lived terminal effector cells derived from T_{EM} cells following persistent or repeated (long-term) antigen stimulation in the context of certain viral infections, particularly CMV and DENV. Using high-resolution single-cell RNA-Sequencing (scRNA-Seq), we unravelled the CD4-CTL heterogeneity and functional properties and discovered a distinct subset of long-lived memory precursors of CD4-CTL effectors distinguished by higher expression of IL7R, a long-term homeostatic survival marker. The identification of precursors of CD4-CTL ($IL7R^{high}$ T_{EMRA}) displaying a molecular program indicative of long-lived memory precursor cells, intermixed with several features of CD4-CTL effectors, is an important discovery from our recent study. Given the established role of the IL7R signalling pathway in homeostatic T cell proliferation and survival, such $IL7R^{high}$ CD4-CTLs are likely to represent long-lived precursors with potential to differentiate to CD4-CTL effectors in the event of repeated infection. Isolation of CD4-CTL precursors based on surface expression of IL7R, will also enable detailed epigenetic studies to define the nature and extent of CD4-CTL reprogramming in such precursor cells generated in response to various viral infections. Our current ongoing efforts are to systematically understand and define molecular mechanisms that drive the development and differentiation of human CD4-CTLs and understand the molecular and functional properties of pathogen-specific human CD4-CTLs (hCMV-, EBV-, DENV-specific) by combining genomics and immunological tools. Briefly, we isolate different CD4 memory subsets (TN, TSCM, TCM, TEM, Precursor-TEMRA and effector-TEMRA) using flowcytometry, and simultaneously perform RNA-Seq, ATAC-Seq nd TCR-Seq to get a comprehensive understanding of the gene expression pattern, open chromatin states and T cell receptor repertoire in human donors with previous history of viral infections (will be tested for virus-specific IgG and IgM in plasma). Parallelly, we will perform scRNA-Seq using Smart-Seq2 that can simultaneously detect gene expression pattern and TCR repertoire, in antigen specific T cells to identify differences and similarities in CD4-CTLs generated in response to different viruses. These studies are likely to provide insights into the molecular mechanisms that govern the early development and function of CD4-CTLs in humans as they make transition from naive to memory precursor to effector cells. The data obtained can serve as important knowledge base in assessing the quality of CD4-CTLs developed following vaccination.

Nanotechnology-based immunotherapeutic platform for cancer

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Cancer is one of the leading causes of death despite the modern surgical interventions followed by chemo/radiation therapy. Host immune system is equipped with essential features for precise removal of the tumor from *in-vivo*, but the tumor cell can successfully survive by deploying a sophisticated and well-coordinated immune-suppressive machinery which includes but not limited to, reduced tumor-associated antigen (TAA) processing ability of professional antigen-presenting cells (APCs), compromised presentation of TAA on class-I major-histocompatibility complex (MHC) of tumor cell, the polarization of macrophages, recruitment of myeloid-derived suppressive cells (MDSC) and T-regulatory cells. Monoclonal antibodies such as anti-cytotoxic T-lymphocyte-associated protein 4(anti-CTLA4), programmed cell death protein 1(PD1) and/or PD1-ligand (PD1-L) show very encouraging results in patients by blocking the immune inhibitory signal exerted by the tumor. However, these treatments are not tumor antigen-specific, and they also only show promising results on a particular type of cancer or patients.

To encounter these challenges, our lab is deploying nanotechnology-based platforms for the development of cancer immunotherapy. We are formulating material based artificial antigen-presenting cells(aAPCs) to induce a sustainable anti-tumor immune response. The essential feature of material based aAPCs is that they remain nonresponsive towards the immune-inhibitory signal exerted by the tumor. We are investigating the role of quantitative measures of the number of tumor-specific peptide-MHC(I/II) along with co-stimulatory molecules on the nanoparticle to boost anti-tumor immune response. Additionally, we are exploring the nanotechnology-based therapeutic interventions to empower the APCs for the efficient presentation of TAA on class-I/II MHC molecules. We are also investigating the role of the chaperone network in the antigen presentation of tumor cell and guided by the data, we will formulate the nano-therapeutic platform for empowering the ability of tumor cell to process and present the TAA on class-I MHC to induce tumor-specific cytotoxic T lymphocytes. Collectively, our study holds promises for the development of therapeutic interventions to prevent/treat cancer growth and metastasis.

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 laboratory is aimed at understanding Sertoli cell (Sc) mediated regulation of spermatogenesis. Sperm production is a complex process that requires coordination between multiple cell types within the testes. Testicular Sc play an indispensible role in regulating germ cell division and differentiation. These cells respond to Follicle stimulating Hormone and testosterone to produce factors which in turn, ensure proper spermatogenic progression. Any defect in either Sc proliferation and/or impaired maturation would adversely affect spermatogenesis and as a consequence, lead to sub fertility or infertility. In such cases, hormone supplementation fails to restore spermatogenesis due to an intrinsic defect in the Sc of the affected individual. Therefore, understanding Sc mediated regulation of spermatogenesis can lead to the identification of genes crucial for spermatogenesis and pave way for the development of therapeutics for the treatment of idiopathic male infertility.

Multiomics studies (downstream *in-vivo* studies)

We have previously used high throughput transcriptomics and proteomics to identify novel transcription factors that are essential for Sc maturation and spermatogenesis (*Mandal et.al, DNA Research, 2017*). Our studies on transgenic mice with Sc specific knock down of specific transcription factors like YY1, RORα and Meis1 have established their roles in Sc mediated regulation of spermatogenesis. Presently, we are trying to decipher the role of CTCF transcription factor in Sc maturation using transgenic mice with Sc specific knockdown of CTCF.

Role of Hippo pathway in Sertoli cell signaling

Our studies on signal transduction in Sc have identified a role of Hippo pathway transducer YAP in regulation of TLR-2 mediated signaling in Sc. TLR-2 signaling is known to play an important role in microbial recognition and initiating immune response in the testes. Pharmacological inhibition of YAP using verteporfin was found to up-regulate the expression of TLR-2 target genes like *Cxcl1* and *Il-6* indicating negative regulation of TLR-2 signaling by YAP in pubertal Sc. Interestingly, YAP inhibition was found to synergize with TLR-2 signaling to increase the expression of these genes. Further investigations revealed a role of protein kinase A (PKA) in verteporfin induced up-regulation in *Cxcl1* and *Il-6*. These results indicated a role of YAP in regulating TLR-2 signaling in Sc. We are also assessing the effect of YAP inhibition on TNFα mediated signaling in Sc in-vitro.

microRNA studies in Sertoli cells

We are also looking into the role of small RNAs in Sc function. Our *in-vitro* studies have identified a number of miRNAs which are differentially expressed in infant and pubertal Sc. We have validated the targets of selected miRNAs like miR-92a-3p, miR-204-5p and miR-382-3p and are

currently trying to decipher their roles in Sc maturation and spermatogenesis using transgenic mouse models.

Publications

Original peer-reviewed article

1. Pradhan BS, Bhattacharya I, Sarkar R, Majumdar SS (2019) Downregulation of Sostdc1 in testicular Sertoli cells is prerequisite for onset of robust spermatogenesis at puberty. **Sci Rep.** doi: 10.1038/s41598-019-47930-x.

Review

1. Bhattacharya I, Sen Sharma S, Majumdar SS (2019) Pubertal orchestration of hormones and testis in primates. **Mol Reprod Dev.** 86:1505-1530.

Regulation of cell death

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Theme of research and objective

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.
Broadly, our research programme explores the underlying mechanisms of cell survival and death in diverse intracellular and extracellular conditions.

A. Autophagy in the *Leishmania* parasite

Our studies show an increase in autophagy during progression of *in vitro* life cycle of the parasite and upon exposure to stress stimuli like starvation, oxidative stress, and drugs. Chemical inhibitors for autophagy increased cell death, indicating the significance of autophagy in cellular defense against adverse conditions. Atg8 is a key autophagy protein contributing to autophagosome formation. A homolog of mammalian autophagy protein LC3, Atg8 was expressed in *Leishmania* parasite. With overexpression of Atg8 (Atg8-OE), the parasites became resistant to stress and their capability to infect macrophages in substantial numbers reduced. Disruption of the Atg8 gene (ΔAtg8) resulting in suppression of Atg8 protein expression, increased susceptibility to stress and reduced the capability to cause infection.

The failure of parasites lacking the Atg8 gene to differentiate into axenic amastigotes *in vitro* as opposed to control and Atg8 overexpressing cells established the involvement of Atg8 protein in differentiation to disease-causing amastigotes. The reduced survival of the parasites lacking the Atg8 gene after infection corroborates with the observation of their inability to differentiate *in vitro*. Understandably, changes in shape and cell size are accompanied by alterations in organelles as well, and therefore it is conceivable that lack of Atg8 prevented those events, stopping conversion to amastigotes. In the *in vivo* observations in a mouse model of leishmaniases where splenic enlargement with sufficient parasite load in splenic aspirates was seen in control mice infected with wild type parasites but were absent in those infected with parasites lacking the Atg8 gene. Interpretation of the data confirms the notion that Atg8 protein plays an important role in the infective abilities of the parasite. In summary, new evidences for a crucial role of Atg8 protein in sustaining *Leishmania* parasite survival during life cycle and stress exposure is supported by these studies.

B. Anti-leishmanial agents

Halictine-2, a novel antimicrobial peptide derived from the venom of eusocial honey bee, *Halictus sexcinctus* was tested for its anti-leishmanial activity *in vitro*. Anti-leishmanial activity was detected. Serine to threonine substitution at position 5 in native peptide enhanced the anti-leishmanial activity of the modified analogue (P^{5T}).

C. Defensive enzymes of *Leishmania*

We analyzed the host interactome of cTXNPx where apoptosis inducing factor (AIF) was one of the abundant fraction. *In* silico studies suggest binding

sites for AIF on cTXNPx. Results suggest that cTXNPx can interact with apoptotic machinery of host to retain the parasite survival intracellularly. This indicates a prominent of role of cTXNPx in preventing cellular apoptosis in addition to its function as a defensive enzyme. Further studies are being carried out.

Publications

Original peer-reviewed article

1. Giri S and Shaha C (2019) *Leishmania* donovani parasite requires Atg8 protein for infectivity and survival under stress. **Cell Death Dis.** doi: 10.1038/s41419-019-2038-7.

Review/Proceeding

1. Shaha, C (2020) Foreword.In. **Clay, craft, music and science: Purnima Sinha's life** (Ed: Rajinder Singh and S.C. Roy). Shaker Dueren, Germany. pp ix-xi.

Biology of trophoblast cells and immunocontraception

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Development of contraceptive vaccine

Female beagle dogs were immunized with i) alum (adjuvant) alone; ii) recombinant dog zona pellucida glycoprotein-3 (dZP3) fused with the promiscuous T cell epitope of tetanus toxoid (TT) separated by a dilysine spacer (TT-KK-dZP3); iii) recombinant protein encompassing T cell epitope of TT followed by dilysine linker, a fragment of dog ZP3 corresponding to the aa residues 307-346, triglycin spacer, T cell epitope of bovine RNase, GnRH, T cell epitope of circumsporozoite protein of *Plasmodium falciparum*, and another copy of GnRH (TT-KK-dZP3-GGG-bRNase-GnRH-CSP-GnRH abbreviated as dZP3-$GnRH_2$), and iv) physical mixture of recombinant porcine ZP3 with promiscuous T-cell epitope of TT and dilysine linker (TT-KK-pZP3) and porcine ZP4 with promiscuous T-cell epitope of bovine RNase and dilysine linker (bRNase-KK-pZP4) in 1:1 ratio. All the animals received 3 intramuscular injections at 4 week intervals. Analysis of pre-immune serum samples as well as those collected 15 days after the first and second boosters revealed good antibody titres in the dogs immunized with dZP3-$GnRH_2$, and physical mixture of TT-KK-pZP3 & bRNase-KK-pZP4. The group of animals immunized with recombinant TT-KK-dZP3 revealed low antibody titres. Mating studies revealed that female dogs immunized with recombinant dZP3-$GnRH_2$ showed promising contraceptive efficacy.

Molecular mechanisms associated with trophoblast migration, invasion and differentiation

i) Trophoblast migration

Previously, we confirmed by qRT-PCR that treatment of HTR-8/SVneo cells with HGF (50 ng/ml) led to up-regulation of miR-33a-5p whereas miR-320c and miR-18a-3p were down-regulated in their expression. Inhibition of miR-33a-5p by inhibitor and over-expression of miR-18a-3p as well as miR-320c by respective mimics led to a significant reduction in HGF-mediated HTR-8/SVneo cells migration.

ii) Trophoblastic cell invasion

To evaluate whether MAPK8 and FAS are direct targets of miR-92a-1-5p, short nucleotides with the predicted wild type (WT) and mutated complementary sequence for both MAPK8 and FAS were synthesized and cloned downstream of firefly Luciferase in pmirGLO Dual-Luciferase reporter vector, and the effect of miR-92a-1-5p overexpression on luciferase activity was evaluated. Transfection of WT MAPK8 and FAS in HEK-293T cells along with miR-92a-1-5p mimic, led to a significant reduction in the luciferase activity, but the mutation in the binding site in the 3'-UTR of both MAPK8 and FAS suppressed the inhibition of luciferase activity caused by miR-92a-1-5p overexpression (Fig. 1). Thus, miR-92a-1-5p inhibits MAPK8 and FAS expression through direct interaction with the predicted complementary sequence present in their 3'-UTR. A direct regulatory

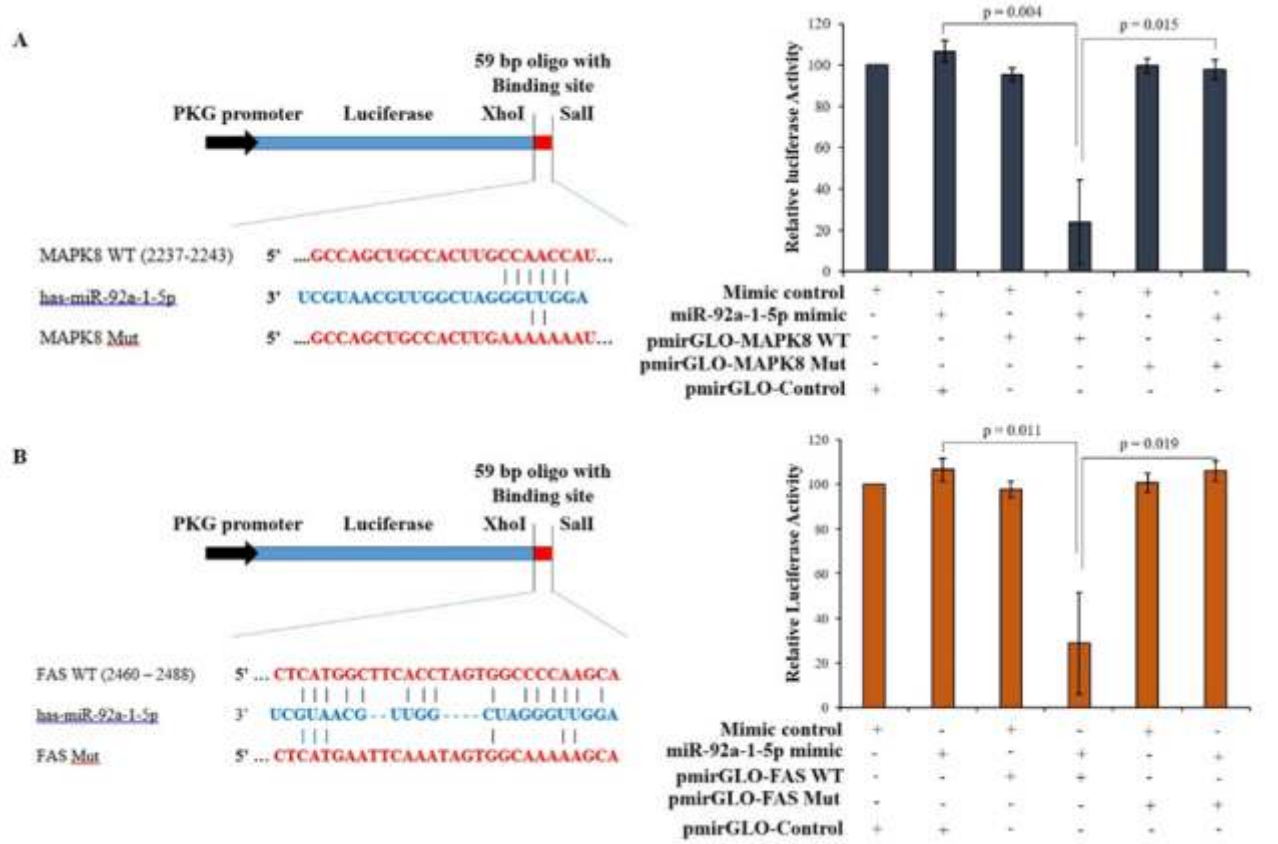

Fig. 1: Direct regulatory relationship between miR-92a-1-5p, FAS and MAPK8. Wild type (WT) and mutated MAPK8 and FAS complementary binding sites to miR-92a-1-5p seed sequence were cloned downstream of the firefly luciferase gene in pmirGLO Dual-Luciferase vector. Panels A and B show the WT and mutated sequences, and luciferase activity determined from HEK-293T cells co-transfected with clones possessing the WT or mutant binding site for miR-92a-1-5p corresponding to MAPK8 and FAS respectively with control or miR-92a-1-5p mimic transfection, as indicated. The data is represented as the mean of four experiments ± S.E.M. performed in duplicates.

effect of the two synthetically synthesized STAT3 binding sites was observed when co-transfection of STAT3 mammalian expression vector and pmirGLO plasmid with STAT3 binding site pertaining to miR-92a-1-5p promoter region was performed.

iii) Trophoblastic cell differentiation

During forskolin (25 µM)-mediated BeWo cell fusion, 3β-hydroxysteroid dehydrogenase/steroid Δ 5,4-isomerase-1(HSD3β1) and WNT2B were confirmed by Western blot and qRT-PCR as the targets of miR-27b-5p. Silencing of both *HSD3*b*1* and *Wnt2B*, led to reduced forskolin-mediated BeWo cells fusion and reduction in the secretion of progesterone/human chorionic gonadotropin.

Publications

Original peer reviewed articles

1. Minhas V, Kumar R, Moitra T, Singh R, Panda AK, Gupta SK (2019) Immunogenicity and contraceptive efficacy of recombinant fusion protein encompassing Sp17 spermatozoa specific protein and GnRH: relevance of adjuvants and micro-particles based delivery to minimize number of injections. **Am J Reprod Immunol.** doi: 10.1111/aji.13218.

2. Verma S, Kang AK, Pal R, Gupta SK (2020) BST2 regulates interferon gamma-dependent decrease in invasion of HTR-8/SVneo cells via STAT1 and AKT signaling pathways and expression of E-cadherin. **Cell Adh Migr** .14: 24-41.

3. Mishra NN, Agarwal A, Polachira SK, Nair R, Gupta SK (2019) Anti-HIV-1 activity and safety profile of polyherbal gel formulation as candidate microbicide. **J Herbal Med.** doi: 10.1016/j.hermed.2019.100284.

Patents

1. Gupta SK, Shembekar N, Mishra A, Varadarajan R, Mallajosyula VMA, Kapre SV, Yoelekar L, Dhere R. Monoclonal antibody specific to hemagglutinin from influenza virus H1-subtype and uses thereof. (Indian Patent Number 318710, Granted on 22nd August 2019).

2. Polachira SK, Nair R, Jayalekha R, Gupta SK, Mishra NN, Agarwal A. Herbal microbicide formulation for preventing HIV
(Indonesian Patent application No. P00201907700 filed on December 9, 2019)

3. Polachira SK, Nair R, Jayalekha R, Gupta SK, Mishra NN, Agarwal A. Herbal microbicide formulation for preventing HIV.
(South African Patent application filed in 2019)

4. Polachira SK, Nair R, Jayalekha R, Gupta SK, Mishra NN, Agarwal A. Herbal microbicide formulation for preventing HIV
(ARIPO Patent Application filed in 2019)

MOLECULAR DESIGN

Structural studies on proteins, dynamics and ligand interactions using NMR

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Theme of research

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

Objectives

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.

Structural studies on the biotin protein ligase of *Leishmania*

This year, we report studies on the biotin protein ligase of *Leishmania,* an offshoot of the fatty acid biosynthesis pathway. The first committed step of fatty acid biosynthesis pathway is the formation of malonyl-CoA from acetyl-CoA, in the presence of the enzyme acetyl-CoA carboxylase. The reaction involves ATP-dependent carboxylation of the biotin prosthetic group, followed by the transfer of the carboxyl group from biotin to acetyl-CoA. Biotin protein ligase can post-translationally modify the BCCP domain of acetyl-CoA carboxylase, methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase, transcarboxylase, oxaloacetate decarboxylase and pyruvate carboxylase by covalently linking biotin to their lysine residue. In the absence of this enzyme, the mitochondrial carboxylases are inactive.

Leishmania biotin protein ligase belongs to Class I, containing an N-terminal catalytic domain, and a C-terminal cap binding domain. Biochemical studies on biotin protein ligase were also carried out. As biotin protein ligase is a 30.5 kDa protein, with no structural information available, crystallization of biotin protein ligase was carried out using hanging drop vapour diffusion method and diffraction images were collected at ESRF synchrotron facility. The data was processed to a resolution of data set was 1.9 A˚ with 99.6 % completeness. Two biotin protein ligase structures PDB 2EJ9 and 2DXU were used to prepare the starting model.

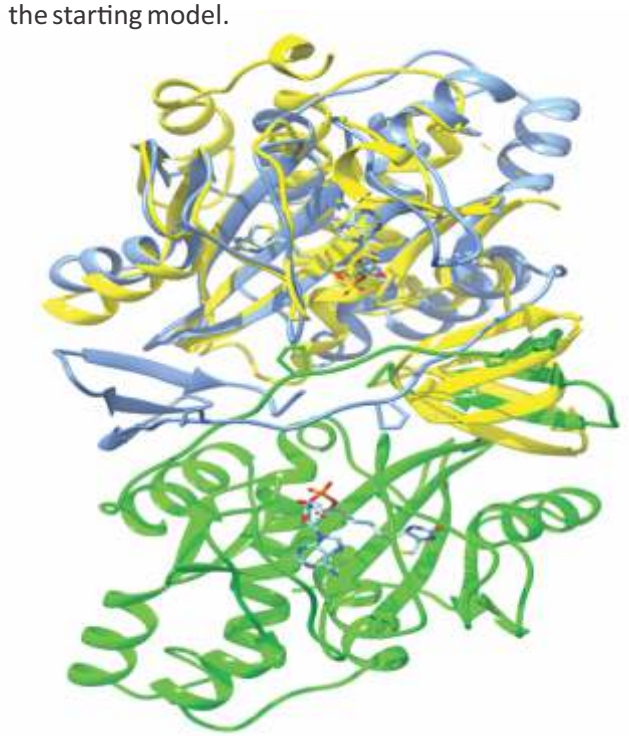

Fig. 1: Crystal structure of the biotin protein ligase of Leishmania major. The two molecules forming a dimer are shown in blue and green. The biotin molecule is shown as ball and stick. The structure of biotin protein ligase of M. tuberculosis is shown in yellow.

The crystal structure of biotin protein ligase of *L. major* comprises an N-terminal catalytic domain, and a C-terminal domain with a long loop. Biotin-AMP was found associated with the active site in all the structures. *L. major* biotin protein ligase forms a unique proline based domain swapped dimer, also called as hand shake interaction, not reported before. The complete data set was collected up to 1.96 Å resolution, and was processed by ESRF auto process software. The protein crystallized in the space group C 121(C24), with unit-cell parameters a = 116.85, b = 46.62, c = 54.57 Å, α-90, β-104.54, g-90. Assuming the presence of one molecule of biotin protein ligase from *L. major* (with calculated molecular weight of 30.5 kDa) in the crystal asymmetric unit, the calculated values of the Matthews coefficient and the corresponding solvent content were 2.40 $Å^3$ Da^{-1} and 48.73%, respectively. The activity of the enzyme was tested against the cognate biotin carboxyl carrier protein domains of methylcrotonyl-CoA carboxylase and acetyl CoA carboxylase. Mutagenesis studies were designed to mutate the two prolines liable for dimerzation of the enzyme. Wild type as well as the double proline mutant appear as monomer in gel filtration, suggesting that the dimer in crystallization is probably concentration dependent.

In an attempt to identify potential inhibitors that target this enzyme of *Leishmania*, AutoDock was used to screen NCI small molecule libraries (Diversity sets II, III and IV), followed by enzyme assay. The shortlisted candidates were tested for their ability to inhibit the growth of *Leishmania donovani* (Ld1S) culture using the alamar blue cell viability against the promastigotes (26°C, pH 6.8), axenic amastigotes (37°C, pH 5.5) and intracellular amastigotes (37°C, pH 5.5), respectively. Two inhibitors were identified that inhibit all three stages of *Leishmania donovani*.

