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3RD RGCB RESEARCH CONFERENCE, 2025

28th - 31st August 2025

ABSTRACT

Rajiv Gandhi Centre for Biotechnology (RGCB),
Thiruvananthapuram, India

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The Evolution of Raman Spectroscopy as a Protein Structure Function Tool for Drug Screening/ Discovery and Understanding the Functions.

Chandrabhas Narayana

Transdisciplinary Biology Division, BRIC-RGCB
Thiruvananthapuram, Kerala, India

Over the past 2 decades, our laboratory has been working on providing an alternative to the golden standard, protein crystallography, for understanding protein structure and function. Since Raman spectroscopy provides molecular signatures, understanding this phenomenon helps in discovering the influence of the environment on the molecules in its physiological conditions. Although the power of protein crystallography is unchallenged, it has not been easy to obtain the protein structure of all essential proteins, despite phenomenal improvements in the technique. With the advancements in computational methods and AI/ML, it is now possible to determine the structure of proteins. But still it has been a challenge to understand the functions of proteins for therapeutics, drug discovery, and understanding the observed phenomena. Our group has been trying to utilize Raman spectroscopy, along with limited knowledge of structure and molecular dynamics simulations, to provide an alternative to this. The talk will expose the audience to how we have provided an understanding of proteins such as p300, aurora kinase, Kpn1, JNK, and Sandracynin, among others.

The Interplay of Membrane Proteins and Lipids: Dynamics Regulates Function

Daniel Huster
Institute of Medical Physics and Biophysics,
University of Leipzig, Germany

The plasma membrane is the most important biological interface for prokaryotic and eukaryotic cells, representing the main barrier that separates the cytosol from the extracellular space. Numerous fundamental processes such as ATP synthesis, cellular communication, signal transduction, active and passive transport, receptor function, proteolysis etc. are carried out by membrane-embedded or membrane- associated proteins. Ever since biological membranes were described as fluid lipid bilayers representing a "two-dimensional oriented solution for integral proteins" in the fluid mosaic model from 1972, an extensive amount of research has advanced our understanding of this interface. Both the lipid phase and the protein components of the plasma membrane have been characterized in great detail and our view on the structure and dynamics of these assemblies is rather comprehensive. Many important new features of lipid membranes have been discovered in the last decades. One milestone in the understanding of the lipid phase was the discovery of the high dynamics and conformational heterogeneity of the lipids in the membrane as shown by diffraction studies, NMR spectroscopy, and molecular dynamics simulations. It is also clear that the lipids in membranes are subject to a dynamic lateral domain structure of which liquid ordered (lo) and liquid disordered (ld) domains have been described in atomistic detail. These heterogeneities in the lateral lipid distribution are not static but highly transient in nature. In spite of all the progress, many issues in the field remain unresolved. (i) There is no good understanding of the biological role of the very large variety of lipid molecules within the membrane, which amounts to hundreds of chemically different lipid species. (ii) There is a well-controlled transbilayer asymmetry of lipids between the two membrane leaflets which is of known importance for processes like blood coagulation or apoptosis. How the lipid asymmetry influences protein function is known only for very few examples. (iii) While the existence of membrane domains is undisputed, there is no satisfactory understanding of the dynamics and lifetime of these fluctuations in the lateral organization of lipids and how they may regulate the function of proteins. (iv) Small lipophilic molecules can partition into the membrane rather nonspecifically and in high concentration leading to significant membrane enrichment. At physiological and pharmacological concentrations of these modulators, membrane properties such as lipid packing, membrane thickness, intrinsic curvature, domain size etc. are modified leading to moderate alterations of membrane structure and dynamics. How these processes influence other membrane properties end even protein function, leading for instance to side effects of drugs, remains largely unknown. Progress, remaining questions and new experimental findings will be discussed in the lecture.

Heart Disease: From Genes to Therapeutics

Deepak Srivastava
Gladstone Institutes and University of California,
San Francisco, USA

Heart disease is a leading cause of death in adults and children. We have described complex signaling, transcriptional and translational networks that guide early differentiation of cardiac progenitors and later morphogenetic events during cardiogenesis. By leveraging these networks, we have reprogrammed disease-specific human cells in order to model genetically defined human heart disease in patients carrying mutations in cardiac developmental genes. These studies revealed mechanisms of dose-sensitivity that involve cellular reprogramming events and have led to new therapeutic approaches, and we demonstrated the contribution of genetic variants inherited in an oligogenic fashion in congenital heart disease. We also utilized a combination of major cardiac developmental regulatory factors to induce direct reprogramming of resident cardiac fibroblasts into cardiomyocyte-like cells with global gene expression and electrical activity similar to cardiomyocytes, and have revealed the epigenetic mechanisms underlying the cell fate switch. Knowledge regarding the early steps of cardiac differentiation in vivo has led to effective strategies to generate necessary cardiac cell types for disease-modeling and regenerative approaches, and are leading to new strategies for human disease.

"DOSA"- Discovery of a new cardiac syndrome by integrating human molecular genetics, stem cells/ organoids and pre-clinical models.

Dhandapany Perundurai
Institute for Stem Cell Science and Regenerative Medicine, (inStem)
Bengaluru, India

Dilated cardiomyopathy (a form of heart failure) leading to thinning of heart muscle affects one in every 200 persons. Various sarcomeric gene mutations have been shown to cause this condition. The molecular causes of such cardiomyopathy are poorly understood. We performed South Asian patients-specific next-generation sequencing, used 2D/3D cellular and organoid models, and fly genetics to explore novel genes and their functions related to such cardiomyopathy. Our results revealed exciting roles for several non-sarcomeric genes in cardiac hypertrophy. My talk will focus on a recently identified novel gene from my group and its functional implications in causing Dilated Cardiomyopathy, Obesity and Sleep Apnea (DOSA) syndrome

Host-pathogen interactions on the innate-immune driveway

Dhiraj Kumar
Cellular Immunology Group,
International Centre for Genetic Engineering and Biotechnology, (ICGEB)
New Delhi, India

Tuberculosis (TB), caused by the pathogen Mycobacterium tuberculosis (Mtb), is a global health challenge. The pathogen is known to alter diverse host physiological processes to establish infection and cause pathology. Curiously, not every individual infected by Mtb develops the disease; rather, most of them end up controlling the initial infection. In some of those, the bacteria may remain silent for a prolonged period as a latent infection before getting activated under circumstances of immune suppression, malnutrition, etc. What contributes to this vast spectrum of host responses remains poorly understood. Our efforts to understand the innate immune mechanisms and their perturbations during Mtb infection have provided insights into factors that could be driving pathogenesis, latency and control of infection. These factors include physiological events like regulation of anti-bacterial functions, innate inflammatory wiring and transcriptional responses due to alternative splicing. Recently, we identified a unique mechanism that empowers Mtb to alter host response machinery in a targeted fashion. These findings help us present a set of unconventional strategies for TB prevention and control.

