BRIC
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RGCB
DISCOVERIES FOR A BETTER TOMORROW

ANNUAL REPORT 2024

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DISCOVERIES FOR A
BETTER TOMORROW

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FROM THE DIRECTOR

"It is not the strongest of the species that survive, nor the most intelligent, but the one most responsive to change."
Charles Darwin

As we navigate through another transformative year, I intent to take a moment to acknowledge and commend the implausible allegiance and passion each of you delivers to our eminent institution. Your unrelenting quest for excellence and innovation is what gages Rajiv Gandhi Centre for Biotechnology (RGCB) a beacon of scientific advancement. Every day, we happenstance intricate scientific defies that entail our ingenuity, resilience, and stanch allegiance. These predicaments are odds to stretch the restraints of what is feasible rather than impediments. We craft imperative advancements in biotechnology and concoct pioneering discoveries only by quelling these obstacles. Our forte abides in our ability to collaborate across varied fields and fetch inclusively unique perspectives. RGCB being a collage of exceptional minds, and our concerted spirit is the cornerstone of our success. Each of you contribute irreplaceable perceptions and proficiency that enterprise our research forward. Let's continue to cherish a milieu of candidness and reciprocal respect, where every contrivance is elicited and valued.

Copious mileposts signpost our progress toward accomplishment and innovation as we preserve our leadership in the scientific community. The commencement of the year is marked with the commissioning of the Biosafety level III (BSL III) facility at our Akkulam campus on the propitious occasion of Makar Sankranti/Pongal. The RGCB BSL III facility is the first of a kind comprehensive Bio-safety Level-3 Laboratory in the state of Kerala to pursue research activities relating to live infectious organisms classified as BSL-3 agents, such as SARS-CoV-2 and influenza.

The scientific investigations at RGCB transcend the pursuit of knowledge; it is a mission to make an eternal impact on society. From emergent novel medical treatments to pioneering sustainable biotechnologies, our research has the clout to advance lives and silhouette a better future. Every experiment, publication, and breakthrough fetch us hither in realizing our vision of a healthier, collateral sustainable world.

The Agasthyalayam Traditional Medicine Unit in Kallar, Ponmudi, Thiruvananthapuram, is one stride en route towards this goal, which was imperatively inaugurated on 22nd February 2024. This initiative is steadfast to practicing and promoting tribal medicinal treatments, explicitly traditional steam baths using medicinal herbs. This program buoyed under the DST - SHRI program ensues the installation of a medicated steam bath facility.

RGCB, a Centre for Excellence, is a covenant to our dedication, perseverance, and passion for effecting prominence. We not only meet standards; but set them. Every encounter we vanquish, every contrivance we initiate, and every milestone we grasp is a collective conquest. Ascending the hierarchies of success and distinction; the Kerala State Cabinet announced the foundation of a Centre of Excellence in Microbiome. This will be a concerted ingenuity of RGCB, Kerala Development and Innovation Strategic Council (K-DISC), and Kerala State Council for Science, Technology and Environment (KSCSTE). Dr.Sabu Thomas, Former Faculty of RGCB, stands as founding director of the centre. The centre will embark research in six domains comprising human microbiome, animal microbiome, plant microbiome, aquatic microbiome, environmental microbiome and data labs. The laboratory for inceptive research activities will be set up at an RGCB-BIC campus situated at KINFRA park, Kazhakoottam.

Reminisce that eminence is not a depot but an incessant journey. It requisites a relentless pursuit of enhancement, a stanch obligation to quality, and a resolute credence in our prowess. Your endeavor, creativity, and resilience are the bedrock of our success.

While we strive for professional perfection, it is equally vital to focus on our personal growth and contentment. We ought to revel on our fruitions, reflect and revive, and endure to nurture your passion for science. Unceasing learning and evolvement are vital to sustaining our momentum and driving innovation. Apart from several project grants and high impact factor publications from our scientists and research scholars, RGCB plays a vibrant role in scientific research augmenting skills in high end technologies.

As part of our commitment to skill development, the DBT-SAHAJ National Facility for 'Mass Spectrometry-Based Proteomics, Metabolomics and Lipidomics' Platforms of our Centre primes a 3-day hands-on training program on "Mass Spectrometry-based Proteomics" A feather in the cap was the inauguration of a central facility for Mass Spectrometry-based Proteomics, Metabolomics, and Lipidomics platforms by Dr Vinod Kumar Paul, Member NITI Aayog, Govt of India in the presence of Dr Rajesh S Gokhale, Secretary, Department of Biotechnology, Govt. of India. The great revolution in DBT was the merger of 14 autonomous institutions of DBT into an apex body called the Biotechnology Research and Innovation Council (BRIC), to streamline governance and outcomes in related research activities. BRIC registered as an autonomous institute on 10th November 2023 was officially launched at the Annual General Meeting of the Society presided by Dr.Jitendra Singh, Hon'ble Minister of Science & Technology in the presence of Dr. Rajesh S Gokhale, Secretary DBT and Directors' of all the fourteen AI's of DBT.

In our quest for transcendence, the profound impact we can have on the world around us warrants significance. Our assurance to social responsibility oblivious of obligation stands as a prospect to make an evocative metamorphosis in our community and beyond. Each one of us has the power to drive positive change. Regardless of innovation, service, or merely by leading with integrity and compassion, our actions ripple outwards, inspiring others and contributing to a better society. Let's harness our collective talents and allegiance not only to achieve professional triumph but also to elevate those in need, champion important causes, and foster a culture of empathy and support. By aligning our goals with a far-flung social mission, we augment our work and craft a legacy that transcends the boundaries of our centre. In unison, let's attempt to be proxies of revolution, steadfast to creating the world a more equitable and compassionate place. Our quality is deliberated not just by our accomplishments, but by the positive impact we make on the lives of others.

Along these lines, I am blissful to apprise that RGCB rolled out a fully-equipped mobile virology testing laboratory, strengthening the efforts to contain the Nipah virus infection reported from parts of the Kozhikode district. DBT with an abiding vision to confront the COVID/Nipah like spread of infection has requested RGCB to set up a

mobile virology lab in the south which can be deployed in remote clusters. Adding, DBT has braced RGCB to set up an NGS facility to study the origin send variant of the infection. This mobile unit will be operated by a team of highly experienced staff with over eight years of expertise in molecular diagnostics from RGCB-LMMD. The unit is equipped with cutting-edge machinery adept at performing several viral tests and also execute testing for bacteria, fungi, and other pathogenic organisms, as well as assessing food quality. The equipment inventory includes PCR machines, gel documentation systems, workstations, high-speed refrigerated centrifuges, minus 80-degree freezers, refrigerators, DNA/RNA extracting machines, and an autoclave, among others. It assists as a self-contained and fully functional diagnostic laboratory. The mobile virology lab is tethered through the internet to RGCB for speedy data analysis and dissemination of results immediately upon authorization.

Rainwater Harvesting at RGCB, Akkulam Campus is hitherto another dedicated project that we have undertaken. The goal of RGCB has been to build a resourceful rainwater harvesting system as an effectual means of garnering the irreplaceable resource of rainfall. Akkulam Campus of RGCB is blessed with a natural water body and a pond. The rainwater harvesting at RGCB can be designed in an ecologically amicable mode so that the flow of rainwater can be congruously delimited to accrue in the said waterbody, the pond. Phase 1 of the rainwater harvesting at RGCB, Akkulam Campus was effectively effectuated within the envisioned time.

Sustaining a healthy mind and body stands decisive for sustained research efforts. It aids emphasis, imagination, and overall happiness.Exercise stances physical and mental health! It's not just about exercise; it also helps relieve stress, improves mood, and boosts overall well-being. Abetted by GATI, RGCB started a Gymnasium for staff and students, which I am fathomlessly delighted to add here.

I am profoundly gratified by your hard work, perseverance, and resolute conviction in our mission. Your inputs are the substratum of our success, and it is your commitment that propels us forward. Let us reveal our attainments, abet each other through defies, and aspire for excellence in all our endeavors.

The imminent holds boundless possibilities, and collected, we have the command to unlock them. Let's embrace each day with inquisitiveness, fortitude, and an unyielding spirit of discovery. Our journey at RGCB is gaining momentum, and I am enthusiastic to perceive the implausible technological contrivances we will achieve together.

With great pride and enthusiasm, I present the Annual Report for the year 2023-24

Jai Hind

Professor Chandrabhas Narayana, FASc, FRSC, FNASc
Director





CANCER RESEARCH



Santhosh Kumar T. R, PhD

Scientist G
Cancer Research

BRIEF THEME OF LABORATORY

The thrust area of our research is to understand molecular mechanisms of chemo resistance and tumor recurrence in solid tumors . We are addressing the chemoresistance through cell based therapy and immunotherapy approaches through inhouse generated biosimilars and recombinant antibodies. Another important focus of the laboratory is development of live cell based assays for identifying molecules that specifically target key pathways in cancer such as cell cycle, proteasome-Ubiquitin pathway, angiogenesis, HIF and tumor stem cells.

MAJOR RESEARCH AREA

- Developed a high throughput assay system to identify lysosomal destabilising agents over mitochondrial targeting compounds to be used as potential agents to target drug resistant cancers.
- The study using cell lines and animal models demonstrated that hypoxia induced mitophagy generates secondary metabolic heterogeneity and its implications in tumorigenesis and metastasis

- **VISUALISING CELL FATES UNDER HYPOXIA RE-OXYGENATION REVEALS TEMPORALLY EVOLVING CELL STATES GOVERNED BY MITOCHONDRIAL DYNAMICS**

Hypoxia is a common tumor micro environmental stress in solid tumors and is an indicator of bad prognosis. Tissue hypoxia determines tumor growth, metastasis, drug resistance and tumor heterogeneity through multiple mechanisms, largely dependent on the extent and duration of hypoxia and also modulated by intermittent re-oxygenation events. The conserved hypoxic stress responses include diverse forms cell death as well as cell survival regulated by HIF dependent and independent signaling. Despite extensive investigation, the nature of hypoxic stress induced cell death and how surviving cells evolve to generate tumor heterogeneity is largely not known. Inorder 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 cells under hypoxia and re-oxygenation. The sensor cells of apoptosis-necrosis revealed a cell death switch from apoptosis to necrosis by hypoxia escaped cells under reoxygenation where mitophagy play a key role in the acquisition of temporally evolving functional phenotypes including metabolic heterogeneity. The hypoxic survival involves mitophagy and the surviving the cells progress through accelerated mitophagy phase to necrosis prone state ultimately regaining initial parental phenotype thereby contributing functional heterogeneity with complex temporally evolving cell states. The impact of such dynamic changes in mitochondria is reflected with cells surviving with mitochondrial permeabilisation, acquiring glycolytic dependent phenotypes, as revealed by single clone expanded ATP sensor cells. Consistent with dynamics of cell death switch, Transcriptomics of cells surviving from hypoxia and re-oxygenation demonstrate temporally evolving genomic landscape supporting complex plasticity of cells as a non-genetic adaptation. Interestingly, cells regained from these distinct stages retained their metastatic potential despite slow growth in animal models. Overall, the study using single cell expanded clones of sensor cells demonstrated that cells acquire distinct functions contributed by changing pattern of tumor hypoxia and oxygenation, secondarily acquiring transient functional traits and metabolic heterogeneity many are governed by non-genetic mitochondrial dynamics. Such temporal alterations in cell states with distinct cell proliferation, apoptosis necrosis switch may be advantages for the growing tumor to evolve under complex micro environmental stress further contributing to tumorigenesis.

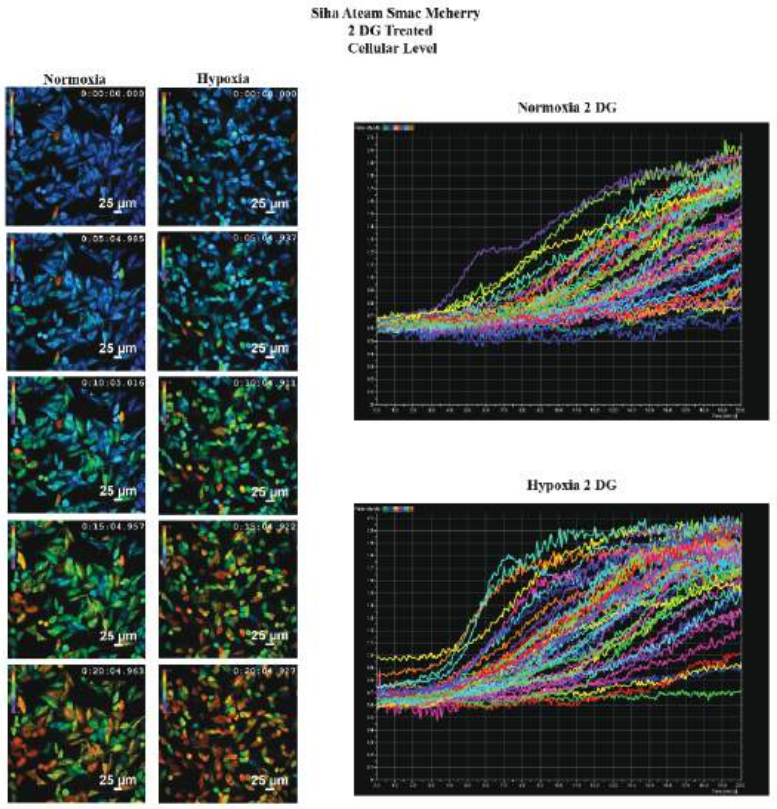


Fig : Fluoroscence Intensity ECFP/EYFP Ratio Changes after 2 DG Treatment in Normoxia and Hypoxia Escaped Cells

Where,
Cells Were 3 Days Hypoxia Exposed and 35 Days Escaped
2 DG Concentration 15.5 mM Used

SiHa cells stably expressing FRET based ATP sensors were exposed to hypoxia following reoxygenation to create surviving cells from these two shocks. The cells were exposed to glucose deprivation by treating with 2DG. Real time FRET ratio imaging was carried out . Cells with low ATP over time is represented with increase in ratio. As shown Hypoxic cels demonstrated faster ATP depletion , however demonstrating higher heterogenic response .



Project Assistant: 2
Technical Assistant: 1
Lab Assistant: 2
Project Associate: 1
Postdoctoral Fellows: 2
PhD Students: 4

Dr Shine V J, Aijaz Ahmad Rather, Dr Kiran S Kumar, Dr. T R Santhosh Kumar, Aman Halikar, Prakash V, Jain Tiffee, Dr Unnikrishnan P S (From L to R)

PUBLICATIONS:

- Sharaf SS, Lekshmi A, S A, K G A, Jyothi S P A, Chandrasekharan A, Somanathan T, Santhosh Kumar TR, K S. A multiplex immunoprofiling approach for detecting the co-localization of breast cancer biomarkers using a combination of Alexafluor - Quantum dot conjugates and a panel of chromogenic dyes. Pathol Res Pract. 2024 ;253:155033.
- Jancy SV, Lupitha SS, Chandrasekharan A, Varadarajan SN, Nelson-Sathi S, Prasad R, Jones S, Easwaran S, Darvin P, Sivasailam A, Santhoshkumar TR. A high-throughput screening system for SARS-CoV-2 entry inhibition, syncytia formation and cell toxicity. Biol Proced Online. 2023;25(1):22
- Chandrasekharan A, Tiwari SK, Munirpasha HA, Sivasailam A, Jayaprasad AG, Harikumar A, Santhoshkumar TR. Genetically encoded caspase sensor and RFP-LC3 for temporal analysis of apoptosis-autophagy. Int J Biol Macromol. 2024;257:128807.
- Jayaprasad AG, Chandrasekharan A, Arun Jyothi SP, John Sam SM, Santhoshkumar TR, Pillai MR. Telomerase inhibitors induce mitochondrial oxidation and DNA damage-dependent cell death rescued by Bcl-2/Bcl-xL. Int J Biol Macromol. 2024;264:130151.
- Jeethy Ram T, Lekshmi A, Darvin P, Rajappan P, Jagathnath Krishna KM, Anoop TM, Augustine P, Mathew AP, Cherian K, Bhargavan RV, Somanathan T, Radhakrishna Pillai M, Santhosh Kumar TR, Sujathan K. Co-expression of galectin-3 and vimentin in triple negative breast cancer cells promotes tumor progression, metastasis and survival. Tumour Biol. 2023;45(1):31-54
- Parambil ST, Thankayyan SKR, Antony GR, Littleflower AB, Augustine P, Somanathan T, Subhadradevi L. YAP transduction drives triple-negative breast cancer aggressiveness through modulating the EGFR/AKT axis in patient-derived xenograft cells. Med Oncol. 2023;40(5):137

INVITED TALKS(PI ONLY):

- Cell Engineering for Drug discovery and Disease modelling .International Conference on New Horizons in Bioengineering: Fostering Academia-Industry Partnership ICB-24, Institute of Science and Technology in Chennai, India, from 14 to 16 February, 2024

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Establishment of life time imaging facility	Department of Biotechnology	2019	5 years	PI



S Asha Nair, PhD

Scientist G & Dean (Research Administration)
Cancer Research

BRIEF THEME OF LABORATORY

Colorectal cancer (CRC) is the 1st and 2nd leading cause of mortality in men and women under the age of 50 respectively. Our team has focused on two aspects for understanding CRC; discerning the role of dietary fibers in modulation of the gut microbiome for better immunotherapeutic response and comprehending the inflammatory pathway gene SERPIN A3 and drug resistant genes STIL in CRC. Our Interdisciplinary research includes PDT and novel photosensitizers in breast cancer therapy.

MAJOR RESEARCH AREA

- **STIL IN CRC**

  Identified STIL to regulate stemness markers CD133 & CD44 and drug resistant markers Thymidylate synthase (TS), ABCB1 & ABCG2, both in in-vitro and in-vivo CRC models.
  STIL could regulate the expression of AKT and its phosphorylation resulted in cleavage and nuclear translocation of beta catenin.

- **PHOTODYNAMIC THERAPY & PHOTOSENSITIZERS**

  Synthesized Aza BODIPY bio conjugate (DPR2b) with biotin moiety for PDT.
  Highest PD efficiency perceived in MDA-MB-231 and MCF7 breast cancer cells.
  Proteomic approach revealed pathways like response to unfolded protein, cell redox homeostasis, regulation of programmed cell death and actin cytoskeletal organization

- **INVESTIGATING THE MOLECULAR ATTRIBUTES OF STIL IN STEMNESS OF CANCER AND TRANSLATIONAL ADVANCES OF PDT EMPLOYING AZABODIPY, A NOVEL PHOTOSENSITIZER.**

STIL - a molecular regulator of Shh and Wnt signaling, mediating stemness in CRC.

Colorectal cancer (CRC), ranking third in incidence and second in cancer associated mortality worldwide, remains the deadliest cancers in late stage of diagnosis. Previous studies from our lab reported that, silencing STIL reduced proliferation and tumor growth in CRC. Also, we observed STIL to regulate stemness markers CD133 & CD44 and drug resistant markers ABCB1 & ABCG2, (Figures 1A) in stable STIL silenced HT29 cells. Silencing STIL gene led to a significant decrease in the size of tumorospheres formed (Figures 1B), as well as the percentage of side population (Figures 1C), indicating the importance of STIL gene in maintaining stem cell population.

Targeted therapies often require molecular information regarding the pathways altered. Therefore, unraveling the pathways and key molecules linking could help develop novel therapeutic targets in CRC. WNT and SHH are the two major developmental pathways altered in CRC. We observed a STIL mediated crosstalk between WNT and SHH the two major developmental pathways, by regulating the effector molecules GLI1 and Beta-catenin (Figures 2A) as well as their target genes (Figures 2B & C). We also found that STIL mediated regulation of GLI and the target genes of SHH could be dependent on SHH pathway. Chemical inhibition of Wnt pathway altered STIL, but not the Beta-catenin levels suggesting a WNT independent regulation of Beta-catenin. We observed that STIL could regulate the expression of AKT, and pAKT (Figures 2D). AKT is a known modulator of Beta-catenin that phosphorylates the Ser552 of beta catenin resulting in cleavage and nuclear translocation of Beta-catenin. Upon STIL silencing, we observed a reduction in short fragment and accumulation of full-length beta catenin in nuclear fraction(Figures 2E). This indicates that STIL might be regulating the phosphorylation and cleavage of Beta-catenin through AKT.

Molecular Efficacy of a Photosensitizer- Biotin-Conjugated Aza-BODIPY in PDT

Photodynamic therapy (PDT) has been an evolving area in cancer research and involves Photosensitizers (PS) as the pro-drugs, which when irradiated by light of a particular wavelength results in the initiation of reactive oxygen species (ROS) leading to cell death. In collaboration with CSIR NEIST Jorhat (Dr. Pranjal Gogoi) we synthesized Aza BODIPY bio conjugate (DPR2b) with biotin moiety for PDT. To analyze the cell line specificity of DPR2b, MTT assay was employed in five cancer cell lines and an immortalized cell line. Our cytotoxicity assay data authenticates the highest photodynamic efficiency of Aza BODIPY in MDA-MB-231 and MCF7 (Figure 3A). Also, cancer stemness markers showed a significant reduction in CD133 expression in breast cancer cells treated with DPR2b and laser irradiation, suggesting a potential reduction in the CSC population. (Figure 3B). DPR2b resulted in reduced SP side population (SP) assay, which potentially identifies drug effluxing cells and CSCs. in MDA MB 231 with mean % population of 0.2% compared to control with 4.5 and 5.7 respectively (Figures 3C).

(Fig 1 A) Immunoblot showing expression of CD133 and CD44 and drug efflux proteins in STIL silenced HT29 cells.

(Fig1 B) Showing reduction in size of tumorospheres formed in control and STIL silenced cells.

(Fig 1C) FACS data showing reduction in percentage of side population with STIL silencing.

Fig 2 - Western blot showing reduction in SHH and WNT effector molecules

(Fig 2A) and target genes

(Fig 2B & C) in STIL silenced cells.

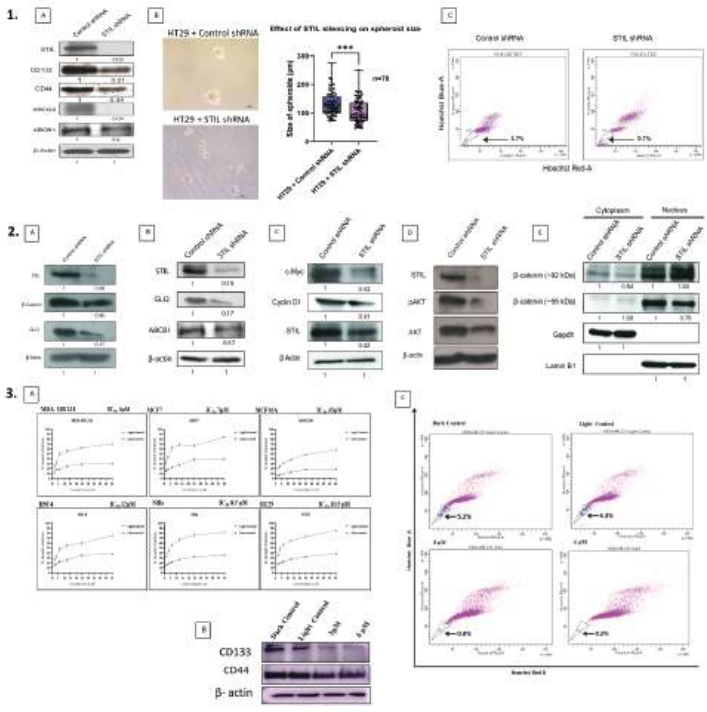
(Fig 2D) Expression of AKT and pAKT reduced upon STIL silencing.

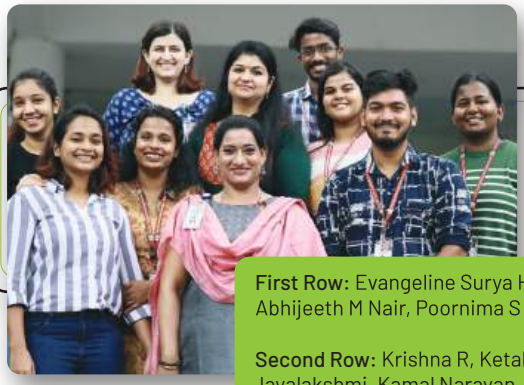
(Fig 2E) Immuno blot showing nuclear and cytoplasmic expression of beta catenin upon STIL silencing.

(Fig 3A) Photocytotoxicity of aza-BODIPY-biotin conjugates in the presence and absence of light in various cancerous cell lines.

(Fig 3B) Western blot of CD133 CD44 expression in MDA MB 231 cells after DPR2b treatment.

(Fig 3C). Reduction in stem cell population post PDT (0.8% & 0.2%.) compared to light (4.3%) and dark control(5.2%).





JRF: 1
Project Assistant: 1
Lab Assistant: 1
PhD Students: 5

First Row: Evangeline Surya Hermon, Meera R Nair, Dr S Asha Nair, Abhijeeth M Nair, Poornima S R (from L to R)

Second Row: Krishna R, Ketakee Mahajan, Divya Jayalakshmi, Kamal Narayan, Aneesha K **(From L to R)**

PUBLICATIONS:

- Dutta D, Nair RR, Mangalath S, Nair SA, Joseph J, Gogoi P, Ramaiah D. Biocompatible Aza-BODIPY-Biotin Conjugates for Photodynamic Therapy of Cancer. ACS Omega. 2023;8(29):26180-26190.
- Dutta D, Nair R, Kayastha N, Nair AS, Gogoi P . Avidin triggered turn-on NIR-fluorescent aza-BODIPY-biotin self-assemblies for cancer cell imaging. New J of Chemistry 2023; 35:16596-16603

INVITED TALKS [PI ONLY]:

- A paradigm shift in the molecular perspective of colorectal cancer:is young old? KTN APSE CON 2023 CONFERENCE, December 21st 2023, Chalavara Palaghat, Kerala.
- Invitation as Resource Person for ACP ANNUAL WOMEN CONGRESS June 3rd 2023 , Perinthalmanna, Malapuram.

CONFERENCE PRESENTATION:

- Rajashree R Nair, Poster Presentation, 'Elucidating the Potential of DPR2b Aza BODIPY: A Novel Photosensitizer for Enhanced Photodynamic Therapy in Breast Cancer at NSW Cancer conference 2023, Sydney, Australia.
- Rajashree R Nair, Oral presentation, "Photodynamic Therapy for Breast Cancer: Molecular Effectiveness of a XPhotosensitizer, Biotin-Conjugated Aza-BODIPY at Kerala Science Congress" 2023, Kuttikanam, Idukki Kerala.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Addressing STIL as a major driver of drug resistance in colorectal cancer, independent of Shh pathway	Indian Council of Medical Research	2023	3 years	PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Samu John	Elucidating role of ETS2 in cell cycle and colorectal cancer progression	University of Kerala	Awarded	2023
02	Anjana Soman	Identification of SERPINA3 in Early onset of colorectal cancer and investigating its molecular manifestation.	University of Kerala	Awarded	2023



Priya Srinivas, PhD

Scientist G & Dean (Academic Affairs)
Cancer Research

BRIEF THEME OF LABORATORY

The major mandate of my laboratory is to understand the molecular mechanism of tumorigenesis in BRCA1 defective cancers and to identify diagnostic and therapeutic options. Other than this, my second major mandate is to develop non-invasive techniques for cancer diagnosis using body fluids

MAJOR RESEARCH AREA

- We developed a stable BRCA1 gene promoter hyper methylation cell line model for the first time, by inducing site-specific methylations in wild type BRCA1 promoter using modified CRISPR and demonstrated the direct impact of promoter hyper methylation on tumor progression through novel cascade of pathways.
- We also delineate potential biomarkers like NBR2 and β-hCG for early diagnosis and prognosis of the disease and propose the use of drugs like biguanides and anti-hCG vaccines for better management of a large proportion of breast cancers.

EPIMUTATIONS IN BRCA1 PROPELS ONCOGENESIS IN SPORADIC BREAST CANCERS

Epimutations have been proposed as a first step towards oncogenesis. One of such epimutations, hypermethylation in the BRCA1 promoter is associated with BRCA1 inactivation in 30-60% of sporadic breast cancers. However, unlike hereditary BRCA1 mutations which account for merely 5-10% of all breast cancer cases, the role of BRCA1 hypermethylation in tumorigenesis remains obscure. To unravel this enigma, we induced site-specific methylations in wildtype BRCA1 promoter using modified CRISPR and demonstrated the direct impact of promoter hypermethylation on tumor formation and progression for the first time. We reveal that BRCA1 hypermethylation effectuates BRCA1 downregulation by regulating ER-α expression and thereby modulating the balance between alternate BRCA1 transcripts. BRCA1 hypermethylation also regulates the transcription of lncRNA, NBR2, that shares BRCA1 promoter. Strikingly, this regulation activates a feedback loop by incrementing BRCA1 downregulation and augmenting tumorigenesis. Further, hypermethylation results in induction of β-hCG & ER-α and downregulation of PR & ER-β in initial stages, which promotes tumor proliferation. However, remodulation of hormone receptors at later stages facilitates the luminal to basal transformation of tumors. Finally, we elucidate the invasiveness and triple-negative phenotype of BRCA1 hypermethylated tumors using the first-ever hypermethylation-based xenograft tumor models and uncover the underlying diagnostic and prognostic markers using proteomics. Altogether, these results describe a novel cascade of pathways that can serve as potential therapeutic targets for BRCA1 defective breast cancers.

FIGURE 1

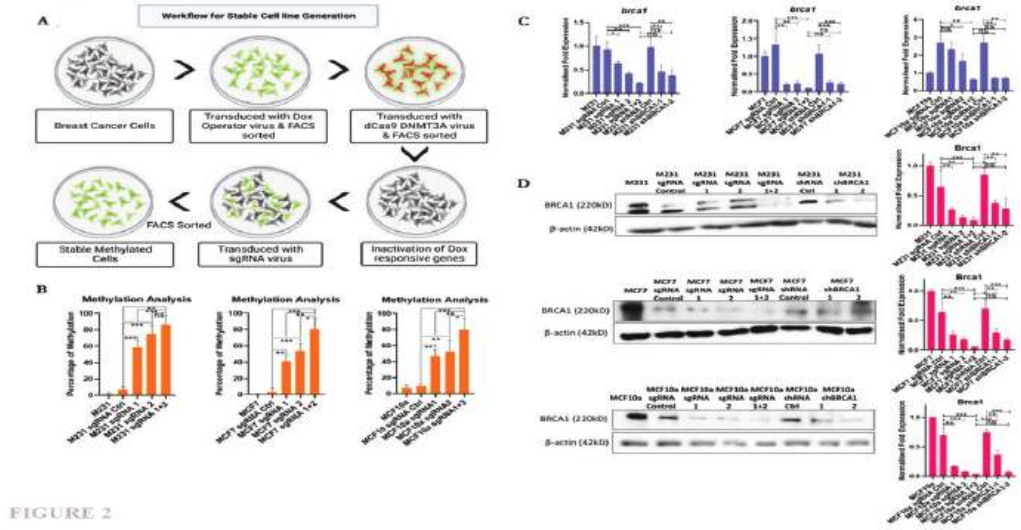


FIGURE 2

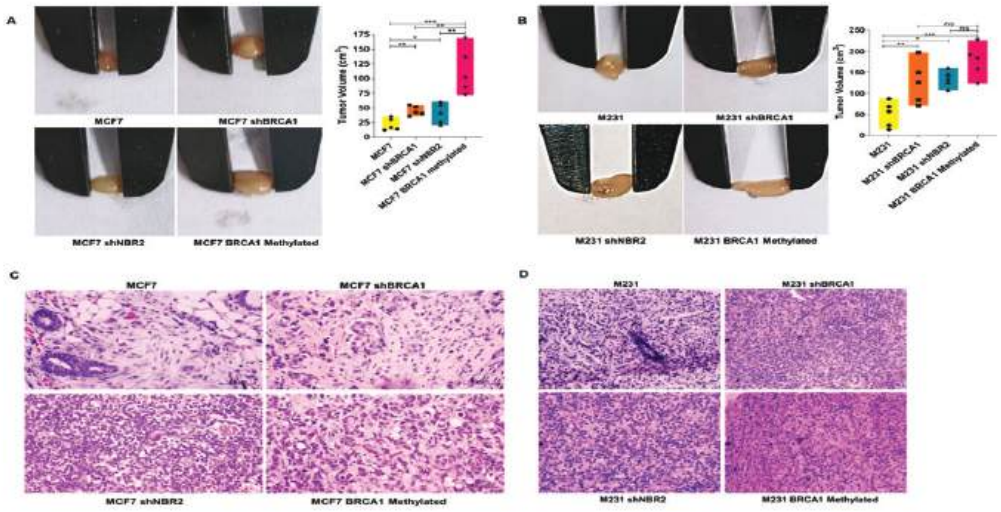


Figure 1: Site-specific Methylation in BRCA1 promoter-α leads to downregulation of BRCA1. A. Workflow for generation of stable BRCA1 methylated cells. B. Methylation levels analysed via MS-qPCR.
C & D depict BRCA1 expression at mRNA (by qRT-PCR) and protein level (by western blot) respectively in the same cell lines. Fold changes were calculated with respect to gapdh for qRT-PCR and β- actin for western blots. shBRCA1-1 & 2 and shNBR2- 1 & 2 represent BRCA1 and NBR2 knockdown cells respectively; sgRNA1, sgRNA2 and sgRNA1+2 represent cells transduced with single sgRNAs (sgRNA1 & sgRNA2) and both sgRNAs against BRCA1 promoter respectively.