Publications

Original peer reviewed articles

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2. Kumar S, Dhembla C, P H, Sundd M, Patel AK (2020) Differential expression of structural and functional proteins during bean common mosaic virus-host plant interaction. **Microb Pathog.** doi: 10.1016/j.micpath.2019.103812.

To develop strategies for making sensors and actuators for biological processes

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A. Effects of immune dysregulation and development of disease regulating strategies for retinitis pigmentosa

Retinitis Pigmentosa (RP) is a heritable ocular disease. The disease causes progressive photoreceptor degeneration due to genetic mutation. 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.

The changes are initiated with the loss of photoreceptor depleting the oxygen requirement in the retinal layer. This depletion attenuates the retinal vessels causing hypoxia in the eye. Studies show that the blood retinal barrier (BRB) is susceptible to hypoxic damage and cause its breakdown.

In the rd1 mouse, the expression profile of inflammatory photoreceptor molecules confirmed that due to the mutational stress on the photoreceptors, they release molecules such as COX2 and iNOS which in turn potentiate the chronic inflammation in the eye. The mRNA and protein expression profiles of pro-inflammatory cytokines such as IL-6, TNF-a, IL-7 was upregulated in rd1 and rd1.NOD mice compared to B6 mice models. Similarly, anti-inflammatory cytokine such as IL-10, TGF-b1 were downregulated in rd1 mice. Further the cytokine profile and Vascular endothelial growth factor (Vegf) levels was also elevated in RP patients (n=30) indicating systemic hypoxia.

The breakdown of BRB due to the ocular inflammatory insult was investigated by Sodium fluorescein angiography. Sodium fluorescein angiography study confirmed the leakage in the BRB as patches of extravasate dye.

Typically, the RPE layer has inhibitory function on peripheral immune cells by mediating MHC-I complexed ocular antigens presentation to the peripheral immune cells. Upon the breakdown of BRB, the mRNA expression profile indicates an upregulation of MHC-II in the RPE layer, which is not classically expressed on RPE. The FACS study on the cell suspension made from RPE layer indicated the significant upregulation of MHC-II. Suggesting that the RPE can potentially function as APC with ocular antigens priming the peripheral immune cells.

B. The utility of reprogrammed monocytes (RM) as a cell-based therapy in sepsis

Generating an animal model of sepsis

To generate a model of polymicrobial sepsis in mice, Cecal Ligation and Puncture (CLP) surgery was performed in BALB/c mice of age 4-6 weeks. The model was validated by hypothermia, hypoglycaemia

and liver specific enzymes. The survival time of animal post-surgery was studied.

Analysing the role of RM in resolution of sepsis

To investigate the effect of RM transplantation on rescue from sepsis, $1x10^6$ cells were transplanted in animals via intraperitoneal route and the percentage survival was considered as the key criterion for rescue from sepsis.

In the survival study, the percentage survival was calculated as survival at 24±2 hrs time point and secondly as the absolute survival. For absolute survival animals were monitored for 5 days post CLP. Amongst all the treatment groups a maximum survival of 66.6% was observed at 24±2hrs in group which received a combination of antibiotic and RM at early stage of disease (*i.e.* 4hrs post-surgery) suggesting that RM transplantation has beneficial effect on survival of septic animals.

Publications

Original peer reviewed articles

1. Nagpal PS, Kesarwani A, Sahu P, Upadhyay P (2019) Aerosol immunization by alginate coated mycobacterium (BCG/MIP) particles provide enhanced immune response and protective efficacy than aerosol of plain mycobacterium against *Mtb*. H37Rv infection in mice. **BMC Infect Dis.** doi: 10.1186/s12879-019-4157-2.

2. Sahay P, Jain K, Sinha P, Das B, Mishra A, Kesarwani A, Sahu P, Mohan K V, Kumar MJM, Nagarajan P, Upadhyay P (2019) Generation of a rat model of acute liver failure by combining 70% partial hepatectomy and acetaminophen. **J. Vis. Exp.** doi:10.3791/60146.

3. Ranjan V, Mishra A, Kesarwani A, Mohan KV, Lal SN, Puliyel J, Upadhyay P (2020) Mother-to-child transfer of reactivated varicella-zoster virus DNA and varicella-zoster IgG in pregnancy. **Viral Immunol.** 33:72-76.

Protease-catalyzed splicing of peptide bond

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We have been exploiting the peptide ligation propensity of transpeptidase sortase for semisynthesis of proteins and well defined bioconjugates. Contemporaneously, we also study interrelationship between structure, function, and dynamics of sortase family enzymes.

Studies on structure, dynamics and function of sortases

Engineering cyclic sortases: We undertook engineering of cyclic sortases through the mediation of isopeptide-linked fragment complementing system (SpyTag-SpyCatcher) present in CnaB2 domain of fibronectin binding protein from *Streptococcus pyogenes.* Two constructs of sortases (cSrt1 and cSrt2) were engineered. Both constructs were found to be active but were produced in low yields. Besides, the presence of linear oligomers posed problems during purification. We explored newer constructs to circumvent these problems. We designed several constructs of the above sortases by varying the GGGGS units in N- and C-terminal region preceding the CnaB2 domain segments to enhance flexibility and facilitate encounter of the SpyTag-SpyCatcher fragments. The studies of sortase constructs are at various stages of expression, purification and characterization.

Structure-function analysis of TfSrtE: In the past year, we reported the expression, purification and characterization of a class E sortase (TfSrtE) from *Thermobifida fusca.* TfSrtE was found to transfer both LPXTG and LAXTG peptides to Gly-nucleophiles with almost equal efficiency. This was an interesting result in view of the fact that class E sortases are known to prefer Ala-substrates (LAXTG) as compared to Pro (LPXTG). Previous work from our laboratory identified a crucial Tyr residue (Tyr112) in *S. avermitilis* SrtE for its Ala-substrate preference. In view of this, we subjected the equivalent residue, Tyr128, in TfSrtE to site-directed mutagenesis and created two mutants, TfSrtE(Y128F) and TfSrtE(Y128A), respectively. The transpeptidation assays of mutants with Ala- and Pro-based peptide substrate yielded interesting results. Y128F mutant was found to be as active or slightly better than the wild type enzyme with respect to the Pro-substrate but its activity against the Ala-substrate was almost abrogated. In contrast, transpeptidation activity against both, Ala- and Pro-substrates, was completely lost in the Y128A mutant. Curiously, Y128A mutant was endowed with high proteolytic activity. Taken together the above results highlight the critical role of the above conserved Tyr residue in fine tuning of substrate preference in sortase family of transpeptidases.

Sortase-mediated protein labeling and conjugation

We had reported the homogeneous preparation of H2BK5Ac and H3K4Ac by sortase-mediated ligation of appropriate complementary fragments generated by chemical synthesis, proteolysis or recombinant expression. The work during this period was focussed on further screening and validation of HDAC and sirtuins as erasers of H2BK5Ac. Accordingly, HEK293 cells were transfected with respective plasmid DNA expressing individual human HDACs / sirtuins, and cell lysates were

prepared for deacetylation assays. The overall data emanating from deacetylation assays with entire gamut of human HDAC/sirtuins, using overexpressed cell lysates, suggested HDAC1 as a prime eraser of K5 acetylation in H2B. That this was indeed the case was further corroborated using a purified preparation of recombinant human HDAC1. Importantly, semisynthetic H2BK5Ac assembled into an octamer, and reconstituted into a nucleosome. Deacetylation of H2BK5ac in nucleosomes was evaluated using cell lysates overexpressed with HDAC1 or HDAC 6, and respective purified recombinant enzymes. The results of assays revealed facile deacetylation of H2BK5ac only in the presence of HDAC1 corroborating the specificity of HDAC1 for K5ac in H2B. Cumulatively, the results show that HDAC1 preferentially deacetylates H2BK5ac in both, isolation as well as in the nucleosomes.

Publications

Original peer-reviewed article

1. Singh S, Gupta K, Shukla S, Sampathkumar S-G, Roy RP (2019) Sortase-click strategy for defined protein conjugation on a heptavalent cyclodextrin scaffold. **PLOS One**. doi:10.1371/journal.pone.0217369.

Patent

1. Roy RP, Samantaray S. India Novel bioconjugates as therapeutic agents and synthesis thereof, (Indian Patent Application No. 320958, granted on 20th September, 2019).

Therapeutic interventions in chronic diseases

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Theme of research

My group is a multi-disciplinary group adapting an integrated approach in drug discovery that combines medicinal chemistry, basic biology and biochemistry principles for efficient drug design process. Interests of the group lie in identifying underlying principles in a disease pathogenesis to discover new targets, designing molecular intervention strategies and confirming the biological/therapeutic activities of the designed compounds. The small molecule regulators contribute to both drug development and understanding biological systems in human body.

Objective

1. To study the Bisphenol A triggered axonal injury and myelin degeneration with concomitant neurobehavioral toxicity in C57BL/6J male mice.

To study the Bisphenol A triggered axonal injury and myelin degeneration with concomitant neurobehavioral toxicity in C57BL/6J male mice

Bisphenol A (BPA) is a ubiquitously distributed endocrine disrupting chemical (EDC). BPA exposure in humans has been a matter of concern due to its increased application in the products of day to day use. BPA has been reported to cause toxicity in almost all the vital organ systems even at a very low dose levels. It crosses the blood brain barrier and causes neurotoxicity. Our study focuses on the adult exposure effects of BPA on axonal and myelin structural proteins and neuroinflammatory marker proteins in adult C57BL/6J male mice with the aim to decipher the role of BPA in demyelinating disorders.We studied the effect of BPA on the cerebral cortex of C57BL/6J mice and examined whether BPA exposure alters the expression of axonal and myelin structural proteins. The oral dose of 40 µg and 400 µg BPA/kg for 60 days BPA exposure resulted in memory loss, muscle coordination deficits and allodynia. BPA exposure also caused degeneration of immature and mature oligodendrocytes. It was observed that subchronic BPA exposure caused neuroinflammation through deregulation of inflammatory cytokines mRNA and protein expression which further resulted into neurotoxicity through axonal as well as myelin degeneration in the brain. BPA also caused increased

oxidative stress in the brain. Our study indicates long-term subchronic low dose exposure to BPA has the potential to cause axonal degeneration and demyelination in the oligodendrocytes and neurons which may have implications in neurological and neuropsychological disorders including multiple sclerosis (MS), neuromyelitis optica and others.

Future plans

- Screening of small molecular inhibitors of amyloid beta, alpha synuclein and transthyretin aggregation and their preventive and therapeutic efficacy in animal models.
- Investigating the role of ERAP as a common cellular player for modulating amyloid load and neurodegeneration.
- Investigating the role of Arl6ip5 in the regulation of autophagy in neuronal cells and neurodegenerative disorders.
- To understand the mechanism of bone loss in AD mouse models.

Publications

Original peer-reviewed articles

1. Pal M, Khan J, Kumar R, Surolia A, Gupta S (2019) Testosterone supplementation improves insulin responsiveness in HFD fed male T2DM mice and potentiates insulin signaling in the skeletal muscle and C2C12 myocyte cell line. **PLOS One.** doi:10.1371/ journal.pone.0224162.

2. Khan J, Salhotra S, Goswami P, Akhter J, Jahan S, Gupta S, Sharma S, Banerjee BD, Parvez S, Gupta S, Raisuddin S (2019) Bisphenol A triggers axonal injury and myelin degeneration with concomitant neurobehavi-oral toxicity in C57BL/6J male mice. **Toxicology.** doi: 10.1016/ j.tox.2019.152299.

Patent

1. Gupta S, Surolia A, Gautam RK and Diwedi VK. Synthetic peptides and random copolymers for the treatment of autoimmune disorders. (Indian Patent Application No. 328267 granted on 27th December, 2019).

Molecular mechanism of enzymatic reactions and enzyme-ligand interactions

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Interdomain interactions mediated by the helical domain of hGBP-2 are not essential for GMP formation

A comparison of the hGBP-1 crystal structure and hGBP2 model structure showed that there were variations in the interdomain interactions. To investigate whether the lower amount of GMP formation in hGBP-2 is because of the absence of specific interdomain interactions essential for tetramerization and whether they can be restored in the presence of the intermediate and helical domain of hGBP-1, two chimeras, CH-I and CH-II were prepared. In these two chimeras, the N-terminal GTP-binding domain of hGBP-2 was kept intact, but the other two regions were gradually exchanged with that of hGBP-1. CH-I produced nearly 4-fold higher GMP and 1.5-fold lower GDP as compared to hGBP-2. However, CH-II produced GMP similar to hGBP-2 with a marginal decrease in GDP production. In CH-I, the k_{cat} value for GMP formation was increased by 2.6-fold, but the $k_{cat}/K_{0.5}$ value for GDP formation was reduced by 4.2-fold as compared to hGBP-2. However, for CH-II the k_{cat} value for GMP formation did not vary, although a marginal decrease in the $k_{cat}/K_{0.5}$ value for GDP formation was observed (1.5-fold). Overall, these data suggest that the helical domain of hGBP-1 in chimeras could not restore the specific interdomain interactions similar to hGBP-1. The data also suggest that unlike in hGBP-1, the helical domain of hGBP-2 has no role in the stimulation of GMP formation.

Tetramer of hGBP-2 has no role in GMP formation

To understand whether the significantly lower amount of GMP in these chimeras as compared to hGBP-1 is due to their inability to form a tetramer, analytical gel-filtration measurements in the absence and presence of either GppNHp or GDP.AlF_4 were performed. Without the analogue, CH-I eluted as a mixture of monomer, dimer and tetramer with monomer being the major form. The elution profile for CH-I with GppNHp showed an increase in the fraction of both dimer and tetramer, but still a considerable amount of monomer was present. However, with GDP.AlF_4 a significant fraction was eluted as a dimer, but the proportion of tetramer did not increase further. Thus, these results suggest that the increased GMP formation in this chimera could be associated with the activity of either substrate- or transition state-induced dimer rather than the activity of dimer or tetramer observed in the absence of analogue.

Without the analogue, CH-II primarily eluted as a monomer. But with GppNHp the elution profile showed the presence of both monomer and dimer along with a marginal increase of tetramer. This profile did not change further with GDP.AlF_4. Despite the absence of the transition state-induced tetramer,

CH-II produced GMP similar to hGBP-2, suggesting that the tetramer of hGBP-2 is not essential for GMP formation. These data also suggest that the interactions mainly between the helical and GTP-binding domains of hGBP-2 are essential for tetramer formation. Overall, these data provide an important insight that unlike in hGBP-1, the tetramer of hGBP-2 has no role in GMP formation.

GTP-binding domain is responsible for the lower GMP formation

A comparison of the sequence of the intermediate region between hGBP-1 and hGBP-2 reveals that hGBP-2 has a Pro instead of a Gln at 285 position. To evaluate the impact of this change in GMP formation, a hGBP-2 Pro285Gln mutant was prepared, which produced GMP similar to wild-type protein. This observation rules out the impact of the amino acid variation in the intermediate region for the reduced GMP formation. Therefore, the lower GMP formation in hGBP-2 as compared to hGBP-1 is mainly due to the difference in their GTP-binding domain. The GTP-binding domain of hGBP-1 (278 residues) structurally resembles the canonical small GTPases (~170 residues), but this domain is relatively larger in size primarily because of the insertion of five motifs (I1-I5). The sequence comparison of this domain in hGBP-1 and hGBP-2 also reveals that the substrate-binding motifs and catalytic residues are conserved. However, the residues present in the insertion motifs of hGBP-2 show variation, which might be responsible for the lower amount of GMP formation, since in hGBP-1 these motifs have been found to be essential for the second phosphate cleavage of GTP.

Publications

Original peer-reviewed articles

1. Rajan S, Pandita E, Mittal M, Sau AK (2019) Understanding the lower GMP formation in large GTPase hGBP-2 and role of its individual domains in regulation of GTP hydrolysis. **FEBS J.** 286:4103-4121.

2. Dutta A, Mazumder M, Alam M, Gourinath S, Sau AK (2019) Metal-induced change in catalytic loop positioning in *H. pylori* arginase alters catalytic function. **Biochem J.** 476:3595-3614.

3. Rajan S, Sau AK (2020) The alpha helix of the intermediate region in hGBP-1 acts as a coupler for enhanced GMP formation. **Biochim Biophys Acta Proteins Proteom.** doi:10.1016/j.bbapap.2020.140364.

Structural and biochemical studies of *Mycobacterium tuberculosis* proteins

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Theme of research

A major project of my laboratory aims at deriving mechanistic understanding of Histidine (His) production by *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB) in humans. Briefly, *Mtb* biosynthesizes His *de novo* from 5-phosphoribosyl-1-pyrophosphate employing 10 enzymes through 10 distinct steps. The essentiality of this pathway for *Mtb* infection combined with its absence in humans suggests that the enzymes of this pathway are important anti-TB drug targets In the past several years we have elucidated the 3D structures of as many as five enzymes of this pathway and have dissected out their biochemical properties. Using structural and biochemical information particularly of HisB (Imidazole glycerol phosphate dehydratase), we have designed specific inhibitors against this enzyme. In another project, we focus on elucidating structure-function relationship of membrane associated proteases and enzymes of glycolysis pathway.

Elucidation of 3D structures of enolase by X-ray Crystallography and Electron Microscopy

Enolase, a key conserved glycolytic enzyme, that catalyzes the reversible conversion of 2 phosphoglycerate (2PG) to phosphoenol pyruvate (PEP), important for energy production is an essential enzyme for mycobacterial growth and virulence. To derive a mechanistic understanding on the action of this enzyme, we sought to determine its 3D structure and carry out biochemical studies. The recombinant *Mtb* enolase we prepared for this purpose comprising of an N-terminal hexa-His tag makes a peptide of 435 (6X His + 429 *Mtb* enolase specific residues) amino acids long. The activity of enolase was determined by monitoring the decrease in absorbance of PEP at 240 nm. We determined its native and 2PG bound crystal structures. The N-terminal domain folds into a central 3-stranded mixed twisted β-sheet resting on two long α-helices. On the other hand, C-terminal domain comprising of a central β-barrel of 8-stranded mixed β-sheet surrounded by nine -helices and three 3_{10} helices forms the characteristic β-a barrel of enolase. In order to elucidate the conformation and oligomeric state of *Mtb* enolase in solution we determined its structure using electron microscopy. The apo-enzyme and PEP-enolase complex at 3.5 mg ml^{-1} were frozen with Quantifoil holey carbon grids (R 0.6/1, Au 300 mesh) and a Vitrobot Mark IV set at 100% humidity and 16°C, with blotting for 3.5 seconds. Data from both the apo and the PEP-enolase were collected with Titan Krios at the National CryoEM facility, Bangalore and Falcon 3 detector in counting mode at

1.07 Å/pixel sampling with images exposed for 60 seconds, with a total accumulated dose of ~27.70 e - /Å 2 and dose fractionated into 25 frames, with each frame having a dose of ~1.1 e - . The data sets were further processed in Relion 3.0 including full frame alignment. The summed images were subsequently used for automated particle picking with Gautomatch, with template derived from manual picking of the particles in Relion 3.0 and CTF was estimated with Gctf. Particles were extracted with a box size of 320 pixels and subjected to two rounds of 2D classification, 3D auto-refinement, per particle CTF refinement, B-factor weighting with Bayesian polishing and refinement, and subsequent 3D classification in Relion 3.0 with D4 symmetry imposed. The nominal resolutions of for apo-enolase

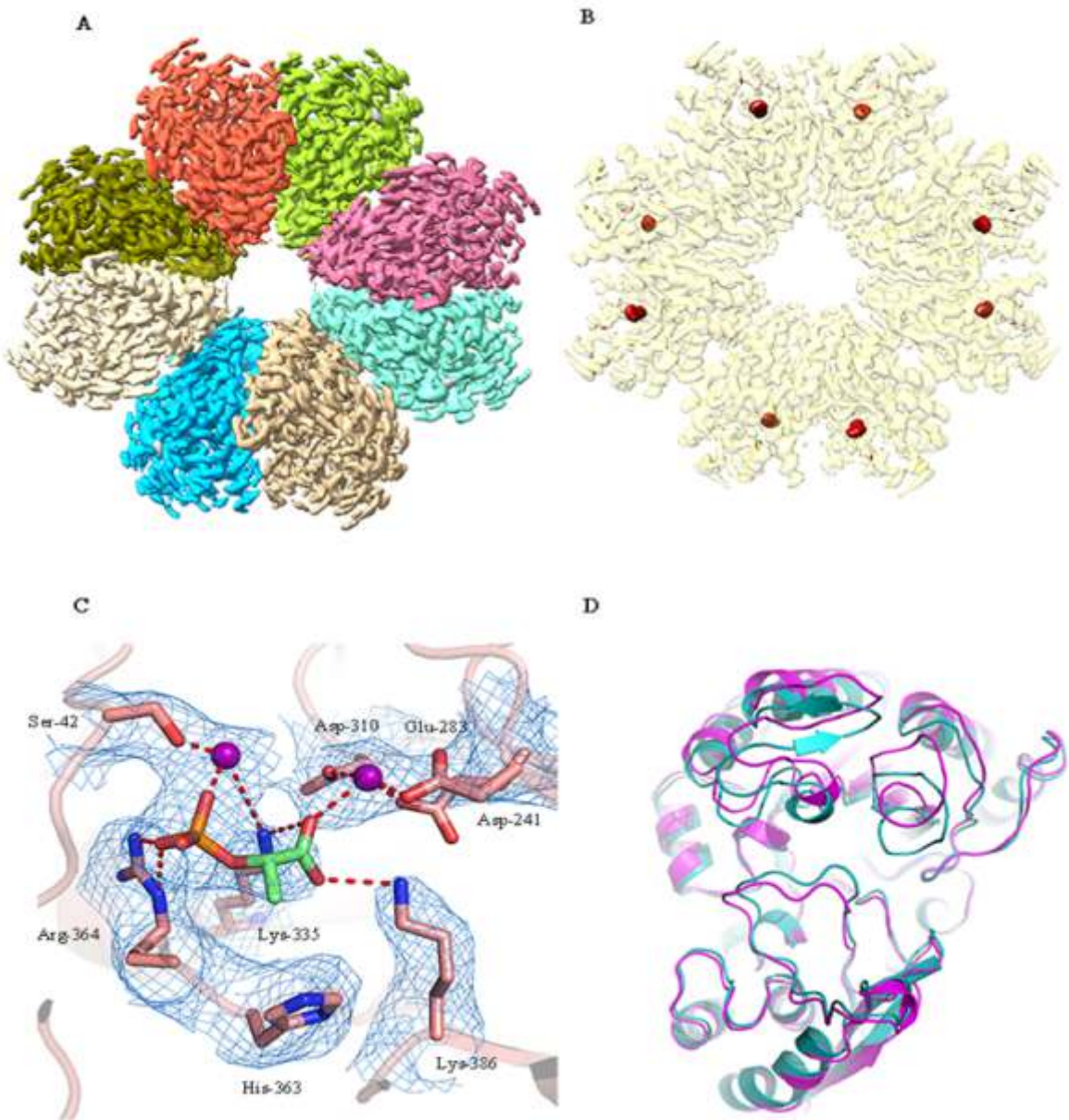

Fig. 1: A. The final B-factor sharpened 3.08 Å Cryo-EM map of the Apo-*Mtb*-Enolase octamer. Each monomer is colored differently. **B.** The difference map (in red) between the PEP bound and apo-Enolase data set shows presence of non protein density in the active site. The apo-Mtb-enolase cryo-EM map (yellow) is shown as reference. **C.** The cryo-EM map around the active site of Mtb-enolase featuring the modeled Mg^{2+} and the ligand, PEP. The residues interacting with the metal and the ligand are represented in sticks. **D.** Superimposition of the crystal structure of 2PG-Enolase (teal) over the cryo-EM structure (magenta) of the apo-enolase shows that except for the ligand binding loops the rest of the structure superimposes with a low rmsd.

(Fig.1A) and PEP-enolase were 3.1 Å and 3.2 Å respectively. Difference maps were calculated with final unsharpened map to verify the presence of PEP in the active site (Fig.1B). Local resolution of the maps was estimated with Resmap. The crystal structure of native enolase (pdb id : 6KKC) was used as the initial model and was docked into the B-factor sharpened map (i.e. after postprocess) and refined using phenix.real_space_refine. Presence of non-protein density in the active site of all the eight monomers led us to model PEP and Mg^{2+} in that location (Fig. 1C). A comparison of the 2.0 Å crystal structure of 2PG-enolase (teal) over the 3.08 Å cryo-EM structure (magenta) of the apo-enolase shows that except for the ligand binding loops the rest of the structure superimposes with a low rmsd (Fig. 1D). Structural and Biochemical characterization of this enzyme aids to design new anti-TB inhibitors.

Publication

Original peer-reviewed article

1. Jha B, Vyas R, Bhushan J, Sehgal D, Biswal BK (2019) Structural insights into substrate specificity of SP_0149, the substrate binding protein of a methionine ABC transporter from *Streptococcus pneumoniae* **Acta Cryst.** doi: 10.1107/S2053230X19009038.