A minimal cell division machinery: Cell division in cell wall-less bacteria

Gayathri Panaghat
Indian Institute of Science Education and Research (IISER)
Pune, India

The *dcw* (division and cell wall) cluster comprise the genes that assist in cell division in bacteria. Mycoplasmas, in the absence of a cell wall, possess the minimum number of genes in the *dcw*cluster. Being a cell wall less organism with a definite shape, the cytoskeletal filaments of *Spiroplasma* FtsZ and the associated proteins FtsA and SepF constitute a minimal cell division apparatus, an ideal system for understanding mechanistic aspects of cell division. Sequence and structural analysis done in our group recently has identified an FtsZ homolog as the only component of the division operon in some of the members of *Mycoplasma*, making it the most minimal cell division machinery. These provide ideal model systems to understand the basic requirements of cell division. A comparative perspective of cell division components across the different domains of life will be presented with an appropriate choice of model systems, as future directions.

Lessons from stem cells research in Ophthalmology

Geeta K Vemuganti
LV Prasad Eye Institute, Hyderabad, India

Stem cells with property to self-renewal and differentiate are explored for various cellular therapies; while the mutated stem cells which generate a tumor, are targeted for elimination to control tumor progression and chemo-resistance. Eye is a unique visual sensory organ that has a direct continuity with the brain and thus shares many common aspects in terms of regeneration and disease spectrum. The regeneration capacity of the 3 layers of eye ie outer coat -cornea and sclera; middle coat- uveal and inner coat retina are distinct hence pose different challenges in planning for cellular therapies. In last 3 decades, our group and many others have made a huge leap in terms of regeneration of ocular surface using cultured limbal stem cells that contributed to restoration of vision to thousands of patients. This has also paved way for understanding of stem cell biology and technique used in stem cell therapy for clinical practice that could be extrapolated to other systems. The pursuit to combat dry eye disease has lead to establishment of human lacrimal gland cultures that not only documented the presence of stem cells, but also identified the tear substances in the secretome, thus opening up new avenues of cell free therapy. Retinal tissue, with 10 layers of cells, requires new strategies including tissue engineering. Attempts have been made to generate retinal cells through BM, cadaveric tissues, fetal tissues and induced pluripotent stem cells- with limited success. On the other hand, the role of cancer stem cells in tumors with its unique challenges of chemoresistance and metastasis, has opened up new avenues of targeted therapies. In Retinoblastoma,the isolation and characterization the cancer stem cells has demonstrated that they play a crucial role in tumor progression. Hence our attempts to reduce the chemoresistance using nano-formulated drugs and targeted delivery of drug loaded-small extracellular vesicles derived from the tumor cells showed promising results. Regenerative potential of normal stem cell, and tumor initiation by cancer stem cells are thus the two contrasting sides of the same coin that warrant exploration.

Emerging Viral Glycoproteins: From Viral Entry and Assembly to Vaccines and Antivirals

Hector Aguilar-Carreño

Division of Life Sciences, College of Letters and Science,
University of California, Los Angeles, USA

My primary research focus is the elucidation of key components in viral glycoproteins and their hosts that: 1] mediate viral entry into mammalian cells, 2] elicit immune responses, and 3] mediate viral assembly and egress from cells. We focus on basic research in viral entry and assembly, as well as preclinical research in vaccine and antiviral development for enveloped RNA viruses, with the strongest emphasis on paramyxoviruses such as the deadly henipaviruses, but also significant emphasis on orthomyxoviruses, coronaviruses, and pneumoviruses. We have developed several new quantitative and kinetic techniques to dissect the mechanistic steps by which glycoproteins modulate viral entry. Further, using viral-like particles and pseudotyped virions we developed highly-neutralizing conformational monoclonal antibodies that block various specific steps in the viral entry process, as well as unique techniques to analyze and characterize such viral particles and the host immune responses they elicit. We study both the viral and cellular factors involved in these processes, and these studies are yielding novel ways to develop broad-spectrum vaccines and antivirals.

Self-organized morphogenesis in plant regeneration: Integrating mechanochemical and geometric cues

Kalika Prasad.
Indian Institute of Science Education and Research, (IISER)
Pune, India

Morphogenesis is driven by intricate mechanical forces that orchestrate cellular and tissue wide deformations. These forces, in concert with cell geometry and biochemical cues, including hormonal signals, propel the morphogenetic process. Utilizing Arabidopsis thaliana as a model system, we investigate morphogenesis through tissue culture-mediated shoot regeneration. Our findings reveal the pivotal role of mechanical forces in guiding the self-organization of shoot progenitors from undifferentiated callus into functional shoot meristems. We propose a "stretch-compress" model, illustrating how mechanical forces induce compression in progenitor cells and expansion in neighboring cells. This dynamic interplay reshapes cell geometry and mechanical tensions, which are essential for the formation of the characteristic dome-shaped shoot meristem. Remarkably, this mechanistic framework is also recapitulated in organ regeneration following injury. In Arabidopsis root tip regeneration, we identify a similar "push-pull" mechanism, facilitating the convergence of longitudinal cell files at the regenerating tip and the reestablishment of the stem cell niche (SCN). Our study highlights the fundamental role of cell geometry and mechanochemical feedback as key regulators of tissue morphogenesis, offering new insights into the forces shaping developmental processes.

Impact of TUBB4B tubulin isotype mutations associated with rare genetic disorders on microtubule dynamics

Kathiresan Natarajan
Pathogen Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

Microtubules, a crucial component of the cytoskeleton, exhibit high structural homology across all eukaryotic organisms. They achieve functional specialization via interactions with microtubule-associated proteins (MAPs) and through tubulin Post-Translational Modifications (PTM). The identification of tubulin isotypic mutations in human brain malformations, collectively known as tubulinopathies, has increased interest in the functional role of tubulin isotypes. Most tubulinopathies arise from a single point mutation in a tubulin isotype, allowing us to investigate its potential role in various neuronal disorders. In this research project, we investigated the role of TUBB4B tubulin isotype mutations associated with rare genetic disorders on the dynamic instability and molecular properties of the microtubules. We generated TUBB4B mutants through site-directed mutagenesis and stably expressed them in the N2A neuroblastoma cell line. Molecular characterization of the mutants were performed using immunoblot analysis, real-time PCR analysis, and microscopy studies through immunofluorescence staining and live super-resolution confocal imaging. The structural perturbations of the mutations were further studied using molecular dynamics simulations. Our findings reveal an unexpected role of the TUBB4B isotype in rare genetic disorders and neurodevelopment, particularly in the polymerization dynamics of microtubules and their stability.

Single-Peptide Assembly of Dual-Diameter α-Helical Nanopores for Pathological Protein Detection

Kozhinjampara R Mahendran

Transdisciplinary Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

Synthetic nanopores are promising candidates for single-molecule protein sensing, with α-helical nanopores offering a powerful platform for chemical modifications and tunable selectivity. Here, we report a synthetic α-helical peptide pore, pPorA, derived from the porin PorACj, which assembles autonomously into octameric pores of large and small conductance states, confirming dual pore architectures. Site-specific incorporation of natural and unnatural amino acids enabled structural control, yielding large and small-diameter pores of identical subunits. Large cyclic sugars bind to small-diameter pores without translocation but traverse through the large-diameter pores, confirming the structural flexibility and size-dependent selectivity of these pores in line with rapid translocation of small unstructured peptides through large pores. Furthermore, using large-diameter pores, we achieved real-time, label-free detection of conformational states of α-Synuclein and its pathological mutants associated with Parkinson's disease. Multiple pathological α-synuclein proteins were simultaneously introduced into the bilayer system and were individually resolved and classified based on their distinct current signatures using machine learning analysis. The small-diameter pores were used to discriminate conformational variants of the mitochondrial peptide Humanin and its disease-associated mutants, offering insight into their apoptotic roles. These findings establish the functional versatility and conformational flexibility of α-helical peptide pores for complex protein sensing and demonstrate their application in developing next-generation nanopore diagnostics and therapeutic screening tools.