Figure 2: BRCA1 hypermethylation leads to xenograft tumors with increased invasiveness.
A & B depict representative image and volume of xenograft tumors generated from MCF7 and MDAMB231 (M231) cells respectively.
C & D represent haematoxylin and eosin staining images of xenograft tumors generated from MCF7 and M231 cells respectively.



JRF: 6
SRF: 1
Project Assistant: 1
Lab Assistant: 1
PhD Students: 7

First Row: Arsha Raj TS, Dr Priya Srinivas, Prianka Kumari, Bhavana Ramachandran, Kaviya S (from L to R)

Second Row: Sansiya Yesudasan, Kavitha UK, Aiswarya M A, Aswathy Ashokkumar, Sheeja B **(From L to R)**

PUBLICATIONS:

- Srinivas P, Varghese GR, Krishnan K, Warrier AV, Kumari P, Ittycheria SS, Neetha RL, Anandan J, Yadev I. Pregnancy hormone can modulate cancer progression in BRCA1 defective cancers. Cancer Lett. 2024; 581: 216528

AWARDS [PI]:

- ICMR-DHR International Fellowship for Senior Scientists 2022-23 (Visiting Scientist, Mayo Clinic, Rochester, Minnesota, USA).

AWARDS [STUDENTS]:

- Neetha R.L, Dr. M. Krishnan Nair Memorial Award (Best Oral presentation), Metastasis Associated Fibroblasts (MAF): A Newsubtype of CAFs in Breast Cancer Metastasis, National Conference on "Emerging Trends in Healthcare Biotechnology: Innovations, Challenges and Future Prospects"; Nov 30 – Dec 02, 2023, Regional Cancer Centre, Thiruvananthapuram, Kerala, India

INVITED TALKS [PI ONLY]:

- Pregnancy hormone can modulate cancer progression in BRCA1 defective cancers, International Ovarian Cancer Research Consortium and The International Society of Precision Cancer Medicine, June 12 – June 13, 2023 Oklahoma City, OK, USA
- 49th Annual Conference of the Association of Clinical Biochemists of India, 49th ACBICON 2023 Kerala,13-16th September, 2023, Al-Saj International Convention centre, Kazhakkoottam, Thiuvananthapuram

CONFERENCE PRESENTATION:

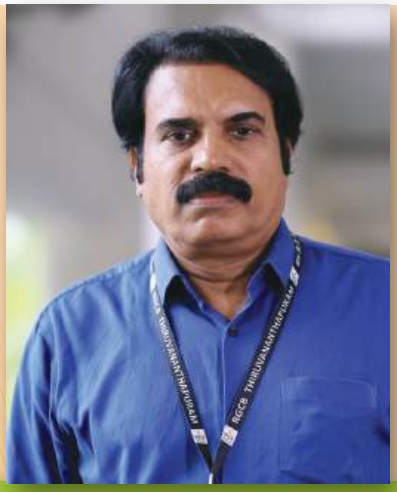
- Neetha R.L, Dr. M. Krishnan Nair Memorial Award (Best Oral presentation), Metastasis Associated Fibroblasts (MAF): A Newsubtype of CAFs in Breast Cancer Metastasis, National Conference on "Emerging Trends in Healthcare Biotechnology: Innovations, Challenges and Future Prospects"; Nov 30 – Dec 02, 2023, Regional Cancer Centre, Thiruvananthapuram, Kerala, India

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Analysis of tumor characteristics and therapeutic strategies for B-hCG positive Breast Cancer Patients from Kerala using microfluidic organ-on-a-chip culture device.	Indian Council of Medical Research	2024	3 years	PI
02	Identification ofxenoestrogen inducedspontaneous mutationsand changes in promotermethylation status in thecancer causing genesBRCA1, BRCA2 and p53in breast, ovarian,prostrate and pancreaticcancers.	Council of Scientific & Industrial Research	2020	3 years	PI
03	Development of low cost diagnostic kit based on miRNA detection for HepatocellularCarcinoma (Co- PI)	Indian Council of Medical Research	2022	1 years	CO-PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Dipyaman Patra	"Epigenetic Alteration of BRCA1 Signalling in Tumorigenesis"	University of Kerala	Awarded	2023



G. S. Vinod Kumar, PhD

Scientist F & Associate Dean (Academic Affairs)
Cancer Research

BRIEF THEME OF LABORATORY

Our group mainly focus on designing and development of peptide synthesis and multifunctional nanostructures for programmed drug delivery targeting systems in cancer and wound healing. Several first and co-authored manuscripts are published in leading scientific journals and in addition hold key patents as inventor on Indian and US patents.

MAJOR RESEARCH AREA

- In wound healing research we have synthesised exosome-laden polysaccharide-based hydrogel dressing to accelerate burn wound healing. The synthesized hydrogel wound dressing showed the following advantages:

  •The hydrogel equipped with dynamically reversible Schiff base linkage possesses easy applicability due to the sprayable property and completely covers irregular wounds.

  • The hydrogel exhibited good antibacterial properties and reduced inflammatory response.

  • In vitro experiments showed good biocompatibility and enhanced wound healing in keratinocyte and fibroblast cell lines.

  • In vivo experiments conducted in partial thickness burn wound model showed enhanced wound closure compared to the control group.

- **A SPRAYABLE POLYSACCHARIDE HYDROGEL WITH DYNAMIC SCHIFF BASE LINKAGE, LOADED WITH ADIPOSE MESENCHYMAL STEM CELL-DERIVED EXOSOMES, ENHANCES BURN WOUND HEALING EFFICIENCY.**

In this study, a thermosensitive sprayable hydrogel was synthesized via a Schiff base reaction between modified natural and synthetic polymers for the sustained release of adipose mesenchymal stem cell derived exosomes to treat severe burn wounds. The synthesized hydrogel was characterized using Fourier Transform Infrared Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), Surface charge analyzer (zeta potential), Scanning Electron Microscopy (SEM), and rheology. Additionally, an antimicrobial peptide, LL18 (KLFKRIVKRILKFLRKLV), synthesized by Solid Phase Peptide Synthesis (SPPS) method and purified by reverse phase HPLC was loaded into the hydrogel to exert antibacterial effect. LL18 peptide loaded hydrogel inhibited the growth of both gram-positive (Staphylococcus aureus) and gram-negative (Pseudomonas aeruginosa) bacteria as confirmed by Kirby disc diffusion assay and release ablation assay. In chronic wounds, LL18 is shown to attenuate oxidative stress, mitigate inflammation, and promote angiogenesis. Furthermore, adipose mesenchymal stem cells were isolated from the peri-renal and inguinal fat tissues of Sprague Dawley rats. The isolated cells were cultured in alpha-MEM media and characterized with flow cytometry and confocal microscopy, which showed the expression of positive surface markers (CD44 and CD90). Subsequently, exosomes from the culture media were isolated using serial centrifugation method, followed by characterization by Dynamic Light Scattering (DLS) showing an average particle size of 100 nm, Transmission electron microscopy (TEM) showing a spherical morphology, and immunoblot showing the expression characteristic tetraspanin surface markers-CD63 and CD81. The isolated exosomes were loaded into the hydrogel and its release profile was evaluated. The biocompatibility, wound healing potential and exosome internalization efficacy were evaluated in keratinocyte and fibroblast cell lines. The result showed good biocompatibility as displayed by MTT and Live/Dead assay. Significant closure of wound area occurred after 24 h in both keratinocyte and fibroblast cell lines. Also, the exosome released sustainable from the hydrogel was successfully internalized by both cell lines revealing that the exosomes were viable inside the hydrogel. In vivo experiments were conducted in partial thickness burn wound model (in Sprague Dawley rats) to evaluate the healing potential of the synthesized hydrogel system. The results showed enhanced wound closure compared to the control group.

Histopathological evaluations also supported the results. Immunofluorescence assays exhibited significant expression of Ki67 (proliferation marker), α-SMA and CD31 (angiogenic marker), also the treatment alleviated inflammation as confirmed by immunofluorescence expression of CD68 (macrophage marker). Overall, the synthesized hydrogel wound dressing showed the following advantages: 1) the hydrogel equipped with dynamically reversible Schiff base linkage possesses easy applicability due to the sprayable property and completely covers irregular wounds; 2) the hydrogel exhibited good antibacterial properties and reduced inflammatory response; 3) the hydrogel maintained the viability of the loaded exosomes along with its sustained release. In this study, a comprehensive examination of the synthesis and characteristics of a thermosensitive sprayable polysaccharide-based hydrogel was conducted, and their effects were studied in vitro. Finally, the wound healing efficiency was investigated using a burn model in rats, expanding the practical use of such wound dressings.

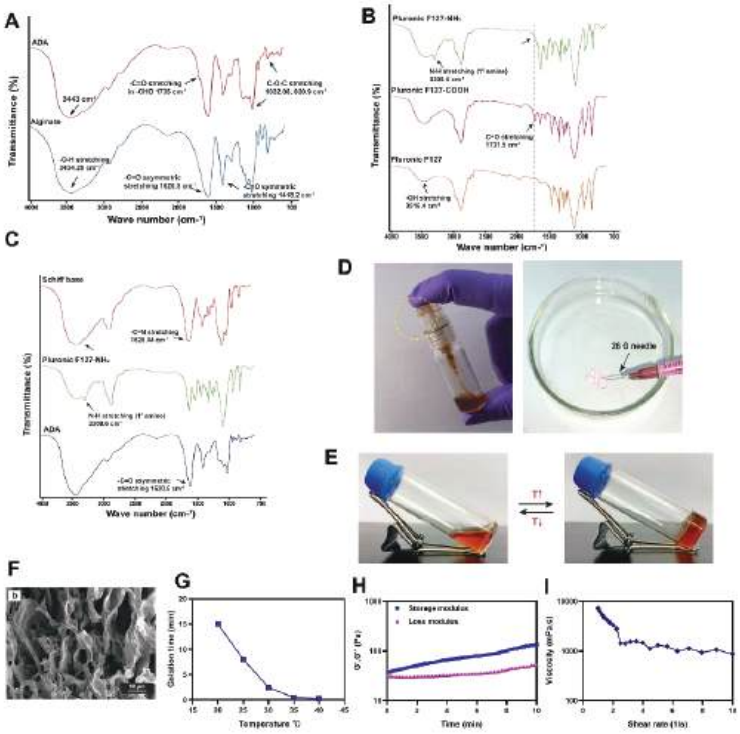


Fig.1. Characterization:

(A) FT-IR spectrum of ADA,

(B) FT-IR spectrum of aminated pluronic F127, (D) (B) FT-IR spectrum of hydrogel,

(D) Sprayability and Injectability,

(E) Thermoresponsive property,

(F) SEM image of freeze-dried hydrogel,

(G) Gelation time of hydrogel at different temperature,

(H) Time-dependent rheological behavior of the hydrogels at 37 °C,

(I) Viscosity of hydrogel with the shear rate from 0.1 to 10 1/s.



Technical Assistant: 1
PhD Students: 4

Arsha UP, L Naresh Goud, Vipin CL, Jyothilakshmi V A, Athira S S

PUBLICATIONS:

- Vipin CL, Priya P, Harikrishnan K, Kumar GSV. Enhanced cutaneous wound healing through MMP-8-responsive release of antimicrobial peptide from a porous chitosan-based scaffold. J Drug Deliv Sci Technol.. 2023;89:105069.

CONFERENCE PRESENTATION:

- Vipin C.L., Poster presentation, Exosome-laden sprayable thermosensitive hydrogel for accelerating burn wound healing, First RGCB Research Conference, 20-23/2023, Kollam



Devasena Anantharaman, PhD

Scientist F & Wellcome Trust-India Alliance Intermediate Fellow
Cancer Research

BRIEF THEME OF LABORATORY

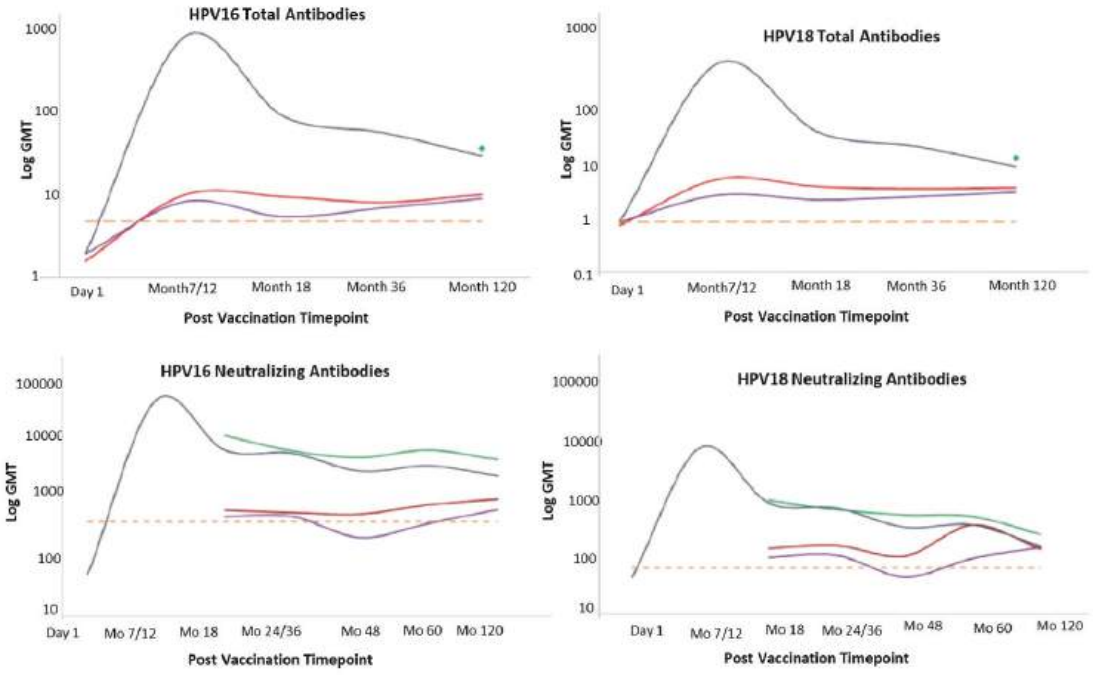
The research focus of the lab is to elucidate the molecular basis for cancer etiology towards an improved disease stratification, particularly for oral and oropharyngeal cancers. In addition, an important thrust area is to understand adaptive immune responses towards human Papillomavirus (HPV) vaccination. We build large epidemiologic studies that integrate high throughput analytical methods to achieve these goals.

MAJOR RESEARCH AREA

- Contribution as subject expert to WHO handbook on oral cancer prevention, summary published in New Eng J Med
- Initiation of National level cancer incidence estimation for HPV positive and negative fractions through collaboration with NCDIR-ICMR
- Certification of HPV genotyping facility in the lab by WHO through participation in Global HPV DNA proficiency test 2023

- **IMPACT OF AGE AT VACCINATION ON NEUTRALIZING ANTIBODIES AT 10 YEARS FOLLOWING HPV VACCINATION**

WHO call for elimination of cervical cancer necessitates catch up vaccination of girls, especially in settings of delayed onset of HPV vaccination and countries with low vaccine coverage. Earlier, we demonstrated non-inferior vaccine response in older girls (15-26 years) compared to younger (10-14 years) based on total and neutralising antibodies against HPV16/18. We examined cervical infections as intermediate end points which also led to similar conclusions. In a follow up analysis, we have examined the impact of age at vaccination on these endpoints in younger and older girls in the context of single dose HPV vaccination. Serology samples at 10 years were obtained from 344 single dose, 324 two doses, 190 three doses, and 167 unvaccinated women. Overall, nearly 60% of all vaccines were immunized between 10–14 years of age. To quantify the total binding antibodies present against HPV 16/ 18, serum samples were tested using the multiplex HPV virus-like particle-based IgG ELISA on Meso Scale Discovery platform (M9ELISA) against a reference standard serum using the parallel line method described in the World Health Organization (WHO) HPV Labnet Manual. Neutralizing antibodies against HPV 16/ 18 L1 proteins were detected using the HT PBNA assay. To assess the effect of age at vaccination, log-transformed GMT were used in linear regression models to obtain GMT ratios and their corresponding 95% CIs. Statistical significance was inferred when the GMT ratio and 95% CI across comparison categories did not include one. Among single dose vaccine recipients at ten years post-vaccination, the younger girls (10–14 years) had detectable binding antibody titers of 97.7% for HPV 16 and 98.2% for HPV 18. For 15–18 year old girls, these proportions were 92.3% and 94.2% respectively. Antibody levels were higher for both age groups compared to unvaccinated women. These data support the speculation when the antibody levels stabilize, there will be very little difference between the single and two/three dose groups or age categories. This data supports the call for catch up immunization of older girls, more importantly with even single dose HPV vaccination.



Evolution of HPV16/18 total binding and neutralization antibodies in single-dose or three doses of the HPV vaccine recipients are shown. Figures are stratified by age groups. Red line depicts single dose at 10-14 years; purple line single dose at 15-18 years; teal line two dose at 10-14 years and blue line two dose at 15-18 years. Dotted orange line depicts antibody titres for natural immunity. For 2-dose group, binding immune responses were assessed only at 10 year time point.



JRF: 2
Project Assistant: 2
Technical Assistant: 2
Lab Assistant: 1
Project Associate: 1
PhD Students: 1

First Row: Kannan TR, Subha S, Dr Devasena A, Sinumol G (from L to R)

Second Row: Jinu A, Anandi S, Kalyani R, Manju V, Simla Rani, Anikesh A, Lekshmy S R (from L to R)

PUBLICATIONS:

- Vijayan Y, James S, Viswanathan A, Aparna JS, Bindu A, Namitha NN, Anantharaman D, Babu Lankadasari M, Harikumar KB. Targeting acid ceramidase enhances antitumor immune response in colorectal cancer. J Adv Res. 2023;S2090-1232(23), pg S2090-1232.
- Bhatla N, Muwonge R, Malvi SG, Joshi S, Poli URR, Lucas E, Esmy PO, Verma Y, Shah A, Zomawia E, Pimple S, Jayant K, Hingmire S, Chiwate A, Vashist S, Mishra G, Jadhav R, Siddiqi M, Anantharaman D, Panicker G, Butt J, Sankaran S, Kannan TPRA, Varghese R, Kartha P, Pillai MR, Waterboer T, Müller M, Sehr P, Unger ER, Sankaranarayanan R, Basu P. Impact of age at vaccination and cervical HPV infection status on binding and neutralizing antibody titers at 10 years after receiving single or higher doses of quadrivalent HPV vaccine. Hum Vaccin Immunother. 2023 ;19(3):2289242.
- Joshi S, Anantharaman D, Muwonge R, Bhatla N, Panicker G, Butt J, Rani Reddy Poli U, Malvi SG, Esmy PO, Lucas E, Verma Y, Shah A, Zomawia E, Pimple S, Jayant K, Hingmire S, Chiwate A, Divate U, Vashist S, Mishra G, Jadhav R, Siddiqi M, Sankaran S, Pillai Rameshwari Ammal Kannan T, Kartha P, Shastri SS, Sauvaget C, Radhakrishna Pillai M, Waterboer T, Müller M, Sehr P, Unger ER, Sankaranarayanan R, Basu P. Evaluation of immune response to single dose of quadrivalent HPV vaccine at 10-year post-vaccination. Vaccine. 2023;41(1):236-245.
- Gheit T, Muwonge R, Lucas E, Galati L, Anantharaman D, McKay-Chopin S, Malvi SG, Jayant K, Joshi S, Esmy PO, Pillai MR, Basu P, Sankaranarayanan R, Tommasino M. Impact of HPV vaccination on HPV-related oral infections. Oral Oncol. 2023;136:106244.

INVITED TALKS [PI ONLY]:

- Sustained Immune Response to qHPV vaccine. Indian Cancer Congress. November 2-5, 2023, Jio World Convention Centre.
- Priorites for Cancer Research in India. 17th December, 2023, National Institute of Immunology.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Human papillomavirus (HPV)-related oropharyngeal cancer burden and the natural history of oral HPV infections: an Indian perspective	DBT/ Wellcome Trust India Alliance (Intermediate Fellowship)	2019	5 years	PI
02	Biomarkers of oral cancer risk prediction	Department of Biotechnology Glue Grant	2018	6 years	PI
03	Accurate and satisfactory analysis of all high risk HPV types and some of the low risks including HPV 6 and 11 antibody titers for the 2-versus 3 dose HPV vaccination clinical trial in India – Follow-up study	International Agency for Research on Cancer - WHO	2020	5 years	PI
04	HPV genotyping for efficacy testing of generic qHPV vaccine development: Serum Institute of India study	Serum Institute of India	2019	5 years	PI



Tessy Thomas Maliekal, PhD

Scientist E-II
Cancer Research

BRIEF THEME OF LABORATORY

Oral cancer is one of the important cancer with respect to mortality, worldwide and in India. The poor prognosis is due to the relapse of the disease or reappearance of the cancer after treatment, which primarily depends on the self-renewal ability of cancer cells. Our lab focuses on identifying the important signaling pathways and their intermediates regulating this property

MAJOR RESEARCH AREA

- Generated pPSmOrange-C1-EPHA2 for photoswitching FRET.
- Generated mTSapphire-C1-EphrinB1 for photoswitching FRET.

GENERATING A PHOTOSWITCHING FRET PAIR TO STUDY THE INTERACTION OF EPHA2 AND EPHRINB1

Foster resonance energy transfer (FRET) is a reliable method to study protein–protein interaction. Phtoswitching FRET is a modification of FRET utilizing the physical properties of the fluorophore. Photoswitchable fluorescent proteins have the capability to switch from one type of distinct fluorescence color to another type of fluorescence color upon excitation with a particular wavelength since it acquires new conformation upon excitation. Thus, the photoswitchable FPs change their fluorescent property and there by their emission wavelength upon irradiation with light of a particular wave length. pPSmOrange-C1 and mT-Sapphire-C1 act as a photo-switching FRET pair.

Eph/Ephrin signaling is a bidirectional pathway, which is involved in various developmental processes. Receptors and ligands of this group are divided into type A and type B depending on their structure. Conventionally Eph receptor and Ephrin ligands of the same family interact with each other, though cross interactions are also reported in certain contexts. Our studies showed that an EPHA2/Ephrin B1 cross signaling regulating CSCs in oral cancer. Our results showed that The expressions of EPHA2 and EphrinB1 are increased in sphere condition that enriches self-renewing cancer stem cells. Immunofluorescence and confocal imaging suggested the interaction of these molecules. To confirm their interaction, we need to perform FRET. For this, we generated pPSm OrangeC1-EPHA2 and mT-Sapphire-C1- EphrinB1 by PCR cloning.

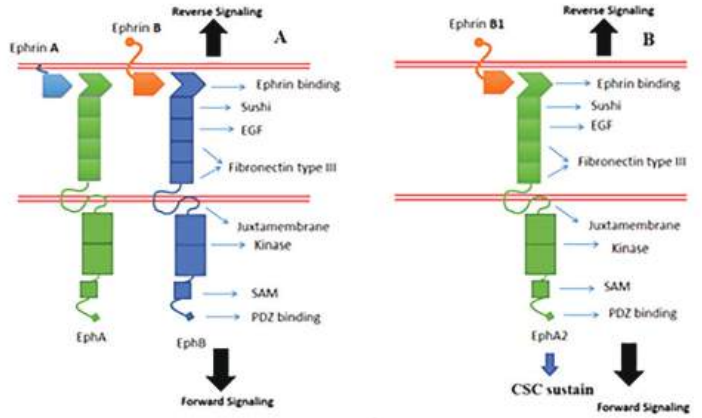
A) The Eph/Ephrin bidirectional signaling

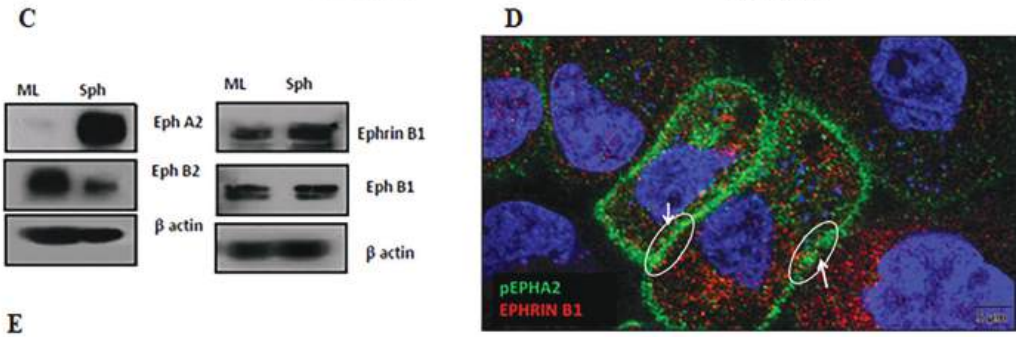
B) The EPHA2/EphrinB1 cross-signaling in CSCs

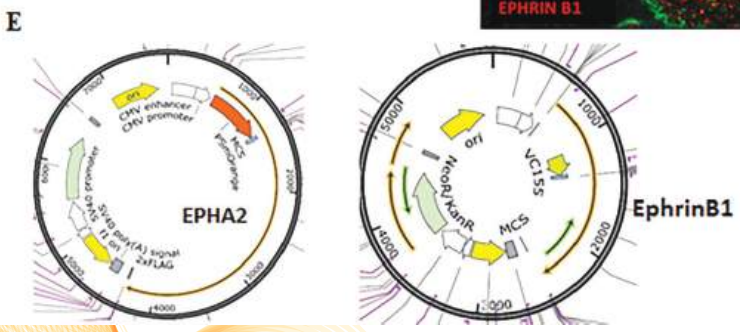
C) The western blot analysis of the components of Eph/Ephrin signaling

D) The confocal images showing the interaction of EPHA2 and EphrinB1. The regions where they interact is shown in circles and arrow marks.

E) The map of the cloned vectors.









JRF: 1
Technical Assistant: 1
Lab Assistant: 1
PhD Students: 5

First Row: Reshma Raj R, Dr Tessy Thomas Maliekal, Nandini Datta, Jannet S (From L to R)

Second Row: Zubair Ahmed Mir, Gayathri Mohan, Padmaja K P (From L to R)

AWARDS [STUDENTS]:

- Reshma Raj R., Best poster, EphrinhB1 mediated EPHA2 signaling regulates cancer stem cells leading to poor overall survival in oral cancer patients, National Conference on "Emerging Trends in Healthcare Biotechnology: Innovations, Challenges and Future Prospects" Thiruvananthapuram from Nov 30 – Dec 02, 2023 at Regional Cancer Centre, Thiruvananthapuram

INVITED TALKS [PI ONLY]:

- TIF1γ drives self-renewal of cancer stem cells by inducing HES1 expression1st RGCB Research conference, September 20-23, 2023, RAVIZ, Kollam.
- TIF1γ drives recurrence in oral cancer, St. Johns Research Institute, St John's National Academy of Health Sciences, February 28, 2023, SJRI, Bangalore.

ONGOING GRANTS:

Sl.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Evaluation of the E3-ligase independent function of Transcription Intermediary Factor1 γ (TIF1γ) in the regulation of cancer stem cells	Science & Engineering Research Board	2023	3 years	PI

PhD AWARDED:

Sl.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Amrutha Mohan	Evaluation of TIF1γ as a mediator of self-renewal of cancer stem cells in Oral Squamous Cell Carcinoma	Manipal Academy of Higher Education	Awarded	2024



Sunil Martin, PhD

Scientist E-II
Cancer Research

BRIEF THEME OF LABORATORY

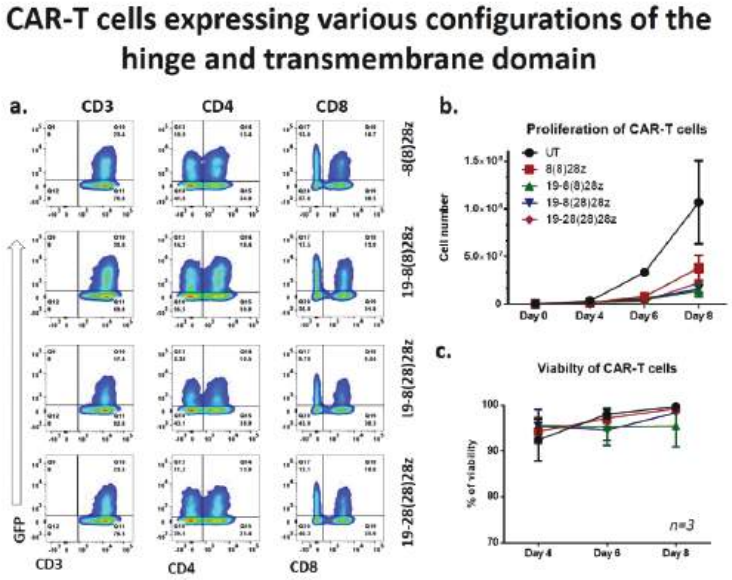
Our laboratory aims to apply synthetic immunology approaches to reprogram the antitumor immunity of alpha beta and gamma delta T cells.

MAJOR RESEARCH AREA

- CARs at various configurations of the hinge and transmembrane domain derived from CD8 and CD28 influence the quality and quantity of cytokine response.
- Pretreatment of tumor cells with zoledronic acid (ZA) was observed to enhance the tumor lysis and IFNg production by gamma delta T cells in a donor-dependent manner.
- Generated CAR T cells targeting CD37 and validated the cytokine production.