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

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

A. Computational methods for linking genes to secondary metabolites

Ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a large class of natural products with diverse structures and bioactivities. RiPPMiner, developed earlier in our group, used for the first time a machine learning (ML) approach to predict RiPP class, leader cleavage site and cross-link chemical structures of RiPPs, utilizing only the sequence of the precursor peptide as input. We have now developed RiPPMiner-Genome, a significantly updated version of RiPPMiner, which can take genomic sequences as input and identify RiPP biosynthetic gene clusters (BGC) and modifying enzymes using HMM profiles. Further, in RiPPMiner-Genome we have also implemented an updated version of ML algorithm which can identify precursor peptide from among multiple small ORFs in the RiPP BGC and subsequently predict its cleavage site and cross-linked chemical structure. In RiPPs, like lanthipeptides, it can also identify other modified residues which are not cross linked. This significantly enhances the accuracy of RiPP chemical structure prediction by RiPP Minoz-Genome compared to the predictions by RiPPMiner. We also have carried out a systematic benchmarking of the prediction accuracy of RiPPMiner-Genome at various levels like BGC identification, precursor prediction, prediction of leader cleavage and cross-link prediction using the dataset of 271 known RiPPs for which BGC information was available.

B. Analysis of protein-protein interaction (PPI) and phosphorylation network in *P. falciparum*

Since experimentally determined 3D structures are available for a very small number of protein-protein complexes from *Plasmodium falciparum*, in this work at attempt has been made to build 3D interactome of *Plasmodium falciparum* using a template based modelling approach. It is encouraging to note that, even though our approach does not utilize any known information about PPIs in *Plasmodium falciparum*, 2690 PPIs predicted by our structure based approach are common to high scoring PPIs in STRING database and our method also predicts 3058 novel interacting protein pairs which are not available in STRING or other PPI databases. Hence, current study not only gives us information about new PPIs which can help in filling the gaps present in currently available PPI networks and signaling

pathways, atomistic details of interaction interfaces predicted by our approach for large number of interacting proteins pairs can help in the design of novel inhibitors/modulators of PPIs as potential new drug candidates for this pathogen. The computational pipeline developed in the current study can be easily applied to model structural interactome of other pathogens and structural elucidation of host-pathogen interactions.

Our ongoing work on phosphosite analysis of plasmodium has been extended to build an integrated phosphosignalling and PPI network. Experimentally identified phosphoproteins as well as proteins harbouring phosphosite motifs have been mapped onto STRING PPI network of plasmodium to build an integrated PPI and phosphosignaling network.

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

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Unlike nucleic acids and proteins, the building blocks for glycan biosynthesis vary widely between species resulting in heterogeneity, complexity, diversity, and self/non-self discrimination. We strive to develop tools to understand the structure and functions of glycoconjugates in living systems. Our goal is to design, synthesize, and characterize novel synthetic hexosamine analogues and investigate their metabolic effects *in vitro* in mammalian cells and *in vivo* in mice.

Mapping of mucin-type O-glycosylation (MTOG) sites on Cd43

CD43 (leukosialin/sialophorin) is present abundantly in all lymphocytes, except naïve B-cells. CD43 is considered to be a negative regulator of immune activation and exhibits a long bottle-brush like structure. The extracellular domain of CD43 ($CD43_{ex}$) consists of 234 amino acids, including 93 Ser/Thr residues, of which 80-90 are estimated to be decorated with *O*-glycans. Towards our goal of comprehensive site mapping of MTOG, we purified $CD43_{ex}$-Fc-His from lentivirally transduced Jurkat cells. $CD43_{ex}$-Fc-His was subjected to reduction, alkylation, and digestion using both trypsin and Glu-C. The glycopeptides were analyzed using nano-LC-ESI-MS/MS system using the HCD-PD-ETD (high energy collision dissociation – product triggered – electron transfer dissociation) methodology. The spectra were analyzed using Byonic and the glycopeptide spectra and site occupancy assignments were manually verified.

The natural diversity in site occupancy was highlighted by the analysis of an undecapeptide 44-MYTTSITSDPK-54 which carries five potential sites. Theoretically this peptide could exist in 32 (2^5) unique glycoforms if each site were allowed to be either unoccupied or occupied with Tn-antigen. Our results showed evidence for the presence of 17 out of 32 glycoforms, including the fully occupied glycopeptide. In summary, we were able to obtain evidence for occupancy of 74-sites, compared to 25-sites reported by an earlier study on galactoglycoprotein (soluble Cd43). HCD-PD-ETD analysis revealed that treatment with Ac_5GalNTGc (**1**), an efficient inhibitor of MTOG, resulted in reduced site occupancy (58 sites compared to 74 sites in wild-type samples) and presence of GalNTGc on 43 sites.

Inhibition of MTOG induced by 1 in mouse T-cells

We investigated the effect of GalNAc analogues in EL-4 (mouse T-cell lymphoma) cells. Treatment of EL-4 cells with **1** resulted in decrease in MAL-II (*Maackia amurensis* lectin-II) epitopes with a concomitant increase in VVA (*Vicia villosa* agglutinin) epitopes. Particularly, **1** induced hyposialylation of CD43 as revealed by the decrease in CD43-S7 (neuraminidase

sensitive) western blots while no change was noticed in the polypeptide levels as revealed by CD43-M19 (glycan independent *C*-terminal epitopes). Next, splenocytes from C57BL/6J mice were harvested and CD90.2+ T-cells were sorted by flow cytometry. CD90.2+ T-cells were either maintained in the presence of IL-2 or stimulated to proliferate with anti-CD3/CD28 *ex vivo* and incubated with GalNAc analogues. Flow cytometry and lectin blots revealed that CD90.2+ T-cells incubated with 1, but not controls, showed a decrease in MAL-II epitopes and a gain in VVA epitopes. These results confirmed the ability of **1** to act as an MTOG inhibitor in a post-translational manner. Studies on the role of MTOG inhibition on the formation of immune synapse and T-cell trafficking *in vivo* are currently underway.

Publication

Original peer-reviewed article

1. Singh S, Gupta K, Shukla S, Sampathkumar S-G, Roy RP (2019) Sortase-click strategy for defined protein conjugation on a heptavalent cyclodextrin scaffold. **PLOS One**. doi:10.1371/journal.pone.0217369.

Delineating immune metabolism interaction in disease pathogenesis of tuberculosis and vitiligo

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

Adaptive responses of pathogens to host-imposed metabolic and nutritional stresses are the key factors influencing host-pathogen dynamics. Our group has been interested in studying the biochemical and functional relevance of a large NRP biosynthetic cluster (Rv0097 to Rv0101) in M. tuberculosis (Mtb) for several years. Over the years this gene cluster has been implicated in Mtb virulence by several groups, without exactly delineating the chemical identity of the end metabolic product and their physiological relevance. Retrobiosynthetic approach suggests an assembly line that initiates with the activation of long-chain a,b-unsaturated fatty acid to its corresponding adenylate by Rv0099, a fatty acyl-AMP ligase (FAAL). This is transferred onto the phosphopatetheine arm of an acyl carrier protein (ACP) (Rv0100). The is then modified by a type II thioesterase (Rv0098)involving Michael addition of glycine at the beta position of a,b-unsaturated fatty acid. Upon which a non-heme iron (II) dependent oxidase (Rv0097) acts to generate a beta-isonitrile fatty acyl moiety. Further RV0101 open reading frame codes for a non-ribosomal peptide synthethase (nrps) protein consisting of two modules, each of which can activate an amino acid. The last domain of NRP contains a reductive that our group had previously shown to catalyze reductive release of acyl chains (Fig.1). In order to identify the metabolite from Mtb, we performed analysis of Mtb biofilm metabolite extract and detected a cluster of unique peaks. MS/MS fragmentation patterns of these peaks revealed common backbone of mass which corresponds to dipeptide of ornithine and phenylalaninol. These metabolites were completely absent in planktonic cultures indicating that they are not required in complete media conditions. In-vitro functional investigation of the role of this metabolite using Mtb strain lacking nrps gene reveal importance of this metabolite in maintaining bacterial fitness under varying zinc environments. Additionally our macrophage studies also suggest dynamic role of these metabolites in zinc metallostasis upon infection. We thus named these metabolites as "Kupyaphores" ('kupya'- sanskrit for metals, 'phores'- latin for carrier). Interestingly these metabolites could be detected early in the infection cycle from lungs of mice infected with wild type Mtb, highlighting a critical role of these metabolites in establishment of infection. We are presently dissecting out the mechanistic regulation of this pathway and their relevance in TB pathology.

B. Understanding mechanisms underlying melanogenesis and depigmenting disorder Vitiligo

The melanocyte proliferation, differentiation and melanogenesis are intricately coordinated through a lineage-specific regulator microphthalmia-associated transcription factor (MITF). Thesetransi-

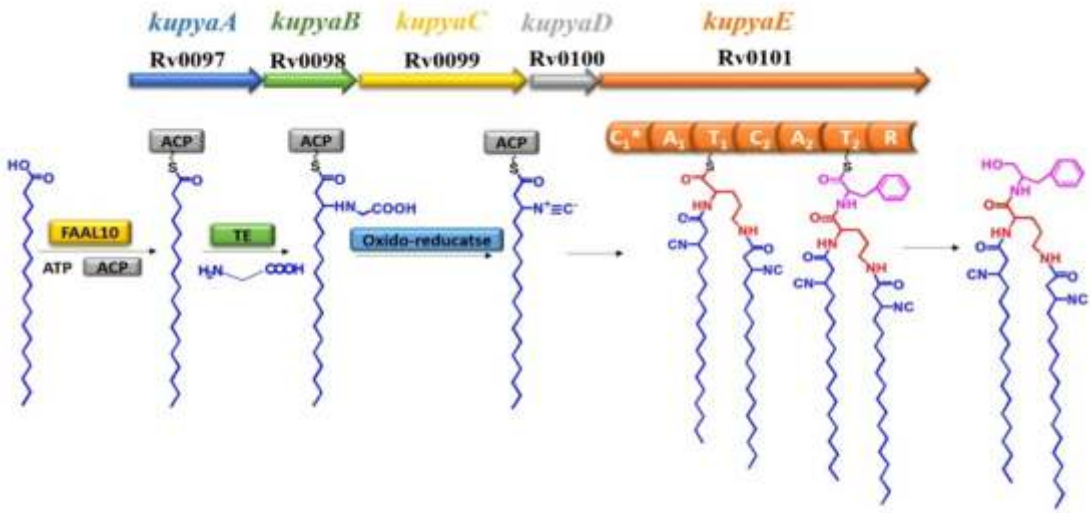

Fig 1: Schematic Representation of biosynthesis of Kupyaphores by Mycobacterium tuberculosis Rv0097-Rv0101 gene cluster. Putative biosynthetic steps are illustrated here.

tions are characterized by a distinctive set of cellular process that requires substantialbioenergetic challenges. To elucidate global changesduring pigmentation cycle, we utilized B16 pigmentation model.Transcriptional profiling was performed from day 3 to day 6 and RNAseq analysis after approriate normalizations showed very good concordance between the two replicates. Unsupervised hierarchical clustering suggested five clusters, which on pathway enrichment analysis revealed top regulated pathways with significant pvalues. Day 3 and 4 showed enrichment of DNA replication and cell cycle, while pathways pertaining to steroid and fatty acid metabolism become top enriched pathways on day 5. Day 6 also have pathways enriched in protein synthesis and mitochondrial changes. These data indicated that

major metabolic changes might be occurring during pigmentation cycle. Cellular and biochemical studies have suggested changes in mitochondrial structures as well as changes in mitochondrial respiration. We are presently addressing these important questions to understand mitochondria-metabolism basis of pigmentation biology.

Publications

Original peer-reviewed articles

1. Raja DA, Gotherwal V, Burse SA, Subramaniam YJ, Sultan F, Vats A, Gautam H, Sharma B, Sharma S, Singh A, Sivasubbu S, Gokhale RS, Natarajan VT (2020) pH-controlled histone acetylation amplifies melanocyte differentiation downstream of MITF. **EMBO Rep.** doi: 10.15252/ embr.201948333.
2. Gupta A, Chauhan A, Priya A, Mantri B, Wadhokar M, Dalave K, Shah B, Gokhale RS, Batra VV, Singh A (2020) Lesional skin in vitiligo exhibits delayed *in vivo* reepithelialization compared to the nonlesional skin. **Wound Repair Regen.** doi: 10.1111/wrr.12798.
3. Vaish U, Kumar AA, Varshney S, Ghosh S, Sengupta S, Sood C, Kar HK, Sharma P, Natarajan VT, Gokhale RS, Rani R (2019) Micro RNAs upregulated in vitiligo skin play an important role in its aetiopathogenesis by altering TRP1 expression and keratinocyte-melanocytes cross-talk. **Sci Rep**. doi: 10.1038/ s41598-019-46529-6.
4. Grover R, Burse SA, Shankrit S, Aggarwal A, Kirty K, Narta K, Srivastav R, Ray AK, Malik G, Vats A,Motiani RK, Thukral L, Roy SS, Bhattacharya S, Sharma R, Natarajan K, Mukerji M, Pandey R, Gokhale RS, Natarajan VT (2019) Myg1exonuclease couples the nuclear andmitochondrial translational programs throughRNA processing. **Nucleic Acids Res.** 47:5852-5866.
5. Jatana N, Ascher DB, Pires DEV, Gokhale RS, Thukral L (2019) Human LC3 and GABARAP subfamily members achieve functional specificity via specific structural modulations. **Autophagy** 14: 1-17.

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 nuclear magnetic resonance spectroscopy and related structural biological methods including enzymology to study the behavior of PGK isoforms in *Leishmania* sp. Phosphoglycerate kinase (PGK) from *Leishmania spp*. which, exists in the cytoplasmic PGKB and glycosomal PGKC isoforms show 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).

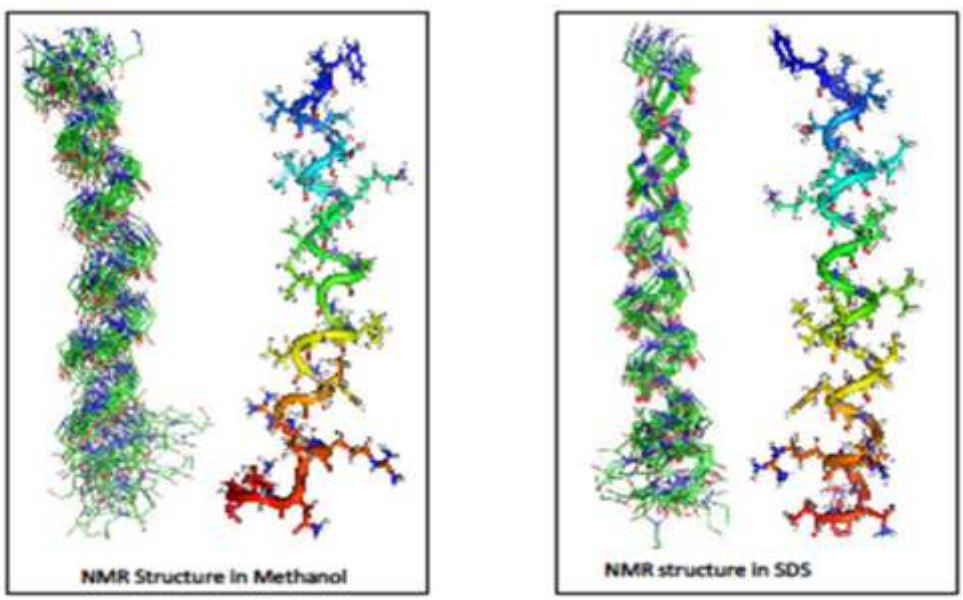

Fig.1: Further, we sought to establish the three dimensional structure of PGKC_*Lmexicana* by homology modelling and biochemical data. We have a final theoretical 3-dimensional model of *L.Mexicana* PGKC (residues 1-479) that enables visualization of the *GXXXG* motif in the enzyme fold (Fig. 2). While supporting our biochemical data, the docking interactions reveal new aspects of the tertiary fold of PGKC.

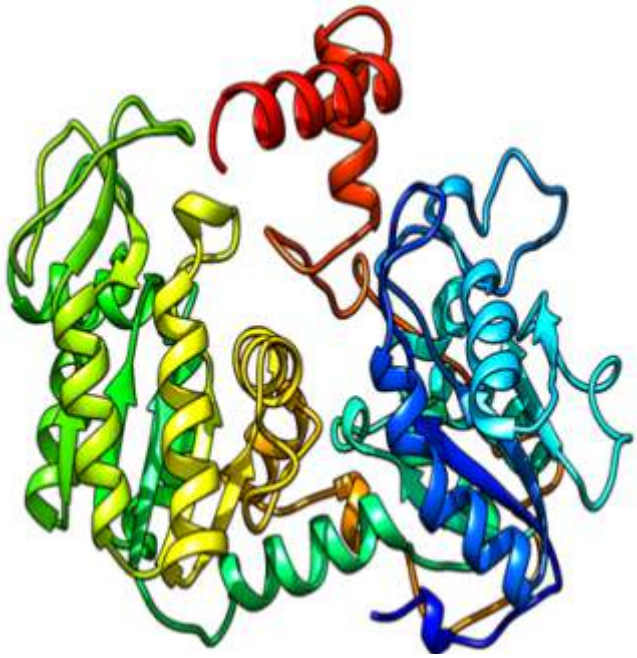

Fig. 2: The helix which corresponds to TMS (Fig. 1) is a discontinuous helix in the model shown in Fig. 2. Furthermore *GXXXG* motif such as is present in our case may have function in stabilizing the protein conformation given the sequence context such as the presence of neighboring β-branched residues. 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.

We are interested in the relevance of information theory (Shannon CE, 1948, Bell Sys Tech J, 27:379-423) used in developmental/evolutionary/genomics biology to protein folding. Coding in protein folding is symbolic but degenerate in that while one sequence does give one structure in the reverse similar folds can be generated from multiple sequences. The probabilistic nature of protein structural code but not the sequence code is evident. The applications of this theory on protein structure/function evolution specifically in the case of PGK of *Leishmania* will be insightful. Residual entropy and shared entropy are unique to non-equilibrium probabilistic systems such as folded proteins, which serve as source of information for directing evolutionary changes of the structure.

GENE REGULATION

Cellular and molecular biology of human cancer

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Breast cancer is the most common cancer in women and second leading cause of cancer related deaths in developing countries. Based on hormone receptors status, around 15% breast cancers are designated as triple-negative breast cancer (TNBC) for which limited treatment modalities are available. In present study, we demonstrated role of AKAP4 in various molecular pathways contributing towards tumor growth that may have clinical relevance for targeting TNBC for better cancer management.

AKAP4 is involved in breast cancer cell growth

AKPA4 protein localization was examined in MDA-MB-231 (TNBC, basal triple negative breast cancer ER-, PR-, HER2-) and MCF7 (luminal breast adenocarcinoma ER+, PR+, HER2-) by confocal microscopy. Confocal images showed AKAP4 protein expression predominantly in cytoplasm and co-localized with various sub-cellular organelles (Figure 1a). Quantitative PCR analysis showed significant reduction ($P< 0.001$) of relative mRNA expression of *AKAP4* in shRNA1, shRNA2 and shRNA3 transfected MDA-MB-231 and MCF-7 cells. Western blot analysis also revealed down regulation of AKAP4 protein in both shRNA2 and shRNA3 transfected MDA-MB-231 and MCF7 cells. We further observed significant reduction ($P<0.001$) of cellular growth and cell viability in AKAP4 ablated breast cancer cells (Fig. 1b-c). Decreased expression of cyclins, cyclin-dependent kinases and PCNA was observed in AKAP4 ablated breast cancer cells (Fig. 1d).

Knockdown of AKAP4 enhances cytotoxic effect of paclitaxel in breast cancer cells

To examine the effect of combination of AKAP4 ablation and paclitaxel on cellular growth and viability in MDA-MB-231, MTT assay was carried out. We observed that combination treatment was significantly more cytotoxic ($P<0.001$) than paclitaxel

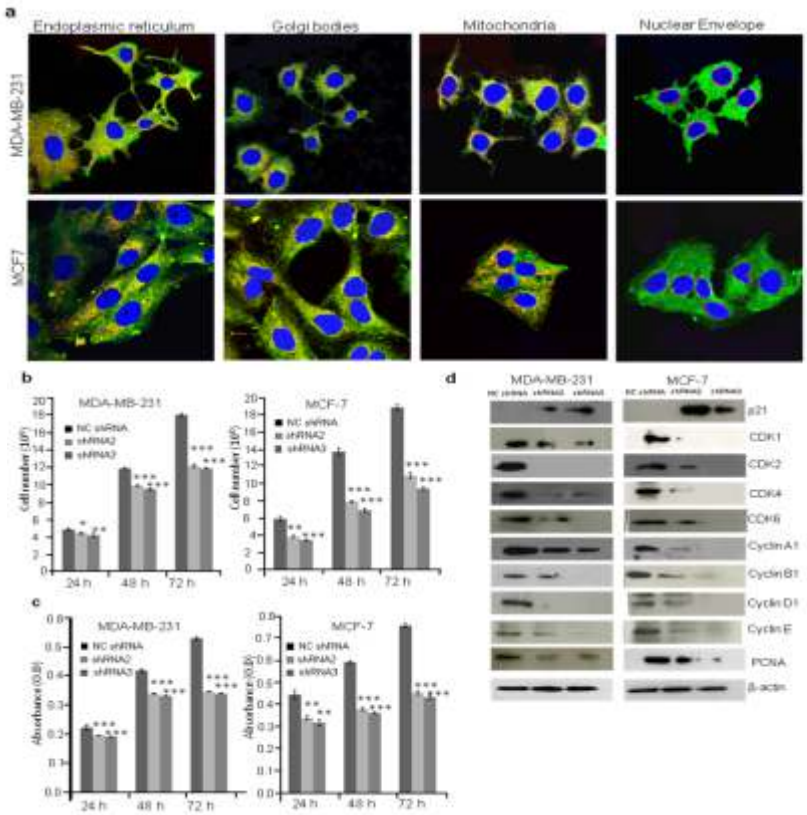

Fig. 1: AKAP4 protein expression is involved in cellular growth in MDA-MB-231and MCF7(**a)** AKAP4 protein expression and co-localization **(b)** Bar diagram shows significant reduction in cellular proliferation **(c)** Cellular viability assay **(d)** Western blot analysis in AKAP4 ablated cells.

alone (Fig. 2a-b). We further assessed AKAP4 ablation and paclitaxel drug combination in non-constant ratio in TNBC cells using the median-effect analysis method. Different concentrations of paclitaxel and AKAP4 shRNA at 48 h showed significant decrease in cell viability in a dose-dependent manner in MDA-MB-231 cells (Fig. 2c). The IC50 values were 3.8 µg/ml, 3.78 µg/ml and 14.17 µg/ml for AKAP4 shRNA2, AKAP4 shRNA3 and paclitaxel

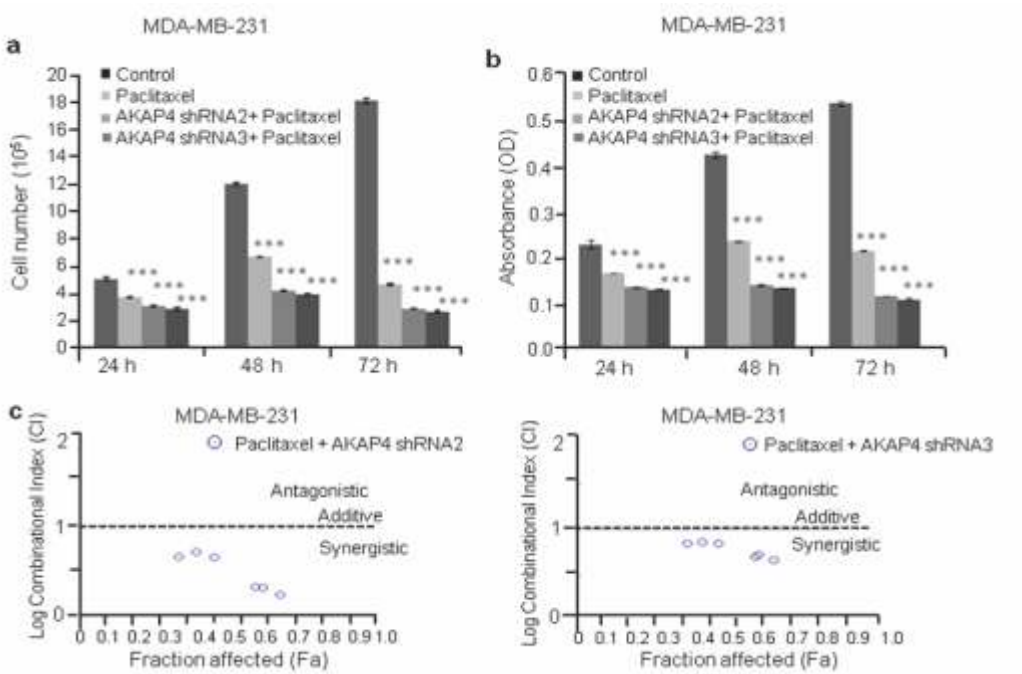

Fig. 2: Combitorial effect of paclitaxel and AKAP4 shRNA results in enhanced cytotoxicity