Lifestyle of fungal phytopathogens: Combating host defences and manipulation through secreted effectors

Praveen Kumar Verma

Plant Immunity Laboratory, School of Life Sciences,
Jawaharlal Nehru University, New Delhi, India

Plant pathogens exhibit biotrophic and/or necrotrophic lifestyles. Biotrophs, which require a living host sustain their infection cycle, often carefully modulate host physiology early in an infection to produce an appropriate niche for themselves. These adapted pathogens exploit plants to get nutrition and shelter. Thus, a pathogen must overcome preformed structural barriers and suppress the immune system of the host by evolving tactics to invade and survive inside the host environment. Pathogen-secreted molecules (termed effectors) manipulate the signalling or metabolic machinery of the host to benefit the pathogen. These secreted effectors can act inside (intracellular) or outside (extracellular) the host cells. The legume crop chickpea (Cicer arietinum) is infected by a devastating fungus Ascochyta rabiei, resulting in Ascochyta blight disease. Although genome sequencing and in planta expression studies have revealed various A. rabiei effectors, their targets inside chickpea remain unclear. We have isolated an early expressed effector A. rabiei PEXEL-like Effector Candidate 25 (ArPEC25) is essential for fungal virulence on chickpea. ArPEC25 is secreted by fungi and moves to the chickpea nucleus where it physically interacts with LIM transcription factors. The chickpea nuclear localization of ArPEC25 is essential for its virulence activity, since it disrupts the DNA-binding activity of a CaβLIM1a factor, resulting in reduced expression of a phenylalanine ammonia-lyase (PAL) gene. The PAL enzyme is an important protein of the phenylpropanoid pathway that produces various molecules including lignin to provide structural strength to the plant cell. Thus, one mechanism by which ArPEC25 manipulates the host is by suppressing lignin levels in chickpea. Some other related mechanisms to combat host generated oxidative stresses will also be discussed in the presentation.

An ER-resident lipid scramblase is crucial for biogenesis and function of apicomplexan parasite secretory organelles

Puran Singh Sijwali

CSIR-Centre for Cellular and Molecular Biology,
Hyderabad, India

Apicomplexan parasites, including Plasmodium falciparum and Toxoplasma gondii, contain specialized secretory organelles such as micronemes, rhoptries, and dense granules, which are essential for parasite motility, host cell invasion, development, and egress. DedA superfamily proteins are implicated in lipid mobilization, which is a key requirement for organelle biogenesis. Herein, we identified and investigated the vacuole membrane protein 1 (VMP1), a DedA superfamily member, of P. falciparum (PfVMP1) and T. gondii (TgVMP1). PfVMP1 and TgVMP1 are ER-localized lipid scramblases. TgVMP1 depletion adversely affected parasite development, motility, host cell invasion, and egress. These phenotypes were consistent with impaired rhoptry and dense granule biogenesis, and decreased secretion of micronemes and rhoptries in TgVMP1-depleted parasites, indicating a crucial role for TgVMP1 in the biogenesis and function of these organelles. TgVMP1 depletion impaired lipid droplet homeostasis, ER organization, and intravacuolar network formation. Restoration of the ER-localized lipid scramblase by complementing TgVMP1-depleted parasites with PfVMP1 or a homolog as distant as human VMP1 rescued the depleted parasites, indicating their functional conservation and a crucial role for ER-resident lipid scramblase activity in the biogenesis and function of secretory organelles. The essentiality of TgVMP1 for parasite development and likely functional conservation of apicomplexan VMP1 proteins highlight their drug-target potential.

Cancer without Disease: Exploring A Novel and Practical Strategy for Prevention and Control of Malignant Cancer

Raghu Kalluri

Department of Cancer Biology, Division of Discovery Science,
The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Cancer is not always lethal. Many cancers remain a contained and dormant group of abnormal cells, never progressing to invasive, malignant, or clinical disease. It is estimated that up to 35% of adults over the age of 40 may already have these contained cancers, or carcinoma in-situ. Individuals with these lesions can be said to have 'cancer without disease.' This talk will explore the question of what factors contribute to cancer remaining silent, and how can we exploit these factors to extend lifespan and continued good health? Early detection of in-situ carcinomas carries the risk of overdiagnosis and overtreatment, and the future of oncology must challenge and redefine cancer classification and treatment strategies. The next leap forward in cancer care will involve approaches to keep cancer contained, stave off clinical illness, and outlive the disease resulting from cancer

Imprecisions in the 3'- untranslated RNA processing and its implication in the regulation of cardiac remodelling in hypertrophy and failure

Rakesh S. Laishram

Cardiovascular Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

The endonucleolytic cleavage step of the eukaryotic mRNA 3′-end processing is considered imprecise, which leads to heterogeneity of cleavage site (CS) with hitherto unknown function. Contrary to popular belief, we show that this imprecision in the cleavage is tightly regulated, resulting in CS heterogeneity (CSH) that controls gene expression in antioxidant response. Globally and using reporter antioxidant mRNA, we discovered an inverse relationship between the number of CS and the gene expression, with the primary CS exhibiting the highest cleavage efficiency. Strikingly, reducing CSH and increasing primary CS usage induces gene expression. Under oxidative stress conditions, there is a decrease in the CSH and an increase in the primary CS usage to induce antioxidant gene expression. Concomitantly, ectopic expression of one of the key antioxidant response gene (NQO1) driven by the primary CS but not from other subsidiary CSs, or reduction in CSH imparts tolerance to cellular oxidative stresses (H2O2 , and NaAsO2). Genome-wide CS analysis of stress response genes also shows a similar result. We establish that oxidative stress induces affinity/strength of cleavage complex assembly, increasing the fidelity of cleavage at the primary CS, thereby reducing CSH inducing antioxidant response. Cardiac hypertrophy in the hearts response to pathological stress leading to cardiac remodelling increasing the cardiomyocyte size and ventricular wall thickness. Hypertrophic stimulus induces oxidative stress in the heart that is countered by cellular antioxidant response. However, at a later stage, notwithstanding the persistent oxidative stress, anti-oxidant response get diminished resulting in hypertrophied heart. We have shown that this overall process is regulated through imprecisions of RNA 3'-UTR processing through Star-PAP. We demonstrated that CSH is compromised at the late state but not at the early sate on hypertrophy induction through Star-PAP. Compromised CSH from Star-PAP down regulation diminishes antioxidant response leading to pathogenesis of hypertrophy in the heart. Thus, our study reports a novel mechanism of cardiac remodelling by the control of oxidative stress response pathway that operates through imprecision of cleavage at the mRNA 3′-end.