ENGINEERING IMMUNE CELLS FOR ADOPTIVE IMMUNOTHERAPY

We are generating synthetic receptors to study the biology of immune effector αβand γδ T cells to boost the specific and durable immune response. The focus of thelab is currently on investigating the CARs targeting CD19 –a pan B cell malignancymarker- to augment the antigen specificity of T cells. CARs are synthetic receptorswith an antigen-binding ectodomain, a co-stimulatory domain, and an endodomainderived from CD3ζ, which signals on behalf of the TCR. This modular designfacilitates HLA-independent antigen recognition and robust T-cell effector functionsdue to the judicious integration of multiple signaling domains. Off-tumor effect and cytokine toxicity remain as major biological limitations that need to be addressed at the level of receptor design.We have previously generated a workflow for the expansion of CD19 CAR T cells inserum-free media and characterized its antitumor function against B cell Acutelymphoblastic leukemia(B-ALL) cell systems. Because the hinge and transmembrane(HTM) domains of CAR influence the efficacy and toxicity, we are now testing various configurations of CAR T cells expressing HTM from CD8 and CD28. We are now charecterising the cellular and molecular aspects of CAR T cells expressing these receptors. . Since γδ T cells can recognize tumor cells in an HLA-independent manner, we are planning todevelop and test CD19 CAR γδ T cells as well. We will carry on with the in vivo testing of CD19 CAR T cells apart from exploring other tumor targets such as CD37 and CD20.



A. The activated T cells were transduced with CAR lentiviral vectors, and the transduction efficiency was assessed by measuring the GFP expression by FACS on day 7 b, c. The proliferation and the viability of CAR-T cells were measured by an automated cell counter.



JRF: 1
SRF: 1
Project Assistant: 2
PhD Students: 3

Muthuganesh M, Praseetha N G, Anjitha S, Archana Praveen, Muhumad Shafi K, Lalitha Priyadharshini S, Dr Sunil Martin, Shubham Parve (From L to R)

PUBLICATIONS:

- Liju VB, Martin S, Nath LR, Gopinadhan Nair GK, Thayele Purayil H. Editorial: Plant-based drugs: the potential novel therapeutic intervention against cancer stemness and metastasis. Front Pharmacol. 2023 Oct 6; 14:1283694.
- Singh A, Alexander SG, Martin S. Gut microbiome homeostasis and the future of probiotics in cancer immunotherapy. Front Immunol. 2023 May 16;14:1114499.
- Ganapathy T, Radhakrishnan R, Sakshi S, Martin S. CAR γδ T cells for cancer immunotherapy. Is the field more yellow than green? Cancer Immunol Immunother. 2023 Feb;72(2):277-286.

INVITED TALKS [PI ONLY]:

- Engineering CAR T cells to target B linegae maligancies. . National Conference on "Emerging Trends in Healthcare Biotechnology: Innovations, Challenges and Future Prospects" in association with Society for Biotechnologists (India) from November 30 – December 02, 2023 (3 days).Regional Cancer Centre, Thiruvananthapuram.
- Synthetic Immunology. Advances in Animal Biology & Conservation (ICRABC) on 19th – 21st December, 2023,Department of Zoology, Kerala University Thiruvananthapuram, Kerala, India

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Engineering NK/γδ T cells with CD19 CAR T cells for adoptive immunotherapy of relapsed or refractory B cell Acute Lymphoblastic Leukemia (B-ALL)	Department of Biotechnology	2018	5 years	PI
02	CD19 CAR T cells to Target Refractory or Relapsed B cell Acute Lymphoblastic Leukemia r/r B-ALL	Department of Biotechnology	2022	3 years	PI
03	CD37 CAR T cells to target Lymphoma	Department of Science & Technology	2022	2 years	PI
04	Affinity-tuned CAR T cells targeting refractory CD20 (+) B lineage malignancies	Department of Science & Technology	2022	4 years	PI



K B Harikumar, Ph.D

Scientist E-II
Cancer Research

BRIEF THEME OF LABORATORY

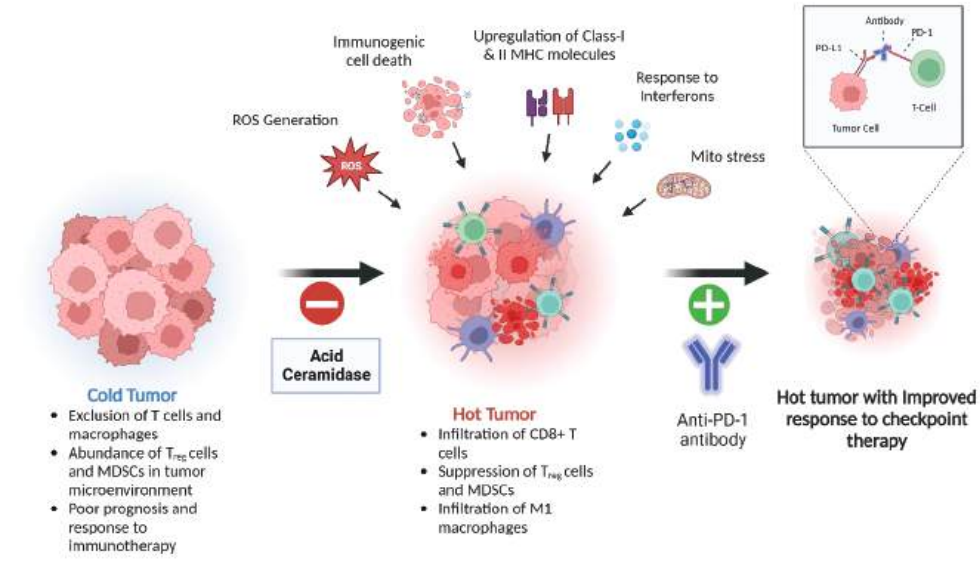
The main focus of the laboratory is understanding the role of inflammation in physiology (innate immune response) and pathophysiology (cancer). We are particularly interested in the roles of Sphingolipids in inflammation and carcinogenesis.

MAJOR RESEARCH AREA

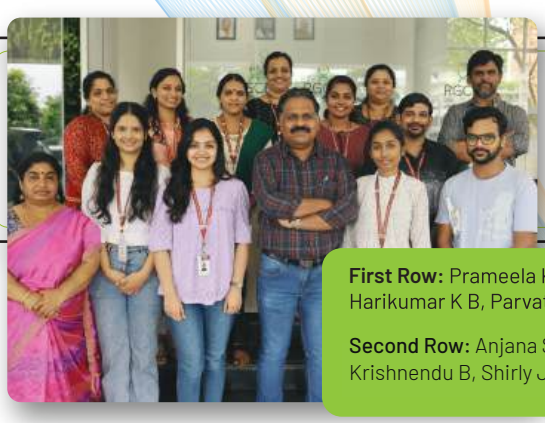
- We reported that silencing the expression ASAH1 resulted in the induction of immunological cell death (ICD) and mitochondrial stress, and small molecule inhibitor of ASAH1 sensitized the checkpoint therapy in colorectal cancer.
- Pancreatic cancer cells modulate the expression of Sphingolipid metabolites and it is used as an immune evasion strategy.

◆ TARGETING ACID CERAMIDASE ENHANCES ANTITUMOR IMMUNE RESPONSE IN COLORECTAL CANCER.

Targeting the tumor microenvironment (TME) and augmenting the host immune response are emerging strategies for managing cancer. Acid Ceramidase (ASAH1) a key enzyme in the sphingolipid metabolism, converts pro-survival ceramide into sphingosine, which in turn is converted into Sphingosine-1-Phosphate (S1P). To elucidate the role of ASAH1 in CRC, we first analyzed the expression of ASAH1 in human tissue array, TCGA data set, and mouse tumor samples and concludes that ASAH1 is overexpressed and associated with the pathogenesis of CRC. Knockdown of ASAH1 expression inhibited tumor growth in immunocompetent mice and but not in immunodeficient mice confirming that ASAH1 mediates the effect through modulation of the host adaptive immune system. We also confirmed that ASAH1 downregulation resulted in the induction of ICD in CRC cell lines, leading to more activation of immune cells and enhanced antigen presentation by APCs, thereby promoting the antitumor immune response. ASAH1 inhibition also resulted in the decreased function of the glutathione system and subsequent activation of ROS production and subsequent activation of ICD. CRC is considered a cold tumor with less infiltration of cytotoxic T cells and the presence of tumor suppressive Treg population. Converting cold tumors to immunogenically hot tumors has been shown to demonstrate effective and measurable clinical outcomes and checkpoint blockade is one such strategy. Targeting ASAH1 enhanced the efficacy of anti-PD1 therapy in colorectal cancer. We noticed that targeting ASAH1 reprograms the highly immunosuppressive tumor microenvironment into an immunoreactive phenotype leading to the inhibition of the tumor. Further analysis revealed that blockade of ASAH1 signaling alone can impact the tumor microenvironment by increasing CD8+ T cell infiltration and higher IFN signaling. Our findings provide strong pre-clinical efficacy data for the combination of ASAH1 inhibitor and ICIs, which led to the sensitization of immunologically cold tumors into immune reactive hot tumors.



ASAH1 is overexpressed in human CRC cases, and silencing the expression resulted in the induction of immunological cell death (ICD) and mitochondrial stress. The ASAH1 inhibitor (LCL-521), either as monotherapy or in combination with an anti-PD-1 antibody, resulted in reduction of tumors and, through induction of type I and II interferon response, activation of M1 macrophages and T cells, leading to enhanced infiltration of cytotoxic T cells. Our findings supported that the combination of LCL-521 and ICIs, which enhances the antitumor responses, and ASAH1 can be a druggable target in CRC.



Postdoctoral Fellows:2
PhD Students: 2
JRF: 1
SRF: 2
Project Assistant: 4
Lab Assistant: 1
Project Associate: 2

First Row: Prameela Kumari T K, Nashat Akhtar, Jinu Sebastian, Dr Harikumar K B, Parvathy G, Rajeev J Thampi, Arun V (From L to R)

Second Row: Anjana S S, Dr Vini Ravindran, Savitha R K, Aparna J S, Krishnendu B, Shirly James, Dr Kalimuthu Kalishwaralal (From L to R)

PUBLICATIONS:

- Mohammed S, Bindu A, Viswanathan A, Harikumar KB. Sphingosine 1-phosphate signaling during infection and immunity. Prog Lipid Res. 2023 ;92:101251.
- Vijayan Y, James S, Viswanathan A, Aparna JS, Bindu A, Namitha NN, Anantharaman D, Lankadasari MB, Harikumar KB. Targeting acid ceramidase enhances antitumor immune response in colorectal cancer. J Adv Res. 2023 :S2090-1232(23)00403-4.
- Thazhackavayal Baby B, Kulkarni AM, Gayam PKR, Harikumar KB, Aranjani JM. Beyond cyclopamine: Targeting Hedgehog signaling for cancer intervention. Arch Biochem Biophys. 2024;754:109952.
- Harikumar KB, Vijayan Y, Sandhu JS. Modulatory Role of Phytochemicals/Natural Products in Cancer Immunotherapy. Curr Med Chem. 2024 Feb 15. (Epub ahead of print).

AWARDS [STUDENTS]:

- Keerthana CK: Oral Presentation, Evaluation of the pharmacodynamics of uttroside B and pre-clinical validation of its therapeutic potential against hepatocellular carcinoma at the International Conference on Emerging Trends in Genomics and Biomedicine, November 15-17, 2023, held at IUCGGT, University of Kerala, Thiruvananthapuram. (Best Oral Presentation Award).

CONFERENCE PRESENTATION:

- Arun. V, Attended hands-on workshop on "Single Cell Transcriptomics with BCR/TCR profiling using 10x Genomics platform", August 28 - 31, 2023, BRIC- Institute of Life Science, Bhubaneswar
- Nashat Akhtar:

  (i) National Conference on "Emerging Trends in Healthcare Biotechnology: Innovations, Challenges and Future Prospects.", held on November, 30 to December, 02, 2023 at Regional Cancer Centre, Thiruvananthapuram, India. Presented poster entitled, "Sphingosine-1-Phosphate (S1P) Signaling: a Key Determinant of Immunotherapy Response in Pancreatic Cancer"

  (ii) IUIS-FIMSA-IIS-IMMUNO-INDIA 2023 course on "Epigenetic Regulation of Immune Responses" held on October 1 to 6, 2023 at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India. Presented an oral talk and poster entitled, "Sphingosine-1-Phosphate (S1P) Signaling: a Key Determinant of Immunotherapy Response in Pancreatic Cancer".

  (iii) Attended the 14th India Probiotic Symposium "Nutrition, Gut Microbiome and Probiotics - The Triple Connection" organized by The Gut Microbiota and Probiotic Science Foundation, held on 3rd and 4th February, 2024, at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India.

- Keerthana C K:

  (i) Oral Presentation, Pre-clinical studies on thepharmacodynamics and therapeutic potential of uttroside B against hepatocellular carcinoma at the International Conference on Recent Innovations in Biotechnology, September 21-23, 2023, held at Sathyabama University, Chennai.

  (ii) Poster Presentation, Mechanistic evaluation and pre-clinical validation of uttroside B as a novel drug against hepatocellular carcinoma at the International conference on Cancer Biology: Molecular Mechanisms, Genomics and Molecular Therapeutics, September 14-16, 2023, held at IIT Madras, Chennai.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Understanding the role of sphingosine kinase isoforms in systemic lupus erythematosus (SLE)	Council of Scientific & Industrial Research	2018	6 years	PI
02	DOT1L regulate the metabolic and epigenetic alterations in pancreatic cancer	Science and Engineering Research Board	2021	3 years	PI
03	A lipid perspective on immune evasion mechanisms in pancreatic cancer metastasis	Indian Council of Medical Research	2023	3 years	PI
04	Sensitizing of immune unresponsive colorectal cancers to checkpoint inhibitors through silencing of Acid Ceramidase expression	Science and Engineering Research Board	2023	3 years	PI
05	Network Pharmacology Approach for Preclinical Evaluation of Novel Phytoconstituents Targeting P13K/Akt Pathway in Lung Fibrosis	Science and Engineering Research Board	2023	3 years	CO-PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Yadu Vijayan	Elucidation of role of acid ceramidase in inflammation driven colorectal cancer	Manipal Academy of Higher Education	Submitted	2024



Parijat Senapati, PhD

Scientist C & DBT-Ramalingaswami Fellow
Cancer Research

BRIEF THEME OF LABORATORY

Our laboratory focuses on unraveling the functional roles of the noncoding parts of the human genome. We study DNA elements such as retrotransposons, long noncoding RNAs and enhancers and their roles in disease pathogenesis. We use a combination of functional genomics and computational approaches to understand the genetic and epigenetic underpinnings of diseases such as cancer with the goal of advancing biomedicine. We also work on collaborative projects focusing on epigenetic mechanisms underlying diabetic complications.

MAJOR RESEARCH AREA

- We have mined the RNA-seq data from The Cancer Gene Atlas and identified several KRAB-ZFP genes whose expression is correlated with patient outcomes such as overall survival and recurrence in breast and head and neck cancers.
- We have successfully made mammalian expression constructs of a few of the KRAB-ZFP genes.

- **MOLECULAR MECHANISMS UNDERLYING RETROTRANSPOSON EXPRESSION AND ITS CONSEQUENCES IN BREAST CANCER PATHOGENESIS.**

Krüppel-associated box domain zinc finger proteins (KRAB-ZFPs/KZFPs) are responsible for transcriptional and epigenetic silencing of retrotransposon elements in normal cells. However, cancer cells frequently show aberrant expression of retrotransposons due to the loss of faithful epigenetic silencing. To identify the mechanisms for the loss of silencing, we mined the transcriptome datasets of the TCGA-BRCA cohort to identify the KZFP genes that have altered expression in breast cancer samples. We have identified several KZFP proteins whose expression is correlated with better prognosis. For example, higher expression of KZFP ZNF649 and ZNF552 predicts longer disease free survival or lower recurrence rate (Figure 1 A and C). To identify the binding

sites and targets of these genes, we analyzed a published ChIP-exo dataset of HA-tagged KZFPs expressed in HEK293T cells. We found that ZNF649 mainly bound to LINE elements of the primate L1 (L1PA) family (Figure 1B). ZNF552 binds to LTR elements mainly LTRs of ERVK families (Figure 1D). We have also identified other transposable elements and gene targets of both KZFPs from this dataset. Furthermore we have constructed FLAG-tagged mammalian expression constructs of these proteins to do some functional assays and characterize their binding sites in breast cancer cell lines. We are currently performing CUT&RUN and other functional assays to study their role in breast cancer progression.

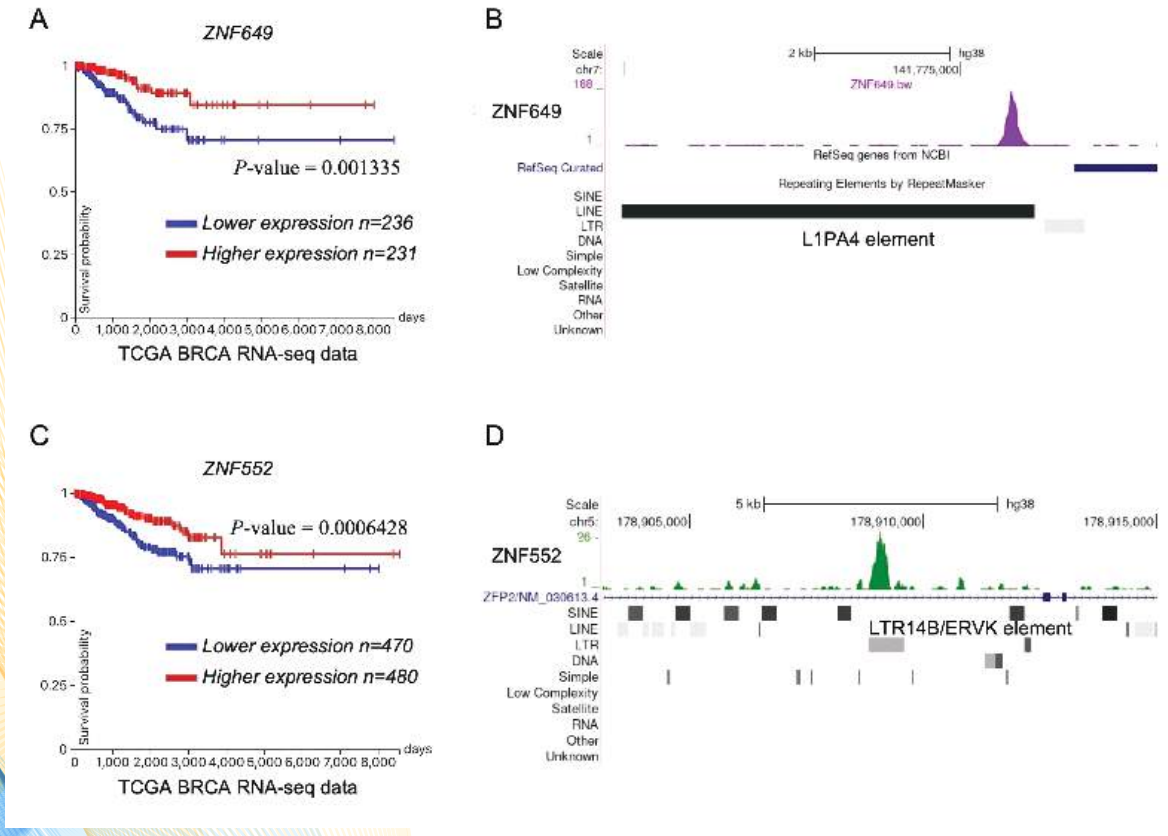


Figure: Decreased expression of KRAB-ZFP proteins ZNF649 and ZNF552 is associated with poorer prognosis.

(A) Survival curves showing disease free interval with patients expressing lower (blue) and higher (red) levels of (A) ZNF649 and (C) ZNF552 gene

AWARDS [PI]:

- Best Poster award at the Young Scientist Conference, IISF2023 held at Faridabad, 16-20th January 2024

INVITED TALKS [PI ONLY]:

- "Epigenome alterations associated with aging: Implications for breast cancer susceptibility"; MBGU seminar on 8th August, 2023 at Jawaharlal Nehru Centre for Advanced Scientific Research
- "Loss of epigenetic suppression of retrotransposons with oncogenic potential in aging mammary luminal epithelial cells"; 8th Asian Chromatin Meeting; on 6th November, 2023 at Jawaharlal Nehru Centre for Advanced Scientific Research

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Molecular mechanisms underlying retrotrans-poson expression and its consequences in breast cancer pathogenesis	Department of Biotechnology	2023	5 years	PI



Ramesh Pothuraju, Ph.D.

Scientist C
Cancer Research

BRIEF THEME OF LABORATORY

The interplay between various factors such as diet, genetics, drugs, environmental influences, and the metabolic state plays a significant role in causing gut dysbiosis (altered gut microbiota). This dysbiosis leads to the infiltration of bacterial components from the gut lumen into the protective mucus layer, causing damage to it. The intestinal mucus layer, which is composed of membrane-bound and secretory mucins produced by goblet cells, serves as a crucial defense against harmful agents and pathogens. Specifically, the compromised mucus layer of the intestine can give rise to inflammatory bowel diseases and can contribute to the development of colorectal cancer (CRC). Further, the connection between obesity and CRC is notable, with research indicating that the relationship is mediated through several mechanisms which includes alterations in adipokines (cell-signaling proteins produced by fat cells), changes in the gut microbiota composition, increased inflammation, and disruption of the intestinal barrier function. Given the importance of these factors, my laboratory is currently focused on gaining a deeper understanding of the role played by the intestinal mucus layer and how alterations in the gut microbiota contribute to the development of CRC and therapy resistance. Our research employs a combination of in vitro and in vivo pre-clinical studies to explore these intricate interactions.

MAJOR RESEARCH AREA

- The combination of a western diet, genetics, drugs, and environmental factors triggers gut dysbiosis, inflammation, and disrupts intestinal balance. My research centers on unraveling the significance of the mucus layer in metabolic disorders, colorectal cancer and drug resistance by using pre-clinical and clinical settings.

- UNDERSTANDING THE ROLE OF MUCIN IN COLORECTAL CANCER THERAPY RESISTANCE.
- ROLE OF DIETARY FIBER IN OBESITY MEDIATED COLORECTAL CANCER.

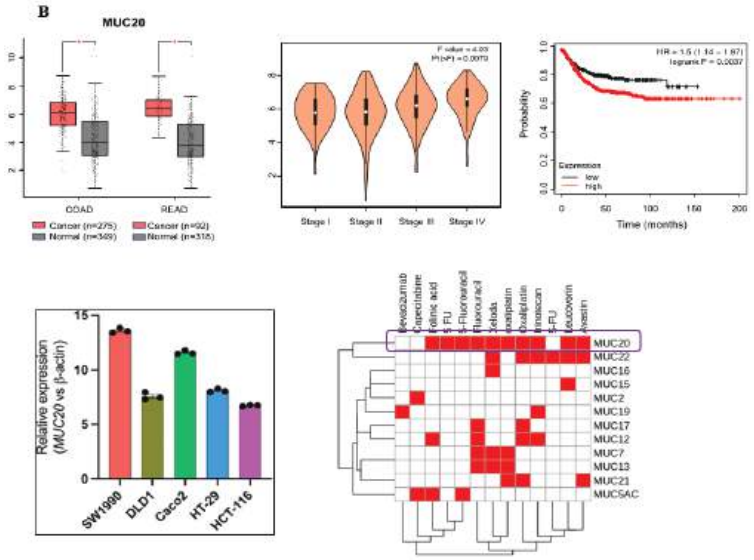
We investigated the potential involvement of transmembrane mucin (MUC20) in the progression of colorectal cancer. We conducted an in-silico analysis using The Cancer Genome Atlas (TCGA) databases and observed that enhanced expression of MUC20 observed in patients with colon and rectal adenocarcinoma, relative to various other types of PAN cancers. Further, TCGA of COAD and READ which includes samples from colon and rectal cancer patients (n=275 and n=92, respectively), as well as normal samples (n=349 and n=318, respectively) revealed a statistically significant ($p < 0.05$) up-regulation of MUC20 expression in CRC patients compared to normal individuals. Additionally, we observed that MUC20 expression levels were higher in a stage-specific manner. Furthermore, we examined the association between MUC20 expression and patient survival in CRC. Analysis of publicly available datasets using Kaplan-Meier curves demonstrated a notable decrease in overall survival among patients with high MUC20 expression ($p<0.0037$), indicating a potential tumor-promoting role for MUC20 in CRC. Subsequently, we employed a selection of CRC cell lines (DLD1, Caco2, HT-29, and HCT-116) to investigate MUC20 expression. We observed a markedly elevated expression of MUC20 at the transcript level within these CRC cell lines.

A). A significant upregulation of MUC20 in CRC patients, compared to normal individuals.

B). An upregulation of MUC20 in a stage specific manner and increased expression of MUC20 is associated with poor overall survival in CRC patients' data set.

C). Expression of MUC20 at transcript levels in various CRC cell lines

D). Individual, or combinatorial treatment of therapy agents showed upregulated majority of mucins in TCGAcolon cancer data set.





Lab Assistant: 1
PhD Students: 1

Dr Ramesh Pothuraju, Vrintha A.M

PUBLICATIONS:

- Choudhury D, Ghosh D, Mondal M, Singha D, Pothuraju R, Malakar P. Polyploidy and mTOR signaling: a possible molecular link. Cell Commun Signal. 2024;22(1):196.
- Abdullah KM, Kaushal JB, Takkar S, Sharma G, Alsafwani ZW, Pothuraju R, Batra SK, Siddiqui JA. Copper metabolism and cuproptosis in human malignancies: Unraveling the complex interplay for therapeutic insights. Heliyon. 2024;10(5):e27496.
- Abdullah KM, Sharma G, Takkar S, Kaushal JB, Pothuraju R, Chakravarti B, Batra SK, Siddiqui JA. α-lipoic acid modulates prostate cancer cell growth and bone cell differentiation. Sci Rep. 2024;14(1):4404.
- Pothuraju R, Khan I, Jain M, Bouvet M, Malafa M, Roy HK, Kumar S, Batra SK. Colorectal cancer murine models: Initiation to metastasis. Cancer Lett. 2024;587:216704.
- Barkeer S, Pothuraju R, Malakar P, Pimentel TC, Siddiqui JA, Nair SA. Gum acacia dietary fiber: Significance in immunomodulation, inflammatory diseases, and cancer. Phytother Res. 2024;38(3):1509-1521.

INVITED TALKS [PI ONLY]:

- Navigating Your Career Compass: Choosing the Right Pathway for your Goals" presented at Animal Biochemistry Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India on August 10th, 2023.



Ani V Das, PhD

Senior Program Scientist
Cancer Research

BRIEF THEME OF LABORATORY

My laboratory focuses on two aspects of cancer stem cells (CSCs): how they are maintained and how they can be targeted. On one hand, we investigate the involvement of Piwi proteins and their associated non-coding RNAs called piRNAs in cancer stem cell maintenance. On the other hand, we are looking at the epigenetic regulation of multidrug resistance proteins in CSCs, so that CSCs could be sensitized towards chemotherapy, which would help eliminating them successfully.

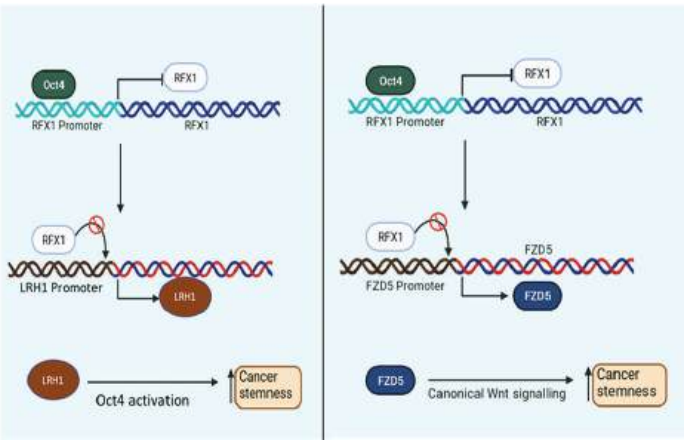
MAJOR RESEARCH AREA

- Role of PiwiL1 in HPV-mediated tumorigeness in cervical cells
- Understanding the regulation of PiwiL1 in cancer stem cells
- Identification of piRNAs and their role in cervical tumorigenesis
- Identification of PiwiL1-associated piRNAs in cervical cancer cells and understanding their role in cervical tumorigenesis
- Epigenetic regulation of multidrug resistance genes in CSCs
- Role RFX1 in the regulation of multidrug genes in CSCs
- Deciphering the role and regulation of RFX1 in CSCs

ROLE OF RFX1 IN THE REGULATION OF CANCER STEM CELLS

Cancer stem cells (CSCs) shows a characteristic higher expression of pluripotency related factors that contributes to the self-renewal and relapse. Regulatory factor X1 (RFX1) is a multifaceted transcription factor that has been demonstrated to exhibit inhibitory effects on its target genes which includes multidrug resistance genes and CSC marker, while also facilitating the process of cellular differentiation. The down-regulation of RFX1 has been observed in numerous cancer types, which hints towards its crucial involvement in the regulation of cancer progression and stemness. RFX1 overexpression compromised the oncogenic and stem-like properties of NTERA-2 cells. The present study reveals that the expression of RFX1 is regulated by the pluripotency factor Oct4 in embryonal carcinoma cells (NTERA2 cells) through an association with Histone deacetylase 1 (HDAC1). In addition, we provide evidences for existence of an inter regulatory mechanism between RFX1 and OCT4 in cancer progression. RFX1 was found to be regulating LRH1(NR5A2), an activator of Oct4 expression and a contributing factor in the development of cancer. Moreover, it also regulates FZD5, which is a receptor of Wnt/β-catenin pathway leading to regulation of Oct4 expression. Here, we elucidate the existence of a regulatory axis between Oct4 and RFX1 in the modulation of cancer stemness. Collectively, the data presented in this study proves that RFX1 functions as a negative regulator of LRH1 and FZD5 expression thereby regulating Oct4 expression and is negatively influenced by Oct4 in the context of cancer progression. The RFX1/Oct4 inter-regulatory axis exhibits potential as a therapeutic target for mitigating the progression of cancer

RFX1-mediated regulation of cancer stem cells. In CSCs, the Oct4 expression is high. Oct4 can directly bind and negatively regulate RFX1, in association with HDAC1. This results in the removal of RFX1-mediated inhibition of LRH1, an Oct4 activator and FZD5, a canonical Wnt signaling receptor, in cancer stem cells, leading to increased stemness. On the other hand, if RFX1 expression is more, that will lead to inhibition of both FZD5 and LRH1, since both are direct targets of RFX1. This will eventually result is down-regulation of Oct4 and other stemness-associated factors, thereby reducing the stemness.