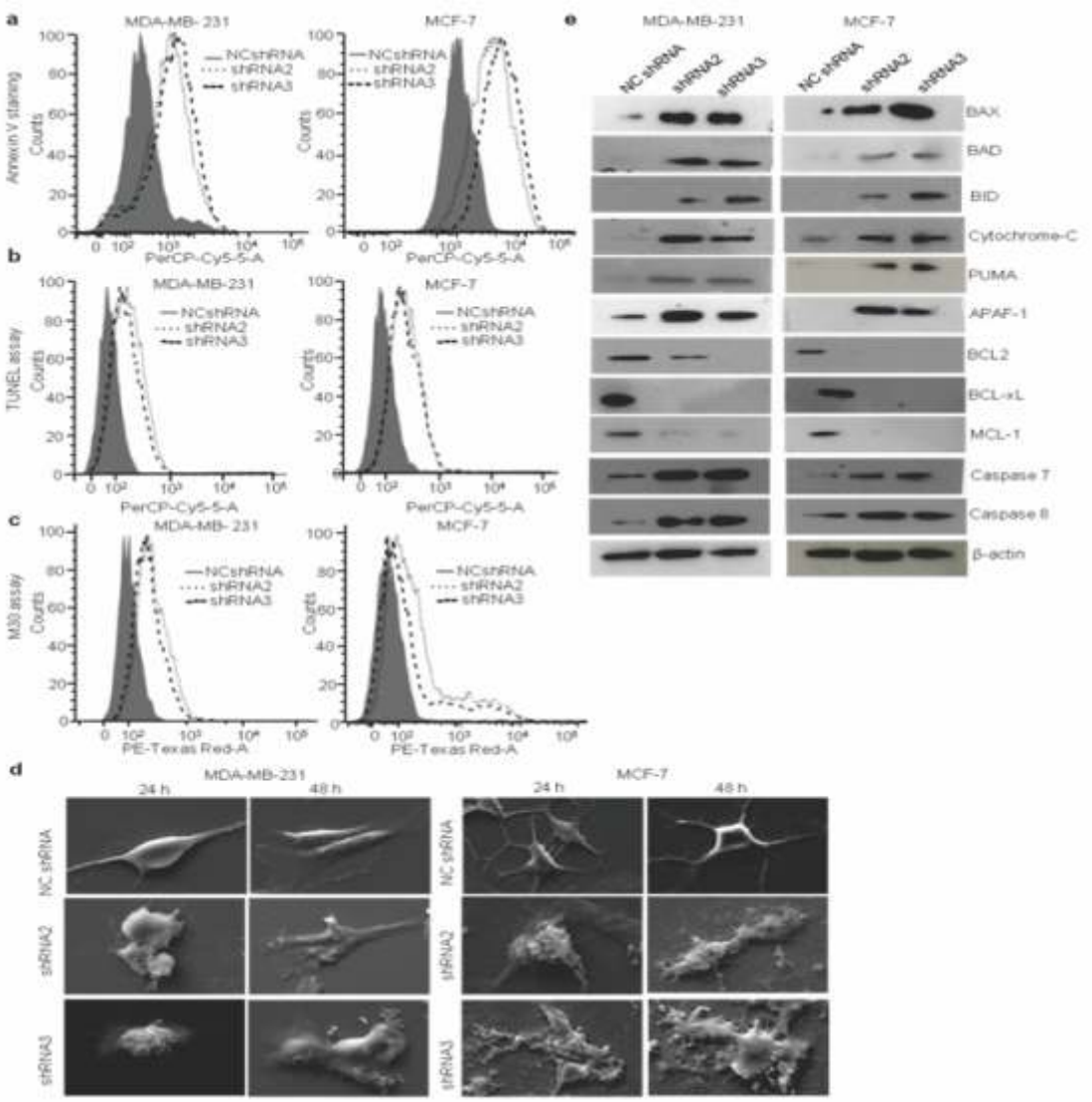

Fig. 3: Knockdown of AKAP4 results in initiation of apoptosis **(a)** Histogram depicts effect of knockdown of AKAP4 on apoptosis by Annexin V assay in MDA-MB-231 and MCF-7 **(b)** Histogram depicts ablation of AKAP4 on DNA damage (c) Histogram depicts ablation of AKAP4 on caspase activation **(d)** Representative images show changes due to apoptosis in AKAP4 down regulated MDA-MB-231 and MCF-7 cells.

treatment respectively. Interestingly, single or combitorial treatment studies revealed that the values of combination index (CI) were <1, (Fig. 2c), indicating that the combination of two treatment resulted in a synergistic effect.

Knockdown of AKAP4 initiates apoptosis

Initially, onset of apoptosis was examined in AKAP4 ablated cells by Annexin-V-PerCPCy5-5-A assay. Flow cytometry analysis of MDA-MB-231 cells revealed 31.15% and 42% annexin-V expression in cells transfected with AKAP4 shRNA2 or shRNA3 respectively compared to 6% with NC shRNA. Similarly, MCF7 cells showed 34.50% and 52.26% of annexin-V expression in cells transfected with AKAP4 shRNA2 or shRNA3 respectively compared to 1.37% of NC shRNA (Fig. 3a). Further, to strengthen our findings, DNA fragmentation using TUNEL assay was carried which showed 42.43% and 31.8% of BrdU positive MDA-MB-231 cells when transfected with AKAP4 shRNA2 BrdU positivity was observed in MCF7 cells transfected with AKAP4 shRNA2 (54.19%) or shRNA3 (51.8%) compared to NC shRNA (2.56%; Fig. 3b). Further to investigate the initiation of apoptosis, M30 assay was used to examine the effect of AKAP4 ablation in breast cancer cells that revealedhigher M30 epitope expression in MDA-MB-231 cells transfected with AKAP4 shRNA2 (53.91%) or shRNA3 (40.63%) compared to NC shRNA (1.83%). Similarly, higher expression of M30 epitope was also observed in MCF7 cells when transfected with

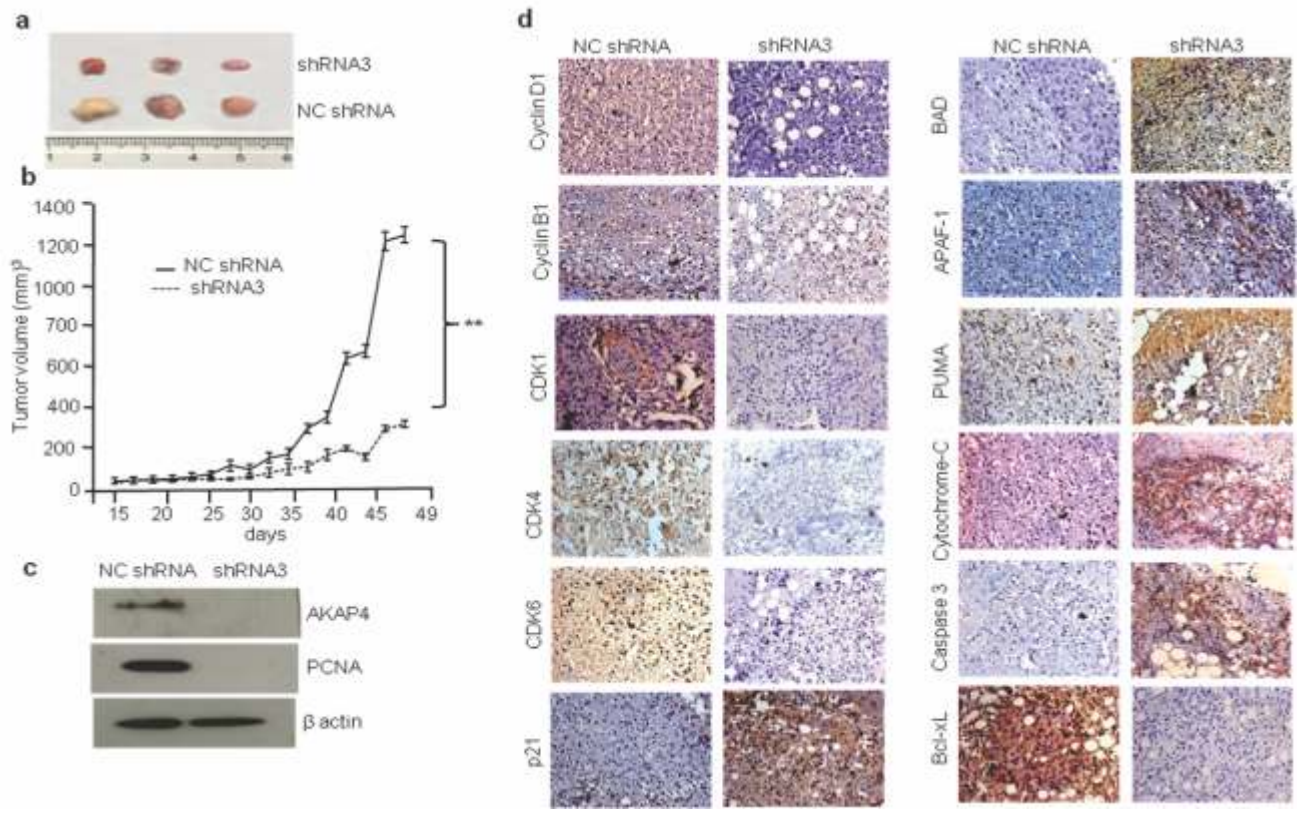

Fig. 4: Knockdown of AKAP4 reduces tumor growth of MDA-MB-231 xenograft. (**a)** Reduced tumor size dissected from animals when treated with AKAP4 shRNA3. **(b)** Tumor volume in AKAP4 shRNA3 treated mice. **(c)** Western blot analysis AKAP4 and PCNA expression in shRNA3 treated mice. **(d)** IHC analyses of various molecules in mice treated with AKAP4 shRNA3.

AKAP4 shRNA2 (33.05%) or shRNA3 (25.02%) compared to NC shRNA (2.26%; Figure 3c). Further, employing scanning electron microscopy (SEM), phenotypic changes associated with apoptosis in AKAP4 down regulated cells were captured. SEM images revealed membrane blebbing and presence of distorted structures in MDA-MB-231 and MCF7 cells post 12 h of treatment with shRNA2 or shRNA3 which continued to increase by 48 h (Fig. 3d). We further investigated various molecules involved in the process of apoptosis in AKAP4 down regulated cells. Western blot analysis revealed up-regulation of pro-apoptotic molecules and down regulation of anti-apoptotic molecules in both the breast cancer cells (Fig. 3e). Our data suggests that AKAP4 plays an important role in cellular growth, viability and apoptosis.

Knockdown of AKAP4 reduces tumor growth

Our *in-vitro* studies on AKAP4 down regulation showed reduction in cellular proliferation in TNBC cells. Hence, in *vivo* studies were carried out using triple negative MD-MBA-231 cells. Knockdown of AKAP4 resulted in significant reduction ($P<0.01$) of tumor size and volume compared to NC shRNA treated group (Fig.4a-b). Also, tumor lysate from mice treated with AKAP4 shRNA3 showed reduced AKAP4 and PCNA protein expression (Fig. 4c). Next, we checked the expression of cell cycle regulators in xenograft tumor sections employing IHC. Our data revealed reduced immuno-reactivity of cyclins (cyclin D1, cyclin B1) and CDKs (CDK1, CDK4, CDK6) in AKAP4 shRNA3 treated group. As expected, up-regulation of CDK inhibitor p21 showed increased immuno-reactivity in AKAP4 down regulated tumor sections (Fig. 4d).

We further investigated the effect of AKAP4 down regulation on pro-apoptotic molecules and anti-apoptotic molecule in tumor section by employing IHC. We observed an increased immuno-reactivity of pro-apoptotic molecules (BAD, APAF-1, PUMA, cytochrome-C) in AKAP4 down regulated tumor sections compared to NC shRNA group. Interestingly, up regulation of caspase 3 was also observed in AKAP4 down regulated tumor sections. In addition, there was down regulation of anti-apoptotic

molecule (Bcl-xL) in the AKAP4 down regulated tumor sections (Fig. 4d). Our *in vivo* studies indicate that ablation of AKAP4 expression does result in reduced tumor growth.

In conclusion, we have demonstrated that AKAP4 plays an important role in TNBC cell growth. Our data suggests therapeutic potential of AKAP4 in breast cancer.

Human clinical trials in cervical cancer patients stage IIIB

"A Phase II double blind clinical trial to assess the efficacy of dendritic cells primed with either patient's own tumor lysate or using rSPAG9 protein in stage IIIB cervical cancer is going on at Cancer Institute, Adyar, Chennai.

Patients screened & enrolment status

Total 182 patients were screened for recruitment of which total of 54 patients were included in this Phase II trials based on inclusion and exclusion criteria. Till date, 48 patients have finished their re-evaluation done employing PET-CT Scan. Number of patients in CR are 43/48 after PET-CT scans evaluation. Five patients who have received 6-10 doses among the randomized arms will complete this year the vaccination regime. No DC Vaccine related toxicity has been observed except for pain at the site of intradermal injection. All the patients will be followed up for 30 months.

Human clinical trials in ovarian cancer patients stage IV (recurrent/metastatic) who have failed two line of systemic therapies - 75 patients

Summary

In our studies we have demonstrated that majority of ovarian cancer patients (90%) express SPAG9 protein. As a result, SPAG9 qualifies to be a novel immunotherapeutic target for ovarian cancer human clinical trials. Standard operative protocols for SPAG9 immunoreactivity have been initiated for recruiting patients as this is one of the inclusion criteria for all patients. (DCGI Approval for the Phase II clinical trial has been granted on 4th March 2020).

Early detection and diagnosis of cervical, ovarian and prostate cancers

Circulating anti-SPAG9 antibody-based detection as well as tissue-based expression of SPAG9 in cancer tissues for early detection and diagnosis of cervical, ovarian, breast and prostate cancers is going on in collaboration with SRCC, Jaipur and Cancer Institute, Adyar, Chennai.

Future plans

1. Ongoing studies on Phase II cervical cancer trials with follow up studies and stability studies of recombinant SPAG9 protein.

2. Ongoing studies on Phase II clinical trials with dendritic cell vaccine for the treatment of recurrent/metastatic reproductive cancer-Ovarian Cancer patients who have failed two line of treatment: Total number of patients to be recruited: 75

3. **Early detection and diagnosis:** The goal is to advance medical research and improve patient outcomes by discovering biomarkers (indicators) for multiple types of cancer. Large scale validation of SPAG9 will be carried out in NCI-AIIMS, AIIMS, Cancer Institute, Chennai and SRCC Jaipur for cervical, ovarian, breast and prostate cancer.

4. **Immunotherapy by Monoclonal Antibodies against SPAG9:** The concept of immunotherapy is based on the assumption that antigenic structures expressed in tumors can be used for therapeutic approaches employing the autologous immune system or by the application of immunotherapeutic reagents. Eight monoclonal antibodies have been generated against SPAG9 peptides which are being tested for efficacy in allograft and xenograft mouse model. These are potentially important studies of clinical relevance and also have a direct translational pathway into the clinic.

Publication

Original peer-reviewed article

1. Jagadish N, Devi S, Gupta N, Suri V, Suri A (2020) Knock down of A-Kinase anchor protein (AKAP4) inhibits proliferation of triple negative breast cancer (TNBC) cells in vitro and in vivo. **Tumour Biol.** doi: 10.1177/ 1010428320914477.

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

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Mycobacterium tuberculosis exploits host ATM kinase for survival advantage through SecA2 secretome

In response to the damage, the host activates an intricate and indispensable signaling cascade entitled "**D**NA **d**amage **r**esponse" (DDR) pathway, which not only detects and repairs the damaged lesions in DNA but also regulates the activation of effectors that determine the fate of the cell. Ataxia telangiectasia mutated (ATM), ATM- and Rad3-related protein (ATR) and DNA dependent protein kinase catalytic subunit (DNA-PKcs) are three drivers of DDR, which belong to the family of phosphoinositide 3-kinase like kinases (PIKKs). ATM is activated through autophosphorylation of S1981 residue and is subsequently recruited to the Double strand breaks (DSBs) through a sensor complex, MRN. Phosphorylation of H2AX at the serine 139(γH2AX), at the site of damage is considered a marker for DNA damage.

One of the remarkable survival strategies of *Mtb* is to intervene with the fundamental signaling events of the host cell and to facilitate these manipulations *Mtb* secretes an enormous number of characterized and uncharacterized effectors inside the host. We designed a study to address the following questions: i) Does *Mtb* target the genomic integrity of the host? If so, do virulent and avirulent *Mtb* target the genomic integrity of the host differentially, and in that case, how? ii). How the host cell responds to the DNA damage mediated through *Mtb*? iii). What is the role of the *Mtb* secretome, if any, in imparting DNA damage? iv). What is the role of ATM kinase activation and subsequent downstream signaling in the survival of pathogen within the host? vi). Is this *Mtb* mediated DNA damage manifested in the mice model of *in vivo* infection?

Results show that,*Rv* infected cells showed considerably higher gH2AX levels compared with the uninfected control. Infection by *Rv* led to significant activation of ATM-Chk2 pathway in both RAW and PΦ cells, suggesting occurrence of DSBs in the host nuclei.The effectors secreted by SecA2 pathway are necessary and sufficient for inflicting genotoxic stress in the host. While ATM activation is essential; *Mtb* survival is not dependent on the activation of downstream effectors. *Rv* inflicts DSBs to activate ATM which in turn activates Akt resulting in anti-apoptotic and pro-survival signals which favors *Mtb*survival.We showed that pathogenic *Mtb* induces genotoxicity both *ex vivo* and *in vivo* resulting in deleterious DSBs in the host genome. We hypothesized that *Mtb* mediated genotoxicity provides a survival niche through activation of ATM kinase. We explored the possibility of utilizing ATM inhibitor towards adjunct host directed therapy (HDT) for TB with the help of murine infection model. We showed that a combination of ATM inhibitor + INH treatment resulted in ~ 1 log fold better clearance compared with INH treatment alone. We believe we have identified a novel survival mechanism utilized by *Mtb*, wherein the pathogen constantly challenges the host genome leading to the activation of pro-survival ATM-Akt signals (Fig. 1). Hence, we propose the use of ATM inhibitors as adjunct for HDT in the treatment of tuberculosis.

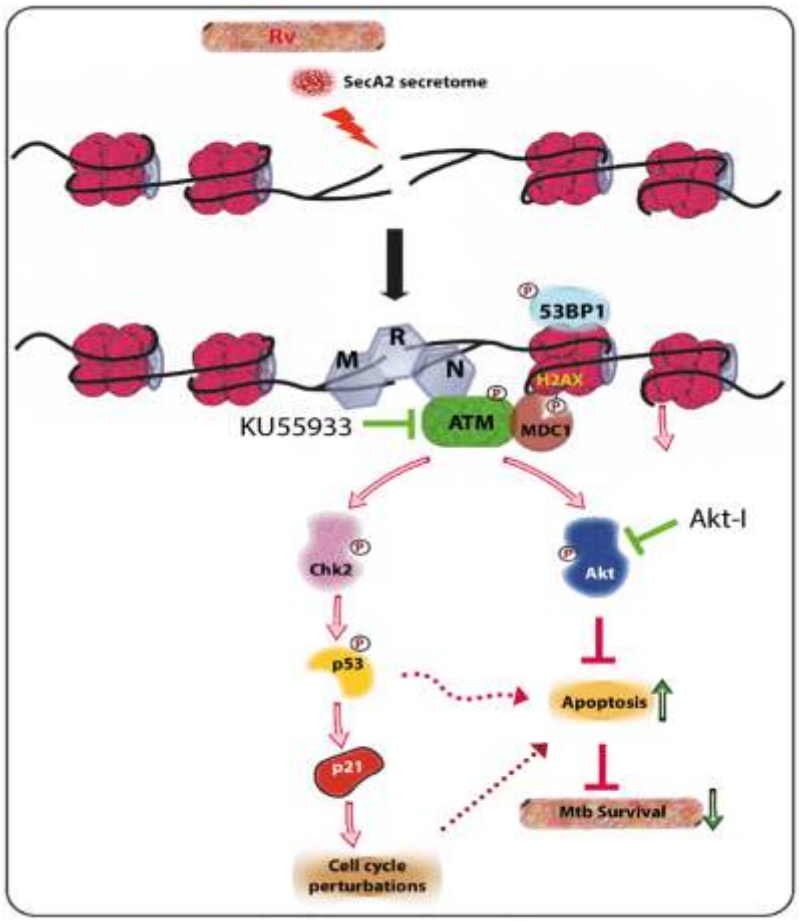

Fig. 1: Model depicting the findings.*Rv* induces genotoxicity and causes deleterious DSBs in the host genome through SecA2 secretome. Host cell in response to the occurrence of DSBs activate ATM kinase and is recruited at the site of damage by the sensor, MRN complex. Activated ATM phosphorylates H2AX in the genome which becomes the foundation for the recruitment of mediator protein MDC1 and amplify signal of DNA damage response pathway (DDR). pATM promote recruitment of 53BP1 at the damage site. pATM as a part of DDR pathway also activates downstream effectors, Chk2 and p53, which are responsible for alterations in the cell cycle progression. pATM in a parallel pathway also activates Akt, which is known inhibit apoptosis and promote cell survival. Activation of ATM and Akt and subsequent inhibition of apoptosis provides survival advantage to *Rv*. Inhibition of ATM or Akt activation through inhibitors promote host cell apoptosis, which impedes the bacilli growth. Phosphorylation status of proteins is depicted with a **P** in a circle.

Publications

Original peer-reviewed articles

1. Lochab S, Singh Y, Sengupta S, Nandicoori VK (2020) *Mycobacterium tuberculosis* exploits host ATM kinase for survival advantage through SecA2 secretome. **eLife** doi: 10.7554/eLife.51466.

2. Prasad D, Arora D, Nandicoori VK, Muniyappa K (2019) Elucidating the functional role of Mycobacterium smegmatis recX in stress response. **Sci Rep.** doi: 10.1038/s41598-019-47312.

3. Srivastava S, Battu M B, Khan MZ, Nandicoori VK, Mukhopadhaya S (2019) *Mycobacterium tuberculosis* PPE2 protein interacts with $p67^{phox}$ and inhibits ROS production. **J. Immunol** 203:1218-1229.

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5. Sevalkar R, Arora D, Singh P, Singh R, Nandicoori VK, Karthikeyan S, Sarkar D (2019) Functioning of mycobacterial heat-shock repressors requires the master virulence regulator PhoP. **J Bacteriol.** doi: 10.1128/JB.00013-19.

Reviews

1. Bhaskar S, Singh B (2019) Immunotherapeutic potential of *Mycobacteriumindicus pranii* against tuberculosis. In: ***Mycobacterium tuberculosis*: Molecular infection biology, pathogenesis, diagnostics and new interventions** (Eds. Hasnain S, Ehtesham N, Grover S, Springer, Singapore) pp 407-417.

2. Bhaskar A, Dwivedi VP, Nandicoori VK (2019) Eliminating mycobacterial persistence: novel targets for anti-TB therapy. In: **Pathogenicity and drug resistance of human pathogens** (Eds. Hameed S, Fatima Z, Springer) pp 57-81.

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

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Theme of research

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 and in interventions that delay it. We are also trying to reposition drugs to treat diabetic complications using mice models.

Objectives

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

Knocking down *cdk-12* in the *daf-2(-)* mutant arrests germline development at the pachytene stage of meiosis that was dependent on DAF-16. In *daf-2;daf-16*, most of the laid eggs fail to reach adulthood, pointing to their poor quality in absence of DAF-16. Using transgenic rescue lines, we found that *daf-16a* is the most important isoform for this germline arrest phenotype. In contrast to mammalian CDK12, in *C. elegans*, *cyclin K* RNAi failed to produce germline arrest, showing that this phenotype may be cyclin-independent or CDK-12 may require a different cyclin.

B. Involvement of novel kinases in DR

We explored the possibility that *flr-4(-)* may be sensitive to differences in Vitamin B12 levels in the two bacteria. We found that HT115 has more B12 compared to OP50. Interestingly, we also found that *flr-4(-)* is more sensitive to B12.

In order to study the role of Vitamin B12, we used the CY573 strain where *cyp-35B1* (a XDP gene) promoter is fused to *gfp.* We found that supplementing Vitamin B12 to OP50 can elicit the same response as that of HT115. Since *E. coli* cannot synthesize B12 and depends on uptake from the media, we used an *E. coli* mutant that fails to take up the vitamin. The parent strain BW25113 elicits expression of CY573 similar to HT115, showing that it has more B12. However, a D*tonB* mutant fails to elicit GFP expression, showing that the uptake is required for activating the pathway leading to expression of XDP genes.

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

Following RIF treatment of db/db mice, western blot showed that the overall levels of AGEs in liver

samples is decreased. The levels of fructosamine in the plasma is also significantly decreased on RIF treatment. Since high glucose level leads to microvascular complications, adversely affecting kidney function, we evaluated the kidney histology from treated and untreated mice to find that the RIF-treated mice were less affected. Increased AGEs also exacerbated incidents of atherosclerosis. RIF-treated *ApoE*$^{-/-}$ mice showed lower atherosclerotic fat deposition in the aorta. Together, these results point towards the protective role of RIF in alleviating many aspects of glucose toxicity in mice.