Malaria's Favorite Hideout: The Mystery of Immature Red Blood Cells.

Rajesh Chandramohanadas
Pathogen Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

During the asexual replicative phase responsible for the clinical manifestations of malaria, *Plasmodium spp.* infect and develop within human red blood cells (RBCs). Among these parasites, *Plasmodium vivax*—prevalent in Asia and South America—exhibits a unique and exclusive preference for immature RBCs known as reticulocytes. While the more virulent *P. falciparum* is capable of invading RBCs of all maturation stages, it also displays a notable tropism for immature RBCs. The molecular determinants driving this host cell preference, as well as the physiological implications of such tropism, remain poorly understood. To address this knowledge gap, we aim to investigate the factors governing host cell selection and the adaptive strategies employed by the parasite in response to host cells with differing metabolic plasticity. Furthermore, emerging evidence suggests that host cell characteristics may contribute to parasite tolerance against external stressors, including frontline antimalarial drugs, highlighting a potential link between host tropism and drug resistance.

Disentangling complex interactions between cancer cells, the matrix microenvironment and the macroenvironment

Ramray Bhat
India Institute of Science, (IISc)
Bengaluru, India

The invasion of cancer cells is driven through a complex and continually changing set of interactions between their genomic properties and the surrounding microenvironment(s). An important contributor to moulding such interactions is the extracellular matrix (ECM), a mixture of secreted proteins that surround the cells and regulate their behavior through biochemical and biophysical cues. I will discuss two stories from my group, one published and the other unpublished, which will seek to showcase novel observations on cancer cell-ECM interactions that in one case sheds light on the resilience of invading cancer, and in other, highlights how they coopt surrounding normal cells to help their travel. Time permitting, I will also discuss a third story about how macroenvironmental metabolites are involved in altering tumor-stromal competitions that has implications for how comorbidities exacerbate metastatic outcome.

Heart Under Pressure: Galectin-3 C-Epitope as a Game-Changer in Cardiac Care

Rashmi Mishra
Neurobiology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

Pressure overload-induced left ventricular cardiac hypertrophy (CH) leads to myocardial thickening and increased risk of heart failure, highlighting the need for novel therapeutic targets and reliable biomarkers. Although galectin-3 is recognized as a serum biomarker in advanced heart failure, its utility in routine-assessment of cardiac dysfunction has been limited. Galectin-3 also exists as N- and C- terminal cleaved epitopes. This study explored the specific utility of Gal-3 C-epitope (Gal-3C) oligomers as therapeutic target and panel biomarker for drug efficacy in CH management. An efficacious cardioprotective Indian traditional medicine, Amalaki rasayana (AR), was administered to rat models of biological aging and pressure overload induced CH to assess correlation between CH regression and decline in Gal-3 C-epitope oligomers. Results showed that in therapy naïve condition, Gal-3C oligomers accumulated in serum and cardiac tissue during CH, contributing to adverse remodeling. AR and its bioactive component, gallic acid (GA), were found to promote galectin-3 phosphorylation, enhancing its intracellular retention and limiting C-epitope oligomer formation. Moreover, AR and GA reduced the binding of Gal-3C oligomers to cardiomyocyte surfaces, aiding CH regression. In summary, Gal-3 C-epitope oligomers are promising therapeutic target in mitigating CH, while they also serve as an effective candidate in development of serum panel biomarker, along with ANP, PPIA and ALB for monitoring CH treatment responses. AR and GA are found to be novel inhibitors of Gal-3 C-epitope oligomers' induced damage in CH.

Hypoxia induced mitophagy generates reversible metabolic and redox heterogeneity with transient cell death switch driving tumorigenesis

Santhosh Kumar TR

Cancer Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

Tumor hypoxia determines tumor growth, metastasis, drug resistance, and tumor heterogeneity through multiple mechanisms, largely dependent on the extent of hypoxia, further modulated by re-oxygenation events. In order to track the cell fates under hypoxia and re-oxygenation, we have developed a sensor cell for real-time tracking of apoptotic, necrotic, and surviving mitophagy cells under hypoxia and re- oxygenation. The study using this sensor revealed a cell death switch from apoptosis to necrosis by hypoxia-exposed cells under re-oxygenation, where mitophagy plays a key role in acquiring temporally evolving functional phenotypes, including metabolic heterogeneity and mitochondrial redox heterogeneity. RNA transcriptomics also revealed a temporally evolving genomic landscape supporting the complex transcriptional plasticity of cells as a non-genetic adaptive event. Interestingly, cells regained from these distinct stages retained their metastatic potential despite slow growth in animal models. Overall, the study demonstrated that cells acquire distinct functions by tumor hypoxia and re- oxygenation, secondarily acquiring transient functional traits and metabolic heterogeneity governed by cell inherent mitochondrial dynamics. Such cell autonomous temporal alterations in cell states governed by organelle integrity with distinct cell proliferation and apoptosis-necrosis switch may be advantageous for the growing tumor to evolve under complex microenvironmental stress, further contributing to tumorigenesis.

Vascular Leakage in Dengue: from signaling pathways to inhibitors

Sreekumar E

Pathogen Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

Shock syndrome can lead to mortality in severe dengue disease. Apart from empirical management with intravenous fluids, there are no specific drugs available at present to treat this condition. Interaction of virus-induced host factors, viral proteins as well as direct virus infection of endothelial cells have been attributed as precipitating causes of enhanced endothelial permeability in dengue. While most studies have focused on the causative factors, our understanding on the mechanistic details are minimal. Endothelial barrier dysfunction has been observed in several other disease conditions including bacterial sepsis and infection with many endotheliotropic viruses. The role of permeability regulating molecules forming the inter-endothelial adherens and tight junctions have been well studied in these conditions. Our studies focused on the role of signaling pathways mediated through Tie-2/Ang receptors and S1P receptors in mediating the cell-membrane expression of VE-Cadherin forming these junctions. We explored in detail how this can be modulated with clinically approved inhibitors in dengue virus infection in cellular and animal models with a goal of drug repurposing for addressing this life-threatening complication of dengue.

Tuning CAR T cells for enhancing anti-tumor functions

Sunil Martin
Cancer Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

Although CAR therapy is clinically approved for blood malignancies, local and systemic multiorgan toxicity due to cytokine release syndrome remains a major challenge. CRS-associated hospitalizations significantly enhance the overall cost of anti-CD19 CAR T cells with CD28 co-stimulatory domain. Fine-tuning the receptor configuration of this modular synthetic receptor is one of the most effective strategies to mitigate the cytokine toxicity. Towards this end, we generated all possible configurations of anti-CD19 CAR with hinge and transmembrane domain derived from both CD28 and CD8α. We observed the differential role of CD28 domains in the cell surface expression, cytokine production, tumor toxicity and induction of IL-1β from autologous myeloid cells. CD28 hinge and transmembrane domains were associated with distinct CAR signaling and immune synapse patterns. In silico studies revealed unique interactions of the HTM-modified CARs with the CD19 antigen, indicative of the distinct role of amino acid residues. The mechanistic basis of the impact of CD28 elements on the anti-tumor functions is being evaluated.