Lab Assistant: 1
PhD Students: 1

Dr Ani V Das, Vidya S

PUBLICATIONS:

- Kunnummal M, Raveendran PS, Basu B, Rani SV, Paul RA, Kuppusamy K, Angelin M, Issac J, James J, Das AV. HPV16 E6/E7-mediated regulation of PiwiL1 expression induces tumorigenesis in cervical cancer cells. Cell Oncol (Dordr). 2024;47(3):917-937.
- Issac J, Raveendran PS, Kunnummal M, Angelin M, Ravindran S, Basu B, Das AV. RXR agonist, Bexarotene, effectively reduces drug resistance via regulation of RFX1 in embryonic carcinoma cells. Biochim Biophys Acta Mol Cell Res. 2023;1870(7):119510

CONFERENCE PRESENTATION:

- Midhunaraj.K, Pooja Sherly Raveendran, Budhaditya Basu, Riya Ann Paul, Krithiga K, Mary Angelin, Sivakumar Krishnankutty Chandrika, Joby Issac, Jackson James, and Dr. Ani V Das. "Role of PiwiL1 in cancer stem cell maintenance and their correlation with HPV mediated tumorigenesis in cervical cancer" Poster presentation, Manipal Research Colloquium-2023 held from 12th -14th April 2023, Manipal Academy of Higher Education, Manipal, India.
- Pooja Sherly Raveendran, Midhunaraj Kunnummal, Yetcherla Raja Aravind, Ayush Dave, Nikitha Reji, Joby Issac, Sheri Vidya Rani, Ani V Das, "Oct4-RFXI inter regulatory mechanism in the maintenance of cancer stenm cells". Oral presentation at International conference on Cancer Biology: Molecular Mechanisms, Genomics and Novel Therapeutics" Organized by Indian Institute of Technology Madras, Chennai India, held on 14-16 September, 2023.
- Pooja Sherly Raveendran, Midhunaraj Kunnummal , Yetcherla Raja Aravind, Nikitha Reji, Nidhi Hallikeri, Ayush Dave, Rahul Jose, Joby Issac , Sheri Vidya Rani, Ani V Das. "RFX1-Oct4 inter-regulatory axis in the maintenance of cancer stem cells". Poster presentation at National symposium on National Symposium on Biotechnology for Sustainable Development 2024 Organized by RGCB held on 20 April, 2024.
- Pooja Sherly Raveendran, Midhunaraj Kunnummal, Yetcherla Raja Aravind, Nidhi Hallikeri, Nikitha Reji, Ayush Dave ,Joby Issac, Sheri Vidya Rani, Ani V Das. "Deciphering the role of RFX1 in regulation of cancer stem cells". Poster presentation at Manipal Research Colloquium-2024 Organized by MAHE Manipal from 12-13 April 2024.

PATENTS APPLIED/ GRANTED:

- Patent Application No. 202341040690
  "Screening Methods For Cancers Using piR-019324 As A Biomarker"

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Deciphering the regulation of PiwiL1 in cancer stem cells	Science and Engineering Research Board,	2021	3 years	PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Midhunaraj K	Elucidating the role and regulation of PiwiL1 in HPV-mediated cervical cancer	Manipal Academy of Higher Education	Awarded	2024



CARDIOVASCULAR DISEASES & DIABETES BIOLOGY



Abdul Jaleel K. A, PhD

Scientist G & Assoiate Dean
(Research Administration & Faculty Affairs)
Cardiovascular Diseases & Diabetes Biology

BRIEF THEME OF LABORATORY

Investigating the metabolic adaptations or alterations responsible for the onset of type 2 diabetes is the main theme of our laboratory. We study normal healthy young people, both men and women, who are having risks for diabetes, such as overweight, family history of diabetes investigating if they already have any metabolic alterations associated with insulin resistance, which is the hallmark of type 2 diabetes. For this we have developed an independent cohort and study them prospectively.

MAJOR RESEARCH AREA

- The laboratory investigates the use of MS-based metabolomics and how independent risk factors for type 2 diabetes evolve over time in developing insulin resistance.
- Besides, we study the functional significance of cardiac mitochondrial subpopulations in type 2 diabetes in our population.
- Our laboratory also investigates on the effect of Lp(a) on angiogenesis and its role in the early appearance of calcification on placenta during pre-eclampsia.

- **ASSESSMENT OF CLINICAL, METABOLIC AND EPIGENETIC VARIATIONS TO EVALUATE THE RISK FOR TYPE 2 DIABETES MELLITUS: A PROSPECTIVE STUDY**
- **COMPREHENSIVE MASS SPECTROMETRY-BASED CLINICAL LIPIDOMICS PLATFORMS FOR PROMOTING BIOMEDICAL RESEARCH AND ADVANCED TRAINING FOR INDIAN RESEARCHERS**
- **FUNCTIONAL SIGNIFICANCE OF CARDIAC MITOCHONDRIAL SUBPOPULATIONS IN TYPE 2 DIABETIC ASIAN INDIANS.**
- **EFFECT OF LIPOPROTEIN (A) IN THE MODULATION OF EARLY APPEARANCE OF CALCIFICATION IN THE PLACENTA.**

1. ASSESSMENT OF CLINICAL, METABOLIC AND EPIGENETIC VARIATIONS TO EVALUATE THE RISK FOR TYPE 2 DIABETES MELLITUS: A PROSPECTIVE STUDY

Our earlier study demonstrated metabolic changes associated with insulin resistance in normoglycemic young adults who have risk of diabetes such as overweight, family history of diabetes etc. This project is a follow-up of those earlier study participants (n=110) after 6 years to see how many of them contracted T2DM or prediabetes and to determine their metabolic alterations retrospectively. So far 94 participants have been studied in the follow-up and found that 9 developed T2DM, 51 had prediabetes and 34 remained normal. All the participants exhibited elevation in BMI, Waist Hip Ratio, Total Cholesterol, TG, and VLDL levels and alteration in Glucose and Insulin response to a mixed-meal challenge. Metabolomics revealed alteration in the plasma metabolites between baseline and follow-up (Figure 1). Levels of twelve metabolites were significantly different in baseline samples of participants who became prediabetic and diabetic during the follow-up. Altered metabolites were involved in beta oxidation of very long chain fatty acids, medium chain saturated fatty acids, and fatty acid and bile acid biosynthesis.

2. COMPREHENSIVE MASS SPECTROMETRY-BASED CLINICAL LIPIDOMICS PLATFORMS FOR PROMOTING BIOMEDICAL RESEARCH AND ADVANCED TRAINING FOR INDIAN RESEARCHERS

The goal of this project is to establish a panel of more than 300 lipid molecules/species, which comes under 25 major lipid classes. At present our panels include 36 lipid molecules, which come under 13 lipid classes and 2 metabolites of one carbon metabolism. These panels are being established in our facility to meet the research needs of investigators.

3. ELUCIDATING THE ROLE OF LIPOPROTEIN(A) IN IMPAIRED ANGIOGENESIS IN PLACENTA DURING PRE-ECLAMPSIA AND ITS IMPACT ON THE VASCULAR HEALTH OF WOMEN

Lipoprotein(a) [Lp(a)], a plasma lipoprotein present only in humans, causes coronary artery calcification and its higher level is an independent risk factor for pre-eclampsia and cardiovascular diseases. Lp(a) deposits were found on both pre-eclamptic placenta and on atherosclerotic plaques but the underlying molecular mechanism is not known. Investigations in human trophoblast cells treated with different concentrations of Lp(a) shows that cell functions such as migration, proliferation and MMP release were dose-dependently inhibited in the presence of Lp(a). Moreover, Lp(a) dose-dependently enhances calcium deposition in trophoblasts. Also, we demonstrated a positive correlation between maternal plasma Lp(a) level and calcification in placenta by studying pregnant women with and without pre-eclampsia.

4. FUNCTIONAL SIGNIFICANCE OF CARDIAC MITOCHONDRIAL SUBPOPULATIONS IN TYPE 2 DIABETIC ASIAN INDIANS

Mitochondrial dysfunction is implicated in cardiac functional impairments and Asian Indians are more prone to type 2 diabetes mellitus (T2DM)- associated cardiovascular diseases. Based on the cellular localization, cardiac mitochondria are classified as subsarcolemmal and interfibrillar subpopulations, which are functionally and metabolically different. The initial findings from high resolution respirometry indicate T2DM adversely affected the subsarcolemmal mitochondrial function whereas the interfibrillar mitochondria were not showing difference in various states of respiration between study groups of non-diabetic and diabetic human participants. Analysis of the untargeted mass spectrometry based proteomic data also revealed altered levels of various protein subunits involved in oxidative phosphorylation.



JRF: 2
SRF: 1
Project Assistant: 1
Technical Assistant: 1
Lab Assistant: 1
Postdoctoral Fellows: 2
PhD Students: 1

Dr Nandini R J, Dr Kalaivani V,
Gopika Satheesh, Dr Abdul Jaleel,
Riya Elizabeth Saji (From L to R)

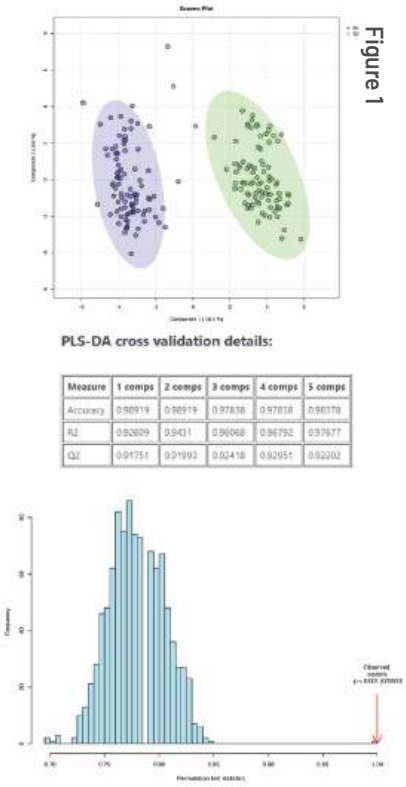


Figure 1

Figure 1: PLS-DA plot of LC/MS/MS data showing clustering of metabolites among baseline (S1) and follow-u (S2). PLS-DA model was evaluated by cross-validation of R2 and Q2 values with the first 5 components. permutation test (permutation numbers-1000) was performed to test the statistical significance and p-valu of <0.05 was considered significant.

PUBLICATIONS:

- Rambaran VH, Ming WSH, Singh N, Nandini RJ, Kai MT, Surendran A, Krishna M, Jaleel A. The design, spectrochemistry and toxicity evaluations of two vanadium (IV) complexes: [V(IV)O(2,6-pyridine diacetatato) (H2O)2] (PDOV) and V2(IV)O2(pyrazine-2,3,5,6-tetracarboxylato)(H2O)4] (PYTOV). Inorganica Chimica Acta. 2023;556:121662.

AWARDS [STUDENTS]:

- Gopika Satheesh, Prof. CC Kartha Travel Grant, "Assessment of the Clinical, Metabolic and Epigenetic Variations to Evaluate the Risk for Type 2 Diabetes mellitus: A Prospective Study", 2nd Joint Annual Meeting of International Society for Heart Research (Indian Section) & International Academy of Cardiovascular Sciences (India Section) held at AIIMS, Jodhpur, India from 16th to 18th February 2024
- Dr. Kalaivani V, Best Oral Presentation Award under Prof. DK Agrawal Award Session, "Elucidating the Role of Lipoprotein(a) in Placental Calcification and Future Cardiovascular Health in Mother and Offspring". 2nd Joint Annual Meeting of International Society for Heart Research (Indian Section) & International Academy of Cardiovascular Sciences (India Section) held at AIIMS, Jodhpur, India from 16th to 18th February 2024
- Dr. Nandini R. J: Best Oral Presentation Award under Prof. S. C Tyagi Award Session, "Effect of type 2 diabetes mellitus on cardiac mitochondrial subpopulations in Asian Indians" 2nd Joint Annual Meeting of International Society for Heart Research (Indian Section) & International Academy of Cardiovascular Sciences (India Section) held at AIIMS, Jodhpur, India from 16th to 18th February 2024

INVITED TALKS [PI ONLY]:

- Title of the talk: Altered Metabolism in Normoglycemic Young Adults Having Risk for Type 2 Diabetes Mellitus
- Joint Annual Meeting of International Society of Heart Research (Indian Section) & International Academy of Cardiovascular Sciences (India Section) ISHR 2024 held at Auditorium, AIIMS, Jodhpur from 16th to 18th Feb 2024

CONFERENCE PRESENTATION:

- Gopika Satheesh, Oral Presentation, Assessment of the clinical, metabolic and epigenetic variations to evaluate the risk for Type 2 Diabetes mellitus (T2DM): A Prospective Study, National Seminar "Epigenetics Meets Metabolomics" held at ICCONS, Shoranur, India from 9th to 10th June 2023.
- Gopika Satheesh, Poster presentation, Assessment of the clinical, metabolic and epigenetic variations to evaluate the risk for Type 2 Diabetes mellitus (T2DM): A Prospective Study, 'RGCB Research Conference 2023' held at The Raviz, Kollam, India from 20th to 23rd September 2023.
- Gopika Satheesh, Oral Presentation. Assessment of the Clinical, Metabolic and Epigenetic Variations to Evaluate the Risk for Type 2 Diabetes mellitus: A Prospective Study, 2nd Joint Annual Meeting of International Society for Heart Research (Indian Section) & International Academy of Cardiovascular Sciences (India Section) held at AIIMS, Jodhpur, India from 16th to 18th February 2024.
- Dr. Kalaivani V, Oral Presentation. "Elucidating the Role of Lipoprotein (a) in Placental Calcification and Future Cardiovascular Health in Mother and Offspring". 2nd Joint Annual Meeting of International Society for Heart Research (Indian Section) & International Academy of Cardiovascular Sciences (India Section) held at AIIMS, Jodhpur, India from 16th to 18th February 2024
- Dr. Nandini R. J: Oral Presentation. "Effect of type 2 diabetes mellitus on cardiac mitochondrial subpopulations in Asian Indians". 2nd Joint Annual Meeting of International Society for Heart Research (Indian Section) & International Academy of Cardiovascular Sciences (India Section) held at AIIMS, Jodhpur, India from 16th to 18th February 2024

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Comprehensive mass spectrometry-based clinical lipidomics platforms for promoting biomedical research & advanced training for Indian researchers	Department of Biotechnology	2021	4 years	PI
02	Genome India: cataloguing the genetic variations in Indians	Department of Biotechnology	2020	4 years	CO-PI



Rakesh S. Laishram, PhD

Scientist F & Swarna Jayanti Fellow
Cardiovascular Diseases & Diabetes Biology

BRIEF THEME OF LABORATORY

Processing and regulation at the 3'-untranslated RNA in gene expression - implications in cardiovascular diseases.

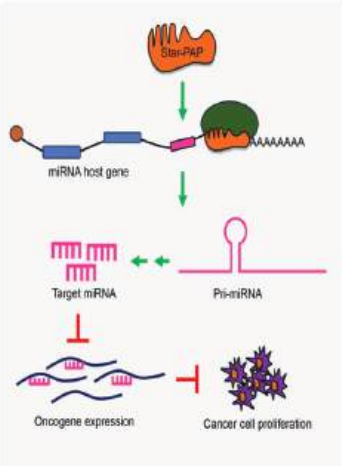
MAJOR RESEARCH AREA

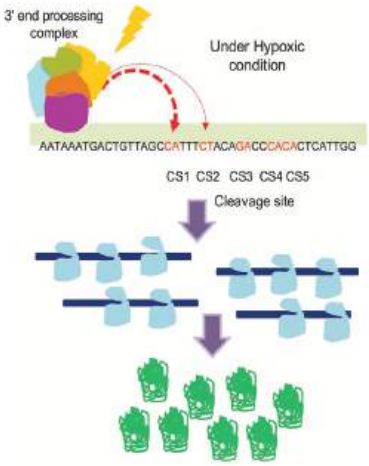
- We discovered that RNA binding protein motif protein 10 (RBM10) phosphorylation enables anti-hypertrophy gene program in the heart.
- cSrc kinase phosphorylates RBM10 and that cSRc inhibition accentuates myocyte hypertrophy and advances development cardiac hypertrophy in the heart.
- Inhibition of ERK has opposite effect on the hypertrophy induction that is mediated through RBM10 phosphorylation.
- Star-PAP, which is typically reduced in breast cancer, has a paradoxical effect on the expression of cancer-causing genes.
- Our research findings hold significant implications in the understanding and therapeutics of breast cancer progression and cardiac hypertrophy.

- **NON-CANONICAL CLEAVAGE AT THE PRE-MRNA 3'-UNTRANSLATED REGION REGULATES GENE EXPRESSION IN OXIDATIVE STRESS RESPONSE AND CELL PROLIFERATION.**

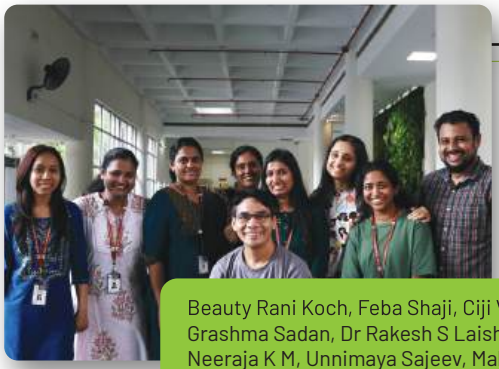
mRNA 3'-end processing is one of the crucial steps in gene expression that is required for the stability and efficient translation. It involves two coupled steps - cleavage followed by addition of a poly(A) tail (polyadenylation), carried out by a cleavage and polyadenylation(CPA) complex assembled at the 3'-UTR. While PA-tails and its regulation are well studied, cleavage site regulation in gene expression is less explored. General notion is that cleavage reaction lacks precision and occurs within 15-30 nucleotides downstream of a PA-signal. Global analysis of cleavage site shows heterogeneity (multiple cleavage sites for one PA-signal) averaging to 6 per poly(A) site. Interestingly, mutational analysis of the cleavage site(s) on NQ01 PA-site revealed specific but stuttering cleavage pattern where the number of cleavage site is inversely related to protein expression. We also showed that in addition to the reduction in the cleavage site, there is increase in the usage of the primary cleavage site that induces gene expression. We show that increased protein expression during oxidative stress and cardiac hypertrophy involves reduction in cleavage heterogeneity. In this report, we primarily focused on how cleavage heterogeneity controls oxidative stress response gene expression. Intriguingly, each PA-site of mRNAs encoding oxidative stress response proteins contains a primary cleavage site, having the highest efficiency of cleavage such that increased cleavage events on other non-primary sites reduce cleavage efficiency thereby limit the protein expression. Stimulation of the cells with oxidative stress reduces cleavage site stuttering resulting in enhanced cleavage at the primary site inducing the protein expression. Genome wide cleavage site analysis also showed reduction in the cleavage heterogeneity globally during oxidative stress response.

In another aspect of the work, We discover that the protein Star-PAP, which is typically reduced in breast cancer, has a paradoxical effect on the expression of cancer-causing genes. Through experiments with breast cancer cells and mouse models, it was observed that Star-PAP negatively regulates the expression of these genes and subsequently affects the proliferation of cancer cells. The study also revealed that the normal function of Star-PAP is essential for controlling the expression of cancer-causing genes, as a mutant version of the protein did not produce the same effect. Furthermore, the research demonstrated that Star-PAP exerts its control over these genes by influencing small RNA molecules called miRNAs, which are down-regulated in breast cancer. By affecting the production of these miRNAs, Star-PAP indirectly impacts the expression of cancer-causing genes, offering a novel insight into the regulation of breast cancer progression. Our research findings hold significant implications in the understanding and therapeutics of breast cancer progression and hypoxic regulation in cardiac hypertrophy.





Model how non-canonical polymerase, Star-PAP negatively regulates cell proliferation through miRNA control (right), and how cleavage heterogeniety reduction controls stimulated stress response genes during hypoxic condition in the cell



JRF: 2
Project Assistant: 1
Technical Assistant: 1
PhD Students: 6
Postdoctoral Fellows: 1

Beauty Rani Koch, Feba Shaji, Ciji Varghese, Grashma Sadan, Dr Rakesh S Laishram, Diksha Singh, Neeraja K M, Unnimaya Sajeev, Malaya Ranjan Behera (From L to R)

PUBLICATIONS:

- Mohanan NK, Shaji F, Sudheesh AP, Bangalore Prabhashankar A, Sundaresan NR, Laishram RS. Star-PAP controls oncogene expression through primary miRNA 3'-end formation to regulate cellular proliferation and tumour formation. Biochim Biophys Acta Mol Basis Dis. 2024;1870(4):167080.
- Shaji F, Mohanan NK, Shahzad S, V P G, Bangalore Prabhashankar A, Sundaresan NR, Laishram RS. Proto-oncogene cSrc-mediated RBM10 phosphorylation arbitrates anti-hypertrophy gene program in the heart and controls cardiac hypertrophy. Life Sci. 2024 15;341:122482.

AWARDS [PI]:

- Elected Fellow of the National Academy of Sciences, India

AWARDS [STUDENTS]:

- Feba Shaji, RGCB merit award
- Diksha Singh , Best poster Award, RGCB symposium

INVITED TALKS [PI ONLY]:

- International Cardiovascular Medicine Summit – March 2024, Instem-Bangalore, India
- Heart failure Conflux (Virtual Platform), Sree Chitra Tirunal Institute, Trivandrum, February – 2024
- RGCB Research Conference, Trivandrum, September 2023
- 11th RNA Meeting, NCCS Pune, December 2022
- One day conference on Exploring Ne Frontiers in Genetics and Genomics, mLAC-Bangalore - 2022
- EMBO meeting on RNA Binding proteins, Pune (hybrid mode), March - 2022

CONFERENCE PRESENTATION:

- Neeraja KM, Poster Presentation, RNA Society meeting, Singapore 2023
- Feba Shaji, Poster Presentation, EMBO Non-coding RNA meeting, Heidelberg, Germany 2023

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Alternative polyadenylation in gene expression – implications in cardiovascular diseases	Science and Engineering Research Board	2020	5 years	PI
02	Cleavage site heterogeneity/stuttering in gene expression and its physiological implications	Science and Engineering Research Board	2024	5 years	PI



Ananthalakshmy Sundararaman, PhD

Scientist C & DBT-Ramalingaswami Fellow
Cardiovascular Diseases & Diabetes Biology

BRIEF THEME OF LABORATORY

Cardiovascular Disease Biology

MAJOR RESEARCH AREA

- Mitochondrial quality control through mitochondria-derived vesicles and its role in cardiovascular disease pathophysiology
- Mito-nuclear communication through direct transit of proteins mediated by mitochondria-derived vesicles.
- Ischaemia-reperfusion injury and the role of Rho family of GTPases in the regulation of actin around the mitochondria.
- Mitochondria-derived vesicle budding and trafficking using proximity labelling techniques

◆ MITOCHONDRIA-DERIVED VESICLE TRAFFICKING IN MITO-NUCLEAR COMMUNICATION

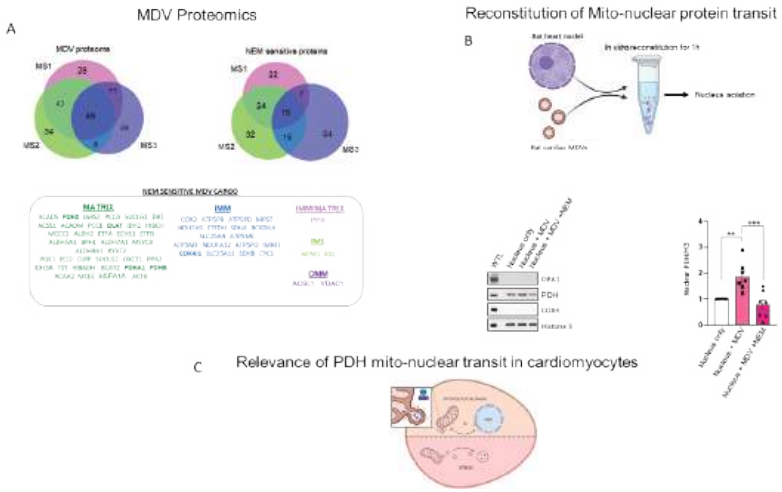
Mito-nuclear communication is key to cellular homeostasis. Metabolites from TCA cycle, calcium, ROS and other small molecules are known to mediate retrograde signalling of mitochondria to the nucleus. Recently, the direct transfer of mitochondrial proteins to the nucleus has been described. However, the mechanism for direct transfer of mitochondrial protein complexes to the nucleus is not understood. We demonstrate the transit of mitochondrial proteins to the nucleus through mitochondria-derived vesicles (MDVs) which represents a novel intracellular trafficking route. Following an unbiased proteomic approach to detecting cargo

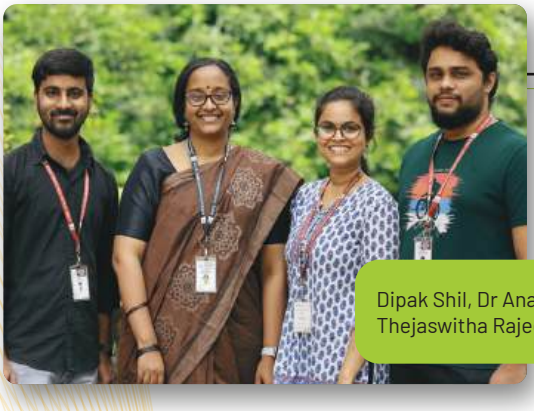
proteins in cardiac MDVs, we screen for the presence of MDV cargo in the nucleus. Pyruvate dehydrogenase complex (PDH) and Cytochrome c oxidase subunit IV isoform 1 COX4I1 are packaged into MDVs in cardiomyocytes but only PDH is targeted to the nucleus in this cell type demonstrating that a subset of cargo-selective MDVs fuse with the nucleus. MDV budding and the subsequent nuclear delivery of PDH is actin dependent in cardiomyocytes. Further, several mitochondrial stresses that are known to increase MDV generation triage these MDVs to other cellular destinations thereby reducing the nuclear pool of PDH. The transit of PDH through MDVs to the nucleus is a basal physiological process in cardiomyocytes. Mito-nuclear trafficking thus represents a physiological pathway in cardiac cells to enable cargo shuttle from mitochondria to the nucleus.

A. Following in vitro budding of MDV from rat heart, we subject MDVs from control and N-ethyl maleimide for proteomics. Hits from 3 runs are represented in the Venn diagram and the list of high confidence MDV cargo which are NEM sensitive are listed.

B. Incubation of isolated MDVs with rat heart nucleus demonstrates in vitro mito-nuclear transit of PDH which is prevented by addition of NEM. Opa1 and COX4, while packaged into MDVs do not transit to nucleus.

C. Graphical representation of the relevance of mito-nuclear PDH transit that oncreases under basal conditions and when cells undergo differentiation but is inhibited in mitochondrial stresses.





JRF: 1
Project Assistant: 1
Lab Assistant: 1
PhD Students: 2

Dipak Shil, Dr Ananthalakshmy Sundararaman, Thejaswitha Rajeev, Mohammed Roshan M (from L to R)

AWARDS [STUDENTS]:

- Thejaswitha Rajeev, Best Poster Award, Transit of Mitochondrial Proteins to the Nucleus through Mitochondria-derived Vesicles (MDVs), RGCB Symposium,2024

CONFERENCE PRESENTATION:

- Thejaswitha, Poster, Transit of Mitochondrial Proteins to the Nucleus through Mitochondria-derived Vesicles (MDVs), Biological Chemistry: Opportunities, Challenges and the Way Forward,18th to 20th December 2023, BITS Pilani K K Birla Goa campus.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Characterising Mitochondria-derived Vesicle Trafficking through a Proximity Labelling Approach- A possible Novel Mito-nuclear Communication Pathway	Science and Engineering Research Board	2023	3 years	PI
02	Role of RhoGTPases in the Intracellular Trafficking of Mitochondria Derived Vesicles (MDVs) and Angiogenesis	Department of Biotechnology	2020	5 years	PI



Sumi S, PhD

Program Scientist
Cardiovascular Diseases & Diabetes Biology

BRIEF THEME OF LABORATORY

The unifying theme of our research program is to delineate the molecular mechanisms underlying endothelial dysfunction, and its impact on the pathogenesis of vascular disease. We aim to specifically address the following aspects.

1. Endothelial mechanobiology and mechanotherapeutics in low-flow and high-flow vascular diseases.

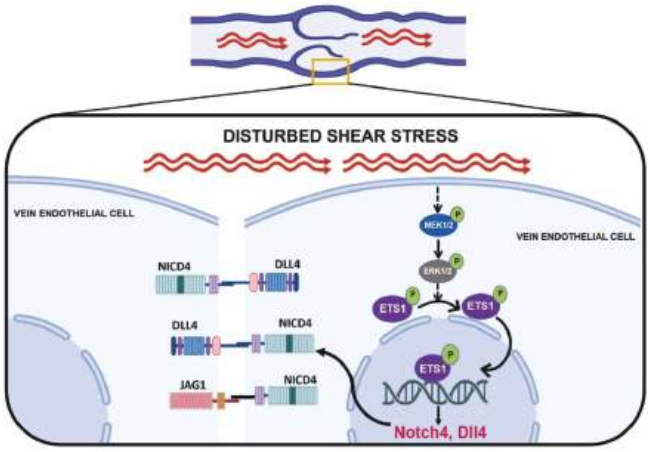
2. Molecular basis of inflammation in the pathogenesis of brain arteriovenous malformations

MAJOR RESEARCH AREA

- We employ tools from microfluidics to comprehend biomedically important processes that occur in the bloodstream, including angiogenesis and endothelial dysfunction in response to hemodynamic alterations.
- We identified the specific role of mechanosensors such as Piezo1, mechanostransducers such as ETS1 and KLF2, as well as mechanosignaling pathways like Notch signaling in the pathogenesis of Chronic venous diseases.
- We have delineated several proteins which can be targeted and screened several compounds to mitigate flow disturbance-induced effects and promote vascular normalization in brain arteriovenous malformations.
- Identified small molecular inhibitors in to reverse endothelial dysfunction in the presence of ensuing disturbed fluid flow.

- **MECHANORESPONSIVE ETS1 CAUSES ENDOTHELIAL DYSFUNCTION AND ARTERIALIZATION IN VARICOSE VEINS VIA NOTCH4 SIGNALING**

Varicose veins are the most common venous disorder in humans and are characterized by hemodynamic instability due to valvular insufficiency and orthostatic lifestyle factors. It is unclear how changes in biomechanical signals cause aberrant remodeling of the vein wall. Our previous studies have shown that arterialization of the saphenous vein contributes to the pathogenesis of varicose veins. Arterial endothelial markers related to the Notch signaling pathway, such as DLL4, HEY2, and ephrin B2, were found to be increased in varicose veins. In the arterial system, mechanoresponsive ETS1 is a transcriptional activator of the endothelial Notch, but its involvement in sensing disrupted venous flow and varicose vein formation has not been investigated. Here, we use human varicose veins and cultured human venous endothelial cells to show that disturbed venous shear stress activates ETS1-NOTCH4/DLL4 signaling. Notch components were highly expressed in the neointima, whereas ETS1 was upregulated in all histological layers of varicose veins. In vitro microfluidic flow-based studies demonstrate that even minute changes in venous flow patterns enhance ETS1-NOTCH4/DLL4 signaling. Uniform venous shear stress, albeit an inherently low-flow system, does not induce ETS1 and Notch proteins. ETS1 activation under altered flow was mediated primarily by MEK1/2 and, to a lesser extent, by MEK5 but was independent of p38MAP kinase. Endothelial cell-specific ETS1 knockdown prevented disturbed flow-induced NOTCH4/DLL4 expression. TK216, an inhibitor of ETS-family, inhibited the acquisition of arterial molecular identity and loss of endothelial integrity in cells exposed to the ensuing altered shear stress. We conclude that ETS1 senses blood flow disturbances and may promote venous remodeling by inducing endothelial dysfunction. Targeting ETS1 rather than downstream Notch proteins could be an effective and safe strategy to develop varicose vein therapies.



Schematic diagram demonstrating the role of disturbed flow in modulating ETS1-mediated Notch signaling in varicose veins



SRF: 2
Lab Assistant: 1
PhD Students: 1

Jeeva Prasannan, Ahalya S, Dr Sumi S, Karthika C L, Aswathy S (From L to R)

PUBLICATIONS:

- Sreelakshmi BJ, Karthika CL, Ahalya S, Kalpana SR, Kartha CC, Sumi S. Mechanoresponsive ETS1 causes endothelial dysfunction and arterialization in varicose veins via NOTCH4/DLL4 signaling. Eur J Cell Biol. 2024;103(2):151420.