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

Quantitative RT-PCR of *cyp-33, cyp-35, cyp-37* and *ugt-16* showed significant upregulation on *drl-1* RNAi that was reduced in absence of *cep-1*. Similar observations were made in the *eat-2(ad465)* mutant. Together, *cep-1* is required for optimal up-regulating XDP genes during DR.

We found that the relative ratio of ROS levels in *drl-1* RNAi-fed worms compared to control RNAi is significantly higher in *cep-1(gk138)* mutant as compared to wild-type. Thus, *drl-1* KD requires s*pace-1 to keep ROS* levels lower during DR, pointing at its anti-oxidant role.

Publication

Original peer-reviewed article

1. Matai L, Sarkar GC, Chamoli M, Malik Y, Kumar SS, Rautela U, Jana NR, Chakraborty K, Mukhopadhyay A. Dietary restriction improves proteostasis and increases life span through endoplasmic reticulum hormesis. **Proc Natl Acad Sci USA**.116:17383-17392.

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

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Theme of research

Specific interactions of myriad *cis*-acting elements and *trans*-acting factors are required within the eukaryotic nuclear milieu to accomplish regulation of nuclear processes critical for development and cellular functions. This is governed by an appropriate organization of chromatin at various levels. CTCF, a DNA binding protein, has emerged as an important contributor to chromatin organization. Intriguingly, CTCF has multifunctional attributes that lead to diversity in the functional outcomes of CTCF binding. To decipher the nature of chromatin domains organized by this multifunctional protein, we are investigating the role of CTCF in regulation of transcription and VDJ recombination at murine TCRb locus.

Objectives

Antigen receptor loci like Igh, TCRa/d, TCRb etc., exhibit exquisitely precise regulation of transcription as well as RAG mediated VDJ recombination during development. This requires appropriate enhancer-promoter interactions and resultant epigenetic alterations at the nucleosomal level. Further, since V, D and J segments are located at vast distances from each other on the chromosome, higher order chromatin reorganization is necessary to bring them in spatial proximity prior to RAG mediated VDJ recombination. In this context, since CTCF is an important global factor contributing to long range interactions of chromatin that are vital for enhancer-promoter interactions as well as other *cis*-DNA interactions, it is of interest to decipher the chromatin structure and organization of the wild type and genetically manipulated AgR loci to understand various aspects of chromatin organization as influenced by CTCF as well as other *cis* and *trans* acting factors.

Based on the analysis of CTCF binding sites and their interactions in relation to regulatory elements and recombining segments, different roles of the CTCF binding sites can be hypothesized that are pertinent for various aspects of regulation of TCRb locus. Our chromosome conformation capture (3C) based analysis suggests that the CTCF binding sites regulate TCRb locus for two key aspects pertinent for VDJ recombination i.e. they aid in organization of the RC and help to define the specific configuration of the chromatin loops necessary to increase the spatial proximity of functionally relevant units like regulatory elements and Recombination Signal Sequences (RSS) associated with V, D and J gene segments. Using ablation of specific CBS, we are testing the contributions of individual CTCF binding sites of TCRb locus for these and other aspects including organization of barrier and/or enhancer blocking insulator near RC, establishment and stabilization of RC and locus contraction.

We have selected the CBS flanking the RC as well as within central V-segment domain that might aid in locus contraction. Using CRISPR/Cas9 based mutagenesis, mouse ES cells were genetically

manipulated to ablate specific CBS. The mutant ES cells were used to generate mutant mice. In an alternative strategy, CRISPR/Cas9 mutagenesis was carried out in the early embryos (1-4 cell stage). Colonies of mouse mutants have been established that carry specific mutations

Chromatin Immunoprecipitation (ChIP-qPCR) analysis was carried out to verify the absence of CTCF binding at mutated binding sites in thymocytes derived from homozygous mutant mice. As predicted, these mice also exhibited altered usage of V segments during V-to-DJ recombination as evidenced by FACS analysis as well as RT-qPCR analysis performed on total thymocytes. Further, the mutations have been bred onto Rag1 null background and double mutant mice have been generated. In the absence of Rag1, the TCRb locus is captured immediately prior to VDJ recombination. Chromosome conformation capture analysis (3C-qPCR) on such thymocytes will help to relate the alterations of the chromatin loopscape to the observed alterations in VDJ recombination profiles. The analysis is being performed to address the special roles of the different CBS that seem to be important for locus contraction in a complex manner.

We have also initiated an investigation to understand the activity of enhancer Eb - an important regulatory element of the TCRb locus. Eb shows enrichment of RNA Polymerase II and it has been suggested that the RNA PolII is first loaded onto Eb and then transferred to the cognate promoters (PDb1 and PDb2). Eb also exhibited presence of H3K4-trimethylation – a histone modification known to be associated with actively transcribing regions of the genome. Several enhancers have been suggested to undergo transcriptions and generate eRNA – an activity that is correlated to their activated status. Analysis of Eb based transcripts will be informative to understand the nature of enhancer-promoter communication.

Role of cell signaling in eukaryotic development

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

A. Phosphoinositide (PIP) signaling in Apicomplexans.

i. Role of a putative PI3-kinase regulator Vps15 in Plasmodium falciparum and Toxoplasma gondii.

It is well known that the activity of class III PI3Ks like VPS34 is regulated by Vps15 which codes for a protein kinase. Since we are interested in phosphoinositide (PIP) mediated signalling in Apicomplexan parasites like *Plasmodium falciparum* and *Toxoplasma gondii*, we started investigating the role of Vps15 in these parasites.

An orthologue of Vps15 seems to be present in both *P. falciparum* and *T. gondii.* PfVps15 is essential for blood stage development as it cannot be disrupted. We generated inducible knock down (iKD) for TgVps15 in *T. gondii*. Plaque assays revealed that parasite growth was suppressed suggesting an important role of Vps15 in *T. gondii* development. Further studies to delineate the processes in which Vps15 is involved are in progress.

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

CDPK7 is conserved in both *P. falciparum* and *Toxoplasma* and our previous studies suggested that CDPK7 interacts with 4'-phosphorylated PIPs and this kinase is essential for replication of both these parasites. Phosphoproteomics revealed that a Glycerol-3-Phosphate-AcylTransferase (GPAT), which facilitates the synthesis of phosphatidic acid (PA) synthesis is differentially phosphorylated upon TgCDPK7 depletion. However, the function of this GPAT has not been reported in *T. gondii*. TgGPAT has close similarity to ER-resident GPATs including one from *P. falciparum*. TgCDPK7 depletion impaired the localization of TgGPAT to the ER and also resulted in loss of its association with the membranes. Collectively, these and other results suggested that TgCDPK7 may regulate TgGPAT localization to the ER, which is likely to impair its function, and may contribute to reduced PE levels in TgCDPK7 depleted parasites.

PfCDPK7 knockout results in reduction of PC levels in *P. falciparum*. Strikingly, phosphorylation of ethanolamine kinase (PfEK) and phosphoethanolamine -N-methyltransferase (PfPMT) was altered significantly in PfCDK7-KO parasites. Strikingly, we found that PfCDPK7 regulates PfPMT stability most likely by mediating its phosphorylation, which is important for PC biogenesis. Collectively, these studies unravel novel mechanisms via which key

processes like trafficking and PL biogenesis is related in apicomplexan parasites.

B. Role and Regulation of key kinase substrates by phosphorylation

RhopH3 is a protein which is critical for host RBC invasion by *P. falciparum*. We had found that RhopH3 is phosphorylated at S804 possibly by PfCDPK1 in the parasite.

A S804A phosphomutant of RhopH3 was created by using CRISPR-Cas9. Using this parasite line (R3_S804A), we were able to demonstrate that RhopH3 phosphorylation was critical for RBC invasion. Live video microscopy revealed that R3_S804 parasites failed to enter the RBCs. Furthermore, phosphorylation at this site also impaired RhopH3 release from the parasite. Collectively, these studies suggested that phosphorylation of RhopH3 at S804 is important for its trafficking to rhoptries, which in turn is important for its release from the parasite and the process of invasion.

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

Previously, we had reported a novel pathway that promotes CRNA by amyloid peptide $A\beta_{42}$, which involves deregulation of miR34a caused by degradation of its transcription factor TAp73. We reported that ubiquitin ligase involved in this process Itch, which is aberrantly activated by $A\beta_{42}$. Furthermore, we identified miR34a as one of the TAp73 targets which was downregulated in response to Itch activation and contributed to CRNA. We have identified other miRNAs like miR449a that may be involved in neuronal differentiation and are deregulated in models of Alzheimer's Disease. Further studies to investigate their role in CRNA are being pursued.

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

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FBW7μ degrades p53 via proteasomal pathway

We wanted to determine whether like Mdm2, FBW7μ can also control p53 levels by targeting it for degradation. Indeed, the protein levels of endogenous p53, like two other FBW7μ targets namely c-Myc and Cyclin E were enhanced in HCT116 FBW7μ KO cells. At a single cell level, stabilization of the endogenous p53 was also observed. Endogenous p53 was also stabilized in HCT116 cells in which FBW7μ has been ablated by a pool of siRNAs. The relative stability of p53 was increased significantly in cells lacking FBW7μ, indicating that FBW7μ is possibly targeting p53 for degradation. This effect on p53 stability occurred only due to FBW7μ #soform expression. The specific effect of FBW7μ expression on p53 was also observed in HCT116 cells in which FBW7μ isoform had been specifically disrupted. Further increasing levels of FBW7μ led to a progressive decrease in p53 levels. Finally, two different proteasomal inhibitors (MG132 and ALLN) reversed FBW7μ dependent p53 degradation in multiple cell types as observed by western analysis and immunofluorescence.

Phosphodegron mediated polyubiquitylation of p53

The consensus phosphodegron for FBW7μ substrate recognition is S/TPXXS/T motif where S/T indicate phosphorylated serine or threonine residues, P is proline while X is any amino acid. In p53 there is only one phosphodegron, ^{33}SPLPS^{37}. FBW7μ binds to p53 WT and not to any of the three p53 phosphodegron mutants (p53 S33A, p53 S37A and p53 S33A, S37A). Consequently, only p53 WT is ubiquitylated via K48 linkage by FBW7μ. This leads to the degradation of p53 WT and not any of the three phosphodegron p53 mutants within the cells. It has been reported that p53 is phosphorylated by GSK3b on Ser33 after pre-phosphorylation by DNA-PK on Ser37. Hence, we hypothesized that Ser33 and Ser37 are phosphorylated by GSK3b and DNA-PK respectively, thereby constituting the functional phosphodegron for FBW7μ. Indeed, depletion of either GSK-3b or DNA-PK stabilized endogenous levels of p53. Specific inhibitors to GSK3b and DNA-PK completely abrogated FBW7μ mediated p53 polyubiquitylation and degradation.

p53 polyubiquitylation by FBW7μ affects its function during DNA damage

Finally, we wanted to know whether this process occurred within the cells after DNA damage, specifically after exposure to two DSB inducers – doxorubicin and IR. Exposure to doxorubicin treatment or 2 hr post-exposure to IR led to increased levels of DNA damage (measured by gH2AX levels) and pSer33-p53. However, as the DNA damage gets repaired, the levels of both gH2AX and pSer33-p53 were decreased. Using immunoprecipitation experiments we found that Ser33 phosphorylated p53 interacted with FBW7α to a higher extent in +Dox condition or 2 hrs after IR exposure when higher levels of damage was present in the cells.

To determine whether FBW7μ indeed ubiquitylates p53 during and/or immediately after DNA damage, FBW7α was depleted from HCT116 WT cells and subsequently exposed to either doxorubicin or IR. Lack of FBW7α led to a robust stabilization of Ser33 phosphorylated p53 and total p53 upon exposure to either types of damages. Immunoprecipitation with anti-ubiquitin antibody revealed increased p53 polyubiquitylation only in doxorubicin exposed cells expressing FBW7μ.

To understand the implication of this regulation, we carried out long-term clonogenic, invasion and soft agar assays in HCT116 p53 -/- cells expressing p53 WT or its different mutants. We found that compared with p53 WT, all the four p53 mutants drastically decreased the ability of cells to form clones and show invasive potential in each of these assays. Hence p53 functions as a tumor suppressor was enhanced when its degradation by FBW7μ was inhibited during or immediately after DNA damage.

Publications

Original peer-reviewed articles

1. Lochab S, Singh Y, Sengupta S, Nandicoori VK (2020) *Mycobacterium tuberculosis* exploits host ATM kinase for survival advantage through SecA2 secretome. **eLife** doi: 10.7554/eLife.51466.

2. Pal S, Medatwal N, Kumar S, Kar A, Komalla V, Yavvari P, Mishra D, Rizvi Z, Nandan S, Malakar D, Pillai M, Awasthi A, Das P, Sharma R, Srivastava A, Sengupta S, Dasgupta U, Bajaj A (2019) A localized chimeric hydrogel therapy combats tumor progression through alteration of sphingolipid metabolism. **ACS Cent Sci**. 5: 1648-1662.

3. Tripathi V, Kaur K, Kharat SS, Hussain M, Damodaran AP, Kulshrestha S, Sengupta S (2019). Abrogation of FBW7α-dependent p53 degradation enhances p53's function as a tumor suppressor**. J Biol Chem.** 294:13224-13232.

Understanding the regulation of DNA replication

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Theme of Research

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 responses to aberrations in replication complexes. The objective is to identify yet unknown checkpoint pathways that monitor the replication apparatus. We are establishing the role of miRNAs and lncRNAs in regulating the cell cycle. We are also interested in establishing the role of replication proteins in the maintenance of centrosome stability. Overall, we are attempting to unravel the protective and regulatory control of mammalian cell cycle, failure of which is likely to cause genomic instability.

Role of non-coding RNAs in carcinogenesis

In order to identify the cancer associated lncRNAs we first, analysed the lncRNA expression in different cancer types which was obtained from TCGA repository and listed the dysregulated lncRNAs. To identify the gene targets of these dysregulated lncRNAs we utilized the data obtained from a technique called MARGI. In this technique ligation between chromatin-associated RNAs with their target genomic sequences is carried out by proximity ligation, forming RNA-DNA chimeric sequences (Fig. 1). We noted that many dysregulated lncRNAs had putative gene targets which are known to regulate cell cycle during oncogenesis. We have also investigated the role miRNA-mRNA regulatory network in oxidative stress. We have identified a miRNA mega-cluster coding approximately 40 miRNAs whichgets induced after oxidative stress. Based on our results we concluded that on sensing

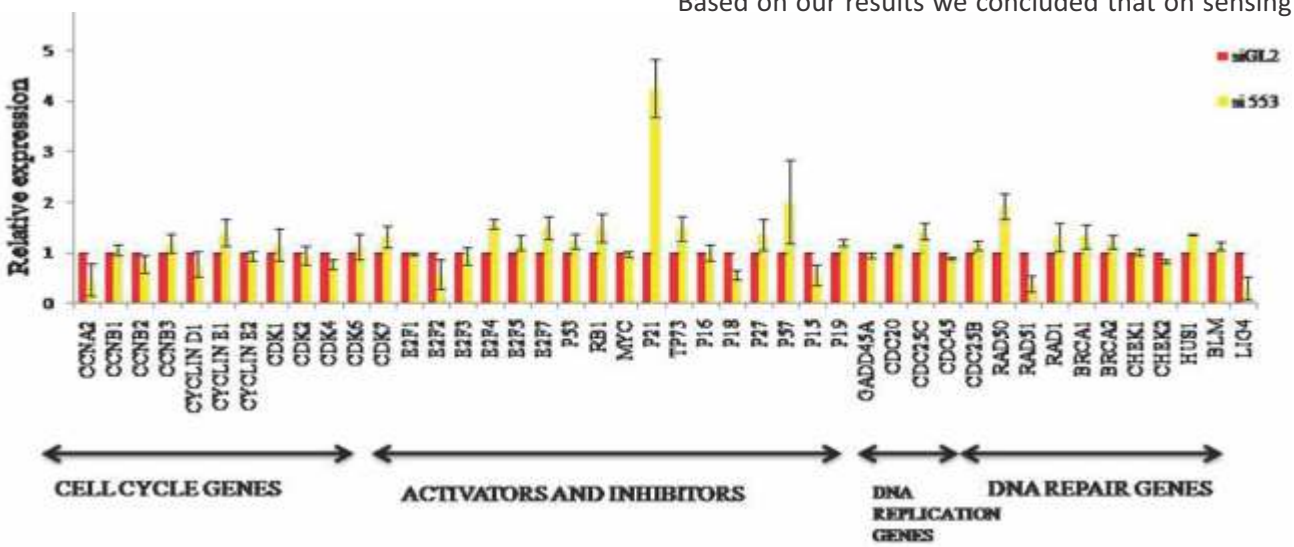

Fig. 1: A screening methodology identifies lncRNAs regulating carcinogenesis. Depletion of lncRNA LNC0002*(si553) leads to the upregulation of cyclin dependent kinase inhibitor p21 resulting in cell cycle block. The figure shows the expression of cell cycle and DNA repair genes.

oxidative stress, the cell induces miR-1* from a megacluster located at 14q32 to target cell cycle promoting cyclins for mediating the cell cycle arrest. *as per DRCCL laboratory inventory.

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 centriolar satellites (CS) get dispersed away from the centrosomes in Sld5-deficient cells, and since the trafficking to centrosomes is dependent on dynein–dynactin-mediated active transport, we studied the function of this complex in HeLa cells. Cytoplasmic dynein is a complex of 11 subunits, composed of a homodimer of motor domain-containing heavy chain (DHC), intermediate chains (IC1 and IC2), light intermediate chains (LIC1and LIC2), and three classes of light chains (LC1, LC2; RB1, RB2;and LL1 and LL2). We assessed the protein levels of individual dynein subunits. Interestingly, we observed that the DHC subunit, which is a motor protein with microtubule binding and ATP hydrolysis activity and generates movement along microtubules, was significantly decreased, a finding that would certainly impair the movement of cargo toward the centrosomes. Moreover, we observed that the entire dynein complex was destabilized, apparently, owing to loss of the major DHC subunit that holds the entire complex together: intermediate chains, IC1 and IC2; light intermediate chain, LIC2; and light chains, LC1 and LC2 displayed significant decrease at protein levels (Fig.2A). The expression of DHC subunit was significantly downregulated at the mRNA level, where as other dynein subunits displayed different levels of downregulation (Fig.2B-D). Thus, it seems that Sld5 deficiency-induced downregulation of DHC expression triggers a sequence of events that destabilize the entire dynein complex.

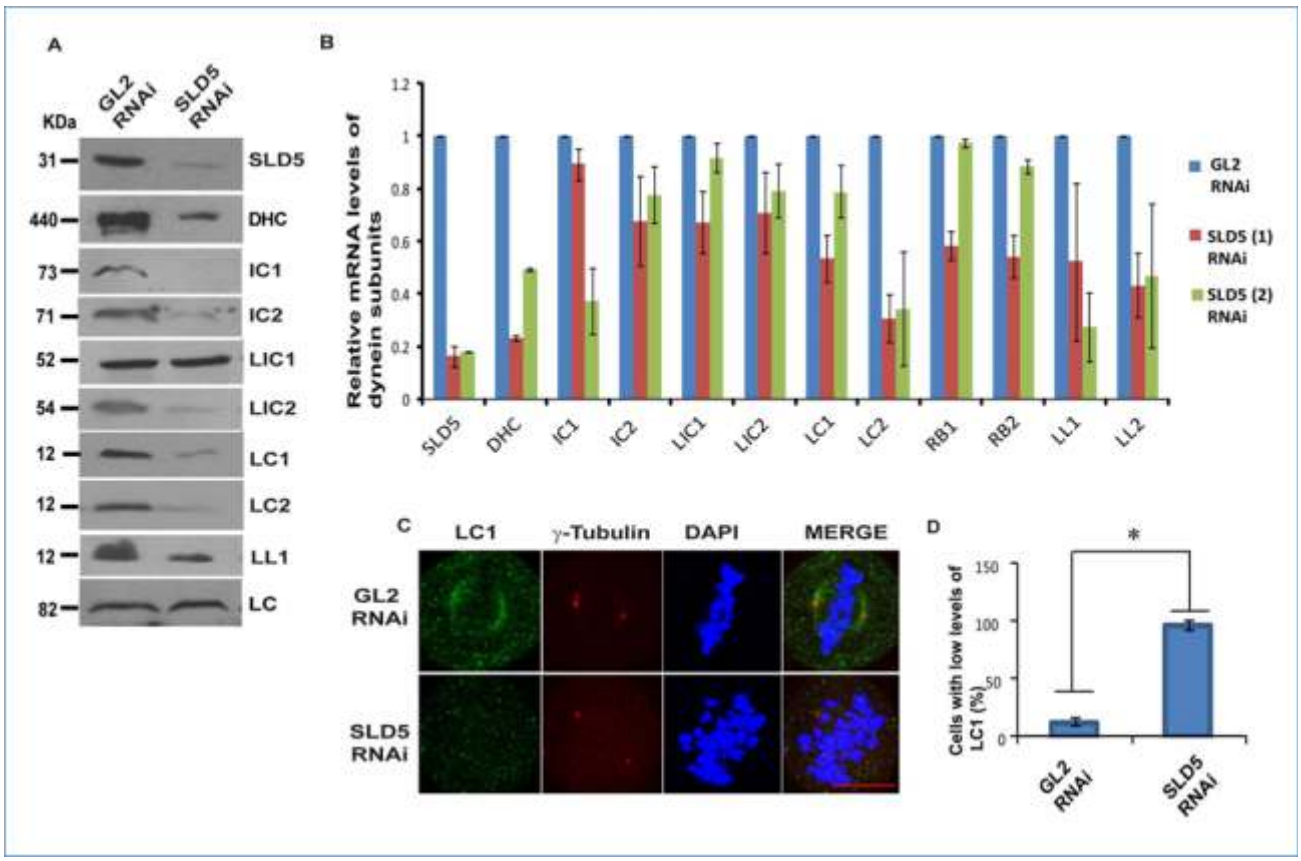

Fig. 2: Dynein complex is downregulated in Sld5-deficient cells.(A) HeLa cells were transfected on three consecutive days with control *GL2*, *SLD5 (1),*or *SLD5 (2)* siRNA and the lysates of transfected samples were immunoblotted with antibodies recognizing the indicated dynein subunits. (B) The mRNA levels of dynein subunits were determined using reverse-transcriptase PCR. The bars indicate the mRNA levels of different dynein subunits following specific siRNA depletions relative to the levels in control *GL2*-transfected cells. β-microglobulin (BMG) served as the internal RNA-loading control. (C) HeLa cells transfected with control *GL2 or SLD5 (1)* siRNA, as indicated in part A, were co-stained for light chain, LC1 (green);-tubulin (red);and DNA (blue). The quantification is reported in part D.

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 proteins viz. caspase-10 and HDAC5 which determine tumorigenesis. Caspase-10 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. HDAC5 is a member of highly conserved class IIa family of ZN^{2+} dependent histone deacetylases. Altered expression of HDAC5 has been linked to tumorigenesis as it regulates transcription of key genes regulating important cellular functions such as cell proliferation, cell-cycle regulation, apoptosis and angiogenesis.

Our proteomics screen identified ACLY as a caspase-10 interacting protein. Caspase-10 cleaves ACLY under conditions of altered metabolic homeostasis. Cleavage of ACLY abrogates its enzymatic activity and suppresses the generation of acetyl-CoA, which is critical for lipogenesis and histone acetylation. To gain further mechanistic insights into the alteration of histone H3 and H4 acetylation status, we next sought to identify the histone acetyltransferase involved. Under metabolic stress conditions, we observed increased histone H3 and H4 acetylation in absence of caspase-10. However, this increase in histone acetylation was abrogated upon co-depletion of GCN5. These results indicate that caspase-10 impairs GCN5 activity to alter global histone H3 and H4 acetylation. GCN5 serves as a coactivator of various oncogenic transcription factors including Myc and E2F. We observed that targets of E2F and Myc involved in cell proliferation and metastasis were specifically responsive to the presence of caspase-10 and GCN5. Concomitant to decline in GCN5 activity upon metabolic stress, the expression of these genes was downregulated. Our data also suggest that under metabolic stress conditions, depletion of caspase-10 results in enhanced invasiveness and migration potential. However, co-depletion of caspase-10 along with ACLY or GCN5 significantly reduced the invasiveness and migration potential. We next investigated the effect of caspase-10 on ACLY-promoted tumorigenesis. We observed that under metabolic stress conditions, caspase-10 depletion results in significantly larger tumors, which was attenuated upon co-depletion of ACLY or GCN5. Since elevated levels of ACLY have been reported in lung adenocarcinoma, we next examined the levels of ACLY and caspase-10 in different grades of lung adenocarcinoma. We observed that consistent with increase in ACLY levels, caspase-10 levels were downregulated with increasing grades of lung adenocarcinoma. Thus our data suggests that dysregulation of caspase-10 expression and consequent upregulation of ACLY is critical for lung cancer progression.