Exosome-Laden Multifunctional Hydrogel Based Delivery System for Enhanced Burn Wound Healing

Vinod Kumar GS
Cancer Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

Severe burns pose significant threats to patient well-being, characterized by pain, inflammation, bacterial infection, and extended recovery periods. While exosome-loaded hydrogels have demonstrated considerable promise in wound healing, current formulations often fall short of achieving optimal therapeutic efficacy for burn wounds due to challenges related to their adaptability to wound shape and limited antibacterial capabilities. Addressing this gap, we developed a novel exosome-laden, sprayable, thermo-sensitive polysaccharide-based hydrogel (ADA-aPF127@LL18/Exo), composed of alginate dialdehyde (ADA) and aminated Pluronic F127 (aPF127), synthesized via Schiff base reaction. The innovative approach of combining ADA and aPF127 through Schiff base reaction results in a hydrogel that is not only effective but also practical for clinical use. Its sprayable nature ensures ease of application and adaptability to various wound shapes, addressing a major limitation of current treatments. The ADA-aPF127@LL18/Exo hydrogel demonstrated sustained exosome release over an extended period ensuring that the therapeutic effects are maintained, reducing the need for frequent dressing changes and minimizing disruption to the healing process. Biological assessments confirmed excellent biocompatibility, with notable improvements in in vitro cell proliferation and migration. These properties are vital for effective wound healing, ensuring that the hydrogel supports cellular activities essential for tissue regeneration. In vivo evaluations using a deep partial thickness burn model revealed that ADA-aPF127@LL18/Exo substantially accelerated the wound healing process. Key observations included rapid epithelialization, which is crucial for forming new skin layers, and robust granulation tissue formation, indicating effective new connective tissue development. The hydrogel also facilitated significant collagen deposition, important for the structural integrity and strength of the newly regenerated tissue. Furthermore, ADA-aPF127@LL18/Exo induced hair follicle regeneration, an important aspect of restoring the skin's normal appearance and function. The hydrogel effectively mitigated inflammatory responses, reducing complications that can prolong healing and contribute to patient discomfort. By promoting enhanced neovascularization, the hydrogel ensured better blood supply to the healing tissue, supporting sustained and robust tissue repair. Overall, ADA-aPF127@LL18/Exo represents a highly promising therapeutic dressing for the treatment of deep burns. Its multifaceted properties, including sustained exosome release, enhanced antibacterial efficacy, excellent biocompatibility, and promotion of critical wound healing processes, position it as an advanced alternative to existing treatments. The hydrogel's ability to mitigate inflammation and facilitate neovascularization further highlights its comprehensive therapeutic benefits. This study contributes significantly to the field of burn wound care, offering a novel solution that enhances patient outcomes and streamlines the healing process.

Structural and Biophysical characterization of Mycobacterium tuberculosis Malate Synthase recognition by an aptamer

Anagha Das, Dileep Vasudevan, Lightson Ngashangva

Transdisciplinary Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Malate synthase G (MSG) is a key enzyme in the glyoxylate shunt pathway of Mycobacterium tuberculosis (Mtb), enabling bacterial persistence during latent infection. High-affinity aptamers targeting MSG offer a promising early detection and therapeutic intervention strategy. This study investigates the structural and biophysical characterisation of a DNA aptamer in complex with Mtb MSG. Biophysical characterisation of MSG was carried out using circular dichroism (CD) spectroscopy, analytical size-exclusion chromatography (SEC), and sedimentation velocity analytical ultracentrifugation (SV-AUC), confirming its secondary structure, oligomeric status, and homogeneity. CD spectroscopy and in silico secondary and tertiary structure prediction tools evaluated the structural features of the aptamer. Mtb MSG was crystallized, and the high-resolution crystal structure of the apo form of MSG was determined. Co-crystallization and soaking of the apo crystals with the aptamer are in progress. Further, the obtained apo structure was used to guide aptamer docking studies. These findings, combined with the crystal structure of the aptamer-MSG complex, would provide a detailed understanding of the molecular recognition mechanism with implications in TB diagnostics and management.

Functional Phenotyping of MMV Pandemic Response Box Identifies Stage-specific inhibitors Against Blood Stage Plasmodium

Akhila T P, Darsana K M, Rajesh Chandramohanadas

Pathogen Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Malaria is a life-threatening infectious disease caused by the protozoan parasites of the species Plasmodium. With the primary goal of identifying new antimalarials belonging to the Pandemic Response Box chemical library (PRB), we conducted a phenotype-based screening, which revealed potent stage-specific inhibitory compounds against the asexual stage of Plasmodium. Sixty out of the 400 molecules from the PRB were active against Plasmodium development in both chloroquine-sensitive and resistant strains, out of which 29 hits acted specifically against the parasite below 3μM. Through phenotype-based screening, we were able to distinguish molecules that interfere with transitions of ring (MMV001014), trophozoite (MMV1593540, MMV1634402), and schizont (MMV1580844, MMV1580496, MMV1580173, MMV1580483) stage parasites through their developmental stages, together with microscopic phenotypes confirming the stage-defined abrogation of growth. The ring stage inhibitor, MMV001014, identified in the screen was irreversible, led to no recrudescence, and showed an antagonistic effect when combined with artemisinin. The trophozoite inhibitors showed nanomolar range IC50 with a non-compromised digestive vacuole, unlike many other trophozoite inhibitors. The 3 schizont stage inhibitors appeared to operate through a mechanism driven by the generation of reactive oxygen species and with molecule-specific effects on iRBC membrane integrity, while 1 schizont inhibitor (MMV1580483) showed no ROS generation, which might operate through target-specific mechanisms. Several of these molecules indicated unique modes of action based on cellular phenotype and other biological phenomena, which warrants detailed mechanistic dissection in future work.

A SERS-based assay aiming early diagnosis of Alzheimer's Disease

Abhirami Ajith, Sudhanshu Mani Tripathi, Poornendhu S J, Atasi Ghanti, Mekhla Chowdhury, Chandrabhas Narayana, Debanjan Bhowmik

Transdisciplinary Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

A defining hallmark of Alzheimer's disease (AD) is the accumulation of amyloid-β (Aβ) fibrillar aggregates (plaques) in the brain. Current diagnostic tools, such as MRI, CT, and PET, primarily detect neurodegeneration or plaque burden, limiting their utility to later stages of the disease. There is a pressing need for sensitive methods capable of identifying AD at its early stages. Aβ aggregation begins with the formation of small, lipophilic oligomers characterized by antiparallel β-sheet structures. Emerging evidence suggests that Aβ oligomers associated with exosomes can cross the blood-brain barrier, and their levels in peripheral blood correlate with disease progression. However, their low abundance poses a significant detection challenge. In this study, we present a highly sensitive, non-invasive platform based on surface-enhanced Raman spectroscopy (SERS) for detecting membrane-bound Aβ oligomers. Monodisperse silver nanoparticles (AgNPs) were functionalized with Rose Bengal (RB), a dye specific to amyloid oligomers. Separately, lipid-bilayer-coated AgNPs were used to capture lipophilic Aβ oligomers. The interaction between the oligomers and RB localized the dye within SERS "hot spots" formed between the nanoparticles, leading to a robust, concentration-dependent enhancement of the RB Raman signal. This approach offers a promising route toward early-stage AD diagnostics via blood-based biomarker detection.