AWARDS [PI]:

- RGCB Best teacher award in 2023

AWARDS [STUDENTS]:

- Ms. Sreelakshmi BJ- Best Poster Award. Aberrant mechanosensitive signaling pathway induce venous endothelial dysfunction in varicose veins. International Conference on Emerging Trends in Genomics and Biomedicine (ETGB 2023) held at IU-CGGT, University of Kerala, Thiruvananthapuram. 15-17 November 2023.
- Mrs. Ahalya S. Best Poster award (3rd price). Oscillatory shear stress abrogates endothelial Piezo1-KLF2 mechanosensitive signalling in venous flow. National Conference on Emerging Trends in Health Care Biotechnology 2023 (ETHB 2023) held at Regional Cancer Centre, Thiruvananthapuram. 30 November -2 December 2023.

CONFERENCE PRESENTATION:

- Ahalya S. Poster presentation, Oscillatory shear stress abrogates endothelial Piezo1-KLF2 mechanosensitive signalling in venous flow. National Conference on Emerging Trends in Health Care Biotechnology 2023 (ETHB 2023) held Regional Cancer Centre, Thiruvananthapuram. 30 November -2 December 2023.
- Sreelakshmi BJ, Poster presentation. Aberrant mechanosensitive signaling pathway induce venous endothelial dysfunction in varicose veins. International Conference on Emerging Trends in Genomics and Biomedicine (ETGB 2023) held at IU-CGGT, University of Kerala, Thiruvananthapuram. 15-17 November 2023.
- Ahalya S. Oral presentation. Altered hemodynamic shear stress promotes endothelial to mesenchymal transition by repressing mechanosensitive Piezo1-KLF2 signalling in venous system. International Conference on Emerging Trends in Genomics and Biomedicine (ETGB 2023) held at IU-CGGT, University of Kerala, Thiruvananthapuram. 15-17 November 2023.
- Karthika CL. Poster presentation. Oscillatory shear stress induces EndMT in cerebral arteriovenous malformations via notch signaling. International Conference on Emerging Trends in Genomics and Biomedicine (ETGB 2023) held at IU-CGGT, University of Kerala, Thiruvananthapuram. 15-17 November 2023.
- Sreelakshmi BJ. Oral presentation. Altered venous shear stress induce endothelial mechanosensitive Ets1-Notch4/Dll4 signaling in varicose veins. International Conference of Advances in Biotechnology: Current Discoveries and Future Perspectives (ICAB-2023), held at Amity University, Mumbai. 17-18 October 2023.

- Ahalya S. Poster presentation. Mechanosensitive Piezo1 ion channel in the pathogenesis of varicose vein. 1st RGCB Research Conference, held at Raviz Kollam. 20-23 September, 2023.
- Karthika CL. Poster presentation. Altered hemodynamic shear stress activates Notch-mediated EndMT in cerebral arteriovenous malformations. National Symposium on Biotechnology for Sustainable Development, held at RGCB, Thiruvananthapuram, 12 April 2023

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Piezo-KLF2 axis in endothelial dysfunction and venous wall remodeling in varicose veins	Science and Engineering Research Board	2021	3 years	PI
02	Molecular Pathogenesis of varicose veins	Dr. N. Radhakrishnan Foundation for Research on Venous Diseases	2018	7 years	PI



PATHOGEN BIOLOGY



Sreekumar E, PhD

Scientist F
Pathogen Biology

BRIEF THEME OF LABORATORY

Our laboratory focuses on developing direct-acting and host-targeted antivirals as well as disease modifiers against dengue and chikungunya, two major mosquito-borne virus diseases of the tropics using host-pathogen interaction studies and molecular approaches.

MAJOR RESEARCH AREA

- NPM1 is a host molecule that restricts Chikungunya virus infection.
- Fingolimod (FTY720) treatment restores endothelial integrity in animal models of Dengue vascular leakage.

- **HOST-PATHOGEN INTERACTION STUDIES ON DENGUE AND CHIKUNGUNYA VIRUS INFECTION TO DEVELOP NOVEL ANTIVIRALS AND DISEASE MODIFIERS**

Chikungunya, an increasingly prevalent mosquito-borne illness caused by a positive-sense, single-stranded RNA virus, has sparked widespread epidemics across tropical regions. In addition to the typical symptoms of acute fever and severe joint pain, cases of neurological complications and mortality have surfaced. Like all viruses, CHIKV hijacks the cellular machinery of its host to establish infection and replicate efficiently. To counter this, the host cell initiates various restriction factors and innate immune responses. Our previous research has illuminated the significant upregulation of Nucleophosmin (NPM1) in CHIKV-infected cells, demonstrating its role in restricting CHIKV infection. We further elucidated the crucial interplay between ADP-ribosylated NPM1 and the CHIKV nsP3 macrodomain in mediating CHIKV restriction. However, the virus may counteract this defense mechanism through its ADP-ribose hydrolase activity, aiding its efforts to perpetuate intracellular replication and evade cellular defenses, including ISG modulation. These findings underscore the multifaceted role of NPM1 in CHIKV restriction, suggesting it as a promising target for host-directed antiviral strategies against chikungunya. Additionally, we have developed ISG reporter cell lines to identify Host-Directed Antivirals (HDA), focusing on ISGs (IFITM1, IFITM2, IFITM3, and IRF1) known for their antiviral activity against enveloped RNA viruses. Screening a flavonoid library with these stable cell lines has yielded lead compounds, which are currently undergoing characterization for their anti-CHIKV and ISG-modulating properties.

Our lab also focuses on another mosquito-borne disease, Dengue, where we look at host-targeted disease pathology modifiers. Endothelial dysfunction leading to plasma leakage is a prominent sign of severe Dengue. There are no effective therapeutic interventions developed for the disease yet. Earlier studies from different research groups, including ours, have shown the role of several kinases and disease modifiers involved in endothelial cell pathways regulating endothelial permeability. Modulating these kinases and disease modifiers could be potential therapeutic targets for treating Dengue virus-induced vascular permeability. Interestingly, we found that administering FDA-approved drugs such as FTY720; and Src-kinase inhibitors has reduced vascular permeability in cellular and animal models of serotype-2 Dengue virus infection. Besides its primary manifestation in the endothelium, Dengue is also known to cause atypical manifestations like intracranial cranial haemorrhages, hepatitis, renal failure, myocarditis, and others. Due to underdiagnosis and lack of awareness of these atypical symptoms, there is a rise in disease burden; and we are trying to unravel how the host and pathogen interact at the molecular level in order to understand the pathogenesis of these conditions.

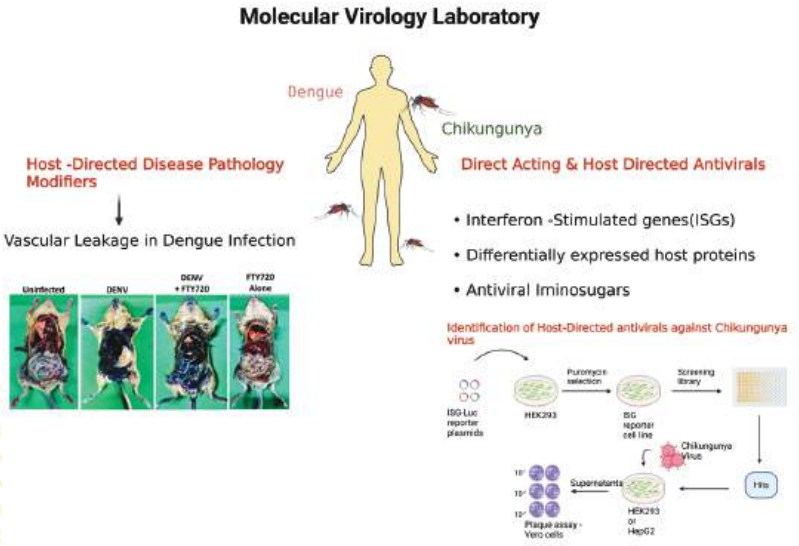


Fig. Thematic areas of Focus by Molecular Virology Laboratory



PhD Students: 4

Guhan KS, Srishti Rajkumar Mishra, Dr E Sreekumar, Mansi Awasthi, Ariya Ajay (From L to R)

PUBLICATIONS:

- Modak A, Mishra SR, Awasthi M, Sreedevi S, Sobha A, Aravind A, Kuppusamy K, Sreekumar E. Higher-temperature-adapted dengue virus serotype 2 strain exhibits enhanced virulence in AG129 mouse model. FASEB J. 2023 Aug; 37(8):e23062.
- Modak A, Mishra SR, Awasthi M, Aravind A, Singh S, Sreekumar E. Fingolimod (FTY720), an FDA-approved sphingosine 1-phosphate (S1P) receptor agonist, restores endothelial hyperpermeability in cellular and animal models of dengue virus serotype 2 infection. IUBMB Life. 2024 May; 76(5):267-285.
- Pradeep P, Sivakumar KC, Sreekumar E, 2023. Host Factor Nucleophosmin 1 (NPM1/B23) Exerts Antiviral Effects against Chikungunya Virus by Its Interaction with Viral Nonstructural Protein. Microbiol Spectr. 11: e05371-22

AWARDS [STUDENTS]:

- Srishti Rajkumar Mishra, Won Best Oral Presentation award for abstract entitled "Evaluation of the effect of Rho-kinase inhibition on Dengue virus-induced vascular permeability" in an international conference, Emerging Trends and Innovations in Biotechnology – BIOSPECTRUM 2023, conducted at MACFAST, Tiruvalla
- Received a Förderverein Grant to attend and present my work entitled "Leveraging the use of FDA-approved Src-kinase inhibitor in suppressing dengue virus-induced vascular permeability" at the 34th European Congress of Clinical Microbiology & Infectious Diseases (ECCMID), 2024 international conference held in Barcelona, Spain.

CONFERENCE PRESENTATION:

Conference attended- (National)

- Parvanendhu P, Presented poster entitled "Host factor Nucleophosmin (NPM1/B23) restricts Chikungunya virus replication by modulating interferon signaling pathway in human astrocytic cells" at 7th Molecular Virology Meeting,IISc, Bangalore September 2023.
- Srishti Rajkumar Mishra, Presented poster entitled "Inhibition of Src-kinase pathway ameliorates endothelial hyper-permeability induced by dengue virus" in 7th Molecular Virology Meeting, 2023, conducted at IISc, Bangalore, in September 2023.
- Ariya Ajay, Presented poster entitled "Host protein PSGL-1 restricts Chikungunya virus infection" at 7th Molecular Virology Meeting,IISc, Bangalore September 2023.
- Guhan KS, Presented poster entitled "Exploring Interferon-Stimulated Genes (ISGs) Modulation as an antiviral strategy against emerging RNA viruses" at National symposium on Biotechnology for sustainable development-2024", RGCB, Thiruvananthapuram, April 2024.

Conference attended- (International -within India)

- Srishti Rajkumar Mishra, Gave Oral presentation entitled "Evaluation of the effect of Rho-kinase inhibition on Dengue virus-induced vascular permeability" in an international conference Emerging Trends and Innovations in Biotechnology – BIOSPECTRUM 2023, conducted at MACFAST, Tiruvalla, in December 2023
- Ariya Ajay, Oral presentation on "Host protein PSGL-1 restricts Chikungunya virus infection" at India International Science Festival 2023 conducted at DBT THSTI-RCB Campus, Faridabad Haryana January 2024.

Conference attended- (International-Outside India)

- Srishti Rajkumar Mishra, Gave Oral presentation entitled "Leveraging the use of FDA-approved Src-kinase inhibitor in suppressing dengue virus-induced vascular permeability" at an international conference, the 34th European Congress of Clinical Microbiology & Infectious Diseases (ECCMID), held in Barcelona, Spain, in April 2024.

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Ayan Modak	Study of Sphingosine signaling pathway in Dengue infection	Regional Centre for Biotechnology	Awarded	2024
02	Parvanendhu Pradeep	Elucidation of the Role of Host-Factor Nucleophosmin (NPM1) in Chikungunya Virus Replication	University of Kerala	Submitted	2023



Rajesh Chandramohanadas, PhD

Scientist E-II
Pathogen Biology

BRIEF THEME OF LABORATORY

The laboratory of red cell diseases focusses on deformations of erythrocytes arising from damages such as Plasmodium infection. We have a specific interest on understanding how various disease states impact biochemical and mechanical properties of RBCs and compromise their function. Our lab is also engaged in the application and/or development of new tools to study the intrinsic properties of red cells in health and disease.

MAJOR RESEARCH AREA

- Identification of new small molecules against Plasmodium from the Medicines for Malaria Venture's Pandemic Response Box chemical library.
- Understanding the molecular determinants of host tropism in Malaria Infection.
- Development of an AI-assisted tool for segregation and scoring of young RBCs from thin blood smears.

◆ HOST-PARASITE INTERACTIONS IN MALARIA AND TARGETING THEREOF

(A) Our laboratory has a focus on identifying novel small molecule inhibitors that can alter stage-transitions in Plasmodium development, such as egress and invasion. We have shown that these cellular processes could be manipulated by small molecule interventions. As a continuation of this, we have recently completed screening and activity determination of a novel compound library (MMV pandemic Response Box) and identified new scaffolds that can interfere with specific stages of the Malaria parasites. We are currently performing various modes of action studies which will allow to further develop these molecules as potential partner drugs for Artemesinin, the frontline antimalarial that is fast losing efficacy.

(B) Plasmodium parasites demonstrate a specific affinity to younger RBCs in circulation, known as reticulocytes. This is an absolute requirement for parasitic species such as P. vivax, but even P. falciparum despite their ability to infect all stages of RBCs, show a tropism towards young reticulocytes. We have developed a method to purify reticulocytes for long term culturing of P. falciparum to understand host dependent adaptations and behavioural changes since the young RBCs are metabolically distinct from mature ones. We have also discovered differential sensitivity of parasites to drugs such as artemesinin when the parasites are within the reticulocytes, possibly indicating a new dimension of drug resistance formation.

(C) Young reticulocytes have a distinct staining pattern, characterized by thread-like reticular matter which is protein-RNA complexes. Reticulocyte counts in several diseases such as anemia are routinely detected by sophisticated analytical methods. We have developed a Convoluted Neural Network (CNN)-based method on CPU to distinguish and count immature human red blood cells from blood smears. We used more than 200 NMB-stained images to train and optimize the model for immature reticulocytes (stained positive with NMB, intensity and pattern of which depends on the developmental stage of the reticulocyte) and mature RBCs (no staining with NMB). Our model was able to successfully count reticulocytes with accuracy more than 90% from unknown samples which were subsequently cross-verified through microscopy and counting.

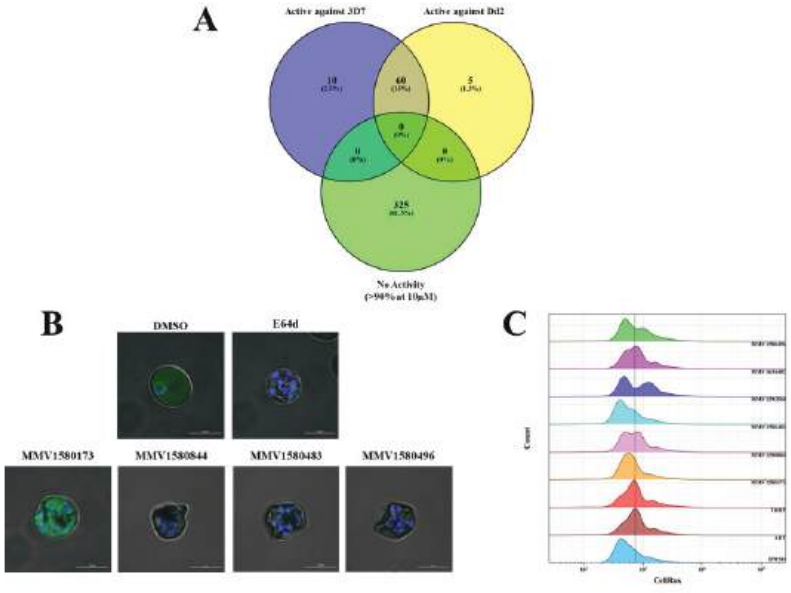


Figure demonstrating the identification of antimalarial molecules from the MMV Pandemic Response Box.
(A) From the library of 400 molecules, we have identified 60 molecules active against wild type (3D7) and CQ-resistant (Dd2) parasites.
(B) Phenotype based characterization aided through confocal microscopy further identified 4 molecules extremely potent against late stage parasite development and egress as indicated by defective vacuole bursting, which is a pre-requisite for parasite release.
(C) The parasites that were irreversibly locked within the RBCs were also damaged through ROS as captured by staining with CellRox.



JRF: 1
Project Assistant: 1
Technical Assistant: 2
PhD Students: 4

Akhila T P,Sreenath M, Dr Rajesh Chandramohanadas, Christeen Davis, Lakshmi V S, Abhinav B R, Lubha Subair (From L to R)

PUBLICATIONS:

- Reghunandanan K, Akhila T P, Krishnan N, K M D, Prasad R, Nelson-Sathi S, Chandramohanadas R. Search for novel Plasmodium falciparum PfATP4 inhibitors from the MMV Pandemic Response Box through a virtual screening approach. J Biomol Struct Dyn. 2024 ;42(12):6200-6211.
- Prasad R, Kadam A, Padippurackal VV, Pulikuttymadom Balasubramanian A, Kumar Chandrakumaran N, Suresh Rangari K, Dnyaneshwar Khangar P, Ajith H, Natarajan K, Chandramohanadas R, Nelson-Sathi S. Discovery of small molecule entry inhibitors targeting the linoleic acid binding pocket of SARS-CoV-2 spike protein. J Biomol Struct Dyn. 2024; 23:1-15.

INVITED TALKS [PI ONLY]:

- "Host Cell Tropism in Plasmodium spp". Regional Young Investigators Meeting, BITS PIlani (Jan 18-20, 2024)
- "Discovery to Translation in Modern Biology: The Malaria Model".20th Refresher Course in Life Science, University of Calicut (04-12-2023- 18-12-2023)

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Deciphering the Role of Novel Reticulocyte surface Proteins in Plasmodium vivax Invasion.	Science and Engineering Research Board	2023	3 years	PI



John Bernet Johnson, PhD

Scientist E-I
Pathogen Biology

BRIEF THEME OF LABORATORY

The innate immune system, the front line immune defense against pathogens, closely interacts with the adaptive arm of the immune system. Using RNA viruses of both veterinary (vesicular stomatitis virus) and human significance (Chandipura and chikungunya virus) our laboratory focuses on deciphering their complex interaction with the complement system and its cross-talk with other immune arms. The emphasis being, unravelling virus-host interactions and develop cutting edge clinically relevant (oncolytic/vaccine) viral vectors.

MAJOR RESEARCH AREA

- Elucidation of the differences in complement activation and modulation across Rhabdoviruses including vesicular stomatitis virus and Chandipura virus.
- Identification of the significance of complement component C1q - Chandipura virus interaction with respect to complement susceptibility and decipher the alternative functions of this interaction including in virus pathogenesis.
- Understanding complement resistance in chikungunya virus by systematically studying the role of viral signatures in modulating complement.
- Generate modalities for the development of an attenuated, safe and potent Chandipura virus platform for oncolytic virotherapy.

◆ COMPLEMENT EVASION BY CHANDIPURA VIRUS – HIJACKING HOST COMPLEMENT REGULATORY PROTEINS TO RESIST NEUTRALIZATION.

Our research is primarily focused on the biology and immune interactions of the members of the Rhabdoviridae and Togaviridae family. Employing representative viruses such as Chikungunya virus (Togaviridae) and Chandipura virus (Rhabdoviridae), we explore the dynamics of host-pathogen interaction. During the reporting period, extensive studies were carried out on how Chandipura virus (CHPV) evades the human complement system (CS). Chandipura virus, a potent human pathogen predominantly reported in the Indian sub-continent, is known to cause paediatric encephalitis with a high case fatality rate. Chandipura virus has a characteristic bullet-shaped morphology and is closely related to the prototypic rhabdovirus, vesicular stomatitis virus (VSV). A previous study from our lab has demonstrated that CHPV activates the classical pathway of CS in an antibody-independent manner. This activation and the resultant neutralization largely depended on the direct interaction of the complement component C1q with CHPV. The mechanism of complement-dependent neutralization can vary significantly among viruses, with viruses like VSV undergoing virolysis while CHPV was neutralized by aggregation.

Despite CS being a potent innate effector system capable of neutralizing a plethora of viruses, many viruses have developed ingenious strategies to circumvent the effect of this evolutionarily archaic system. Even though CHPV was found to be susceptible to the CS in fluid phase experiments, it was imperative to identify if CHPV also has mechanisms to evade the neutralizing effects of complement. Viruses have evolved an array of complement evasion strategies during co-evolution. RNA viruses are known to incorporate both soluble and membrane-bound host regulators of complement activation (RCAs) to the viral envelope during and upon egress, exploit RCAs as entry tools or decoys etc. We hypothesized that CHPV being an enveloped virus will incorporate the host membrane-associated RCAs. CHPV was cultured and purified from cell lines like A549 and HeLa that expressed RCAs like CD46, CD55 and CD59 differentially, wherein A549 cells express more CD46 while the HeLa cells have more CD55. Immunoblotting experiments and transmission electron microscopy analysis carried out with sucrose gradient purified CHPV generated from these cell lines revealed that CHPV could incorporate at least three important membrane-associated RCA namely, CD46, CD55 and CD59. Functional assays specific for CD46 and CD55 revealed that the virion-associated RCAs were biologically active, and conferred protection to the virus.a Infection by rhabdoviruses is known to destabilize the host transcription and translation. As RCAs exploited by the viruses are also host proteins, we sought to understand whether viruses egressing at different time points post-infection resisted complement differentially. We subjected CHPV collected from infected A549 cells to virus neutralization assay. We found that viruses collected at different time points post-infection were differentially susceptible to serum. Currently, we are studying the expression profile of RCAs at different time points post-infection to corroborate this finding with the ultimate goal of identifying the most dominant regulator in conferring protection to the virus. These results will have far-reaching implications not only in understanding the basic biology of the virus but also in engineering novel CHPV-based oncolytic and vaccine vectors.

Figure IA. Sucrose-gradient purified CHPV was subjected to transmission electron microscopy to detect the presence of membrane-associated RCAs CD46, CD55, and CD59. Immuno-gold-labelled antibodies were used to detect the presence of regulators on CHPV negatively stained with phosphotungstic acid (Scale-50nm).

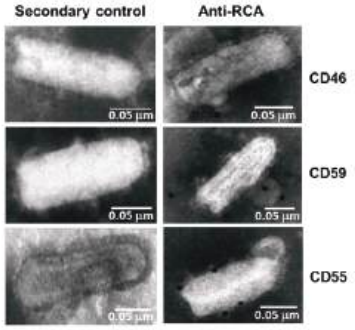
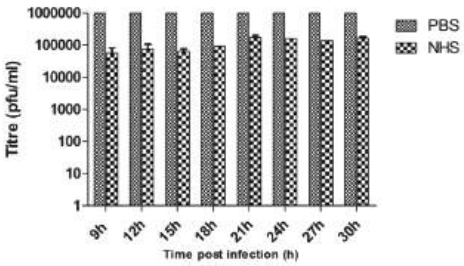


Figure 1B. Human lung cancer cell line,A549 was infected with CHPV at 1MOI and crude virus samples were collected at different time points post-infection. The virus stock was titrated and equal titres of each time-point virus were subjected to neutralisation assay. Virus treated with PBS only served as the negative control. The protection conferred by RCAs is not uniform across time points.





PhD Students: 4
Lab Assistant: 1

Priti Kumar, Dr John B Johnson, Karthika Rajeevan, Devika S R **(From L to R)**

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Molecular characterization of the cytopathic property of Chandipura virus to engineer an attenuated oncolytic vector.	DST-Science and Engineering Research Board	2023	3 years	PI
02	Understanding measles vaccine failure (and success) in Southern India.	National Institute of Health-USA	2017	5 Years	CO-PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Umerali K	Interaction and modulation of complement responses by Chandipura virus.	University of Kerala	Awarded	2023



Karthika Rajeeve, PhD

Scientist E-I
Pathogen Biology

BRIEF THEME OF LABORATORY

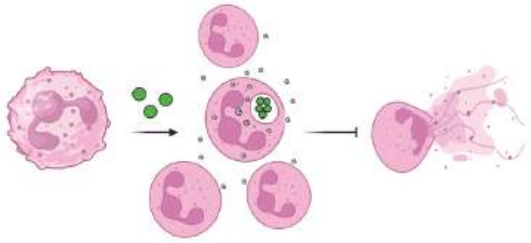
Our laboratory is focused on the pathogenesis and immune evasion mechanisms of human pathogens Chlamydia trachomatis and Mycobacterium tuberculosis. We use cutting-edge technologies like ex vivo human models to study host-pathogen interaction to facilitate drug discovery.

MAJOR RESEARCH AREA

- We report the surprising observation that the mechanism of restriction of bacterial growth is through the inhibition of c-Myc signaling by IFNγ as opposed to IDO-dependent depletion of tryptophan levels, as had been previously suggested.
- We have discovered a novel protein SCA with the potential to generate stem cells
- We discovered a novel role of immune cells in the spread and dissemination of Chlamydia trachomatis

- **DECIPHERING THE NOVEL ROLE OF IMMUNE CELLS IN THE SPREAD AND DISSEMINATION OF CHLAMYDIA TRACHOMATIS.**

Neutrophils are the most abundant, short lived terminally differentiated leukocyte with distinct tiers of arsenals to counter pathogens. They are characterized by their potent antimicrobial and antifungal capacity, which is granted by the cellular components: antimicrobial peptides, neutrophil-specific proteolytic enzymes, as well as their production of ROS and neutrophil extracellular traps (NETs). In this study we have discovered that neutrophils as such are a cluster of 15 different cell types. Chlamydial infection can increase the lifespan of the neutrophils and can enrich or shift the population of neutrophils towards one of this cluster with a long living phenotype. This is the first discovery that defines a heterogeneous sub-population of "pathogen associated neutrophils" (PAN) that serve as a Trojan horse for pathogens. This finding will be a paradigm shift in neutrophil biology that could be exploited for crucial therapeutic interventions.



Chlamydia trachomatis infect a population of human neutrophils which serve as a trojan horse for the bacterium.



JRF: 1
Project Assistant: 1
Technical Assistant: 1
Lab Assistant: 2
PhD Students: 2

Paridhi Agarwal, Dr Karthika Rajeeve, Smitha R P, Nijisha M (From L to R)

AWARDS [PI]:

Selected to serve as a Guest Editor for Communications Biology (Nature Publishers).

AWARDS [STUDENTS]:

- Nijisha M, Best poster award- Deciphering the role of Rv0464c, a hypothetical protein, in the reactivation of Mycobacterium tuberculosis. National Symposium on Biotechnology for Sustainable Development-2024, 20th April 2024. Rajiv Gandhi Centre for Biotechnology (RGCB)

INVITED TALKS [PI ONLY]:

- Invited talk at the Indo-US seminar on Recent Biochemical Approach in Therapeutics (RBAT-IX 2023), University of Kerala.

CONFERENCE PRESENTATION:

- Nijisha M, Poster presentation, Deciphering the role of Rv0464c, a hypothetical protein, in the reactivation of Mycobacterium tuberculosis. National Symposium on Biotechnology for Sustainable Development-2024, 20th April 2024. Rajiv Gandhi Centre for Biotechnology (RGCB).

PATENTS APPLIED/ GRANTED:

- Patent Application No. 202341056900 "A NOVEL STEM CELL ACTIVATOR (SCA) PROTEIN AND ITS PREPARATION METHOD THEREOF" (Provisional)

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Deciphering the novel role of immune cells in the spread and dissemination of Chlamydia trachomatis.	DST-Science and Engineering Research Board	2021	3 years	PI
02	Unravelling the role of Chlamydial deubiquitinase in evading the host innate immune system.	DST-Science and Engineering Research Board -SRG	2022	2 years	PI



Karthik Subramanian, PhD

Scientist C & DBT-Ramalingaswami Fellow
Pathogen Biology

BRIEF THEME OF LABORATORY

The respiratory bacterial pathogen, Streptococcus pneumoniae colonizes the nasopharynx of healthy children and adults and kills over 1 million people worldwide annually due to pneumonia, septicemia and meningitis. The focus of my laboratory is to study molecular mechanisms of bacterial pathogenesis and identify therapeutics targeting key bacterial virulence factors. A major project in the lab investigates the host extracellular vesicles shed in response to infection and engineer them for potential vaccine platforms.

MAJOR RESEARCH AREA

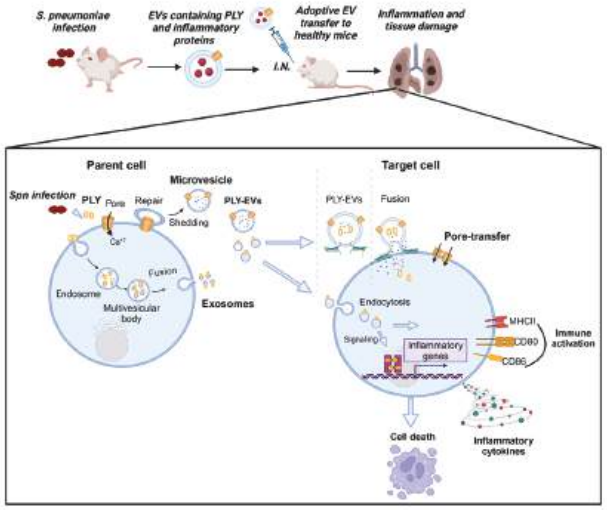
- Established methodology for isolation and characterization of extracellular vesicles from human cell cultures and murine broncholalveolar lavage samples.
- Identified that bacterial pore-forming toxin, pneumolysin induces EV shedding and transferred pore to recipient cells upon fusion.
- Identification of proteins differentially enriched in EVs shed upon challenge with pneumolysin toxin by quantitative proteomics.
- Using adoptive EV transfer experiments in zebrafish and mouse models we showed that host EVs shed in response to bacterial toxins disseminate pathogencity in vivo.
- The manuscript from this study is currently under revision in iScience (Cell Press).

◆ INVESTIGATING THE ROLE OF HOST EXTRACELLULAR VESICLES IN HOST-PATHOGEN INTERACTIONS.

Streptococcus pneumoniae colonizes the human upper respiratory tract and is a major cause of life-threatening diseases such as pneumonia, septicaemia and meningitis, resulting in death of over 800,000 children annually. Pneumococci express several virulence factors such as the polysaccharide capsule and the cholesterol dependent pore-forming cytotoxin, pneumolysin (PLY) to evade immune response and establish disease. Pneumolysin toxin binds to cholesterol present in the plasma membrane of host cells, oligomerizes and form trans-membrane pores, which eventually lead to Ca2+ influx and cell death. Cellular membrane repair mechanisms limit the extent of damage and shed the toxin-damaged membrane in the form of extracellular vesicles (EVs). During an infection, variety of EVs are released from infected cells and contain both bacterial virulence factors such as toxins as well as host factors that could modulate the course of the infection and host immune response. In this project, we aim to isolate and investigate the structure, protein composition and pathological function of EVs produced by human immune cells upon infection by the respiratory tract bacterial pathogen, Streptococcus pneumoniae and its pore-forming toxin, pneumolysin (PLY).