Dysregulation of HDAC5 has been implicated in development of many pathogenic conditions including cardiac hypertrophy and cancer. A greater insight is needed on the molecular mechanisms

underlying the role of HDAC5 in tumorigenesis attributed to its deacetylase activity. To identify novel substrates of HDAC5, we performed a proteomic screen. Among the novel interactors, SATB1 was of particular interest. SATB1 (Special AT rich binding protein 1) is a global genome organizer that recruits chromatin remodeling proteins to epigenetically regulate several genes. It has been recently reported to be overexpressed in various cancers. We plan to further investigate whether SATB1 is a HDAC5 substrate and its implications for neoplastic transformation.

Publication

Original peer-reviewed article

1. Kumari R, Deshmukh RS and Das S (2019) Caspase-10 inhibits ATP-citrate lyase-mediated metabolic and epigenetic reprogramming to suppress tumorigenesis. **Nat Commun.** doi: 10.1038/s41467-019-12194-6.

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

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Regulation of protein homeostasis and energy metabolism are intertwined: increased availability of nutrients tend to increase protein synthesis while a deficit in energy levels leads to increased protein degradation. Amino acids generated by protein degradation, in turn, contribute to energy homeostasis. Though disruption of this cross-talk is observed in several diseases, the mechanisms by which these processes regulate each other at the cellular and organismic levels are not fully understood. Our research focuses on understanding various mechanisms that are involved in reciprocal regulation of metabolism and protein synthesis.

Major projects underway in our laboratory are as follows:

A. Translation regulation in immune cells

We have previously found that resting B cells have a higher rate of protein synthesis compared to resting T cells, which primes the resting cells for activation. Follow up of this work identified the differential regulation of two arms of the mTOR pathway to be responsible for the increased protein synthesis in B cells. AKT, which activates the mTORC1 pathway is more active in resting B cells compared to resting T cells. Moreover, phosphorylation levels of S6, a downstream marker for mTORC1 activity is also high in B cells. In spite of the higher activity of mTORC1 in B cells, translation levels of TOP mRNAs are low in B cells suggesting the 4EBP arm of mTORC1 is somehow inactivated. Indeed, B cells achieve this by increasing the total protein levels of 4EBP, an inhibitor for ribosomal protein-coding mRNAs, leading to the repression of ribosome biogenesis.

B. Metabolic regulation in skeletal muscle protein synthesis

Skeletal muscle is an organ that directly links the energy metabolism with protein homeostasis. Vitamin D deficiency associated muscle atrophy is a major healthcare problem but the etiology is unknown. Our data show that vitamin D receptor-deficient mice (VDR-/-) indeed has a deficiency in protein synthesis during the fasting stage while no observable difference in the postprandial stage. Moreover, proteasome-mediated protein degradation is also high in these mice indicating that there indeed is an imbalance in proteostasis in VDR-/- mice. These defects in protein synthesis and degradation correlate with decreased mTOR activity in these muscles.

C. Regulation of translation by pathogens

Mycobacterium tuberculosis is a human pathogen that claims the maximum number of lives than any other infectious agent. The emergence of multidrug-resistant *M. tb* is a major concern, especially for India, which has the highest *M. tb* burden. A number of antibiotics that are used to treat tuberculosis as well as other bacterial infections target ribosomes, the protein synthesis factory in the cell. But ribosomal dynamics in *M. tb* is not yet understood.

We developed a new version of the cutting edge technology, ribosome profiling, that enables us to analyze the ribosome dynamics in *M. tb* at a high resolution. We found that the ribosome dynamics of *M. tb* as evidenced by the widespread ribosome pausing is dramatically different from that of well-studied model organisms such as *E. coli*.

Publications

Original peer-reviewed articles

1. Chawla AS, Khalsa JK, Dhar A, Gupta S, Umar D, Arimbasseri GA, Bal V, George A, Rath S. (2020) A role for cell-autocrine interleukin-2 in regulatory T cell homeostasis. **Immunology.** doi: 10.1111/imm.13194.

2. Khalsa JK, Chawla AS, Prabhu SB, Vats M, Dhar A, Dev G, Das N, Mukherjee S, Tanwar S, Banerjee H, Durdik JM, Bal V, George A, Rath S, Arimbasseri GA (2019) Functionally significant metabolic differences between B and T lymphocyte lineages. **Immunol**. 158: 104-120

3. Bhalla P, Shukla A, Vernekar DV, Arimbasseri AG, Sandhu KS, Bhargava P (2019) Yeast PAF1 complex counters the pol III accumulation and replication stress on the tRNA genes. **Sci Rep.** doi: 10.1038/s41598-019-49316-5.

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Understanding the sources and mediators of meta-inflammation that aggravate inflammatory bowel disease (IBD) pathogenesis during Obesity/Lean Metabolic Obesity (LMO)

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Theme of research

Inflammatory bowel disease (IBD) is an idiopathic disease which is a combination of two sets of pathologies; chronic inflammation of the entire gastrointestinal tract (Crohn's disease; CD) and inflammation of the colon (Ulcerative colitis; UC).Diet-induced obesity is one of the risk factors for the development of IBD. Although the parallel rise in obesity and IBD has been well documented, the causative relationship between obesity and IBD is far from understanding. In response to nutrient surplus,various organs secrete multiple plasma soluble factors called organokines that act on other organs to modulate target organ functions. However, the role of such organokines and their source in the development of IBD is less explored. In view of these observations, in our laboratory, we use *in-vitro* cell culture-based assays and genetic mutant mouse models to understand the role of multi-organ cross-communication via secretoryorganokines that are critical in the development of IBD.

We ask the following key questions:
(1) Does HFD induced obesity exacerbate colon meta-inflammation? (2) Is there a functional cross talk between other organs and colon influencing IBD development? (3) What are the potential organokines that are critical for the development of IBD during obesity? (4) What are the molecular mechanisms underlying organokines mediated exacerbation of IBD?

Objectives

(1) Development of a colitis mouse model to study the role of meta-inflammation

To understand the correlation between IBD and obesity, we do not want to have a mouse model with overwhelming inflammation. Severe damage to the epithelial barrier and/or heightened inflammatory response may mask the regulatory mechanism in the development of meta-inflammation-associated IBD. To overcome this, we have established a mouse model of colitis that displays low-grade inflammation by administering a group of mice with 3 different doses of DSS (1%, 3%, and 5% of DSS) for 5 days. After analyzing macroscopic and microscopic criteria of colitis assessment in these mice, we concluded that the administration of 1% DSS for 5 days induce low-grade inflammation in mice and decided to use this mouse colitis model with low-grade of inflammation for investigating the role of HFD induced meta-inflammation in colitis.

(2) Role of HFD feeding in the colon meta-inflammation

Having established a colitis mouse model of sub-optimum inflammation, we asked the next important question. Does feeding of HFD induce colon inflammation? and what is the role of HFD in DSS induced colitis? Lean mice were fed HFD for 10 weeks to induce obesity-insulin resistance and then divided into 2 groups. One group was administered with 1% DSS for 5 days and others received water as vehicle control. we compared these two groups of obese mice with lean mice that were administered 1% DSS or water. Our study in consistent with data observed in human cohorts suggests that the HFD diet-induced meta-inflammation increases the risk of IBD development.

Having established the mouse model of HFD induced meta-inflammation and validated the human obesity and IBD correlation, we next plan to screen the secretory organokine-genes datasets, identify and functionally validate organokines that could play a key role in colon meta-inflammation.

ANCILLARY RESEARCH

Production of transgenic and other animal models for biomedical research

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Objective of the research is to generate transgenic animals for study of functional genomics, mammalian development and for the use in biomedical research. To provide services of making transgenic animals to various laboratories of the nation.

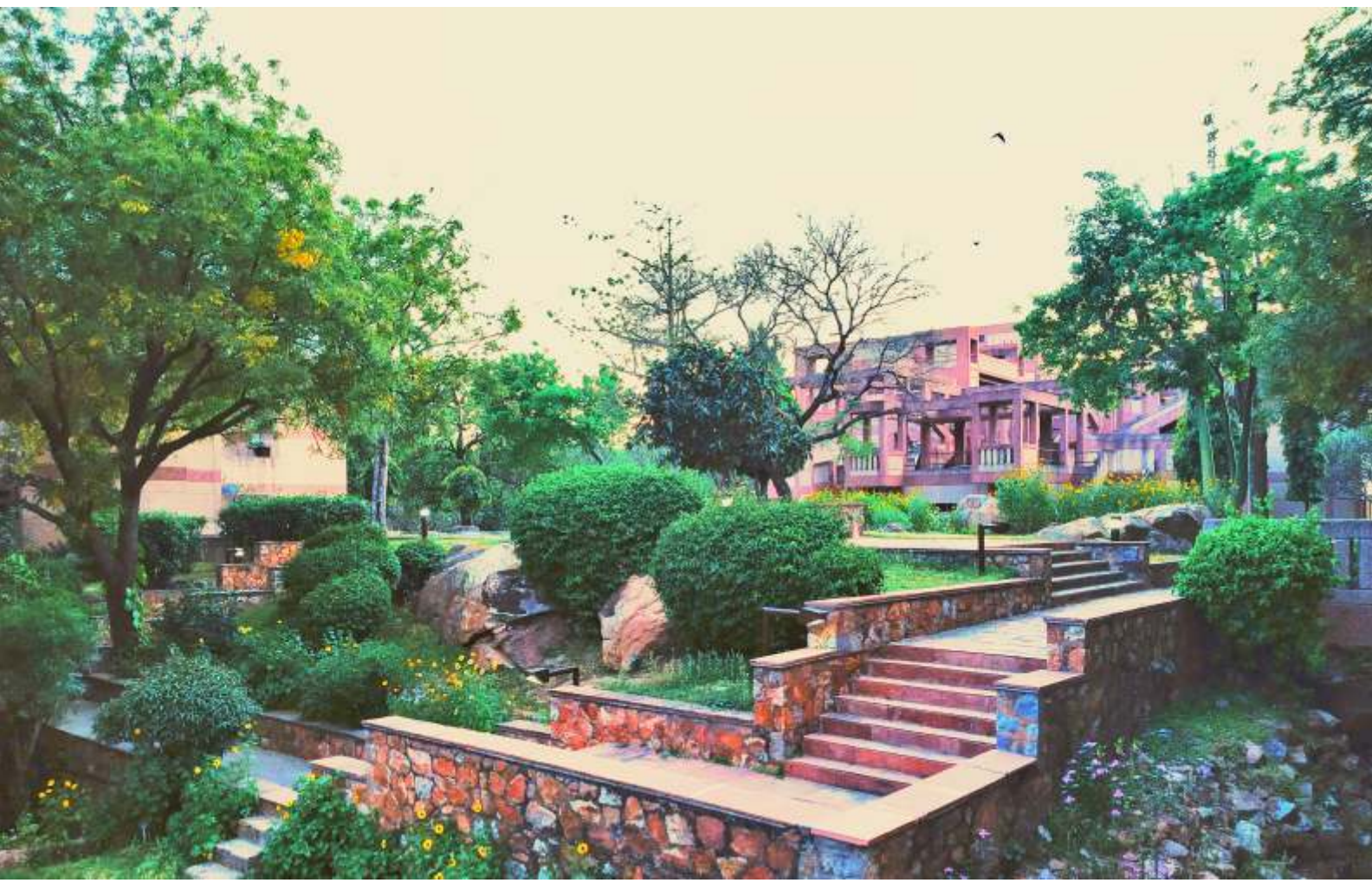
Generation of transgenic mice having Sertoli Cell specific knock down of Long noncoding RNAs

Long noncoding RNAs (lncRNAs) are an emerging class of RNA molecules being known for their importance in maintaining cellular physiology and association with various diseases. The knowledge about the identity and physiology of lncRNAs in testes, and particularly Sertoli cells is scarce. To understand the role of lncRNAs in Sertoli cell function, transgenic mice of Gas5 and Tug1 lncRNAs were generated to understand their role *in vivo.* The plasmids containing the gene specific shRNA were electroporated in the testes of 30 days old mice. Two shRNAs for Tug1 and one shRNA for Gas5 were electroporated. The electroporated males were mated with wild type females and F1 pups were obtained. The pups are being analysed by PCR and Slot Blot.

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.

To understand the Role of Homeobox Gene HOXA10 in Pathogenesis of Endometriosis, we have generated transgenic mice carrying an HOXA10 shRNA (Dr. Deepak Modi, NIRRH, Mumbai). Endometriosis is characterized by the presence of endometrial-like tissues outside the uterine cavity. While many factors are associated with endometriosis, the cause of the disorder is unknown. Previous studies have demonstrated that expression of the transcription factor HOXA10 is reduced in endometrium of women with endometriosis. However, the functional significance of this downregulation in pathogenesis of endometriosis is unknown. The results of Dr. Modi revealed that the HOXA10 hypomorphs have increased number of KI67 positive cells in both epithelium and stroma indicative of hyperproliferation; these animals had higher expression of IL1 β and ERβ indicative of higher inflammatory activity. Further studies are ongoing to evaluate how loss of HOXA10 contributes towards pathogenesis of endometriosis.



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REVIEWS/PROCEEDINGS/CHAPTERS

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5. Garg LC, Dixit A, Gopal K. Recombinant nontoxic protein vaccine against *Clostridium perfringens* infection and epsilon toxin Intoxications. (Indian Patent Application No. 319556 granted on 30th August, 2019).
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8. Polachira SK, Nair R, Jayalekha R, Gupta SK, Mishra NN, Agarwal A. Herbal microbicide formulation for preventing HIV (Indonesian Patent Application No. P00201907700 filed on 9th December, 2019)
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12. Solanki P, Sri S, Lakshmi GBVS, Kumar A. Novel fluorescence-based toggle sensor for detection of antibiotics (Indian Patent Application No. 201911042412 filed on 18th October, 2019)
13. Singh A, Verma J, Ahuja R, Panda AK. A method for fabrication of bilayered PDLLA Gelatin composite scaffold for tissue regeneration. (Indian Patent Application No. 201911036222 filed on 9th September, 2019)
14. Meena J, Panda AK. A highly efficient polymer particulate vaccine formulation entrapping admixture of alum and antigen. (Indian Patent Application No. 201911036242 filed on 9th September, 2019)
15. Singh A, Panda AK. A process for fabrication of transparent polymeric scaffold for tissue engineering applications. (Indian Patent Application No. 201911036223 filed on 9th September, 2019)

AWARDS AND DISTINCTIONS

Distinctions/Honours/Fellowships

Dr. Vinay Kumar Nandicoori was conferred the JC Bose National Fellowship 2019.

Dr. Nirmala Jagadish was conferred OPPI Woman Scientist Award.

Dr. Soumen Basak received Shanti Swarup Bhatnagar Prize (SSB) in Biological Sciences (2019).

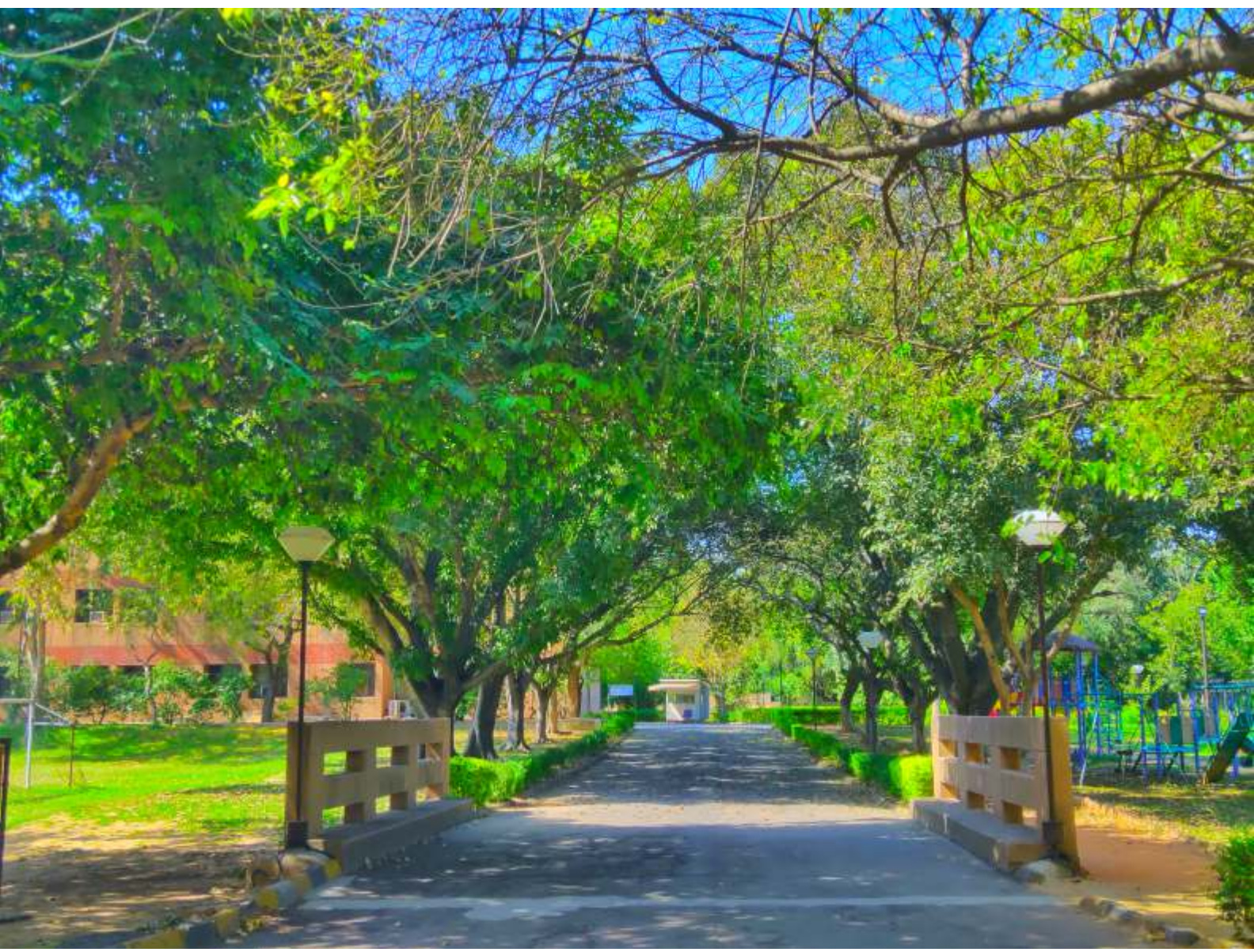
Dr. Arnab Mukhopadhyay was selected as fellow of The National Academy at Science, India (NASI). He also received the Science and Technology Award for Research - SERB STAR.

Ph.D. DEGREES AWARDED TO NII SCHOLARS

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. Bhavya Jha	Structural and biochemical studies of histidinol phosphate phosphatase from *Mycobacterium tuberculosis*	Dr. B.K. Biswal
2	Mr. Zaffar Equbal	Stage specific differentiation of human mesenchymal stem cells into neurons and their characterisation	Dr. Madhulika Srivastava Dr. Pushkar Sharma
3	Ms. Surbhi Goswami	Chemical and biological modulation of glycoforms of sialoglycoconjugates and their functional consequences	Dr. SG. Sampathkumar
4	Ms. Basanti Malakar	Elucidating phosphorylation mediated regulation of secretion and function of effectors, CFP10 & ESAT6 in *Mycobacterium tuberculosis* virulence	Dr. Vinay K.Nandicoori
5	Mr. Shubhendu Trivedi	Modulation of innate immune signaling by HIV-1 via TRAFs	Dr. P.B. Tailor Dr. A.C. Banerjea
6	Ms. Sonam Verma	Role of interferon gamma in regulating invasion of trophoblastic cells	Dr. Rahul Pal Dr. S.K. Gupta
7	Ms. Saishruti Kohli	Unravelling the molecular determinants of SIRT6 tumor suppressor functions	Dr. Sanjeev Das
8	Ms.Usha Yadav	Understanding the lipoic acid synthesis pathway of *Leishmania major*	Dr. Monica Sundd
9	Ms. Sonia Verma	Molecular characterization of a fluoride resistance gene in *C. elegans* longevity	Dr. Arnab Mukhopadhyay
10	Ms. Mansi Grover	In silico analysis of long non-coding RNAs and architectural proteins associated with epigenetic regulation	Dr. Debasisa Mohanty
11	Ms. Irene Saha	Understanding the role of NF-kB family member RelB in dendritic cell diversity	Dr. P.B. Tailor
12	Mr. Sachendra Singh Bais	Investigating the role of canonical NF-kB signaling in host cell responses to RNA viruses	Dr. Soumen Basak
13	Mr. Mohd. Anees Ahmed	Understanding specificity of anti-*Salmonella* immune responses and their role in immunity	Dr. Ayub Qadri
14	Ms. Vandita Dwivedi	Modulation of glycoforms of cell surface molecules by N-acetyl-D-galactosamine (GalNAc) analogues and its functional consequences	Dr. S.Gopalan Sampathkumar
15	Ms. Raksha Devi	Understanding the role of Sld5 in maintaining centrosome structure	Dr. Sandeep Saxena
16	Mr. Praveen Kumar	Understanding the microRNA-gene regulatory networks during DNA damage	Dr. Sandeep Saxena

S.No.	Student's Name	Topic of Research	Guide
17	Ms. Suman Gupta	Analysis of innate and adaptive immune components in the maintenance of gut homeostasis	Dr. Anna George
18	Ms. Beneeta Kalha	Delineation of molecular pathways involved in gonadotropin-mediated chemoresistance in tumor cells	Dr. Rahul Pal
19	Ms. Afshana Quadiri	Characterization of the host immune responses against a malaria liver stage subunit vaccine candidates SLTRIP	Dr. Agam P. Singh
20	Mr. Danish Umar	Analysis of temperature-mediated modulation of T-cell functions	Dr. Anna George
21	Ms. Hritika Sharma	Consequences of immune recognition of hemoglobin in lupus	Dr. Rahul Pal
22	Ms. Anita Goyala	Deciphering the role of *Caenorhabditis elegans* p53 like-1 gene (cep-1) in dietary restriction-mediated longevity assurance	Dr. Arnab Mukhopadhyay
23	Mr. Pitale Durgesh Manohar	Understanding the early events in *Leishmania* infection	Dr. Soumen Basak
24	Ms. Himanshi Agarwal	Understanding the role of BLM helicase during DNA double-strand break response	Dr.Sagar Sengupta
25	Mr. Vineet	Human arginase-I: revealing the role of metal ions, heat activation and macromolecular crowding on enzyme stability and activity	Dr. Apurba Kumar Sau
26	Ms. Mehak Zahoor Khan	Unraveling the functional facets of protein kinase G (PknG) and *actinomycetes* oxidative stress response regulator (AosR) in mycobacterial stress response.	Dr. Vinay K. Nandicoori
27	Ms. Akansha Singh	Design and evaluation of composite scaffold for tissue engineering applications	Dr. Amulya K. Panda
28	Ms. Preeti Attri	Investigating the role of BLM in the regulation of histone modifications after DNA damage	Dr. Sagar Sengupta
29	Ms. Ankita Dutta	Understanding the structure, function and regulation of *helicobacter pylori* arginase	Dr. Apurba K.Sau
30	Ms. Chandni Sood	Role of Rab18 in regulation of phagosome maturation in macrophages	Dr. Soumen Basak
31	Ms. Richa Kumari	Deciphering the role of PKM2 in oncogenesis	Dr. Sanjeev Das
32	Ms. Prity Yadav	Functional and structural aspects of sortases	Dr. R.P. Roy



LECTURES AND SEMINARS

Foundation Day Lecture

The 33rd Foundation Day of NII was celebrated on 6th October, 2019 at the Institute. **Prof. Anil K. Gupta** (Visiting Faculty, IIM Ahmedabad & IIT Bombay, Founder, Honey Bee Network, SRISTI, GIAN and NIF, CSIR Bhatnagar Fellow) was invited as a Guest of Honour. He delivered a lecture on **"Scientific innovation with social and empathetic responsibility"**.

Dr. Amulya K. Panda, Prof. G.P. Talwar, Prof. Anil K. Gupta and Dr. Renu Swarup (Secretary, DBT) on the occasion.

The NII Auditorium was re-christened the "GP Talwar Auditorium" in recognition of Prof. Talwar's contributions to science and to the establishment of the Institute.

Memorable moments with Prof. G.P. Talwar, Mrs. Talwar, Dr. Renu Swarup (Secretary, DBT) and Dr. Amulya K. Panda at the re-christening ceremony of the NII Auditorium.

DBT Foundation Day Celebrations

The 34th Foundation Day of the Department of Biotechnology was celebrated on 26th February, 2020 at NII.


Dr. Harsh Vardhan (Minister of Health and Family Welfare) with other dignitaries on the occasion.

Science Popularization Programme

NII conducted a science popularization programme for school and college-going children on 30th May, 2019. The students of Delhi Public School, Mason's Public School and Deen Dayal Upadhyaya College were appreciative of both the lectures delivered by members of the NII Faculty and the lab visits.


Dr. Amulya K. Panda with students and members of the NII Faculty on the occasion.

Road Show: Global Bio India 2019

As a prelude to Global Bio India 2019, a Road Show was held at NII on 11th November, 2019. Lectures by members of the NII Faculty as well as laboratory visits were organized.

Dr. Amulya K. Panda, Dr. Anil K Suri, Dr. Gopalan Sampathkumar and Lt. Col. (Dr.) D. K Vashist with participants.