IFNγ induced immunomodulation to guide the navigation and design of immune effector T cells for adoptive immunotherapy

Archana Praveen, Sunil Martin

Cancer Research Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

IFNγ is a proinflammatory cytokine with pleotropic effect on tumor immune microenvironment. Nonetheless, the detailed cellular and molecular basis of IFNγ induced regulation of TME remains to be investigated. In this context, we explored the dose-dependent expression dynamics of immunomodulators at lower and higher doses of rhIFN-γ in vitro by qPCR and flow cytometry. We observed a distinct enhancement in the expression of CXCL9, BTN3A1 and PD-L1 by IFN-γ whereas with no significant changes in the tumor intrinsic expression of CXCR3 - common receptor of these chemokines. Interestingly, metabolic pathways are involved in regulating immunomodulators. Finding reveals that pharmacological inhibition of glycolysis enhances the sensitivity of interferon gamma signaling to induce CXCL9. On the other hand, inhibition of fatty acid oxidation reduces IFNγ induced expression of CXCL9. Noteworthily, the majority of the CXCR3 expression is in CD8+ T cells followed by CD4+ T cells, CD11b+ Myeloid cells, and γδ T cells. IFNγ activated PBMCs were found to enhance the release of IL-6 and IL-1β in a dose-dependent manner which can contribute to inflammation and toxicity. Currently, we are optimising the 3D culture system of oral cancer in vitro to investigate the impact of IFNγ signaling in the infiltration and functions of CXCR3+ve T cells.

STAT3 Modulation in Obesity-Driven Pancreatic Cancer: Implications for T Cell-Mediated Immunity and Tumor Progression

Arun V, Shirly James, Aparna J S, Harikumar K B

Cancer Research Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

The global obesity epidemic has been linked to increased incidence and poorer outcomes in pancreatic ductal adenocarcinoma (PDAC). This study investigates the role of Signal Transducer and Activator of Transcription 3 (STAT3) in obesity-driven PDAC, focusing on its impact on immune function and tumor progression. Using genetically engineered mouse models with STAT3 deficiency and high-fat diet-induced obesity, we observed reduced precancerous lesions and stromal alterations compared to STAT3-intact controls. Molecular analysis revealed decreased expression of inflammatory markers, immune checkpoint molecules, and metabolic regulators in STAT3-deficient models. Examination of human PDAC transcriptomic data stratified by body mass index (BMI) uncovered obesity-related changes in T cell receptor complex gene expression, immunoglobulin related genes and distinct pathway activation patterns. Gene set enrichment analysis suggested a more active anti-tumor immune response in normal-weight patients compared to obese individuals. Furthermore, JAK/STAT pathway was found to be elevated in obese and overweight patients. These findings collectively indicate that STAT3 plays a critical role in modulating immune function and tumor progression in obesity-associated PDAC, potentially through regulation of inflammation, T cell exhaustion, and metabolic reprogramming. Our research highlights the complex interplay between obesity, inflammation, and immune dysfunction in PDAC progression, positioning STAT3 as a promising therapeutic target for obesity-driven pancreatic cancer.

ERK-mediated regulation of Star-PAP in pathological cardiac hypertrophy

Beauty Koch, Rakesh S. Laishram

Cardiovascular Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Star-PAP is a non-canonical poly(A) polymerase found in nuclear speckles. Regulated by PIPKIα. It adds poly(A) tails to specific mRNAs, particularly those involved in stress responses. Our research uncovers how Star-PAP, a key enzyme in gene expression, is regulated, particularly in pathological cardiac hypertrophy. We found that the ERK signaling pathway negatively controls Star-PAP levels. Specifically, when ERK is active, Star-PAP is reduced. We discovered that ERK directly phosphorylates Star-PAP, especially within its zinc finger (ZF) domain. This phosphorylation event appears to be a critical signal for Star-PAP's degradation. Our experiments show that Star-PAP is broken down through a ubiquitin-mediated process. Importantly, a specific phosphorylation at serine 10 on Star-PAP is essential for it to bind to the E3 ubiquitin ligase, an enzyme that tags proteins for degradation. These findings were consistent in both cell line and an animal model of cardiac hypertrophy, where we observed increased ERK activity and reduced Star-PAP. In conclusion, our work reveals that ERK-mediated phosphorylation leads to the breakdown of Star-PAP, a crucial event in cardiac hypertrophy.

Characterization of a nucleoplasmin isoform from Leishmania major

Bimal Jana, Dileep Vasudevan

Transdisciplinary Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Nucleoplasmin is a major family of histone chaperones well known for its roles in histone binding, storage, and nucleosome assembly. Herein, we have identified and are characterizing a nucleoplasmin isoform from Leishmania major, a protozoan parasite with a complex life cycle requiring tight chromatin regulation. Sequence analysis of the N-terminal domain (NTD, residues 1–98) showed low identity with known nucleoplasmins but revealed hallmark nucleoplasmin features such as the presence of C-terminal acidic stretches and a predicted pentameric assembly. The codon-optimized gene was cloned into a pET-22b(+) vector, expressed in E. coli BL21(DE3) cells, and purified using Ni-NTA affinity and size-exclusion chromatography (SEC). Analytical SEC and sedimentation velocity – analytical ultracentrifugation (SV-AUC) indicated a homogeneous pentameric oligomer, while CD spectroscopy confirmed a predominantly β-sheet organization and high thermostability (Tm ~81 °C). Chemical stability (salt and urea) of the domain was also confirmed. Interaction studies showed interaction with histone oligomers (H2A/H2B dimer and H3/H4 tetramer). These features of the protein are consistent with those of nucleoplasmin family proteins, confirming it as a nucleoplasmin. Moreover, EMSA studies suggest its assembly with the in vitro reconstituted nucleosome core particle (NCP). Crystallization trials yielded crystals that need to be tested for diffraction limits and data collected.

SSTP1, A Novel HDP, Triggering Apoptosis in Triple Negative Breast Cancer

Gayathri Mohan , Madhumathy G Nair, Jyothy S Prabhu, Hafsa Shabeer and Tessy Thomas Maliekal

1 BRIC-RGCB, Thiruvananthapuram, Kerala, India

2 St.John's Research Institute, Bangalore

Background: SSTP1, a novel HDP that our group has discovered has been demonstrated to cause apoptosis in oral cancer cells. Subsequent investigations have shown that the peptide stimulates apoptosis by shifting the JAK/STAT proliferative pathway to the apoptosis inducing IL6/JNK1/AP1 pathway. Further experiments suggested that SSTP1 exhibits efficacy against cancer cells that overexpress IL6Rα such as triple negative breast cancer (TNBC). Though IL6Rα is a druggable target in TNBC, blockers of the JAK/STAT did not show an increase in overall survival. Thus, our study focusses on investigating whether SSTP1 can be used as a therapeutic agent in TNBC.

Methodology: We investigated the intermediates linking gp130 to JNK1 activation through Co-Immunoprecipitation and western blots using specific inhibitors in MDA-MB-231. To increase the stability of the peptide, we have modified the peptide.