We found that PLY-induced host EVs (PLY-EVs) are selectively enriched in innate immune and inflammatory proteins, which induce human dendritic cell (DC) maturation and alert them to infection. In vivo, administration of PLY-EVs induced mortality and inflammation in zebrafish embryos. Using an intranasal challenge mouse model, we found that infection with wild-type pneumococci induces higher EV release in the lungs as compared to PLY-mutant strain. Adoptive transfer of EVs isolated from infected mice to healthy recipients induced pathology in a PLY-dependent manner. Our findings suggest that expelling PLY toxin pores through vesicles works as double edged sword. While cells remove the membrane assembled toxin pores through vesicles to evade lysis, the released EVs harboring PLY and other inflammatory proteins induce cytotoxicity and inflammation within the host. Thus, host-EVs disseminate pore-forming toxin and other inflammatory cargo during infection that has dire consequences during infection.

Host extracellular vesicles shed during infection induce toxicity and inflammation in a pneumolysin dependent manner. During infection, S. pneumonia releases the pore-forming toxin, PLY that oligomerizes to form pores on the plasma membrane of eukaryotic host cells. At sublytic toxin doses, Ca2+-dependent intrinsic cell membrane repair mechanisms are proposed to remove toxin pores by shedding the damaged membrane as microvesicles. Concurrently, PLY can also be endocytosed into endosomes that form multivesicular bodies through internal budding mediated by ESCRT protein complex and fuse with the plasma membrane to release exosomes bearing PLY. We found that PLY upregulates shedding of host EVs containing membrane bound PLY that induce cytotoxicity and hemolysis upon fusion with target cells. Using intranasal challenge mouse model, we showed that transfer of EVs from infected mice induced pathogenicity in healthy recipients in a PLY-dependent manner highlighting the significance of EVs shed during infection.





JRF: 2
Project Assistant: 1
PhD Students: 1

Joel Abraham, Aswathy C S, Dr Karthik Subramanian, Himani Dhyani, Charmi Panchal (From L to R)

PUBLICATIONS:

- Subramanian K, Banerjee A. Editorial: Deceiving the host: mechanisms of immune evasion and survival by pneumococcal bacteria. Front Cell Infect Microbiol. 2023 Jun 19:13:1231253

AWARDS [PI]:

- Joined Early Career Board member at ACS Infectious Diseases journal- January 1, 2024
- Invited member of the Indian Society for Extracellular Vesicles- Dec 15, 2023
- Member, Indian Immunology Society
- Life-member, Kerala Academy of Sciences

CONFERENCE PRESENTATION:

- Chinmayi Bhat, Poster Presentation, Bacterial toxin pneumolysin drives systemic pathogenicity through shedded extracellular vesicles, National Symposium on Biotechnology for Sustainable Development on 12th April 2023, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Investigating pneumococcal adaptation to intracellular survival within the host and characterization of macrophage extracellular vesicles for novel vaccine development.	DBT-Ramalingaswami Re-entry Faculty award	2021	5 years	PI
02	Disarming bacterial pathogens using novel peptides that target pore-forming toxins: from in silico to in vivo	Department of Science & Technology [DST]-INSPIRE grant	2020	5 years	PI



Arun Sankaradoss, PhD

Scientist C
Pathogen Biology

BRIEF THEME OF LABORATORY

The laboratory focuses on developing circular RNA and DNA-based vaccines for Dengue virus and other infectious diseases. Our lab's long-standing research interest is to elucidate the protective role of resident, effector memory T cells in protective immunity following infection and vaccination.

MAJOR RESEARCH AREA

- Dengue virus serotypes 1-4 infection model development using IFNAR1-/- and IFNAR1-/-IFNGR1-/- knockout mice models.
- Developing nanoDNA-LNP-based vaccine for dengue. Preclinical studies are ongoing.

- **DEVELOPING CUTTING–EDGE NUCLEIC ACID–BASED VACCINE PLATFORM FOR DENGUE AND OTHER INFECTIOUS DISEASES.**

The lab focuses on developing both DNA and RNA-based vaccines.

DNA vaccines: The widespread use of DNA vaccines has been hampered by low protective efficacy in humans. We aim to improve the DNA vaccines by developing an adjuvanted nano DNA vaccine in conjunction with genetic adjuvants and delivering them using LNP formulation. Here, we describe the development of a nano DNA- LNP vaccine for dengue virus encoding the EDIII-NS1 region of the viral proteins as responses to this segment typically do not mediate ADE of infection, a challenge to developing vaccines for this infection. The in vitro transfection efficiency of the candidate showed a significantly increased percentage of antigen-positive cells than conventional DNA plasmids. Furthermore, vaccination of mice with this construct induced significantly increased antigen-specific IgG binding antibody responses than conventional pVAX1 DENV DNA vaccines. Further studies are underway to investigate the neutralizing antibody potential, protective efficacy and ADE abrogation of antibodies induced by the candidate against DENV 1-4 serotypes.

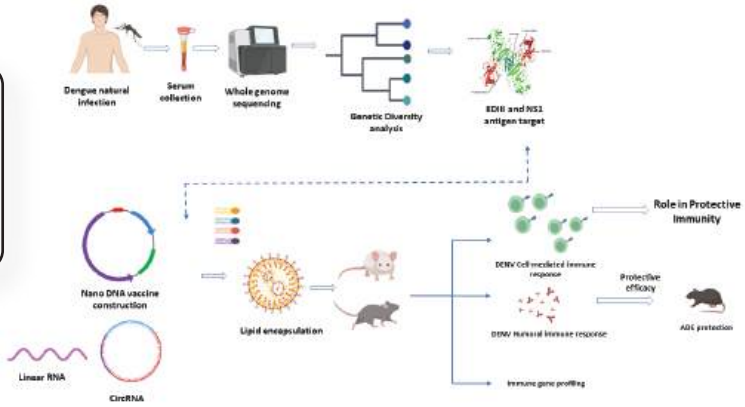
RNA platform: Vaccine antigens can be delivered in an mRNA modality through multiple approaches. i.e. unmodified non-replicating mRNA, a nucleotide-modified mRNA and circular RNA (CircRNA). In this context, we will be developing these platforms with the same dengue viral antigens and intend to investigate differences in the vaccines' efficacy. More specifically, we intend to elucidate the protective role of CD4 and CD8- T cell phenotypes in response to these platforms using various preclinical models.



Ramya, Dr Arun Sankaradoss, Thiriveni V (From L to R)

Project Assistant: 1
PhD Students: 1

Illustration of work plan



INVITED TALKS [PI ONLY]:

- Next-gen vaccine platforms for infectious diseases, Vellore Institute of Technology, 13th July 2023. Online mode

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Novel Adjuvanted Nano plasmid-based DNA Vaccine Development for dengue virus : Opportunities to Expand the platform for Emerging Infectious Diseases and one health	Biotechnology Industry Research Assistance Council-BIG	2023	18 months	PI



Santanu Chattopadhyay, PhD

GN Ramachandran Fellow
Pathogen Biology

BRIEF THEME OF LABORATORY

We work on genetic geography, virulence and antibiotic resistance of the gastric pathogen Helicobacter pylori and its interaction with various microbes in gastrointestinal microbiota in the context of severe gastric diseases. We use next-generation sequencing (NGS) based approaches to study microbial genomics and metagenomics, which are typically followed by in vitro microbe-microbe interactions and antimicrobial susceptibility assays.

MAJOR RESEARCH AREA

- Detection of Western-specific non-synonymous mutations in Eastern-cagA of H. pylori.
- Identification of similar Western-specific non-synonymous mutations in H. pylori vacA and recA.
- Detection of Western-specific synonymous mutations in the housekeeping and virulence genes of H. pylori.
- Appreciation of genome-wide Western-specific mutations in Eastern-H. pylori.

POINT MUTATIONS IN THE GENOMES OF GASTRIC PATHOGEN HELICOBACTER PYLORI

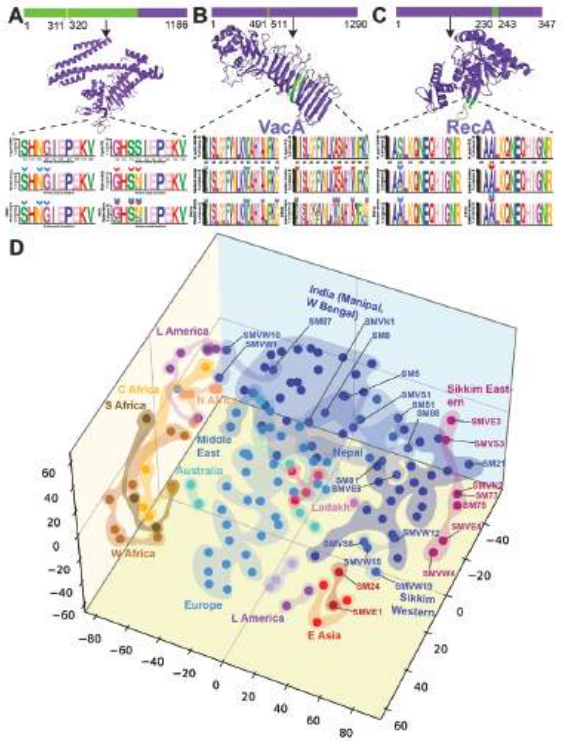
Helicobacter pylori colonizes in the human stomach for decades before causing severe diseases like peptic ulcer and gastric cancer. During the long-term colonization, the bacterium adapts to the changing milieu of the stomach and gradually co-evolves with the respective human. Owing to the higher rates of recombination and point mutation than most bacterial species, H. pylori genomes evolve remarkably fast and are known to show panmictic population structures. A unique DNA polymerase I and faster mutation rate also allow the bacterium to form quasispecies. No two H. pylori strains isolated from two individuals are exactly same. Despite the tremendous variations in the genomes, one of the major virulence effectors of H. pylori, the oncoprotein CagA, which is encoded by the cytotoxin associated gene A (cagA), are either of 'Eastern' or of 'Western' types. Our analysis revealed that the H. pylori strains isolated from a Himalayan, North-East Indian state, Sikkim, which shares borders with China, Nepal and Bhutan, carry Eastern-CagA with Western-specific mutations (Fig 1A). We wanted to test whether the accumulations of Western-specific point mutations are restricted to the cagA gene, or similar point mutations are also incorporated in other H. pylori genes. Our results show that like Eastern-CagA, Eastern-VacA (encoding vacuolating cytotoxin, a virulence effector protein) and Eastern-RecA (encoding recombinase, required for recombination and DNA repair) also have Western-specific substitutions ((Fig 1B and C). Next, to test if the Eastern-H. pylori housekeeping genes also get westernized, we made a SNP distance matrix based on the concatenated 7 housekeeping genes (trpC, urel, atpA, mutY, ppa, yphC, efp) of 342 H. pylori strains from different parts of the world. This matrix was used to generate a t-distributed Stochastic Neighbor Embedding (t-SNE) plot (data not shown). The Western-strains from Sikkim were mostly associated with hpEurope and hpAsia2. The Sikkim-typical Eastern strains, were clustered along with the hpEAsia strains. However, the Sikkim atypical Eastern-strains, which showed westernization in cagA, vacA and recA, formed a significantly distinct cluster. The calculated Euclidean distances between the H. pylori populations further confirmed the finding. Finally, we tested if the atypical Eastern-strains also carry Western-specific mutations throughout their genomes. We generated a similar t-SNE plot based on the SNP distance matrix of 747 concatenated core genes (distributed throughout the genome) of 156 H. pylori strains (Fig 1D). The Sikkim typical Eastern-strains, SM24 and SMVE1, were seen distinctly in the plot close to strains from East Asia and Latin America. Seven Sikkim atypical Eastern strains (SM75, SM77, SMVN2, SMVE3, SMVS3, SMVE4 and SMVW4), which showed the accumulation of Western-specific mutations in previous analyses, formed a distinct cluster (cluster II) between the Sikkim Eastern Cluster I and the Sikkim Western Cluster. Euclidean distances between the Sikkim Eastern Cluster I and Sikkim Eastern Cluster II is 7.4, while the distance between the Sikkim Eastern Cluster II and Sikkim Western Cluster is 9.3. All these findings showed that some of the Sikkim Eastern-strains, which express the atypical CagA, have accumulated genome-wide non-random, Western-specific adaptive point mutations.

(A) Mutations within a stretch of ten amino acids (311 to 320) at the N-terminal end region of CagA.

(B) Nonrandom Western-specific mutations in VacA for the region 491 to 511.

(C) In RecA, the typical Western strains carry A and S at position 232, while the Eastern strains carry A at the position.

(D) t-SNE plot based on the core genes showing the different H. pylori populations along with the strains from Sikkim.





JRF: 3
PhD Students: 1

First Row: Dr Santanu Chattopadhyay, Pahil Sen, Doryl Infanta, Anagha K B

Second Row: Retnakumar R J, Nikita Agarwal

PUBLICATIONS:

- Chattopadhyay S, Murugaiyan G, Hajela N and Ramakrishna BS. Editorial: Benefiting from microbes: challenges in getting the 'pros' and avoiding the 'cons'. Front. Gastroenterol. 2023. 2:1293448. doi: 10.3389/fgstr.2023.1293448.
- Chouhan D, Retnakumar RJ, Devi TB, Dharmaseelan S, Alexander SM, Devadas K, Chattopadhyay S, Nair GB, Pillai MR. Unusually high clarithromycin resistance in Mycobacterium abscessus subsp. abscessus isolated from human gastric epithelium. Front Microbiol. 2023 Aug 28;14:1193380.
- Ngashangva L, Chattopadhyay S. Biosensors for point-of-care testing and personalized monitoring of gastrointestinal microbiota. Front. Microbiol. 2023. 14. 1114707. (IF: 6.06).
- Nath AN, Shaji AM, RJ Retnakumar, Nair GB, Chattopadhyay S. Battling Helicobacter pylori with Our Microbial Weapons: The Emerging Era of Novel Microbiome Based Probiotics. Chapter 5. In Cutting edge science and applications: Intestinal Microbiota and probiotics. Ed. Sesikeran B and Lahiri K. Jaypee. 2023. Page 45-68.

AWARDS [PI]:

- Best Teacher Award (Selected by the RGCB MSc 2021-2023 students).

AWARDS [STUDENTS]:

- Retnakumar RJ, PhD student in my lab received best poster award in National Symposium on Biotechnology for Sustainable Development-2023 held at Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum on April 12, 2023.
- Retnakumar RJ, PhD student in my lab obtained the first prize for oral presentation at the International Conference on 'Innovations in the Sphere of Biological Sciences' at AIHSHE, Coimbatore on 15-16 February, 2023.

INVITED TALKS [PI ONLY]:

- Delivered a popular talk entitled 'Antimicrobial resistance in bacterial pathogens: why and how?' at the Ezhuthachan college of Pharmaceutical Sciences, Trivandrum in the 'One day program on antimicrobial resistance' on December 20, 2023.
- 'Helicobacter pylori infection and gastric diseases: not a straightforward story' at the International Conference on 'Innovations in the Sphere of Biological Sciences' at AIHSHE, Coimbatore on 15-16 February, 2023.

CONFERENCE PRESENTATION:

- Chattopadhyay S. 'Helicobacter pylori infection and gastric diseases: not a straightforward story' at the International Conference on 'Innovations in the Sphere of Biological Sciences' at AIHSHE, Coimbatore on 15-16 February, 2023.
- Retnakumar RJ, PhD student in my lab obtained the first prize for oral presentation at the International Conference on 'Innovations in the Sphere of Biological Sciences' at AIHSHE, Coimbatore on 15-16 February, 2023.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Genomic and metagenomics insights on Helicobacter pylori virulence and antimicrobial resistance	DST- Science & Engineering Research Board	2023	2 years	PI
02	Evaluating the possibility of using new Bifidobacterium strains as probiotic supplements along with standard antimicrobial therapy against Helicobacter pylori and understanding the mechanism	Department of Biotechnology	2023	2 years	PI
03	To assess the impact of gut microbiome and associated bacteriophages in non-alcoholic fatty liver disease and its stages	Kerala State Council for Science - Technology and Environment	2023	2 years	PI



PLANT BIOTECHNOLOGY & DISEASE BIOLOGY



Soniya E. V, PhD

Scientist G

Plant Biotechnology & Disease Biology

BRIEF THEME OF LABORATORY

Our laboratory concentrates on elucidating the molecular mechanisms governing plant interactions in black pepper with biotic and abiotic factors and also focus on the genomic, metagenomic and transcriptomic aspects of this interactions. Specifically, we investigate plant-pathogen interactions, plant stress responses, plant – microbe interactions and delve into the intricate molecular pathways involved in the synthesis of secondary metabolites.

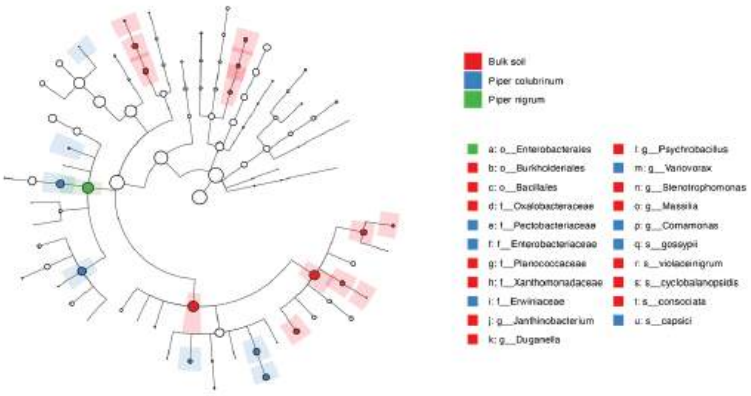
MAJOR RESEARCH AREA

- The bacterial microbiome of the susceptible and resistant Piper species has been elucidated using amplicon based metagenomics sequencing. The significant differences in the differential abundance of rhizobacteria in the susceptible and resistant varieties of Piper sps provides the influence of plant-host genotype in recruiting the bacterial communities during P capsici infection.

- **UNLOCKING DEVELOPMENTAL PATHWAYS AND DEFENCE MECHANISMS IN PIPER NIGRUM L. THROUGH INTEGRATED OMICS ANALYSIS AND IDENTIFICATION OF NOVEL TYPE III POLYKETIDE SYNTHASES FOR MEDICINALLY IMPORTANT NATURAL COMPOUNDS**

One of our primary areas of research is centred on Piper nigrum, commonly known as black pepper, a spice crop of significant medicinal and economic value cultivated in tropical regions worldwide. We are particularly concerned with combating Phytophthora capsici, an oomycete pathogen responsible for quick wilt in black pepper, posing a substantial threat to this important crop. Through various molecular approaches, we aim to develop resistance against Phytophthora capsici in black pepper. In order to gain a better understanding of Phytophthora capsici resistance in Piper spp., we performed the whole genome sequencing of Piper nigrum (Panniyur 1; susceptible species) and Piper colubrinum (resistant species). Our research has led to the identification and validation of novel miRNA, lncRNAs, and N6-methyladenosine modifications that play crucial roles in combating pathogen invasion Additionally, we have conducted high-throughput tRF-target cleavage validations to elucidate the involvement of tRFs in stress response mechanisms. Simultaneously, we investigate the small RNAs produced by Phytophthora capsici during its interaction with black pepper plants, with the aim of devising strategies to reduce disease severity. Furthermore, we analyse the root and rhizosphere bacterial microbiome profiles of Piper spp. to better understand their impact on disease resistance induction, particularly in resistant cultivars compared to susceptible ones. The role of nitric oxide during stress response of P. capsici infection in black pepper, and key genes involved in nitric oxide biosynthesis and their post-transcriptional regulatory mechanisms were also studied. We have also explored the use of green nanoparticles for disease management. Moreover, our research extends to the study of the colonization of Piper nigrum by Piriformospora. indica and its effects on growth advantages, floral induction, and evocation. Through these investigations, we aim to provide valuable insights into combating this detrimental oomycete pathogen. In addition to our work on black pepper, we are also involved in identifying and modulating type III polyketide synthases (PKS) involved in the production of therapeutically relevant secondary metabolites from the medicinal plants. The yeast expression of Quinolone synthase from Aegle marmelos for the production of secondary metabolites is ongoing. Through these multifaceted research efforts, our laboratory aims to provide unique strategies for combating plant pathogens and enhancing the production of beneficial secondary metabolites. Genome India is a national initiative funded by DBT involving 20 institutes aimed at investigating genetic variations within the Indian population. The project's objective is to conduct whole genome sequencing (WGS) to establish a comprehensive catalogue of genetic variations among diverse population groups across the country and also to construct a reference genome specifically tailored to Indians. This dataset will serve as a valuable national resource, catering to the requirements of both the scientific and medical communities. As part of this effort, RGCB has gathered blood samples from five distinct ethnic groups in Kerala and secondary data analysis is progressing.

Cladogram of Linear discriminant analysis (LDA) effect size (LEfSe) analysis of bacterial abundance in the resistant and susceptible Piper spp.





SRF: 1
Project Assistant: 2
Technical Assistant: 1
Lab Assistant: 2
PhD Students: 2

First Row: Sinsha Prakashan, Dr E V Soniya, Aparna S A **(From L to R)**

Second Row: Vasudev Paveri, Himaparvathy A, **Amal T S (From L to R)**

PUBLICATIONS:

- Vijayanathan M, Vadakkepat AK, Mahendran KR, Sharaf A, Frandsen KEH, Bandyopadhyay D, Pillai MR, Soniya EV. Structural and mechanistic insights into Quinolone Synthase to address its functional promiscuity. Commun Biol. 2024 14;7(1):566.
- Santhoshkumar R, Hima Parvathy A, Soniya EV. Biocompatible silver nanoparticles as nanopriming mediators for improved rice germination and root growth: A transcriptomic perspective. Plant Physiol Biochem. 2024;210:108645.
- Asha S, Kattupalli D, Vijayanathan M, Soniya EV. Identification of nitric oxide mediated defense signaling and its microRNA mediated regulation during Phytophthora capsici infection in black pepper. Physiol Mol Biol Plants. 2024;30(1):33-47.

AWARDS [STUDENTS]:

- Hima Parvathy A, PhD scholar: Best Paper Presentation Award -Oral presentation- "Unravelling Microbiome Diversity: A Comparative Microbiome Exploration of Piper Genus Reveals Novel Insights for Crop Resilience and Bacterial Population Dynamics" at International Conference on Emerging Trends in Genomics and Biomedicine (ETGB 2023), Inter-University Centre for Genomics and Gene Technology (IU-CGGT), University of Kerala from 15th -17th November,2023

INVITED TALKS [PI ONLY]:

- Invited speaker (2023) at International conference on Plant Biotechnology and Genome Editing during 27-29 June 2023 organised by Kakatiya University in collaboration with Aberystwyth university.
- Invited speaker (2023) at International conference on Emerging Trends in Genomics and Biomedicine (ETGB) conference Organised by Inter-University Centre for Genomics & Gene Technology (IU-CGGT) for the, 2023.
- Distinguished speaker (2024). The 125th-anniversary lecture series on 'Women in STEM on17th January, 2024 at 2PM. Organised by Govt. College for Women, Vazhuthacaud, Thiruvananthapuram
- Unravelling Genetic Diversity: The power of DNA Fingerprinting. 27th January, 2024, organized by Indian Society of Analytical Scientists.

CONFERENCE PRESENTATION:

- Hima Parvathy A, PhD scholar: "Unravelling Microbiome Diversity: A Comparative Microbiome Exploration of Piper Genus Reveals Novel Insights for Crop Resilience and Bacterial Population Dynamics" at International Conference on Emerging Trends in Genomics and Biomedicine (ETGB 2023), Inter-University Centre for Genomics and Gene Technology (IU-CGGT), University of Kerala from 15th -17th November,2023.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Genome India: Cataloguing the genetic variation in Indians	Department of Biotechnology	2020	3 years initially, now extended upto July 31st, 2024	PI
02	DBT BUILDER: RGCB Interdisciplinary Life Science Program for Research Based Learning	Department of Biotechnology	2021	5 years	PI



George Thomas, PhD

Scientist G & Dean (Research Administration)
Plant Biotechnology & Disease Biology

BRIEF THEME OF LABORATORY

Our lab primarily investigates the extensive array of pathogenicity factors synthesized by the oomycete pathogen, Pythium myriotylum, during its compatible interaction with spice crop ginger and their corresponding targets (susceptibility gene) in the host plant genome. We also aim to understand the key differences in the expression signature of these genes in both the susceptible ginger and the resistant Zingiber zerumbet in the context of the rhizome rot disease.

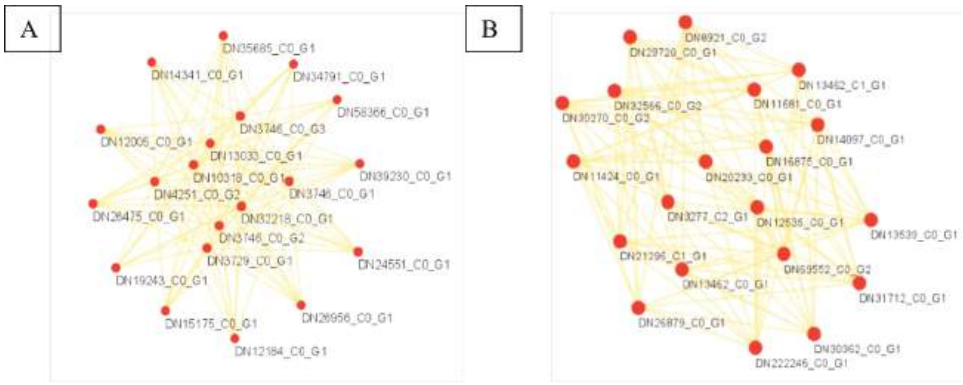
MAJOR RESEARCH AREA

- Characterized the transcriptome of Pythium myriotylum during its infection process in ginger and Z. zerumbet.
- dentified putative susceptibility targets of P. myriotylum in the ginger host through protein-protein interactome analysis.
- Determined the expression modulation of the interacting pathogenicity genes and susceptibility genes in the host.

ELUCIDATING THE PROTEIN-PROTEIN INTERACTOME OF THE PYTHIUM MYRIOTYLUM-ZINGIBER OFFICINALE PATHOSYSTEM AND IDENTIFICATION OF THE SUSCEPTIBILITY TARGETS IN THE HOST

Ginger has been grown worldwide for its medicinal benefits and use as a spice. India, being a leading producer, accounts for 45% of the world's ginger production. Ginger is heavily prone to infection by Pythium myriotylum, causing the soft rot or rhizome rot disease. It causes an annual yield loss of 50-90 %. Obligatory asexual reproduction exclusively by rhizomes impedes breeding for soft-rot resistance in ginger through sexual hybridization. Clonal selection for soft-rot resistance is also impossible, as all ginger cultivars available today are equally susceptible to soft rot. Limited knowledge exists regarding the pathogenicity factors employed by Pythium myriotylum to render the ginger host susceptible to disease. These pathogen molecules effectively manipulate host machinery to facilitate pathogen growth and survival. Current crop protection strategies increasingly focus on identifying genes or factors within the host that support pathogen survival and proliferation, termed susceptibility factors. Mutations in these genes have been shown to diminish pathogen virulence. Furthermore, infection is an outcome of a compatible host-pathogen interaction brought about by various protein-protein interactions, which act as key players in the disease cycle.

In our previous study, we conducted dual transcriptome sequencing on collar tissues sampled from P. myriotylum inoculated ginger and Z. zerumbet, resulting in the identification of 1896 and 218 transcripts originating from the pathogen, respectively. Characterization of these transcripts revealed a greater number, as well as the induction, of pathogenicity genes during pathogenesis in the susceptible host compared to the resistant one. In-silico analyses unveiled putative effectors and pathogenicity-associated genes in P. myriotylum, notably Carbohydrate Active Enzymes, which exhibited higher expression in susceptible plants than in resistant Z. zerumbet. Subsequent protein-protein interaction network analysis, following cluster analysis, identified putative susceptibility genes in the host, which are currently undergoing validation through virus-induced gene silencing experiments.



Representative protein-protein interaction network obtained by analyzing the host transcripts with the P. myriotylum transcripts using the MCODE plugin in Cytoscape. A- ginger transcripts with pathogen transcripts; B- Z. zerumbet transcripts with pathogen transcripts. Panels C and D represent the expression signature of an interacting pathogenicity gene identified in ginger and Z. zerumbet, respectively in network analysis. Green bars: expression of the pathogenicity gene during the infection in ginger; Blue bars: expression of the pathogenicity gene during the infection in Z. zerumbet. Statistical significance ($p < 0.05$) is denoted by symbols.



Technical Assistant: 1
Lab Assistant: 1
PhD Students: 2

Lini Varghese, Vinitha M R, Dr George Thomas, Jiya Rose (From L to R)

PUBLICATIONS:

- Augustine L, Varghese L, Kappachery S, Ramaswami VM, Surendrababu SP, Sakuntala M, Thomas G. Comparative analyses reveal a phenylalanine ammonia lyase dependent and salicylic acid mediated host resistance in Zingiber zerumbet against the necrotrophic soft rot pathogen Pythium myriotylum. Plant Sci. 2024 Mar;340:111972.



Manjula S, PhD

Scientist F

Plant Biotechnology & Disease Biology

BRIEF THEME OF LABORATORY

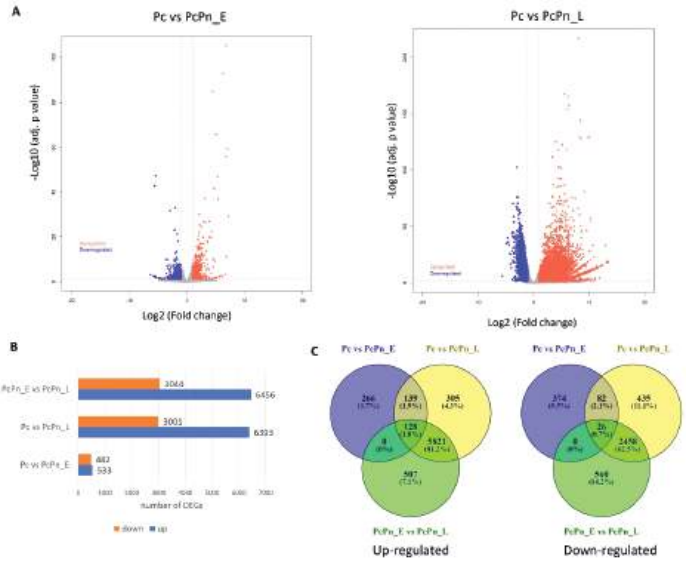
The major focus of the lab is to gain molecular insights into the Piper nigrum L. X Phytophthora capsici Leonian pathosystem through 'omics' and ensuing functional approaches with the ultimate aim of devising better crop protection strategies in black pepper against 'foot-rot' disease.