5th International Yoga Day

On the occasion of the 5th International Yoga Day (21st June, 2019). Dr. Guru Deo, (Morarji Desai National institute of Yoga (MDNIY), New Delhi) delivered a lecture on the **"Role of diet in yoga practice".** Mr. Tanmay demonstrated Yoga asanas.

Scientists, students and administrative staff participated in a one-hour long Yoga session. Apart from conducting the exercises, Dr. Deo also spoke about the health benefits of each asana. The instructors enlightened the participants about the importance of stress-free living and appropriate diet in Yoga.

Dr. Guru Deo and Mr. Tanmay with Lt. Col.(Dr.) D.K. Vashist (Senior Manager) and Ms. Chandresh Bhagtani (Administrative Officer) on the occasion.

INVITED SEMINARS

S. No.	Topic	Speaker Detail	Date
1	Emerging advances in cellular immunotherapy: Challenges in meeting global demand	Prof. Rajiv Khanna Group Leader, Tumour Immunology Laboratory QIMR Berghofer Medical Research Institute, Australia	02.04.2019
2	Ser/Thr/Tyr kinase and phosphatase-mediated post-translational modifications in *Streptococcus pyogenes*: Implications on bacterial virulence and pathogenesis	Dr. Vijay Pancholi The Ohio State University Columbus, OH, USA	09.04.2019
3	Arising frontiers in the robustness of bacterial-bacterial and gut bacterial-host cell communication	Dr. Gyanendra P. Dubey Molecular Microbial Pathogenesis Unit, Institut Pasteur, Paris	25.04.2019
4	Distinct roles of PD-1 in T cell exhaustion and memory	Dr. Vandana Kalia University of Washington and Seattle Children's Research Institute, USA	26.04.2019
5	A molecular journey to free energy landscapes of amyloid forming α-synuclein fibrils	Dr. Divya Nayar Centre for Computational and Data Sciences (CCDS), IIT Kharagpur, Kharagpur	24.05.2019
6	Mycobacterial biofilms: Are they relevant *in vivo?*	Dr. Ashwani Kumar Principal Scientist Institute of Microbial Technology, Chandigarh	07.06.2019
7	Analysis of antigen receptor sequences of a unique lymphocyte reveals a T cell-neoantigen encoded in a public BCR of TD patients	Dr. Rizwan Ahmed Post-Doctoral Fellow Department of Pathology Division of Immunology, Johns Hopkins University , Baltimore, USA	18.06.2019
8	A philosophical reconstruction of clinical science in cancer: Are personalized treatment plans application or construction of knowledge?	Dr. Alok Srivastava CeeTOC Inc., San Francisco & Visiting Researcher Philosophy, UC Davis, USA	26.07.2019
9	Innate immune route to inflammation	Dr. Vijay Rathinam, D.V.M., Ph.D. Assistant Professor of Immunology and Associate Director of Immunology Graduate Program University of Connecticut Health School of Medicine, Farmington, USA	26.07.2019
10	Intestinal infection regulates immunity, behavior and learning in *C. elegans.*	Dr. Jogender Singh Department of Molecular Microbiology & Immunology Oregon Health & Science University, Portland, USA	02.08.2019

S. No.	Topic	Speaker Detail	Date
11	Integrating computational methods and experimental data for understanding the binding affinity of protein-protein complexes	Prof. M. Michael Gromiha Department of Biotechnology IIT Madras, Madras	02.08.2019
12	Cancer: Molecular mechanisms and therapeutic implications	Dr. Archita Ghoshal Penn State College of Medicine, USA	08.08.2019
13	O-GlcNAcylation: linking metabolism to epigenetics	Dr. Vaibhav Kapuria Center for Integrative Genomics University of Lausanne, Switzerland	23.08.2019
14	Innate-like T cells: Moving at the frontline	Dr. Shilpi Chandra Division of Developmental Immunology La Jolla Institute for Allergy and Immunology San Diego, USA	06.09.2019
15	HIV envelope-based vaccine design for the induction of broadly neutralizing antibodies	Dr. Rajesh Ringe Ramalingaswami Fellow Institute of Microbial Technology, Chandigarh	13.09.2019
16	MechanoImmunology – Exploring physical drivers of immune cell polarization during inflammation and disease	Dr. Nikhil Jain Department of Health Science and Technology ETH Zurich, Switzerland	17.09.2019
17	Oncogenes and their role in shaping the tumor-microenvironment	Dr. Sushil Nehra Department of Obstetrics and Gynecology University of Pennsylvania Philadelphia, PA, USA	24.09.2019
18	Understanding of phospho-signalling pathways in regulation of malaria parasite survival	Dr. Mahmood M. Alam Lord kelvin/Adam Smith (LKAS) fellow Wellcome Centre for Integrative Parasitology Institute of Infection, Immunity and Inflammation, University of Glasgow, UK	25.09.2019
19	Host pathogen interaction and mathematical modeling	Dr. Koushik Roy Department of Microbiology, Immunology and Molecular Genetics University of California, Los Angeles, USA	04.10.2019
20	Mechanistic insight into understanding the translational aspects of autophagy in cancer	Dr. Suresh Kumar Department of Molecular Genetics and Microbiology University of New Mexico, Albuquerque, USA	11.10.2019
21	Improving the efficacy of immune checkpoint blockade in lung cancer	Prof. Vivek Mittal Director Neuberger Berman Lung Cancer Laboratory Weill Cornell Medical College of Cornell University, New York, USA	22.10.2019
22	A network perspective on biologically-relevant chemical spaces	Dr. Areejit Samal, Reader Computational Biology Group, The Institute of Mathematical Sciences Chennai	05.11.2019

S. No.	Topic	Speaker Detail	Date
23	DNA repair, neurodegeneration and aging	Dr. Vilhelm Bohr Chief Laboratory of Molecular Gerontology National Institute of Aging National Institutes of Health, USA	21.11.2019
24	A CRISPR-Cas9 screen reveals novel mechanisms of PD-L1 regulation	Dr. Shruthy Suresh Aggarwal Department of Molecular Biology, University of Texas Southwestern Medical Center Dallas, Texas, USA	05.12.2019
25	Congenital disorders of glycosylation: Structure and function of oligosachharyl transferase function	Prof. Smita Mohanty Professor of Chemistry Oklahoma State University USA	09.12.2019
26	Microbiome and metabolome as drivers of HIV persistence	Prof. Rafick-Pierre Sékaly Case Western Reserve University School of Medicine Department of Pathology Cleveland, OH, USA	19.12.2019
27	Extraterrestrial viruses, fishy research, cancer models and health without borders - a professional trajectory of interconnectedness. And beyond…	Dr. Alok Deoraj Florida International University Miami FL, USA	20.12.2019
28	Targeting NF-kB for cancer immunotherapy	Dr. Sankar Ghosh Chairman and Silverstein and Hutt Family Professor Department of Microbiology & Immunology Columbia University, New York, USA	27.12.2019
29	Heme trafficking from the ground-up: Lessons from bloodless worms	Dr. Iqbal Hamza Professor Dept. of Animal & Avian Sciences University of Maryland, MD, USA	08.01.2020
30	Understanding the immune mechanisms of neuroinflammation and neurodegeneration	Dr. Deepak Kumar Kaushik Hotchkiss Brain Institute University of Calgary, Canada	24.02.2020
31	Dynamic activation and regulation of MAP kinase p38	Dr. Senthil K. Ganesan University of Arizona, Department of Chemistry and Biochemistry, Tucson, AZ, USA	02.03.2020
32	From structure to drug discovery	Dr. Pankaj Kumar Chauhan Department of Biochemistry Jamia Hamdard University Hamdard Nagar, New Delhi	06.03.2020
33	Therapeutic targeting of pericytes in vascular diseases	Dr. Mitrajit Ghosh Centre for Healthcare Science and Technology (CHST) Indian Institute of Engineering Science and Technology (IIEST), Shibpur, Howrah	12.03.2020

NII FACULTY AND Ph.D SCHOLARS LECTURE SERIES

April 2019 to March 2020

S. No.	Topic	Speaker Detail	Date
1	Bacterial inclusion bodies	Dr. Amulya K. Panda	05.09.2019
2	Evaluation of long non-coding RNA (lncRNA) mediated regulation of sertoli cell function	Ms. Ayushi Jain Ph.D Scholar, 2015 batch	12.09.2019
3	Dissecting out the functions of *M. tuberculosis* membrane proteases: A relatively unexplored area	Dr. Bichitra Biswal	19. 09.2019
4	hDAC5 deacetylates SATBI To duppress tumorigenesis Investigating the role of RelB in anti-viral immune response	Ms. Shalakha Sharma Ph.D Scholar, 2015 batch Ms. Yashika Ratra Ph.D Scholar, 2015 batch	26. 09.2019
5	Cell signaling in *Mycobacterium tuberculosis*	Dr. Vinay K. Nandicoori	03.10.2019
6	CD8 T-Cell influences the humoral immunity establishment to live attenuated Japanese Encephalitis vaccine Understanding the mechanisms for mitochondrial dysfunction in cells lacking RECQL4, the gene mutated in Rothmund Thomson Syndrome	Ms. Anurag Kalia Ph.D Scholar, 2015 batch Mr. Aamir Khan Ph.D Scholar, 2015 batch	10.10.2019
7	Metabolic control of cellular signaling and gene expression	Dr. Arnab Mukhopadhyay	17.10.2019
8	Immunological evaluation of nanoparticle-based pneumococcal antigens Gleaning insight into B-cell biology: Preliminary comparative sequence analysis of anti-polysaccaride antibody response in vaccinees	Ms. Mamta Ph.D Scholar, 2015 batch Ms. Hema Sori Ph.D Scholar, 2015 batch	24.10.2019
9	Altering glycosylation in mammalian brain by stealth	Dr. S.G. Sampathkumar	31.10.2019
10	Understanding the role of monocytes in hepatitis B infection Role and regulation of E3 ligase itch in neuronal apoptosis	Ms. Prakriti Sinha Ph.D Scholar, 2015 batch Ms. Monika Chauhan Ph.D Scholar, 2015 batch	07.11.2019
11	Experiences and learning from clinical diagnostics	Dr. P K Upadhyay	14.11.2019
12	Insight into the management of histidine requirements by *Mycobacterium tuberculosis* during the course of infection Investigating the role of Sld5 in regulating the centriolar satellites	Ms. Anam Ashraf Ph.D Scholar, 2015 batch Mr. Vipin Kumar Ph.D Scholar, 2015 batch	21.11.2019
13	Understanding the regulation of arginine metabolism in *Helicobacter pylori*: A Structure-function study	Dr. A K Sau	28.11.2019

S. No.	Topic	Speaker Detail	Date
14	Profiling b-O-GlcNAc modifications on FoxP3 Understanding of hemoglobin receptor recycling in *Leishmania*	Ms. Ahana Addhya Ph.D Scholar, 2015 batch Mr. Irshad Ansari Ph.D Scholar, 2015 batch	05.12.2019
15	Human $CD4^+$ T cell memory and infectious diseases	Dr. Veena Patil	12.12.2019
16	Study the macrophage function and underlying mechanism in TB-IRIS development using T cells deficient mice as animal model In silico analysis of mycobacterial lipid remodeling enzymes	Mr. Lalit Pal Ph.D Scholar, 2015 batch Mr. Bhushan Dhamale Ph.D Scholar, 2015 batch	19.12.2019
17	The art of breaking and making peptide bonds	Dr. R.P. Roy	26.12.2019
18	Analysis the influence of CTCF on regulation of TCRB locus Investigating the role of CDK-12 in aging and germline development under low insulin signaling condition	Ms. Monika Ph.D Scholar, 2015 batch Mr. Gautam Chandra Sarkar Ph.D Scholar, 2015 batch	02.01.2020
19	From immunological memory to protective immunity: Our journey with *Streptococcus pneumoniae*	Dr. Devinder Sehgal	09.01.2020
20	Understanding the role of osmolality in the process of osteogenesis Understanding the role of GTB hydrolysis by hGBP3 and its splice variant hGBP3 C in antiviral activity	Ms. Madhu Baghel Ph.D Scholar, 2015 batch Ms. Sowmiya Gupta Ph.D Scholar, 2015 batch	09.01.2020
21	Peptide-MHC based nanomedicine for reprogramming of regulatory network	Dr. Santiswarup Singha	23.01.2020
22	Effect of glycosylation on HAL-2, an alpha helical antimicrobial peptide GXXGXXXG motif Plays a role in acyl chain sequestration by fungal type I acyl carrier protein	Ms. Gagandeep Kaur Ph.D Scholar, 2015 batch Ms. Garima Ph.D Scholar, 2015 batch	30.01.2020
23	Novel patterns of ribosome pausing in *Mycobacterium tuberculosis*	Dr. Aneeshkumar A.G.	06.02.2020
24	Organizing the loops of life: Understanding CTCF at work	Dr. Madhulika Srivastava	05.03.2020
25	Analyzing the signaling network regulating inflammation: A computational biology approach Effects of immune dysregulation and development of disease regulating strategies for retinitis pigmentosa	Mr. Manti Kumar Saha Ph.D Scholar, 2017 batch Ms. Varsha K. Mohan Ph.D Scholar, 2017 batch	12.03.2020

CONFERENCES/SYMPOSIA/WORKSHOPS

DBT-BIRAC Leadership Programme

Prof. Eric Green (Director, National Human Genome Research Institute, National Institute of Health, Bethesda, USA) delivered a DBT-BIRAC Leadership Dialogue Series lecture on **"From the human genome project to precision medicine: A journey to advance human health"** on 8th January, 2020.

Prof. Eric Green, Dr. Renu Swarup and Dr. Amulya K. Panda with other scientists.

DBT-NIAID Vaccine Adjuvant Science Collaboration Training Workshop

A DBT-NIAID Vaccine Adjunt Science Collaboration Training Workshop was held from 10th to 11th April, 2019.

Participants at the Workshop.

World Immunology Day - 'Immunology Today' Symposium

World Immunology Day was celebrated on 29th April, 2019 at the Institute theme of the inaugural celebration was 'Immunology Today'. Students from all across the Delhi-NCR region spanning several colleges and Institutes participated in the Symposium. After the introductory speech by Dr. Amulya K. Panda, five lectures on topics in contemporary immunology were presented. Five Ph.D. scholars were also given the opportunity to showcase their work. Dr. Nimesh Gupta acted as moderator and facilitated interaction between school students and the speakers. The day concluded with an immunology quiz.

Dr. Amulya K. Panda with members of the Faculty and other scientists on the occasion.

Hands-on Training Workshop for FACS Data Analysis

A hands-on Training Workshop on flow cytometer data analysis was organised by Dr. Nimesh Gupta on October 11th, 2019. The Workshop focused on the advances in FACS data analysis and hands-on training on FlowJo Software. A total of 75 participants (Ph.D. scholars and young scientists) from across research Institutes in the Delhi-NCR region received training. Dr. Keefe Chee (Life Sciences Institute, National University of Singapore) and Dr. Nimesh Gupta delivered the lectures.

Participants at the data analysis software training session.

National Science Day

NII celebrated National Science Day on 28th February, 2020. Ph.D. students and research scholars observed the event with great fervour. The day constitutes a major science festival at NII, during which Ph.D. students present posters describing their research. Prof. Jitendra P. Khurana (Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi), delivered a lecture on **"Molecular insight in to plant vision"**. The day concluded with distribution of awards for the best posters.

Prof. Jitendra P. Khurana and Dr. Amulya K. Panda presenting the Best Poster award to Tripti Nair.

India EMBO Symposium

'Mycobacterial Heterogeneity And Host Tissue Tropism'.

The Symposium, jointly organized by NII and the International Centre for Genetic Engineering and Biotechnology from 11th- 15th February, 2020, focused on neglected and poorly-managed aspects of TB pathogenesis. An interdisciplinary and international group of 40 speakers and chairpersons, along with nearly 150 participants from different countries, were in attendence. Novel scientific insights that could form the basis for future modalities of treatment and diagnosis were discussed.

Dr. Renu Swarup and Dr. Amulya K. Panda with other participants during the inaugural function.

MoU SIGNED AND VISITORS TO THE INSTITUTE

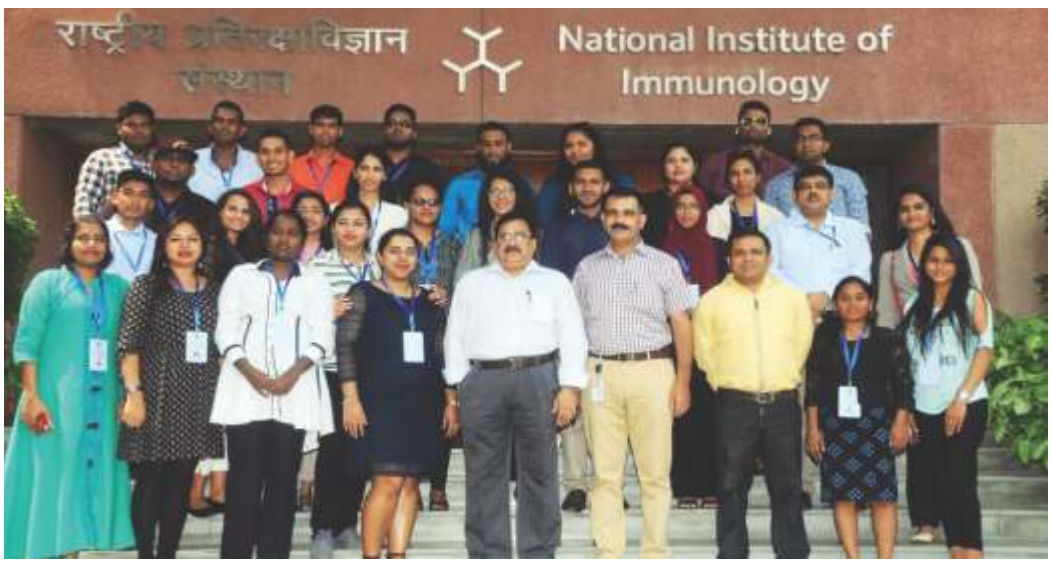
MoU signed with INMAS (DRDO)

NII signed an MoU with the Institute of Nuclear Medicine of Allied Science (DRDO) on May 29th, 2019, to carry out collaborative research. The signatories were Dr. Amulya K. Panda and Dr. Tarun Sekhri (Director, INMAS).

Dr. Amulya K. Panda, Director, NII and Dr. Tarun Sekhri, Director, INMAS with the team.

Visitors under the Know India Programme

NII organized a visit for 54 overseas participants under the Know India Programme of the Ministry of External Affairs for Young Diaspora on August 13th, 2019.



Dr. Amulya K Panda and Lt. Col. (Dr.) D.K. Vashist with participants of the Know India Programme during their visit.

FAREWELL TO Ph.D. STUDENTS

The farewell function for Ph.D. students (Batch of 2014) with Staff Scientists was marked by the planting of a tree.

Ph.D. students of the 2014 batch planting a tree alongside Dr. Amulya K. Panda and Dr. Rahul Pal.

SUPPORTING UNITS

Small Animal Facility

The Small Animal Facility of the Institute is devoted to the humane care and breeding of experimental animals used in approved research. At present, the Small Animal Facility holds 104 mouse strains, including 89 mutant strains, 16 inbred strains and 1 outbred strain. In addition, the Facility also houses 6 rat strains, a stock of rabbits as well as guinea pigs.

The propagation of all defined strains is done in a three- tier system i.e., the Foundation Stock (FS), Pedigreed Expansion Stock (PES) and Production Stock (PS). Genetically modified mouse strains are bred either by 1.homozygous mutant (-/-) x homozygous mutant (-/-) 2. heterozygous mutant (-/+) x homozygous mutant (-/-) 3. heterozygous mutant (-/+) x heterozygous mutant (-/+).

Defined breeding protocols and careful management and husbandry procedures are followed to ensure the purity of murine stains. To maximize genetic purity and uniformity of mice, inbred strains are propagated and replaced periodically in a manner that minimizes the genetic drift and inbreeding depression. A random sample from a few breeders of foundation, expansion and production stocks are monitored with the help of a few microsatellite markers to ensure genetic purity. Several principal investigators also assist in the genotyping of transgenic and knockout mice strains.

Health monitoring program includes regular screening for pathogens, including hepatitis virus, parvovirus, norovirus, pnemonia virus, mycoplasma and Sendai virus; ELISA and PCR are employed. Bacterial pathogens such as *Pseudomonas aeroginosa, Streptobacillus moniliformis, Bordetella, Bronchiseptica, Citrobacter rodentitium, Pasteurella pneumotropica, Staphylococci* and *E.coli* are screened for using culture, using biochemical methods and PCR. Faecal samples are screened for the presence of sedimentation method for the presence of the syphacia and aspicularis species of endoparasites. Periodic FACS analyses are also carried out on immunodeficient mice to assess leakiness.

Procedures are in place to prevent transmission of infection between cages; these include careful handling of animals, washing using automated cage and bottle washers, use of sterilized corn cob bedding, autoclaving of cages and use of acidified autoclaved drinking water. The breeding and experimental colonies are maintained within a barrier system with individual ventilated cages. A veterinarian carries out necropsy/autopsy procedures on infected animals, if indicated. A recommended preventive schedule of medication is strictly followed to reduce the infections to the extent possible.

Primate Research Centre

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

Under the breeding programme, group mating is facilitated in large open pens. In these semi-natural conditions, food and water is provided *ad libitum*. Infants are weaned at the age of six to twelve months (depending on season and weight of infants) after which they are transferred to open enclosures/semi-natural housing for optimal growth, the development of the bones and muscles, and enhanced motor coordination. Monkeys are independently housed at puberty. To prevent cross-cage contamination or infection, strict hygienic procedures are followed. Animals are regularly monitored for tuberculosis, simian herpes virus and

simian hepatitis virus. Animals who are unwell are isolated and treated. Primates are fed with standardised pellet feed. In addition, bread, soaked Bengal gram, vegetables and/or fruits are also given daily. Diet is regularly with vitamins and calcium in bread are given. The staff at PRC undergo an annual preventative health check up. All surgeries, treatments for injury and the administration of medication are performed by a registered veterinarian, and all procedures such as immunizations, and biopsies are aided by experienced technical staff. A research laboratory at the Centre provides basic services to investigators. Clearance of the research proposals by the CPCSEA (after initial clearance from the Institutional Animal Ethics Committee) is necessary for conducting research on primates. Macaques have been employed in research related to infectious diseases, reproduction, endocrinology, immunology and contraception. Staffs ensure that all procedures are pain-free, with minimum stress to the animal, and all effort is made to keep the animals comfortable. There are seventeen open enclosures with swings and shelters, which are used for rehabilitation or socializing, All attempts are made to maximize residence in such enclosures.

OTHER SERVICE UNITS

Establishment, Personnel and General Administration Services

The Division continued to provide key support for optimally utilizing and integrating human and administrative resources aimed at realizing the vision of the Institute. During the reporting period, administrative support was provided for formulating policies and ensuring their effective implementation. Other key areas include handling service matters, recruitments, career development, foreign visit of scientists for training/ conferences/ bilateral exchange visits exchange visits, staff welfare. staff welfare, post retirement dispensation, preparation and submission of periodic reports to the administrative ministry, liaisoning with them and preparing responses to parliament questions. To bolster capabilities and enhance productivity, the Institute periodically sponsors administrative and technical staff for training in recognized training Institutes.

The Institute's RTI Cell files the quarterly reports on the RTI portal. The Institute also has an effective grievance redressal mechanism to deal with public as well as staff grievance petitions, ensuring timely disposal.

Financial and Accounting Services

The Division has been responsible for preparation of the annual budget, management of fund utilization, receipt and disbursement of all payments, internal auditing, getting accounts audited by statutory and CAG auditors, sending reports to funding agencies and recovery and remittance of TDS from salary and contractors, filing institutional income tax return, obtaining required exemptions from 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 & Purchase Department of NII is responsible for procurement of chemicals, consumables, glassware, plasticware along with other required miscellaneous items. Items are procured by Stores Department-1 from local as well as international sources. The procurement function is overseen by a Purchase Committee comprising three or more Scientists, the Finance & Accounts Officer and the Stores & Purchase Officer.

Engineering, Maintenance and Instrumentation Services

The Engineering Department of the Institute has been entrusted with all the engineering activities involving maintenance, services and capital works. It has always been the endeavor of the Department to provide the best of services with the use of the latest/modern technology; as a result, systems are being continuously modernized. Major activities undertaken during the reporting year are as follows:

- Installation of 10 KW rooftop grid sharing solar system.
- Setting up of new laboratories and offices.
- Installation of LED lighting fixtures & retrofitting of LED lamps in existing fixtures.
- Installation of CCTV cameras.
- Replacement of PVC fills in cooling towers.
- Miscellaneous HVAC/Civil/Electrical work in the Small Animal Facility.
- Installation of floodlights in the playground.
- Replacement of compound lighting fixture with LED lighting fixtures.
- Replacement of cooling coil of AHUs.
- Creation of new parking space near JNU Gate and New Guest House.
- Finishing/painting works at terrace level of Main Building.
- Renovation works in a few apartments.
- Upgradation work in a few laboratories.
- Upgradation of Gym space in the community facility block.
- SITC of air washer for Small Animal Facility.
- Kitchen renovation work in the NII staff quarters at Dwarka.