Results: Our results confirmed that IL6Rα, gp130 and ASK1 are the upstream mediators of the JNK1/AP1 apoptotic pathway. The conventional IL6 blockers do not activate this JNK1/AP1 pathway. The modified peptide showed increased stability and activity.

Conclusion: Our study revealed that SSTP1 induce apoptosis by activating JNK1 through L6-IL6Rα-gp130/ASK1 axis. The modified peptide with increased stability and activity could be used as a therapeutic agent which requires further validation.

Identification and functional characterization of the CHPV and complement interacting partners.

Karthika R, John B Johnson

Pathogen Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Chandipura virus (CHPV) is a neurotropic arbovirus causing paediatric encephalitis. Upon transmission by sandflies into the human host, the virus faces the complement system (CS), which is one of the major arms of the innate immune system and the frontline defence against pathogens. It has been demonstrated that in the fluid phase CHPV activates the classical pathway of CS and undergoes neutralisation via aggregation. However, the key CHPV and complement interacting partners, and the mechanism of interaction remains unidentified. Using biochemical approaches, this study aims to investigate the nature and effect of the interaction of CHPV proteins with the complement system, critical for gaining a deeper understanding of CHPV pathogenesis.

Injectable biopolymer based integrated nano drug delivery implant system to treat breast cancer

L. Naresh Goud, G.S. Vinod Kumar

Cancer Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Triple-negative breast cancer (TNBC) treatment relies on paclitaxel (PTX), doxorubicin (DOX), and cyclophosphamide (CYP), but systemic delivery often leads to toxicity and poor efficacy. This study develops an injectable silk fibroin-based hydrogel integrated with drug (DOX and PTX)- loaded PLGA nanoparticles (DNPs), with cyclophosphamide directly loaded into the hydrogel (Hydrogel@DNPs+CYP) due to its high clinical dosage, to enhance localized TNBC therapy. Silk fibroin and drug loaded PLGA nanoparticles were synthesized and characterized. In vitro studies using MDA- MB-231 TNBC cell lines showed superior nanoparticle efficacy versus free drugs, confirmed by MTT, rhodamine phalloidin, live dead, acridine orange, annexin V/PI, and clonogenic assays. Western blot revealed PARP and caspase-3 cleavage, indicating apoptosis. Tumor spheroid assays demonstrated enhanced nanoparticle penetration and toxicity. In in-vivo study, tumor inhibition was most significant in the Hydrogel@DNPs+CYP group, which displayed reduced tumor volume and weight, suggesting efficient localized drug action. In contrast, tumors in the Control, Blank Hydrogel, and Free Drug groups grew progressively. This one-time injectable system offers sustained, non-invasive drug delivery, overcoming systemic toxicity and bioavailability limitations. It holds promise for revolutionizing TNBC treatment and may extend to other cancers.

Differential proteomics analysis of Dengue virus-infected cardiomyocytes

Mansi Awasthi, E Sreekumar

Pathogen Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Severe cases of Dengue may present atypical manifestations involving cardiac complications, observed in 8-25% of patients as reported across different studies. We aim to understand the molecular mechanisms of DENV-induced cardiac manifestations by exploring the role of differentially expressed proteins and altered cellular pathways in cardiomyocyte damage. To initiate the study, infection of Dengue virus serotype 2 (RGCB880) was established in Human cardiomyocyte cell line AC16 and confirmed by immunofluorescence and western blot. Identification of differentially expressed proteins was done by proteomic analysis of the lysates of DENV-infected and uninfected AC16 cells collected at 48h post infection. The results so obtained were analysed using DEqMS package in Rstudio after log2 transformation, normalisation and missing value imputation. Out of 3990 proteins, 119 proteins were found to be significantly upregulated and 29 were downregulated. Upon enrichment using GSEA, Metascape and DisGeNET analyses, these altered proteins were found to be associated with cellular processes like regulation of viral replication, cardiac events and cardiomegaly. In a DENV-2 animal infection model, we identified histopathological changes in cardiac tissues by H&E staining and observed the presence of DENV-2 antigen by immunohistochemistry. The differential protein expression patterns observed in AC16 cells are being further evaluated in the animal model.

The first secretome landscape of Phytophthora capsici Leon: Discovery of a key effector in Piper nigrum pathogenesis

Mookul Samader, S Manjula

Plant Biotechnology & Disease Biology Division, BRIC-RGCB,
Thiruvananthapuram, Kerala, India

Phytophthora capsici, a hemi-biotrophic oomycete, causes quick wilt disease in Piper nigrum. The crop is indigenous and originated in the Western Ghats, which contributes to 95% of the India's area of production. Root rot is responsible for up to 30% of vine mortality and crop losses of up to 40% in Kerala. Phytophthora species causes infection in the host plant by modulating the plant innate immune system through various secreted effector proteins. However, the underlying mechanisms by which Phytophthora capsici effectors manipulate host plant defense remain largely unknown. Here, we have done the first complete characterization of the secreted proteins of P. capsici by LC-MS/MS. More than 200 secreted proteins were identified after 12 days of incubation under two conditions. The mycelia were either grown in PDB media, or were first allowed to infect Piper nigrum for 6 hours before incubation in PDB. We selected one effector protein Glucanase inhibitor protein 4 (UniProt ID: B1AC89) for functional studies. The effector gene (714 bp) was cloned into pCAMBIA 1302 binary vector which was transformed into Agrobacterium tumefaciens GV3103 strain. Transient foliar overexpression in Nicotiana benthamiana provided evidence for the critical role of this effector in the pathogenicity of P. capsici in the host P. nigrum.

Rv0464c: A Peroxidase-Like Protein Driving Reactivation of Dormant Mycobacterium tuberculosis

Nijisha M, Surya P R, Yogita Kapoor, Vinay Kumar Nandicoori[1], Karthika Rajeeve.

Pathogen Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

[1]Centre for Cellular & Molecular Biology, Hyderabad.

Reactivation of dormant Mycobacterium tuberculosis (Mtb) is a critical step in tuberculosis (TB) pathogenesis. Throughout infection, Mtb endures oxidative stress from both aerobic respiration and host-derived reactive oxygen and nitrogen species, necessitating robust redox defense mechanisms. Proteomic profiling identified Rv0464c as significantly upregulated during early reactivation. This hypothetical protein contains peroxiredoxin-like domains, belongs to the carboxymuconolactone decarboxylase family, and shares similarity with alkyl hydroperoxide reductase (AhpD). Our study confirms these findings and demonstrates the robust peroxidase activity of Rv0464c. Overexpression of Rv0464c in Mycobacterium smegmatis enhanced growth, improved survival in human macrophages, reduced intracellular ROS, and inhibited dormancy and helps in faster reactivation, suggesting a role in Mtb virulence and reactivation. Rv0464c localizes to the membrane, forms oligomers in the plasma membrane, and is secreted via vesicles into the extracellular matrix. To determine the importance of oligomerization, cysteine-to-alanine mutants were generated. These mutants showed disrupted oligomer formation and significantly reduced peroxidase activity and survival advantage, highlighting that cysteine-mediated oligomerization is critical for Rv0464c function. Our findings position Rv0464c as a key regulator of redox balance and reactivation, and a potential biomarker or therapeutic target for shortening TB treatment.