MAJOR RESEARCH AREA

- Major genes of Phytophthora capsici with critical roles in pathogen growth and virulence have been identified.
- Generated first secretome library of P. capsici infecting black pepper.
- The promising role of defense priming for crop protection in black pepper has been established across varieties that show gradation in response to P.capsici.
- Identified metabolite biomarkers associated with priming in black pepper, with potential scope of translation

- **INSIGHTS INTO PATHOGENICITY MECHANISMS OF PHYTOPHTHORA CAPSICI LEON. IN BLACK PEPPER (PIPER NIGRUM) AS REVEALED BY TRANSCRIPTOMICS APPROACH**

Phytophthora capsici has a broad host range that includes several important vegetable crops like tomato, pepper, cucumber, eggplant, and, lima beans and is ranked the world's fifth most destructive oomycete . P.capsici exhibits a hemibiotrophic lifestyle with an initial asymptomatic biotrophic phase followed by a necrotrophic phase during which it kills the host cells . Once the soil is infected, controlling P.capsici becomes challenging as the pathogen persists in the form of oospores or mycelium in the soil and plant remnants. Like in other susceptible crops, P.capsici can infect every part of P.nigrum, including the roots, stems, leaves, and fruits, and inflict substantial damage resulting in significant yield losses in black pepper . Since no varieties of P.nigrum are entirely resistant to P.capsici, farmers typically rely on chemical fungicides to control the spread of this disease. Besides the toxicity caused by these chemicals on farmers as well as on the ecosystem, this rapidly evolving pathogen is developing resistance to these treatments thereby reducing their effectiveness as fungicides. The details on the mechanism of interaction of P.capsici and P.nigrum remain poorly understood, hindering efforts to enhance disease resistance. To address this knowledge gap, we conducted an RNA-seq analysis to investigate the gene expression profile of P.capsici while infecting P.nigrum. Comparative transcriptome analysis between axenic culture, and early and late infection stages of P.capsici revealed a substantial number of differentially expressed genes. Our findings demonstrate the induction of metabolic pathways, signaling cascades, and crucial pathogenicity-related processes during infection of P.nigrum by P.capsici. Specifically, we observed orchestrated expression of cell wall-degrading enzymes, effectors, and, detoxifying transporters at different infection time points, implicating their roles in pathogenicity. The expression patterns of key pathogenicity-associated genes including effectors were validated using reverse transcription quantitative real-time PCR. This study establishes a strong foundation for further studies elucidating the pathogenic mechanisms employed by P.capsici during infecting P.nigrum and for developing effective disease management strategies. Future investigations building upon these findings are essential for advancing our understanding of this pathosystem and for implementing targeted approaches to mitigate black pepper foot rot.



(A) Volcano plots depicting the proportion of DEGs in the pairwise comparisons Pc vs PcPn_E and Pc vs PcPn_L. Significantly upregulated and downregulated genes are represented by red and blue dots, respectively, and grey dots represent genes without a significant differential expression.

(B) Bar plot depicting the number of DEGs in each pairwise comparison.

(C) Venn diagram represents the summary of the overlap between DEGs.



SRF: 3
Technical Assistant: 1
PhD Students: 3

Gayathri G S, Dr S Manjula, Mookul Samader (From L to R)

PUBLICATIONS:

- Vijayakumar S, Sakuntala M. Validation of reference gene stability for normalization of RT-qPCR in Phytophthora capsici Leonian during its interaction with Piper nigrum L. Sci Rep. 2024;14(1):7331.

AWARDS [STUDENTS]:

- Saranya.V. Best Oral Presentaion award, 'Understanding Phytophthora capsici pathogenesis in Piper nigrum L. through gene expression profiling and effector functional studies' National Symposium on Plant Health Management: Current Trends and Novel Mitigation Strategies, September 11 to 12, 2023 at ICAR-CTCRI, Trivandrum
- Liya Kurien. Best Oral Presentation Award. 'Insights into the role of Receptor-like Kinases in essential oil mediated 'Defense Priming' against Phytophthora capsici' National Symposium on Plant Health Management: Current Trends and Novel Mitigation Strategies, September 11 to 12, 2023 at ICAR-CTCRI, Trivandrum

INVITED TALKS [PI ONLY]:

- 'Molecular mechanism of plant-pathogen interaction and 'defense priming' in Piper nigrum x Phytophthora capsici pathosystem',International Conference on Emerging Trands in Genomics and Biomedicine. Inter University Centre for Genomics and Gene Technology, University of Kerala, Kariavattom Nov 15-17, 2023.
- 'Mutiomics approach for deciphering defense priming and innate immunity during Piper nigrum(L) - Phytophthora capsici interaction'.National Conference on Integration of Multi-omics Approaches for Crop Improvement, TNAU, Coimbatore, March 5-6, 2024

CONFERENCE PRESENTATION:

- Indu.M. Oral Presentation. 'Priming' Induced Metabolic Reprogramming Imparts Improved Resilience to Piper nigrum L. from Phytophthora capsici infection. National Symposium on Plant Health Management: Current Trends and Novel Mitigation Strategies, September 11 to 12, 2023 at ICAR-CTCRI, Trivandrum.
- Indu.M. Poster Presentation. 'Elucidating the molecular basis of 'Priming' in Piper nigrum L. for exploring its potential in crop protection' International Conference on Recent Trends in Biological Sciences 2023. January 17-19, 2023. Inter University Centre for Evolutionary and Integrative Biology, University of Kerala, Thiruvananthapuram.

- Saranya.V. Poster Presentation. 'Time course transcriptomic analysis reveals orchestrated expression of pathogenicity related genes in Phytophthora capsici during its interaction with Piper nigrum L'. International Conference on Recent Advances in Biological Science. January 17-19, 2023. Inter University Centre for Evolutionary and Integrative Biology, University of Kerala, Thiruvananthapuram.
- Saranya.V Poster Presentation. 'Molecular dynamics of Piper nigrum- Phytophthora capsici interaction: the pathogen's perspective'. International Conference on Emerging Trends in Plant Science', March 6-7, 2023. Catholicate College, Pathanamthitta, Kerala.
- Liya Kurien. Poster Presentation. 'The potential role of Piper nigrum Receptor-Like Kinases (RLKs) proteins in fine-tuning host immunity to the 'foot-rot' causing oomycete pathogen Phytophthora capsici'. International conference on 'Current trends and future prospects of Plant Biology'. February 23 to 25, 2023. Department of Plant sciences, School of Life Sciences, University of Hyderabad, Telengana.
- Mookul Samader. Poster Presentation ' Insights into the secretome of Phytophthora capsici Leonian during its infection of Piper nigrum L through LC-MS/MS approach' International Conference on Fungal Biology and Plant-Microbe Interactions 2024" (ICFBPMI-2024), 16-18 February 2024, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi.

PATENTS APPLIED/ GRANTED:

- Patent Applied: An Antifungal Synthetic Peptide Derived from Osmotin Protein (INDIA)

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Exploiting Defense Priming in Tea (Camellia Sinensis) for crop protection against major insect pests Tea mosquito bug (Helopeltis theivora) & Red spider mites (Oligonychus Coffeae) and diseases (Blister blight, Exobasidium vexans and grey blight, Pestalopsis theae) and metabolome-assisted identification of biomarkers for quality improvement	National Tea Research Foundation (NTRF)	2024	March	PI



Anish Kundu, PhD

Scientist C
Plant Biotechnology & Disease Biology

BRIEF THEME OF LABORATORY

To study defense and growth related metabolic alteration of plant systems (both model and crop plants) during interactions with the ecologically important biotic factors, such as growth promoting endophytes, pathogens and insect herbivores.

MAJOR RESEARCH AREA

- To study metabolic alteration in Ginger upon growth promoting Piriformospora indica colonization which showed significant growth promotion.
- To study metabolic alteration in Rice upon growth promoting Piriformospora indica colonization which showed significant growth and yield associated trait promotion in this crop.
- Role of Jasmonic acid signalling in primary metabolism alteration upon herbivory in Plants.

- **ELUCIDATING MOLECULAR MECHANISM OF DEFENSE AND GROWTH RELATED METABOLIC ALTERATION OF PLANT SYSTEMS DURING INTERACTIONS WITH THE ECOLOGICALLY IMPORTANT BIOTIC FACTORS.**

We have devided the research theme in three parts. In the first part, we have established the optimized protocol of colonization of Piriformospora indica, a growth promoting endosymbiotic fungus in Ginger (Zingiber officinale) roots . This colonization showed significant growth promotion in Ginger plants (Fig. 1A and B). Our next aim will be to identify whether P. indica can increase tolerence in Ginger against devastating soft rot creating pathogen Pythium myriotylum.

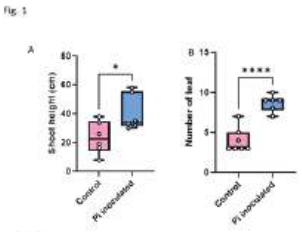
Next, we have optimized colonization of P. indica in indigenous rice vareity (indica) Dani Gora. P. indica showed a siginificant growth promotion in roots and shoots upon 15 day of post inoculation. We also checked the five yield associated phenotypes (i.e. panicle per plant, panicle length, seeds per panicle, 1000 grain weight, leaf width) in matured rice colonized with P. indica and observed significant induction of these phenotypes (Fig. 2A and B). Next, we shall aim to go for root and shoot specific global metabolomics and will identify the metabolie and pathway biomarkers, those are crucially responsible for this growth and yeild promotion.

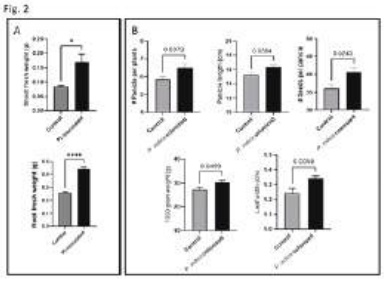
In the third part of the research theme we showed imparement of Jasmonic acid signalling differentially altered primary metabolic pathway. We observed differential enrichment of sugar and amino acid metabolism in JA signalling mutants coi1-1 and myc2. Methionine (Met) induction was also found to be inturrupted in coi1-1 which indicated less biosynthesis of aliphatic glucosinolate during herbivory (Fig. 3 A, B and C). Aliphatic glucosinolates are crucial defense metabolites against herbivores in Arabidopsis and other Brassicaceae plants. This suggests that jasmonate signaling is crucial for herbivore mediated induction of precursor amino acids for aliphatic glucosinolates biosynthesis.

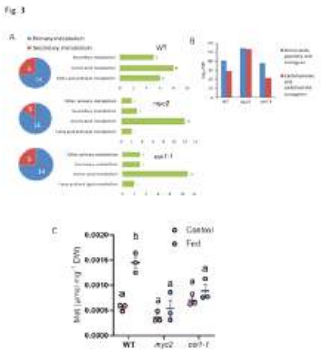
Fig. 1 Quantitative analyses of (A) shoot height and (B) leaf numbers in control and P. indica colonized Ginger. Significant analysis was done by t-test, P*<0.05, P**<0.01, P***<0.001.

Fig. 2. (A) Quantitative analyses of Dani Gora root and shoot phenotypes in control and P. indica colonized plants. Significant analysis was done by t-test, P*<0.05, P**<0.01, P***<0.001. (B) Quantitative phenotypic alteration in matured P. indica colonized and control rice.

Fig 3. (A) Broader classification of herbivore-induced significantly enriched metabolism classes in WT, myc2 and coi1-1; (B) Significantly enriched metabolite classes (P<0.05, FDR<0.05) which contributes mostly in perturbation of primary metabolism upon herbivory in jasmonate mutants (myc2 and coi1-1) in compare to WT. (C) Mean ±SE Met content in all the three genotypes. Statistical significance was estimated by two-way ANOVA. Different letters indicate significant differences, P<0.05.









Technical Assistant: 1
Lab Assistant: 1
PhD Students: 1

Dr. Anish Kundu, Manjima A.M

PUBLICATIONS:

- Kundu A. Antimicrobial to anti-herbivore: Sakuranetin in rice efficiently inhibits brown planthopper by targeting their beneficial endosymbionts. Physiol Plant. 2023 Nov-Dec;175(6):e14110.

INVITED TALKS [PI ONLY]:

- Anish Kundu, Grow better with mutualism: Piriformospora indica employs host's putrescine for growth promotion in plants, SERB sponsored National Seminar on Advancements in Plant Sciences, 3-4 January 2024, St. Berchmans College, Changanassery, Kerala

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Exploiting Defense Priming in Tea (Camellia sinensis) for crop protection against the major insect pests(Tea mosquito bug (Helopeltis theivora) & Red spider mites (Oligonychus coffeae) and diseases (blister blight, Exobasidium vexans and grey blight, Pestalopsis theae) and metabolome-assisted identification of biomarkers for quality improvement	National Tea Research Foundation (NTRF)	2024	3 Years	Co-PI



Moumita Srivastava, PhD

Scientist C
Plant Biotechnology & Disease Biology

BRIEF THEME OF LABORATORY

Our lab studies molecular mechanisms of plant immune responses – how the response of individual cells regulates the intracellular signalling events triggered after the detection of a molecule (effector) of pathogen /host origin – using the post-translational modifier protein SUMO as a starting point. We are interested in understanding the mechanism and regulation of SUMO proteases, the enzymes which cleave-off the SUMO from their substrate.

◆ EXPLORING THE ROLE OF SUMOYLATION IN REGULATING PLANT GROWTH AND DEVELOPMENT

We are currently a new lab working on establishing our hypothesis. I am working on plants like Arabidopsis, black pepper and rice. In Arabidopis, we are currently exploring the molecular mechanisms that regulate blue light mediated endoplasmic reticulum stress response. Our preliminary results give strong evidence that blue light promotes endoplasmic reticulum stress responses. With the current progress in the investigation, MYC2 seems to have a crucial role in regulating these responses, however, more investigation is required to confirm the hypothesis.

In black pepper, we are currently working to identify the components of the SUMOylation machinery. Our computational analysis has identified one SUMO isoform that is closely related with Arabidopsis SUMO1. We have also identified SUMO conjugation machinery including the SUMO activating enzyme (SAE1a, SAE1b and SAE2), SUMO conjugating enzyme (SCE1) and SUMO ligating enzymes (SIZ1 and HPY2) in black pepper. Currently, we are working on characterising these components biochemically.

Another part of the work focusses on identifying the WRKY transcription factors in plants. WRKY transcription factors are an important group of proteins that are mainly involved in plant immune responses. Our computational analysis has identified the members of the WRKY transcriptional factors family in black pepper. Currently we are working to identify the WRKY transcription factors involved in regulating plant immune response against the pathogen Phytophthora capsici through biochemical and phtysiological analysis.

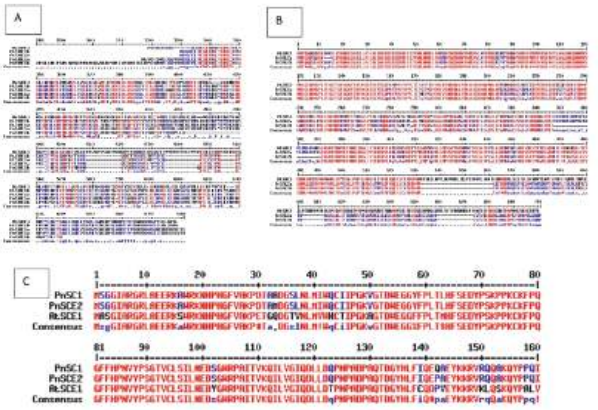


Figure 3: Sequence alignment of SUMO activating enzymes, SAE1 (A) and SAE2 (B) and SUMO conjugating enzyme SCE1 (C) in black pepper (*Piper nigrum*) with *Arabidopsis thaliana*.

Sequence alignment of SUMO activating enzymes, SAE1 (A) and SAE2 (B) and SUMO conjugating enzyme SCE1 (C) in black pepper (Piper nigrum) with Arabidopsis thaliana.



Lab Assistant: 2
PhD Students: 1

Dr Moumita Srivastava,
Priyanka T (From L to R)



NEUROBIOLOGY REGENERATIVE BIOLOGY & REPRODUCTION BIOLOGY PROGRAM



Malini Laloraya, PhD, FNASc

Scientist G
Reproduction Biology

BRIEF THEME OF LABORATORY

My laboratory mainly focuses on understanding molecular events crucial for embryo implantation since Failed implantation represents a major obstacle in assisted reproduction and is called 'black-box of assisted reproduction'. Polycystic Ovarian Syndrome (PCOS) which is associated with sub-fertility due to a higher risk of implantation failure, is our second focus of research. In this, we use a systems biology approach in tandem with immune profiling and impact of circadian cues on PCOS pathogenesis

MAJOR RESEARCH AREA

- Embryo implantation – Created human Endometrial Organoids (EMOs) to assess uterine receptivity changes in Recurrent Implantation Failure.
- Polycystic Ovary Syndrome -Deciphering the altered immune landscape in PCOS Pathogenesis:
  (i) Identified an increase of an exhaustion phenotype "PD1+ve T cells" in PCOS. Treatment with PD1 antibody rejuvenated Treg from their exhausted phenotype.
  (ii) Treg phenotypes in PCOS is of the type PD1high T-betlow.

- **UNDERSTANDING THE PATHOGENESIS OF RECURRENT IMPLANTATION FAILURE (RIF) AND POLYCYSTIC OVARIAN SYNDROME (PCOS).**

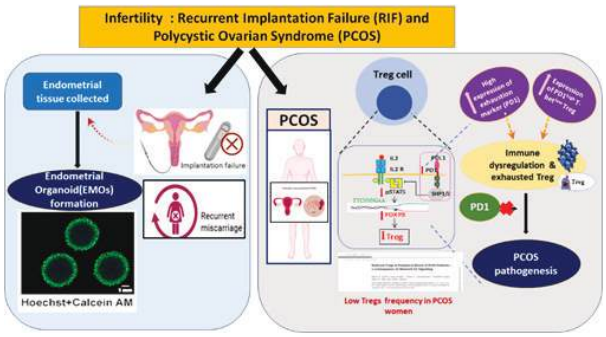
CREATION OF ENDOMETRIAL ORGANOIDS AS A TOOL TO STUDY RECURRENT IMPLANTATION FAILURE-

Pregnancy is central to this process in mammals. Despite giant leaps in technologies in assisted reproduction, our success rate has remained low. This is due to the fact that in women being treated for infertility, even when healthy embryos are transplanted, the failure rate is high: "up to 70 % fail to dock successfully in the uterine lining". This is largely due to our inability to understand human embryo implantation and identify molecular markers, of uterine receptivity status. We have used recurring implantation failure(RIF) for this as RIF subjects are known to exhibit decreased endometrial receptivity. To gain a better insight into the problem associated with RIF we created Endometrial Organoids (EMOs) derived from these Endometrial biopsy(EB) endometrial organoids as Human EMOs show all the physiological, cycle-mimicking responses to hormones – viz., proliferative, secretory and menstrual phases thereby recapitulating that of endometrium. We successfully created EMOs (Figure 1A). The creation of EMOs would enable the use of organoids for testing drugs for RIF treatment. (Funding DBT-EMF)

PCOS PATHOGENESIS IS ASSOCIATED WITH REGULATORY T CELL EXHAUSTION:

PCOS is one of the major endocrine-metabolic disruptions in fertile-aged women that is manifested with immune complications such as low-grade inflammation, autoantibodies, and reduced immune tolerance. Immune tolerance is given by a specific T cell subset "regulatory T cells" which are capable of highly immunosuppressive actions and can regulate immune homeostasis, thus maintaining self-tolerance. Earlier, we had reported reduced peripheral Treg levels in PCOS women compared to normal females (Krishna MB et al 2015), which signifies an altered immune tolerance in PCOS. Interestingly, we witnessed a significant increase of an exhaustion phenotype "PD1+ve T cells" in the PBMCs of PCOS women via epigenetic, gene expression analysis. T cell exhaustion critically impairs the effective action of T cells to control proinflammation and immune suppression. Further increased PD1+ve Treg population

in PCOS *women via FACS analysis confirmed Treg exhaustion. Additionally, we observed heterogeneous exhausted Treg phenotypes showing a significant difference in PD1high T-betlowTreg populations in PCOS conditions compared to control. A minimalistic expression of Eomes+veTreg was seen in both control and PCOS subjects. As T-bet represses the expression of PD1 and maintains the effective function of CD8+ T cells, a low T-bet expression on the exhausted Tregs may be another potential cause behind the overexpression of PD1, subsequently leading to Treg reduction in PCOS pathogenesis. Upon blocking the overexpressed PD1 with its antibodies, the PBMCs of PCOS subjects show an expansion of the Treg population, confirming a critical role of higher PD1 in lowering the Treg population. This suggests that Tregs can be rejuvenated from the exhausted state. Hence, overexpression of exhaustion phenotype "PD1" signifies Treg exhaustion in PCOS condition, which hinders Treg differentiation and thus reduces the Treg population in PCOS women. (Funding DBT-IMF/ICMR-EMF).*



Cartoon showing causes of Infertility during Recurrent Implantation Failure (RIF) and Polycystic Ovary Syndrome(PCOS) Pathogenesis.



PhD Students: 1
JRF: 5
Lab Assistant: 2

First Row: Jaskirat Singh Sandhu, Barsha Bharati, Atheena Menon G
Second Row: Adithian M S, Malini Laloraya, Lipika Priyadarsini Patra, Deepthi Prakash C, Anshad A

PUBLICATIONS:

- Krishna MB, Johnson BS, Vasudevan M, Pillai SM, Laloraya M. miRNA-mRNA Network in PBMCs of PCOS Women Identifies Overactivated Stress-Activated Kinases. Cell PhysiolBiochem. 2023 Apr 30;57(2):137-156

AWARDS [PI]:

- Awards Committee Member of The American Society for Reproductive Immunology (2022-2025).

AWARDS [STUDENTS]:

- Atheena Menon G was awarded the Prof.ShamimHaider memorial gold medal award for the best oral presentation under the Auspices of Indian Society of Comparative Endocrinology (ISCE) organized at the Department of Zoology, SavitribaiPhule Pune University, Pune, India during 12th –14th October, 2023.
- Lipika Priyadarsini Patra was awarded the best poster presentation in the RGCB National Symposium on Biotechnology for Sustainable Development – 2023 on 12th April 2023.

INVITED TALKS [PI ONLY]:

- PCOS-where endocrinology meets immunology. S-15: REPRODUCTIVE IMMUNE-ENDOCRINE CROSS-TALK: IMPLICATIONS FOR WOMEN'S HEALTH at the 42nd Annual Meeting of the American Society for Reproductive Immunology held during May 20, 2023 to May 25, 2023 at La Fonda on the Plaza, Santa Fe, New Mexico, USA.
- The Many Facets of Oxyradicals – From Stem Cells to Embryo Implantation with Implications for Assisted Reproduction" at International Conference on Molecular Medicine, Reproduction and Endocrinology (ICMMFE-23) and as part of the 40th annual meeting of the Society for Reproductive Biology and comparative endocrinology (SRBCE), during 14-16 September 2023 at Division of Life Science, School of Science, Navrachana University, Vadodara.
- Managing PCOS - All about resetting your circadian rhythm" Resource person for National workshop on Circadian Biology during 11-12th September 2023 organized by Department of Zoology, The Maharaja Sayajirao University of Baroda, on the theme "Molecular Circadian rhythms in health and disease".
- "Reproduction Research - essence of Life" at Biotechnology Department, Motilal Nehru National Institute of Technology, Allahabad on 26th October 2023.

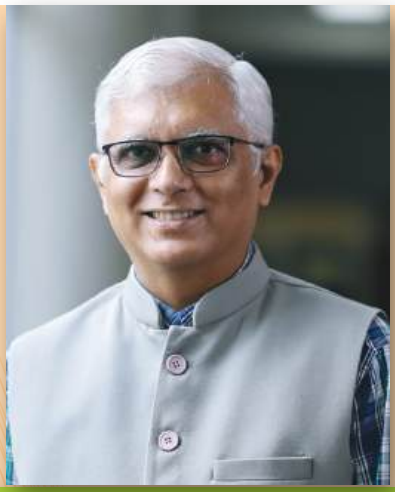
- "Towards the development of Newer strategies for improved Infertility Management" at the Trivandrum OBGyn Meeting on Nov 16 2023, Trivandrum.
- "Cognition and spatial memory dysfunction in PCOS-a consequence of altered brain wiring?" at the International Symposium on Recent Trends in Comparative Endocrinology and Physiology: from brain to behaviour; under of Auspices of Indian Society for Comparative Endocrinology during 12014 October 2023 held at Department of Zoology, S. P. Pune University, Pune.
- Cracking the Immune Landscape of PCOS at International Conference on Molecular Signaling, 2024, February 1-3, 2024 at the Department of Animal Biology, School of Life Sciences, University of Hyderabad, Hyderabad, India.

CONFERENCE PRESENTATION:

- LipikaPriyadarsiniPatraposter presentation of her work entitled "Phosphorylation defects of STAT5 signaling: a probable reason for low Treg differentiation in PCOS" in the RGCB National Symposium on Biotechnology for Sustainable Development – 2023 in 12th April 2023, RGCB, Thiruvananthapuram, Kerala.
- LipikaPriyadarsiniPatra poster presentation of her work entitled "PCOS is associated with Treg heterogeneity with presence of PD1 high T-bet low Tregs." in the 1st RGCB conference – 2023 (20th-23rd September 2023), Hotel TheRaviz, Kollam, Kerala, India.
- Atheena Menon G oral presentation of her work entitled "Deciphering the Frequency of Different Subtypes of CD19+ B Cells in Polycystic Ovarian Syndrome" presented at International Symposium om Recent Trends in Comparative Endocrinology and physiology: from Brain to Behaviour" under the Auspices of Indian Society of Comparative Endocrinology (ISCE) organized at the Department of Zoology, SavitribaiPhule Pune University, Pune, India during 12th –14th October, 2023.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Investigating the role of superoxide in regulating the major events during embryo implantation.	Science & Engineering Research Board	2020	3 years 7 months	PI
02	Molecular Analysis of Circadian Rhythm in Polycystic Ovarian Syndrome Patients.	Department of Biotechnology	2020	3 Years 4 months	PI
03	Ascertaining the causes of low tregs in polycystic ovarian syndrome patients.	Indian Council of Medical Research	2021	3	PI
04	Creation of patient derived endometrial organoids for understanding the underlying causes of RIF.	Department of Biotechnology	2022	3	PI



Moinak Banerjee, PhD

Scientist G
Neurobiology

BRIEF THEME OF LABORATORY

Investigating complex disorders of brain with specific interest on genetics and epigenetics and developing tools for personalised medicine. Fundamental aspects of complex disorders lies in its population and its environment, which is responsible for its phenotypic, metabolomic and biochemical diversities.

MAJOR RESEARCH AREA

- Resolved genetic diagnosis for more than 100 rare disease condition.
- Identified Antiepileptic drug specific proteome profile.
- Developed pharamcogenetic profiles of antihypertenives.
- Developed deep learning tool for predicting the regulatory functions of non coding variants and their significance in neurological conditions.

- **GENOMICS AND EPIGENOMICS OF COMPLEX DISEASES OF BRAIN**

Mission program on PediatricRare Genetic Disorders (PraGeD) PRaGeD program at RGCB, 120 rare condition probands were recruited from accross Kerala. We have resolved 76 cases using candidate gene genotyping, WES, MLPA, Arrayand 44 are under analysis.

Genetics of Developmental and Epileptic Encephalopathies (DEE): Whole exome sequencing analysis of 155 DEE probands including 138 trios has been completed and genetic test reports have been issued to these patients. The diagnostic yield (61/155=39.3%) was investigated based on the number pathogenic or likely pathogenic variants identified. The most common type of disease-causing variant identified was missense variants (72%), and the remaining included frameshift variants (15%), non-sense variants (8%), splice site variants (3%) and CNVs (2%).

Pharmacoepigenetics of antiepileptic drugs: The present study involves assessing the epigenetic modifications and proteome profile induced by antiepileptic drugs in cell culture model. We identified 111 unique proteins exclusive to the Carbamazepine, 110 unique proteins exclusive to the Phenytoin and 119 unique proteins exclusive to the Valproic acid treated group. The proteins linked to antiepileptic drug treatment are significantly connected to various metabolic processes, including RNA and non-coding RNA metabolism, regulation of carbohydrate catabolism, ribosome biogenesis, translational initiation and peptide biosynthesis.

Pharmacoepigenetics of antihypertensive drugs: The "trial and error" strategy of antihypertensives results in adverse effects and treatment-resistant hypertension. Pharmacogenetics explores how genetic makeup affects medication outcomes, allowing personalized treatment. We performed genotyping array with 750000 SNVs, in patients with and without hypertension. We observed 20 reported pharmacogenetic variations involving transporter, metabolizing, and drug target genes. Examining these variations showed that a substantial percentage of patients had genetic profiles linked to decreased or zero response to specific medications. The study also found that, for various pharmacogenes in the Indian population, the haplotype

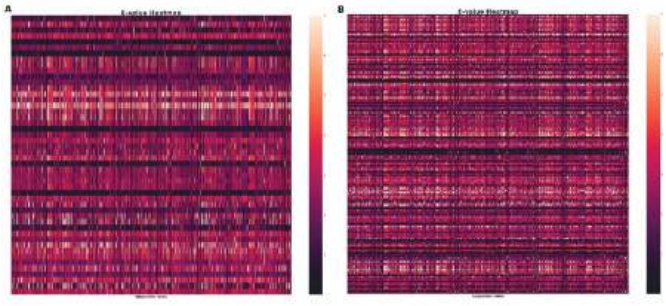
frequency for poor and non-responsiveness varied.

Genetics and epigenetics of Hearing loss: Nearly 150 genes were reported to be associated with NSHL and most of the genetic variations identified in NSHL-associated genes are in the noncoding regions. These noncoding variants can affect gene expression directly by disrupting the interaction between the regulatory elements (distal and proximal enhancers) or indirectly by disrupting the TADs and the chromatin organization. Using genotyping array with 650000 we identified significantly associated SNV and predicted the CNVs. CNVs were predicted computationally using two different tools using different prediction algorithm. The filtered CNVs are mapped to the reference genome, and CNVs in the 1M neighborhood of NSHL-associated genes were selected. The candidate CNVs that overlap with known regulatory elements and those commonly predicted will further be used for identifying their role in TAD disruption.

Artificial intelligence in identifying regulatory interactions: We developed a deep-learning model named Neur-Ally, which was trained on epigenomic datasets from nervous tissue and cell line samples. The model predicts differential occurrence of regulatory features like chromatin accessibility, histone modifications and transcription-factor [TF] binding on genomic regions using DNA sequence as input. The model was used to predict the regulatory effect of neurological condition specific non-coding SNPs using in-silico mutagenesis. The effect of associated SNPs reported in Genome-wide association studies [GWAS] of neurological condition, Brain eQTLs, Autism Spectrum Disorder [ASD] and reported probable regulatory SNPs in neurological conditions were predicted by Neur-Ally.

Fig A. Unique proteins peculiar to Valproic acid-treated samples and their biological process.

Fig B. E-value heatmap showing significant variants A.Neurological condition specific GWAS SNPs, B. Neurological condition specific eQTL SNPs. Variants in rows and labels in columns.





JRF: 2
SRF: 1
Project Associate: 1
Postdoctoral Fellows: 1
PhD Students: 4

Ruchira Menghal, Rashmi Sukumaran, Ardra M, Dr Moinak Banerjee, Archisha Banerjee, Shabith Raj, Neethu Mohan, Samyukta Bhass (From L to R)

PUBLICATIONS:

- Prakash A, Banerjee M. An interpretable block-attention network for identifying regulatory feature interactions. Brief Bioinform. 2023 Jul 20;24(4):bbad250.
- Mullapudi T, Debnath M, Govindaraj R, Raj P, Banerjee M, Varambally S. Effects of a six-month yoga intervention on the immune-inflammatory pathway in antipsychotic-stabilized schizophrenia patients: A randomized controlled trial. Asian J Psychiatr. 2023 Aug;86:103636.
- Krishna S, Fasaludeen A, Jose M, Banerjee M, Sundaram S, Radhakrishnan A, Menon RN. Impact of variant subtype on electro-clinical phenotype of Dravet syndrome- a South Indian cohort study. Seizure. 2024;115:81-86.
- Jose M, Fasaludeen A, Pavuluri H, Rudrabhatla PK, Chandrasekharan SV, Jose J, Banerjee M, Sundaram S, Radhakrishnan A, Menon RN. Metabolic causes of pediatric developmental & epileptic encephalopathies (DEE)- genetic variant analysis in a south Indian cohort. Seizure. 2024;115:20-27.
- Polakkattil BK, Vellichirammal NN, Nair IV, Nair CM, Banerjee M. Methylome-wide and meQTL analysis helps to distinguish treatment response from non-response and pathogenesis markers in schizophrenia. Front Psychiatry. 2024 Mar 7;15:1297760.
- Sukumaran R, Nair AS, Banerjee M. Ethnic and region-specific genetic risk variants of stroke and its comorbid conditions can define the variations in the burden of stroke and its phenotypic traits. eLife 2024: 13:RP94088.
- Thanseem I, Banerjee M, Melempatt N, Prakash A, Iype M, Anitha A. Comprehensive Genetic Study of a Monozygotic Triplet Discordant for Autism Spectrum Disorder. Neurol India. 2024 Mar 1;72(2):384-387.