The Department is currently working on the following projects:

- Re-carpeting & repairing of roads.
- Installation of LED lighting fixtures & retrofitting LED lamps in existing fixtures.

- Installation of a rain harvesting system.
- Replacement of cooling coil of AHUs.
- Refurbishment of an conditioning system with allied works at the New Animal Facility.
- Renovation work in a few existing apartments.
- Silicon treatment on exterior grit plaster.
- Upgradation work in a few laboratories.

The Swachta Pakhwada team of NII with Dr. Amulya K. Panda.

Library and Documentation Services

The Library And Documentation Department is a service oriented supportive unit which works 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, plus, a plethora of resources that are accessible online by scientific staff and students. NII is a member of the DeLCON consortium project of the Department of Biotechn-ology. The Library coordinates procedures (both online and print) for the subscription of journals, as well as processes the payment of journal publication charges. The Library has computerized all its housekeeping activities. A searchable database, Web-Online Public Access Catalogue (Web-OPAC), is being maintained.

The Library has been involved in compiling, designing and printing the Parliamentary and Scientific Annual Reports of the Institute in Hindi and English. Additionally, the Library also prepares monthly pictorial research publications bibliometrics reports. Information on subscriptions, new procurements and publications is regularly updated on the NII website.

The Library maintains a searchable Institutional Digital Repository, containing full texts of articles published from NII from the year 2008. The Library also conducts an induction programme for new- comers, as well as workshops on various subjects such as the use of plagiarism and Scopus software.

The Library is responsible for all the binding and photocopying work of the Institute. A Hindi Library, which houses a collection of Hindi-books (including those dealing with administrative practices) and magazines has been set up for popularizing the official language amongst amongst the Institute staff.

Resources

Eight reference books and ninety-three Hindi books have been added to the Library collection. The Library participates in organizing Institutional lectures such as the Foundation Day Lecture and the National Science Day Lecture National Science Day Lecture, amongst many others.

As a new initiative, the Library has set up Twitter, Blog and Facebook accounts of the Institute and posts regular updates. User awareness training on "Web of Science" and "EndNote" was organized on 28th August, 2019.

Lecture on use of "Web of Science" and "EndNote."

Academic and Training Services

The activities of the Academic & Training Department have three major groups viz. Students Affairs, Outside Training and In-House Training. The Academic Department has been involved in organizing Ph.D. admissions, pre-Ph.D. registration courses, Doctoral Committee meetings, Academic Committee meetings and also coordinates the disbursal of fellowship to scholars. The Institute takes in scientists who have been awarded independent fellowships from the following institutes/organisations which then work

under different Principal Investigators: Indian Institute of Science Bangalore (DBT-RA), ICMR (SRF/RA), DST-SERB (NPDF) DST-Inspire Faculty, DST (WOS) and CSIR (SRA/RA); DHR-young scientists and holders of the Ramalingaswami Re-entry Fellowship are also eligible. The Institute also imparts short-term training to post-graduate students from different Universities/ Institutions who are sponsored by the Indian Academy of Science Bangalore for six-month project work. Under-graduate students belonging to different colleges also receive training under the Science Setu Programme. The Department has also been involved in arranging the participation of scientific, technical and administrative personnel in different training courses.

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 to their primary responsibilities. The Cell has followed various instructions issued by the CVC from time to time to ensure effective implementation of the measures outlined in the instructions for strengthe-ning 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 Institute 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. The staff members employed in areas prone to corruption are rotated frequently. Sizeable purchases of chemicals, consumables and instruments are handled through various Purchase Committees of the Institute, thus eliminating the possibility of collusion detrimental to quality and price of purchases. Periodically, the Institutional Committees are reconstituted. The Cell has been rendering periodical reports and returns on vigilance activities to the administrative machinery and the CVC.

'Vigilance Awareness Week' was observed in the Institute from October 28th, 2019 to November 02nd, 2019. A banner announcing the observance of the Vigilance Awareness Week was put up at the main entrance of the Institute. Placards bearing slogans against corruption were displayed inside the premises. A pledge to fight corruption was taken by the NII community on October 29th, 2019. An Integrity Pledge was taken on the CVC website by members of the community. As essay writing competition was organized on October 31st, 2019 on the theme "Integrity- A Way Of Life". Shri T. P. Sharma (Under Secretary from the CVC) delivered a lecture on October 31st, 2019 on the theme of the Vigilance Awareness Week 2019.

Vigilance Officer Shri T. P. Sharma, (Under Secretary from the CVC) with Dr A. K Sau, (CVC , NII), Dr. A.K. Panda, Director, Lt. Col. (Dr.) D.K. Vashist, Senior Manager and Ms. Daisy Sapra Section Officer.

Computer Centre

The Computer Centre has been providing Information Technology-related support to the Institute, which involves managing switches and Wi-Fi controllers in a 1000 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 Centre staff facilitates day-to-day troubleshooting, maintenance and anti-virus support of about 850 PCs and other peripheral devices. In addition, the Computer Centre also provides specialized services like management of HPC clusters, managing floating licenses for access to bioinformatics softwares over LAN and IT support for developing in-house software for payroll and maintenance of employee databases.

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. From a large number of applicants from across the country, every year, 30-35 scholars are admitted to this Programme on a competitive basis after an examination and interview.

The Ph.D. programme of the Institute was launched in the academic year 1986-87. So far, 478 students have been awarded the Ph.D degree, including 32 who obtained the degree in academic year 2019-20.

In addition, the Institute accepts students from various Universities/Institutions as Summer Research Fellowship Awardees. The Institute also accepts students for the project work during the last semester of their Post Graduation course.

PUBLICATIONS

Eighty research papers by the scientists and scholars of the Institute were published this year. Of these publications, seventy were published in journals as peer-reviewed research papers and the others were published as reviews/proceedings. Details are listed in Annexure-I.

PATENTS AND TECHNOLOGY TRANSFER

The Institute has a policy of protecting intellectual property rights of inventions made within 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, the Institute has filed, eight patent applications, while seven patents were granted/ issued.

LECTURES DELIVERED ON INVITATION/PAPERS PRESENTED

The scientists of the Institute continued to deliver lectures including 'Keynote Addresses and Inaugural Addresses' and 'Serial Lectures' at various institutions, conferences, symposia, workshops and training programmes in India and abroad.

LECTURES/SEMINARS BY VISITING SCIENTISTS/ GUEST INVESTIGATORS

The Institute continued to receive visiting scientists and guest investigators from all over the world. Thirty six seminars were organized by the Institute in various areas of interest. These seminars were attended not only by the scholars and scientists of the Institute, but also by the investigators from other institutions.

ANTI-TERRORISM DAY

Anti-Terrorism Day was observed by employees on 21st May, 2019. At 11:00 AM, anti-terrorism/ violence pledge was taken which stated, “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 aim of promoting 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 20th August, 2019 by taking the pledge, “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, 2020 in 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, 2019. At 11:00 AM, a pledge was taken which stated, "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, 2019. 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.

Dr. Amulya K. Panda addressing members of the Institute on Independence Day.

REPRESENTATION OF SCHEDULED CASTES, SCHEDULED TRIBES, OTHER BACKWARD CLASSES AND ECONOMICALLY WEAKER SECTIONS

The Institute complies with reservation orders, as per the directives of Government of India, while making appointments, to ensure representation of Scheduled Castes, Scheduled Tribes, Other Backward Classes and Economically Weaker Sections (EWS).

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 appropriate representation.

IMPLEMENTATION OF OFFICIAL LANGUAGE POLICY

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

1. To promote Hindi as official language in official work, Hindi Pakhwara (Hindi Fortnight) was celebrated in the Institute with great zeal from 1st to 14th September, 2019. During this period, 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 in which a large numbers of faculty members, staff members and students had participated; a Kavi sammelan was also held. Hindi Diwas (Hindi Day) was celebrated on 14th September, 2019 at the culmination of Hindi Pakhwara.

2. In order to reduce hesitation while doing official work in Hindi, the Institute organized quarterly Hindi workshops/lectures for employees during the year.

3. The Institute has implemented the Govt. of India scheme the writing of notes and drafts originally in Hindi. An incentive scheme for encouraging the writing of articles and research papers in Hindi on scientific and technical subjects was also implemented.

4. The Institute published the 3rd edition of its in-house magazine "JAIPRATIRAKSHA DARPAN" in Hindi in December, 2019 and the process for publishing the next edition is underway.

Organizers and participants at an event of the Hindi Pakhwara and inauguration of the National Book Trust book stall by Dr. P. Tailor.

RTI ANNUAL RETURN INFORMATION SYSTEM (2019-2020)

NATIONAL INSTITUTE OF IMMUNOLOGY
NEW DELHI

Report on Monthly Disposal of Cases
2019-2020

Sl. No.	Year	Month	Opening Balance	Receipt	Disposal	Closing Balance	Cumulative Disposal
1.	2019	April	303	2	1	305	304
2.	2019	May	305	1	2	306	306
3.	2019	June	306	3	1	309	307
4.	2019	July	309	4	4	313	311
5.	2019	August	313	2	3	315	314
6.	2019	September	315	0	2	315	316
7.	2019	October	315	0	0	315	316
8.	2019	November	315	2	2	317	318
9.	2019	December	317	2	1	319	319
10.	2020	January	319	12	1	331	320
11.	2020	February	331	5	13	336	333
12.	2020	March	336	6	6	342	339

RTI ANNUAL RETURN INFORMATION SYSTEM (2019-2020)
ANNUAL RETURN FORM

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

Year 2019-2020 (upto March 2020)
Insert Mode (New Return)

		Progress in 2019-20			
	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	303	44	0	0	44
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. 30	0	0

Last date of Uploading the Pro-active Disclosures on the website of PA	(Format 28/03 /2020)

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

ORGANIZATION

NII SOCIETY

Prof. G. Padmanaban
President, NII Society
INSA Senior Scientist, Former Director,
IISc Bangalore & Senior Science
Innovation Advisor BIRAC, DBT

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

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

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

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

Prof. (Dr.) Rajiv Garg
Director General
Directorate General of Health Services
Ministry of Health & Family Welfare
Government of India
New Delhi

Prof. Balram Bhargava
Secretary, Department of Health Research &
Director General, Indian Council of Medical
Research, New Delhi

Prof. Randeep Guleria
Director
All India Institute of Medical Sciences, New Delhi

Prof. M. Jagadesh Kumar,
Vice-Chancellor
Jawaharlal Nehru University, New Delhi

Prof. M. Radhakrishna Pillai
Director
Rajiv Gandhi Centre for Biotechnology, Kerala

Dr. Debashis Mitra
Director
Centre for DNA Fingerprinting and Diagnostics,
Hyderabad

Ms. Kiran Mazumdar-Shaw
Chairperson & Managing Director
M/s Biocon Limited
Bangalore

Dr. Shiv Kumar Sarin
Director
Institute of Liver & Billary Sciences
New Delhi

Dr. Balvinder Shukla
Vice-Chancellor
Amity University, Noida, Uttar Pradesh
Senior Vice President, RBEF

Dr. Amulya K. Panda
Director
National Institute of Immunology
New Delhi

GOVERNING BODY

Dr. Renu Swarup
Chariperson
Secretary
Department of Biotechnology
Ministry of Science & Technology
Government of India, New Delhi

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

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

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

Prof. (Dr.) Rajiv Garg
Director General
Directorate General of Health Services
Ministry of Health & Family Welfare
Government of India
New Delhi

Prof. Balram Bhargava
Secretary, Department of Health Research &
Director General, Indian Council of Medical
Research
New Delhi

Prof. Randeep Guleria
Director
All India Institute of Medical Sciences
New Delhi

Prof. M. Jagadesh Kumar,
Vice-Chancellor
Jawaharlal Nehru University, New Delhi

Prof. M. Radhakrishna Pillai
Director
Rajiv Gandhi Centre for Biotechnology, Kerala

Dr. Debashis Mitra
Director
Centre for DNA Fingerprinting and Diagnostics,
Hyderabad
Ms. Kiran Mazumdar-Shaw
Chairperson & Managing Director
M/s Biocon Limited
Bangalore

Dr. Shiv Kumar Sarin
Director
Institute of Liver & Billary Sciences
New Delhi

Dr. Balvinder Shukla
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Ex-Officio Director
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New Delhi

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DBT or his nominee
Member (Ex-Officio)
Ex-Officio Joint Secretary (Admin)
DBT, New Delhi or his nominee

Shri M.K. Gupta
(Member)
Ex-Engineer (Civil)
IUAC
Nominees of PMC of
Architect Special Invites

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ICGEB, New Delhi

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Member (Ex-Officio)
Ex-Officio Executive Director
Regional Centre for Biotechnology
Faridabad

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Member (Ex-Officio)
Ex-Officio Director (Finance)
DBT, New Delhi

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Nodal Offcer for NII
Ex-Officio DBT
Scientific Co-ordinator/
Nodal Officer for NII

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Member Secretary (Ex-Officio)
Ex-Officio Senior Manager
National Institute of Immunology
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Dr. Debasisa Mohanty
Academic/Student Affairs
Staff Scientist
National Institute of Immunology
Aruna Asaf Ali Marg, New Delhi

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Staff Scientist
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Dr. P. Nagarajan
(Member)
Staff Scientist
National Institute of Immunology
New Delhi

**On deputation at NIAB, Hyderabad*

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Jawaharlal Nehru University, New Delhi
Dr. Mohammed Faruq

Bio-safety Officer
IGIB, New Delhi
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International Centre for Genetics and
Engineering Biotechnology
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New Delhi

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New Delhi

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National Institute of Immunology
New Delhi

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All India Institute of Medical Sciences
New Delhi

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(Alternate Chairperson)
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Technology Institute
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(As on 31.03.2020)

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Dr. Subeer S. Majumdar, Staff Scientist-VII (on Deputation)
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Dr. Pushkar Sharma, Staff Scientist-VII
Dr. Debasisa Mohanty, Staff Scientist-VII
Dr. Mohd. Ayub Qadri, Staff Scientist-VII
Dr. Rahul Pal, Staff Scientist-VII
Dr. Pramod K. Upadhyay, Staff Scientist-VII
Dr. Madhulika Srivastava, Staff Scientist-VII
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Ms. Neerja Wadhwa
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Mr. T. Khaling

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Mr. Nand Lal Arya
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Mr. Surinder Singh Rawat
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Mr. Bhan Singh
Mr. Chatter Singh
Mr. Krishan
Mr. Jawahar Singh
Mr. Rakesh Kumar II
Mr. Raj Kumar
Mr. Ram C. Singh Rawat
Mr. Vijay Pal

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Section Officer
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Skilled Work Assistant
Ms. Rupinder Kaur

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Ms. Sunita Sachdev

Technical Officer II
Mr. Naveen Chander

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Mr. Raj Kamal Singh

Senior Technical Officer
Mr. Mukesh Chander

Assistant Engineers
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Mr. Tarsem Singh
Mr. Amar Nath Sah
Mr. Netra Pal Singh
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Mr. Rambir Singh

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Mr. Deen Mohd
Mr. Sharwan Kumar
Mr. Rajiv Kumar
Mr. Brahm Dev

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Mr. Surender Kumar Kalra
Mr. Prabhu Dayal
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Mr. Ranjiv Mahajan

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Mr. Rajesh Kumar

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Mr. Sadhu Ram
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Mr. Surender Singh
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Mr. Dinesg CPS Negi

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Mr. Ram Bhool
Mr. Ram Dev Yadav
Mr. Ram Surat
Mr. Subhash Chand III

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Manager (A&E)
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Mr. Ranbir Singh

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Ms. Sheela Satija
Ms. Daisy Sapra

Accounts Offier
Mr. Dev Dutt Sharma

Management Assistants
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Mr. Sant Lal
Mr. Dharambir
Mr. Siddharth Sharma

Junior Translator
Ms. Nisha

Junior Assistant I
Mr. Alam Singh
Mr. Mohan Lal

Drivers
Mr. Madan Lal
Mr. Mahender Singh
Mr. Satyabir Singh
Mr. Suti Prakash

Technician II
Mr. Puran Singh

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Mr. Nand Lal Malakar
Mr. Rajeev Kumar
Mr. Ajay Kumar

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Finance & Accounts Officer
Mr. Padam Singh Rawat

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Mr. Om Prakash

Skilled Work Assistant
Mr. Naveen Negi

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Mr. Rakesh Satija
Mr. Aslam Ali

Management Assistant
Mr. Than Singh

Junior Assistant I
Mr. Debarshi Deb

Junior Assitant II
Mr. Balraj

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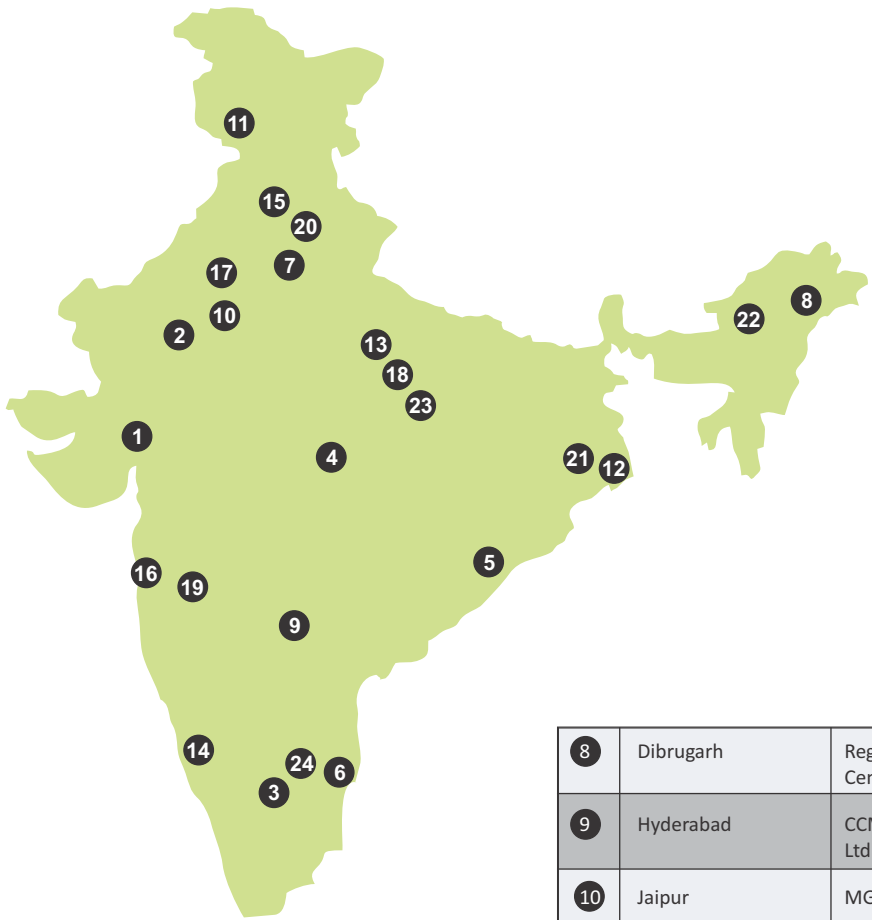
Mr. Manoj Kumar Rajak, Mr. Owais Rashid Hakiem, Mr. Vipin Kumar and Mr. Phunglianpau

A page from history

Dr. S. Ramachandran (Secretary, DBT) and Prof. G.P. Talwar (Founder Director, NII) with Mr. J.R.D. Tata on the latter's visit to NII in 1987.

NII Collaborations

National

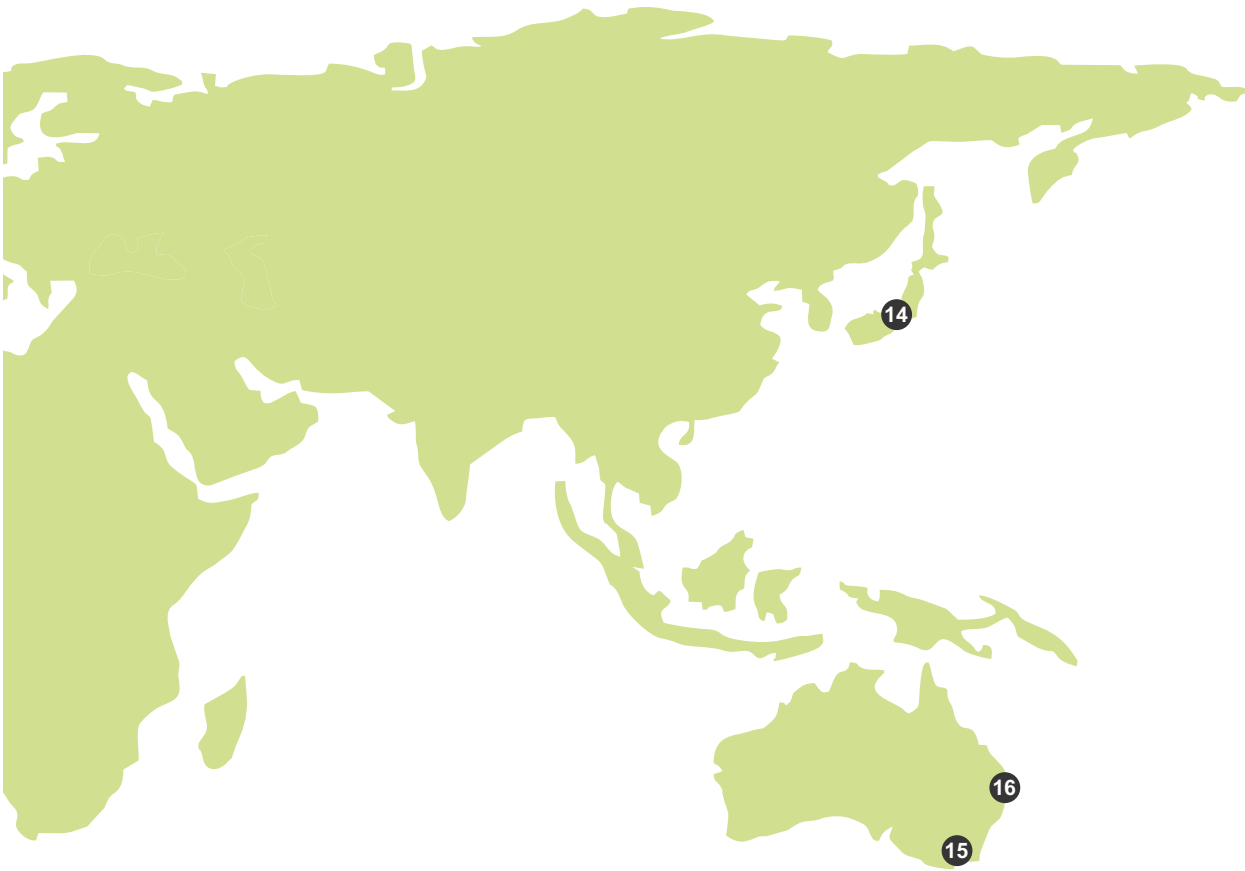


1	Ahmedabad	Nirma University
2	Ajmer	CURAJ
3	Bangalore	Christian Blind Mission IISc, IOB, JNCASR, St. Johns Research Institute, NCBS
4	Bhopal	NIREH, IISER (Bhopal)
5	Bhubaneswar	ILS
6	Chennai	Cancer Institute (WIA), TRPVB
7	Delhi/NCR	DU, Dr. Shroff Charity Eye Hospital, IGIB, RML Hospital St. Stephen's Hospital, UCMS/GTB Hospital, RCB, THSTI, Amity University, NBRC, AIIMS, JNU, TRF, NIP, IITD, SH &VMMC, VIMHANS Hospital, NPL, Jamia Hamdard, NSIT, DIPSAR, ILBS, NSIT, ICGEB, Jamia Millia

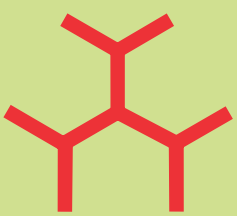
8	Dibrugarh	Regional Medical Research Centre
9	Hyderabad	CCMB, Vitane Biologics Pvt. Ltd.
10	Jaipur	MGMCH
11	Jammu	IIIM
12	Kolkata	Bose Institute
13	Lucknow	SGPGI
14	Manipal	MAHE
15	Mohali	IISER
16	Mumbai	TIFR, NIRRH, ACTREC
17	Pilani	BITS
18	Prayagraj	IIT-Allahabad
19	Pune	ARI, CSIR-NCL, IISER, TCS
20	Roorkee	IIT
21	Shantiniketan	Vishwabharti University
22	Tezpur	Tezpur University, Assam
23	Varanasi	BHU Medical College, IIT
24	Vellore	CMC

International

1	Washington State University
2	La Jolla Institute for Immunology
3	Barrow Neurological Institute
4	University of Nebraska Medical Center
5	University of Minnesota
6	Loyola University
7	State University of New York (SUNY), Buffalo
8	University of Delware



9	National Institutes of Health, Bethesda
10	North Eastern University
11	Universite de Rennes
12	Imperial College
13	University of Grenoble
14	Kumamoto University
15	Walter and Eliza Hall Institute of Medical Research
16	University of Queensland



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