Millets in augmenting colorectal cancer immunotherapy: study on identifying its molecular manifestation

Poornima S.R, S. Asha Nair, Ramesh Pothuraju

Cancer Research Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Millets are rich in bioactive compounds especially flavonoids and phenolic acids with high antioxidant and anti-inflammatory effects. Millets can be harnessed based on these functional properties as a dietary intervention, focusing on inflammation-related conditions particularly colorectal cancer (CRC). Cancer still persists as a global burden, with CRC ranking third globally in terms of mortality. A transition of dietary pattern towards the modern dietary habits has resulted in an increased prevalence of CRC in younger population. With evidence suggesting healthy diet and lifestyle can reduce chronic inflammation and improve CRC prognoses, millets can be a healthier alternative to now present staple crops. SCFA, byproducts of dietary fibre fermentation have shown significant immunomodulatory properties. Millets being a rich source of dietary fibre, exploring their immunomodulatory properties in accordance with enhancing the immune checkpoint inhibitor therapy in CRC could prove an effective dietary strategy in tackling CRC.

Heterogeneous neural stem cells of embryonic cortical niche vary in potency and lineage commitment

Rahul Jose, Paul Ann Riya, Nair Pradeep Jyothi, Ramankunju Aryasree , Suresh Surya Sivadasan Bindu Dhanesh, Budhaditya Basu, Jackson James

Neurobiology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Neural stem cells (NSCs) in the developing cortex exhibit heterogeneity in Hes1 gene activation. This defines two subtypes: Notch-dependent Hes1-expressing (NDHes1) NSCs, corresponding to well-characterized radial glial cells (RGCs), and a distinct population—Notch-independent Hes1-expressing (NIHes1) NSCs. Unlike RGCs, NIHes1 NSCs maintain Hes1 expression without Notch signaling and cell-cell interactions. Using single-cell RNA sequencing (scRNA-seq), we identified NIHes1 NSCs and uncovered their unique transcriptional profile. These cells display elevated expression of proneural genes, active Wnt and Hippo signaling, and higher proliferative capacity than NDHes1 NSCs. Pseudotime analysis indicates NIHes1 NSCs are early precursor NSCs that give rise to both RGCs and intermediate progenitor cells (IPCs). Conditional knockout of the Notch-independent Hes1 promoter (NIHes1 cKO) in Nestin+ NSCs disrupts the niche, causing excessive gliogenesis and abnormal migration of projection neurons. Additionally, NIHes1 knockdown results in increased RGCs and reduced IPCs. Our findings reveal that the Hes1+ NSC pool is more diverse than previously recognized. NIHes1 NSCs represent a functionally distinct subtype essential for establishing the embryonic NSC niche and ensuring balanced neurogenesis and gliogenesis during cortical development.

Impact of TUBB4B Tubulin Isotype Mutations on Microtubule Dynamics.

Thasni Fazil, Kathiresan Natarajan

Transdisciplinary Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Background: Microtubules have an exceptionally high degree of structural homology across all eukaryotic organisms, making them an important component of the cytoskeleton. In recent years, the identification of tubulin isotypic mutations in human brain malformations, which are collectively known as tubilinopathies, has increased interest in the functional role of tubulin. Additionally, a spectrum of neurological malformations that can be attributed to mutations in several different tubulin isotypes has been identified. The other neurological consequence of the TUBB4B mutations, on the other hand, has not been found yet. Therefore, it is plausible that the TUBB4B isotype plays a crucial part in the development of the nervous system. Furthermore, any mutation of it might modify the dynamics of the microtubules, which could potentially have ramifications for several neurological disorders.

Methodology: In this research project, we investigated the impact of TUBB4B tubulin isotype mutations on microtubule dynamics through site directed mutagenesis and microscopy studies. The experimental results were further confirmed by molecular dynamic simulations.

Results and Conclusion: Our findings might provide an unsuspected role of the TUBB4B isotype in neurodevelopmental disorders. In particular, the TUBB4B mutant might affect the polymerization dynamics of microtubules and their stability.

Breaking Boundaries: Mitochondria-derived vesicles Deliver Cargo to the Nucleus

Thejaswitha Rajeev, Dipak Shil, Ananthalakshmy Sundararaman
Cardiovascular Biology Program, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Cardiomyocytes rely on mitochondria for energy production and metabolic homeostasis. Increasing evidence shows that enzymes such as pyruvate dehydrogenase (PDH) can localize to the nucleus, where they contribute to the nuclear acetyl-CoA pools and influence gene regulation. The physiological relevance of this nuclear pool, and the mechanisms facilitating its redistribution, remain largely unclear. We aimed to investigate the translocation of PDH to the nucleus under physiological and stress conditions, and the trafficking mechanisms that facilitate this process. Mitochondria-derived vesicles (MDVs) are a selective, early-response quality control pathway. These 70–150 nm vesicles bud from mitochondria and transport specific cargo to organelles such as lysosomes and peroxisomes. We hypothesized that MDVs might mediate PDH nuclear delivery. We confirmed nuclear PDH in cardiomyocytes by Western blotting. Proteomic analysis of MDVs identified PDH as part of their cargo, and reconstitution of purified MDVs and rat heart nuclei revealed reduced nuclear PDH-E1 upon vesicle fusion inhibition, supporting a vesicle-based delivery route. Importantly, nuclear PDH rises under physiological conditions but drops under stress, suggesting that stress-induced MDVs do not contribute to PDH nuclear delivery. These findings propose a new role for MDVs in mitochondrial–nuclear communication, expanding their functional scope beyond quality control.

Functionally Active Synthetic α-Helical Pores

Varsha Shaji, Neilah Firzan C A , Swati Lakshmi , Kozhinjampara R Mahendran
Transdisciplinary Biology Division, BRIC-RGCB, Thiruvananthapuram, Kerala, India

Synthetic nanopores are promising candidates for single-molecule protein sensing, with α-helical nanopores offering a powerful platform for chemical modifications and tunable selectivity. We designed synthetic α-helical peptide pores based on the sequence of naturally occurring transmembrane α-helices. Our lab has developed stable synthetic pores, inspired by PorACj, a porin from Corynebacterium jeikeium, capable of single-molecule sensing of cyclodextrins and polypeptides. Notably, this is the first report on the assembly of a large stable synthetic transmembrane pore with single oligomerization and a steady large-conductance state. We also constructed mirror-image pores and enhanced their performance by altering charge distribution, resulting in improved conductance, selectivity, and selective cytotoxicity toward cancer cells with minimal impact on normal cells. Site-specific incorporation of natural and unnatural amino acids facilitated structural tuning, producing both large- and small-diameter pores with the same sub-unit composition. Large pores enabled label-free detection of conformational states of α-Synuclein and its Parkinson's-associated mutants, while small pores differentiated structural variants of Humanin, shedding light on their apoptotic roles. Additionally, we investigated α-helical antimicrobial peptides (AMPs) from frog skin, which disrupt bacterial membranes. By assembling these AMPs into defined stoichiometries using cyclodextrin scaffolds, we aim to overcome the challenges in studying their pore-forming states. These synthetic pores have potential as components of nanodevices and therapeutic agents targeting pathogens and cancer cells.