INVITED TALKS [PI ONLY]:

- "Integrating creatitivity and critical thinking in the learning process." FDP on "Possibilities and Potentials of National Education Policy 2020 (NEP 2020)" Internal Quality Assurance Cell (IQAC), The Neotia University (TNU) in association with Guru Angad Dev Teaching-Learning Centre of MHRD, Govt. of India (under PMMMNMTT) at SGTB Khalsa College, University of Delhi. June 20-July 3, 2023.
- Epigenetic dissection of Neuropsychiatric Diseases: Hypes and hopes. International GATC conference in genomics, INSTEM, Bengaluru, April 7-9, 2023
- Pharmacoepigenetics of antipsychotics may resolve precision medicine in schizophrenia. Center for Human Genetics, Bengaluru. 10th April 2023.
- Resolving conflicts in developmental disorders using genetic and epigenetics. Association of Child Neurology of India, AIIMS, New Delhi. 19th April 2023.
- Deriving hopes in autism spectrum disorder: Challenges and opportunities. National Seminar "Epigenetics meets Meets Metabolomics", at Institute for Communicative and Cognitive Neurosciences (ICCONS), Shoranur, on 09-10th June 2023
- Genetics, epigenetics and metabolomics of iSAH: an Indian perspective. 16th Intl. Subarachnoid Hemorrhage Conference, Duke University, Durham USA. 16-18 June 2023.
- Dissecting dilemmas in neurodevelopmental disease. 8th Annual (International) Conference of Board of Genetics Counseling, Genetics and Genomics in Health and Diseases. University Of Hyderabad, 7-9 July 2023.
- Paradigm shift in understanding genetics and epigenetics of Schizophrenia. Asian genetics Section: International Conference of the Genetics Society of Korea. BEXCO, Busan, Korea. Oct. 18 - 20, 2023.
- Decoding the hypes and hopes of drug discovery and precision medicine in Schizophrenia: A genomic and epigenomic perspective Theme: Applications of Bioinformatics in Health Care, Indian Conference on Bioinformatics 2023 (#InBix2023), SBST, VIT, Vellore, 24-26 Nov., 2023.
- How to dissect a complex disease. National seminar on "NanoGenPro Triad: Exploring the future science" SB College Changanasserri. 10th Jan 2024.
- Genetic and Epigenetic Dissection of Complex brain disorders. Central University of South Bihar, Gaya, 11th March 2024.
- Dilemma's in biomedical research and way to resolve. ICAR-Indian Institute of Agricultural Biotechnology, Ranchi. 12th March 2024.
- Resolving complexities of stroke needs a precise stratification approach. GATC2024, ICGEB, New Delhi. 12-14th April, 2024.

CONFERENCE PRESENTATION:

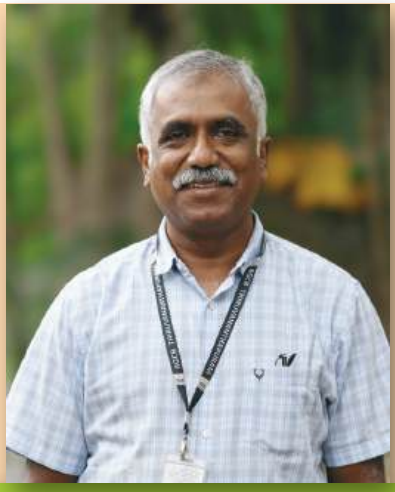
- SamyuktaBhass: Oral presentation for the National Seminar "Epigenetics meets Meets Metabolomics", June 2-3, 2023, Institute for Communicative and Cognitive Neurosciences (ICCON), Shoranur, Kerala.
- Alfiya F: Digital poster presentation "Functional and structural characterization of variants of uncertain significance identified from whole exome sequencing of developmental and epileptic encephalopathies in an Indian cohort: an In Silico approach" at the 35th International Epilepsy Congress, 2-6 September, 2023, Dublin, Ireland.
- Alfiya F: Oral presentation "Diagnostic yield of Trio-Whole exome sequencing and Clinical exome sequencing in children with Developmental and epileptic encephalopathy" at Epilepsy conference, ECON23, 21st to 23rd July, 2023, Marriot hotel, Jaipur, Rajasthan.
- Alfiya F: Oral presentation "Pathogenic Metabolic gene variations in Developmental and epileptic encephalopathy" at the National Seminar: Epigenetics meets Metabolomics, June 2-3, 2023, Institute for Communicative and Cognitive Neurosciences (ICCON), Shoranur, Kerala.
- Alfiya F: Platform presentation "Comparing the diagnostic yield and clinical utility of Trio-Whole exome sequencing and Clinical exome sequencing in children with Developmental epileptic encephalopathy" at International Child Neurology Congress 2024 (ICNC2024), May 6-10, 2024, Cape Town, South Africa.
- Neethu Mohan: Oral Award paper presentation titled " EvaluatingPharmaco-epigenomic response of Antiepileptic drugs" at the Epilepsy Conference, ECON23, 21st to 23rd July, 2023, Marriot hotel, Jaipur, Rajasthan.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Mission mode program on rare genetic disorder	Department of Biotechnology	2023	5 year	PI
02	Novel derivatives of Tacrine—- a preclinical study	Indian Council of Medical Research	2022	3 year	Co-PI
03	Genetics of Moya moya Disease	Department of Biotechnology	2023	3 year	Co-PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Anil Prakash	Evaluating an integrative model for genotypic heterogeneity in Autism Spectrum disorder"	University of Kerala	Submitted	2023
02	Binithamol K Polakkattil	Identifying The Epigenetic Signatures Of Treatment Response In Schizophrenia	University of Kerala	Submitted	2024
03	Rashmi Sukumaran	Identifying genetic and functional correlates of comorbidity factors in Stroke	University of Kerala	Submitted	2024
04	Thrinath M	Understanding the Effects of Add-on Yoga Therapy on Immune-Inflammatory Markers in Schizophrenia	NIMHANS	Submitted	2024



Jackson James, PhD

Scientist G
Regenerative Biology

BRIEF THEME OF LABORATORY

The main focus of our lab is to understand the early developmental cues that promote neural stem cell maintenance and fate specific differentiation which will shed light in developing possible therapeutic strategies against neurodegenerative diseases.

MAJOR RESEARCH AREA

- Functional relevance of a unique subclass of Notch independent Hes-1 (NIHes-1) expressing neural stem cells in developing/adult cortex.
- Regulation of stemness by Pleiotropic Hes-1 expression in neuroblastoma/glioblastoma.
- Role of canonical and non-canonical Notch signaling associated molecules in understanding the development of quiescent neural stem cell pool.
- Guiding retinal ganglion cell axons to brain visual centers.
- Functional characterization of Homeobox gene Tlx3 (Hox11L2) in cerebellar development and its link to Autism Spectral Disorder (ASD).

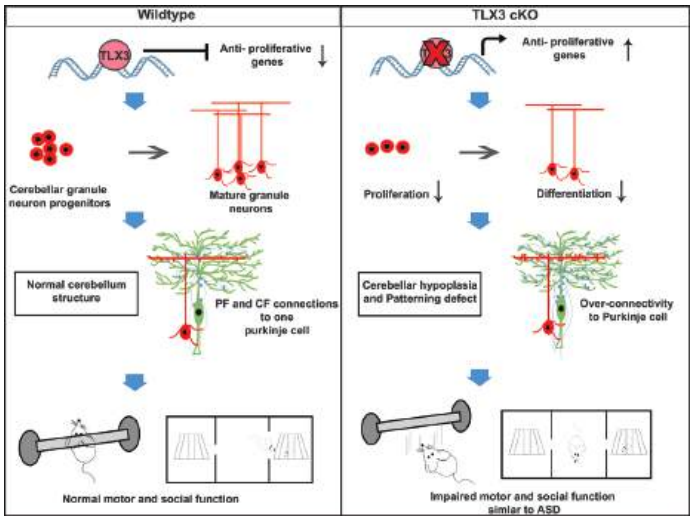
◆ TLX3 IS A CRUCIAL REGULATOR OF GRANULE NEURON PROGENITOR PROLIFERATION DURING EARLY CEREBELLUM DEVELOPMENT AND ITS DYSFUNCTION CAN LEAD TO AUTISM

TLX3 is a Homeobox domain transcription factor, initially discovered and implicated in T cell leukaemia, and thereafter, identified and revealed its prominent role in the development of the peripheral and central nervous system (PNS and CNS). TLX3 is well known as a master regulator of the fate specification of excitatory neurons and is primarily known to function in post-mitotic cells. However, previous work from our lab has shown that TLX3 is expressed in the proliferating granule neuron progenitors (GNPs) of the cerebellum. In addition, we have also demonstrated that PAX6 regulates the expression of TLX3 in the posterior cerebellum, which in turn controls a few autism spectrum disorder (ASD) candidate genes. These findings are intriguing since we demonstrated a previously unidentified association between TLX3 in development and disease. Previous studies on TLX3 in the PNS and CNS did not show an association with any pathological condition. The only pathological association of TLX3 has been reported in T-cell leukaemia due to a recurrent interchromosomal translocation in T-cell acute lymphoblastic leukaemia cells. Therefore, we conducted this study to understand the role of TLX3 in proliferating GNPs, its primary role in cerebellum development, and its association with ASD.

To achieve this, we used cGN-specific Tlx3-conditional knock-out mice that were only affected in the cerebellum. We found that loss of TLX3 in the early stage of cerebellum development results in decreased proliferation of cerebellar GNPs by directly targeting anti-proliferative genes such as Tgfb2 (TGF β 2), Cadm1, and Cdk2ap1. Consequently, the altered generation of cerebellar GNPs has led to cerebellar hypoplasia along with severe defects in the cerebellar patterning. We also observed cell type proportion changes upon TLX3 deletion, where the granule neuron proportion in the Tlx3 cKO was significantly decreased whereas Purkinje cells (PC) were drastically increased. Following this, we assessed the primary synaptic connections of the cerebellum. PC forms the sole output of the cerebellum, where it is innervated and synaptically connected by climbing (CF) and granule neuron extension parallel fibres (PF). We found that synaptic connections PC-PF and PC-CF were increased and that each Purkinje cell was highly innervated and connected with enhanced PF and CF puncta following Tlx3 deletion.

Having established that TLX3 dysfunction from the GNPs caused significant structural and molecular changes in the cerebellum, we next evaluated the various behavioral deficits in Tlx3cKO. The cerebellum is associated with discrete behavioral activities such as fine motor coordination, cognitive abilities, and social functions. Furthermore, the posterior cerebellum is implicated in cognition and social development, whereas the anterior cerebellum contributes to fine motor development. We found that TLX3 cKO mice exhibit a typical ASD-like phenotype with observable impairments in motor, cognitive, and social functions. Finally, we assessed the potential possibility of TLX3 mutation in ASD human subjects and found the presence of TLX3 mutation associated with ASD patients.

Altogether, our findings establish the crucial role of TLX3 in the development of the cerebellum, and its absence could be considered one of the causative factors for ASD pathogenesis and manifestation.



Graphical abstract showing the summary of our findings. In the wild type, TLX3 directly suppresses anti-proliferative genes and promotes normal proliferation and differentiation of granule neurons. Hence, the deletion of TLX3 withdraws the suppression over anti-proliferative genes, resulting in decreased proliferation and differentiation of granule neurons. This altered homeostasis culminates in cerebellar hypoplasia, patterning defect, and primary synaptic connections within the cerebellum. Finally, this leads to the atypical ASD-like behavior in mice, together with motor dysfunction.



JRF: 1
Project Assistant: 1
Technical Assistant: 1
PhD Students: 6

First Row: Sreedevi L R, Jyothi P Nair, Aryasree R, Dr Jackson James, Biju S Nair, Rahul Jose (From L to R)

Second Row: Meera v, Parvathy Surendran, Surya Suresh (From L to R)

CONFERENCE PRESENTATION:

- Riya Ann Paul ,Young Scientist Conference (9th India International Science Festival) held on January 17-20, 2024-, Differential Hes1 promoter activation maintains neural stem cell heterogeneity in developing neocortex (Oral Presentation)
- Surya Suresh,XLI Annual meeting of Indian academy of Neurosciences (IAN) and International Conference on "Brain: Chemistry to cognition" held during October 4-6, 2023 at Jiwaji University, Gwalior, India- Does Presenilin I have a role in Neural Stem Cell quiescence? (Poster Presentation)
- Jyothi P Nair ,XLI Annual meeting of Indian academy of Neurosciences (IAN) and International Conference on "Brain: Chemistry to cognition" held during October 4-6, 2023 at Jiwaji University, Gwalior, India- Identifying transcriptional factors involved in non-canonical Hes1 activation (Poster Presentation)

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Functional relevance of a unique subclass of Notch independent Hes-1(NIHes-1) expressing neural stem cells in developing/adult cortex	DST-Science and Engineering Research Board	2022	3 Years	PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Dr. Riya Ann Paul	Differential Hes-1 promoter activation in regulating stem-ness in neocortical development and neuroblastoma	University of Kerala	Awarded	2024
02	Dr. Parvathy Surendran	Functional Characterization of TLX3 in Cerebellum	University of Kerala	Awarded	2024
03	Budhaditya Basu	Elucidation of the role of circular RNA in retina and Hes1 expres-sion in maintaining neural stem cell heterogeneity	Regional Center for Biotechnology (BRIC-RCB)	Submitted	2024



Debasree Dutta, PhD

Scientist E-II
Regenerative Biology

BRIEF THEME OF LABORATORY

The laboratory's primary objective is to exploit cues derived from research conducted during pre- and post-implantation development to gain insights into disorders including pre-eclampsia, leukemia, and breast cancer. These cues are being utilized to develop more effective methods for disease detection, assessment of new biomarkers, and the creation of therapeutic interventions.

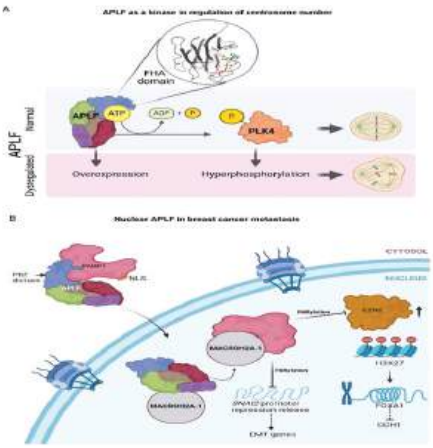
MAJOR RESEARCH AREA

- For the first time, identification of a histone chaperone APLF as a kinase.
- Nuclear and cytosolic shuttling of APLF regulate EMT associated breast cancer metastasis.
- Loss in histone chaperone HIRA contribute to chromatin compaction in Chronic Myeloid Leukemia cells.

- **HISTONE CHAPERONE AND DNA REPAIR FACTOR APRATAXIN PNK LIKE FACTOR IS A KINASE AND ITS NUCLEAR VS CYTOSOLIC SHUTTLING CAN DICTATE EMT ASSOCIATED BREAST CANCER METASTASIS**

Our recent studies revealed the role of mouse Aprataxin PNK-like Factor (APLF) in development. Nevertheless, the comprehensive characterization of mouse APLF remains entirely unexplored. Based on domain deletion studies, here we report that mouse APLF's Acidic Domain and Fork Head Associated (FHA) domain can chaperone histones and repair DNA like the respective human orthologs. Immunofluorescence studies in mouse embryonic stem cells showed APLF co-localized with γ-tubulin within and around the centrosomes and govern the number and integrity of centrosomes via PLK4 phosphorylation. Enzymatic analysis established mouse APLF as a kinase. Docking studies identified three putative ATP binding sites within the FHA domain. Site-directed mutagenesis showed that R37 residue within the FHA domain is indispensable for the kinase activity of APLF thereby regulating the centrosome number. These findings might assist us comprehend APLF in different pathological and developmental conditions and reveal non-canonical kinase activity of proteins harbouring FHA domains that might impact multiple cellular processes.

Metastases from breast cancer account for most fatalities associated with the disease. Our previous report indicated the involvement of APLF in epithelial-to-mesenchymal transition (EMT) associated metastasis of triple negative breast cancer (TNBC) cells. However, non-metastatic cells also exhibited the expression of APLF, the implications of which in disease advancement remain uncertain. Here, we demonstrate that the metastatic prognosis of breast cancer cells may be determined by the cellular localization of APLF. Using TNBC patient samples and cell lines, we discovered that APLF was localized in the nucleus and cytoplasm, whereas other subtypes of breast cancer had cytosolic or perinuclear localization. To investigate metastatic properties in vitro and in vivo, we modeled APLF differential localization by stably producing APLF-tagged nuclear localization signal (NLS) in the luminal subtype MCF7 breast cancer cell line in the absence of putative APLF NLS. We obtained the mechanistic insight from the functional and molecular studies that PARP1 facilitates the transport of APLF from the cytosol to the nucleus, assisting in the metastasis of TNBC cells linked with EMT. We anticipate that the inhibition of cytosolic PARP1-APLF interaction may potentially aid in the prevention of breast cancer metastasis in TNBC patients.



A.Schematic representation on the role of APLF as a kinase in the regulation of phosphorylation of PLK4 in the maintenance of centrosome number in mouse Embryonic stem cells.

B. Schematic representation on the shuttling of APLF through PARP1 from cytosol to nucleus in triple-negative breast cancer cells to regulate EMT associated breast cancer metastasis.



JRF: 2
SRF: 1
Lab Assistant: 1
PhD Students: 5

First Row: Fathima Hameed, Anasua Dey, Namrata Chatterjee, Anjali Devarajan, Debparna Nandy **(From L to R)**

Second Row: Mayur Balkrishna Shirude, Dr Debasree Dutta, Sruthy M R, Bindhu M S **(From L to R)**

PUBLICATIONS:

- Varghese PC, Dutta D. Protocol for isolation of mouse pre-implantation embryos for gene expression analysis. STAR Protoc. 2023;4:102479.
- Baral I, Shirude MB, Jothi DL, Mukherjee A, Dutta D. Characterization of a Distinct State in the Continuum of Pluripotency Facilitated by Inhibition of PKCζ in Mouse Embryonic Stem Cells. Stem Cell Rev Rep. 2023;19:1098-1115.
- Hameed JSF, Devarajan A, M S DP, Bhattacharyya A, Shirude MB, Dutta D, Karmakar P, Mukherjee A. PTEN-negative endometrial cancer cells protect their genome through enhanced DDB2 expression associated with augmented nucleotide excision repair. BMC Cancer. 2023;23:399.

AWARDS [STUDENTS]:

- Mayur B Shirude, best oral presentation, HIRA mediated chromatin architecture in regulation of cell fate decision in Chronic Myeloid Leukemia, RGCB Merit award, 16th November 2023, MR Das auditorium RGCB, Trivandrum.

INVITED TALKS [PI ONLY]:

- Epigenetics in development to disease biology, IMMUNO-INDIA 2023:Epigenetic Regulation of Immune Responses , October 01-06, 2023, RGCB, Trivandrum.
- Hematopoiesis to leukemogenesis: through the lens of histone epigenetics, Colloquium Lecture_IHS, Presidency University, 15th February, 2024, Presidency University, Kolkata.

CONFERENCE PRESENTATION:

- Mayur B Shirude, Poster presentation, Histone Chaperone HIRA dictates cell fate by modulating chromatin architecture in Chronic Myeloid Leukemia. RGCB Research Conference 2023, The Leela, Kollam, 20-23 September 2023.
- Mayur B Shirude, Poster presentation, 8th Asian Forum for chromosome and chromatin Biology Conference, held at Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore, 4th-6th November 2023.
- Mayur B Shirude, Talk presentation, 45th Annual International Asilomar Chromatin, Chromosomes, and Epigenetics Conference, held at Asilomar Conference Center" at Pacific Grove, in the Monterey Bay Area, California, USA. 7th December 2023 and concludes on 10th December 2023 (could not attend).
- Mayur B Shirude, Poster presentation, Histone chaperone HIRA regulates chromatin architecture in chronic myeloid leukemia. Genome Organization & Nuclear Function conference, at Cold Spring Harbor Laboratory from April 30 – May 4, 2024.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Normal vs. abnormal hematopoiesis- result of a deregulated chromatin regulated by Histone chaperone HIRA?	Science and Engineering Research Board	2022	3 years	PI
02	Epigenetic regulation of trophoblast stem cells involved in women reproductive health and disease: an observational and functional study.	Indian Council of Medical Research	2023	3 years	PI
03	Dissecting domains of APLF chaperoning EMT in development	Science and Engineering Research Board	2023	3 years	PI
04	Evaluation of histone chaperone APLF as a novel biomarker in triple negative breast cancer.	Department of Biotechnology	2018	6 years	PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Pallavi Chinnu Varghese	Epigenetic modifiers in regulation of development.	Manipal Academy of Higher Education	Awarded	2023
02	Ishita Baral	Role of PKC Signaling in Naive vs. Primed States of Pluripotency	Manipal Academy of Higher Education	Awarded	2023



Rashmi Mishra, PhD

Scientist E-II
Neurobiology

BRIEF THEME OF LABORATORY

The goal of the translational mechanobiology lab is to understand 'how cells sense and respond to the biomechanical microenvironment' with a therapeutic implications to cancers, neuro-cardio-degenerative diseases and abnormal ageing. The workflow involves examining the mechanical stresses at the cellular level via physico-chemical and multi-omics analysis. The results obtained are escalated to the animal models for validation and to the clinical samples for examination of the bench-to-bedside relevance.

MAJOR RESEARCH AREA

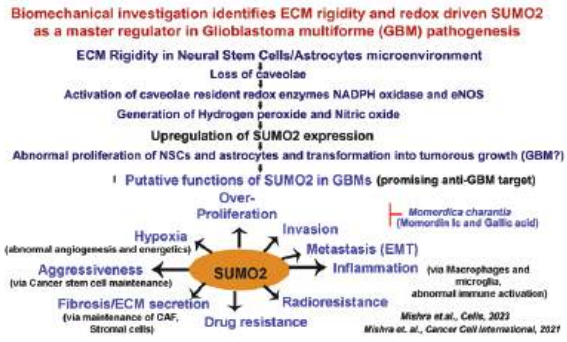
- Bacopamonnieri and its active component can effectively eradicate peri-tumoral cancer cells via catastrophic macropinocytosis.
- SUMO2 is a master regulator of tumour cell adaptation to harsh biomechanical microenvironment. Momordicacharantia and its active components mediate aberrant SUMO2 conjugation in cancer cells, suppressing the biomechanical adaptation.
- GM3-IRE1-sXBP1-SREBP2-ACSS2-Excess Cholesterol synthesis pathway targeting phyto-drugs can effectively attenuate adaptation of cancer cells to harsh low pH mechanical microenvironment.
- GAP-43 is a plasma membrane resident sensor of rigid mechanical microenvironment in neurons.

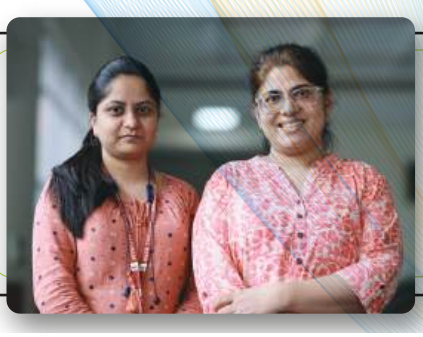
- **SUMO2 IS A MASTER REGULATOR OF GLIOBLASTOMA MULTIFORME (GBM) TUMOUR CELL ADAPTATION TO HARSH BIOMECHANICAL MICROENVIRONMENT AND EMERGES AS A KEY TARGET IN ANTI-GBM THERAPEUTICS.**

Human body runs on an intricate design of biophysical forces. Disturbances in the normal biomechanical tissue homeostasis are emerging as a key cause of deadly brain tumors-the Glioblastoma multiforme. Glioblastoma multiforme (GBM), a type the most malignant of all neoplasm and is associated with neural stem-like cells and astrocytes. In India, the young population in the age group of 20-34 is consistently showing higher risk for GBM development. Besides, brain cancers have emerged as an important risk factor for cognitive diseases and paediatric development. Besides, the tumour relapse generally occurs in the inoperable regions surrounding the ventricles, optic nerves, corpus callosum, pituitary glands etc., and is refractory to most treatment modalities. Hence, newer chemotherapeutic targets need to be delineated for timely patient management with both primary (pGBM) and recurrent (rGBM) glioblastoma tumours.

Our study puts forth the therapeutic insights into killing both primary and recurrent GBMs of pediatric and adult origin via targeting of the biomechanical adaptations that drives the cancer growth. Our initial results showed that that excessively rigid microenvironments around neural stem-like cells (NSCs) and astrocytes leads to loss of caveolae, the membrane modules that regulate the activity of key redox associated enzymes-the NADPH oxidase and eNOS. This enables rise in hydrogen peroxide and nitric oxide redox species, causing abnormal and excessive proliferation of NSCs and astrocytes. Our mass spectrometry assisted proteomics identified high SUMO2 expression in caveolae depleted NSCs and astrocutes. SUMO2 was further deduced to be the downstream target of hydrogen peroxide and nitric oxide, which suggests that the loss of caveolae leads to redox species generation that activate SUMO2. Through clinical meta-analysis, we further found that SUMO2 isoform was significantly over-expressed in both primary and recurrent GBM tumour tissue. Cellularly, bulk and glioblastoma cancer stem-like cell population as well as pro-tumourigenic stromal cells over-expressed SUMO2 isoform. Molecularly, all major kinases and other genes involved in GBM formation and maintenance were found to be targets of SUMO2 conjugation. Clinically, SUMO2 isoform over-expression was significantly correlated with shorter survival rates in GBM patients of both adult and paediatric origins. In summary, SUMO2 is identified as the ECM rigidity and redox-driven master regulator of GBM growth. Hence, precise spatio-temporal SUMOylation drives GBM. Therefore, SUMO2 is a promising anti-GBM drug target. We further found that aberrant SUMO2 substrate conjugation along with inhibition of excessive growth factor signaling, autophagy and glycolysis, by extract of Momordicacharantia, enriched in MomordinIc and gallic acid, turn abnormally growing NSCs and tumour cells vulnerable to death and clearance.

SUMO2 is a master mechano-effector in the survival adaptation of glioblastoma multiforme tumour cells to rigidity-fibrosis biomechanical stress





JRF: 1
SRF: 1
Lab Assistant: 1
PhD Students: 1

Pooja Shinde, Dr Rashmi Mishra

PUBLICATIONS:

- Gayathri K G, Shinde PL, John S, Sivakumar KC, Mishra R.Understanding The Combined Effects Of High Glucose Induced Hyper-Osmotic Stress And Oxygen Tension In The Progression Of Tumourigenesis: From Mechanism To Anti-Cancer Therapeutics. Cells. 2023;12(6):825.

INVITED TALKS [PI ONLY]:

- How do brain astrocytes and astrocytic cancer cells sense and respond to the extracellular acidification stress?" at RGCB Research Conference on 21stSeptember 2023, Thiruvananthapuram

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Understanding and targeting the loss of caveolae landscape of Glioblastoma multiforme-Novel implications on anti-GBM therapeutics.	Council of Scientific and Industrial Research	2024	3 years	PI
02	Identification of the Role of Redox Signaling Pathways in the Mechanobiology of Glioblastoma multiforme.	Department of Biotechnology	2018	4.5 years	PI
03	How Galectin-3 Drives Pressure Overload Mediated Cardiac Hypertrophy and Heart Failure	Indian Council of Medical Research	2021	3 years	PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Gayathri KG	Understanding the Combined Effects of High Glucose Induced Hyper-Osmotic Stress and Oxygen Tension in the Progression of Tumourigenesis: From Mechanism to Anti-Cancer Therapeutics.	Manipal Academy of Higher Education	Awarded	2023



Rajeeve Sivadasan, PhD

DBT- Ramalingaswami Faculty Fellow
Neurobiology

BRIEF THEME OF LABORATORY

Unraveling the Mechanisms Underlying Neurodegenerative Disorders

MAJOR RESEARCH AREA

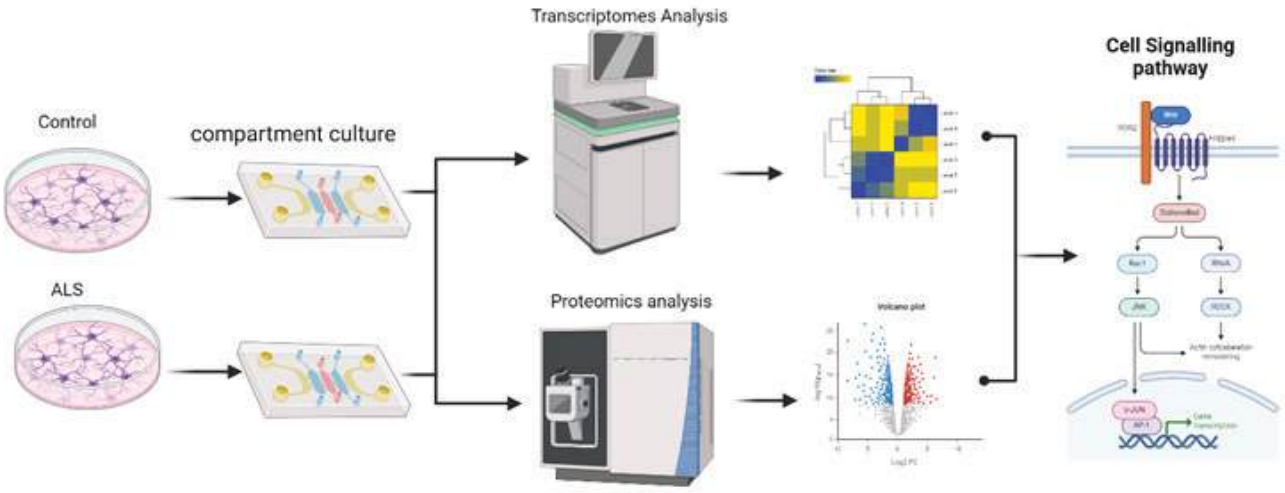
- We developed a compartmentalized system to clarify the RNA enriched in axons.

- **"THE INFLUENCE OF AXONAL RNA AND PROTEINS ON SYNAPTIC PLASTICITY"**

The nervous system, the most advanced and intricate biological system in living organisms, relies on many neuronal and glial cell subtypes. These cells are precisely localized and form dynamic neural networks that respond to various stimuli, facilitating higher-order cognitive and behavioural functions. ALS (Amyotrophic Lateral Sclerosis) and FTLD (Frontotemporal Lobar Degeneration) are neurodegenerative diseases linked by clinical and pathological similarities. Both diseases, with numerous associated genes, occur in sporadic and familial forms and are characterized by pathological protein aggregations. Proteins such as TDP-43 (TAR DNA-binding protein 43-kDa) and FUS/TLS (fused in sarcoma/translocated in liposarcomas) are common to ALS-FTLD and SMA (Spinal Muscular Atrophy). Research has highlighted novel mechanisms of protein aggregation involving RNA-binding proteins. Typically found in the nucleus, these proteins translocate to the cytoplasm under neurodegenerative stress, forming stress granules. These granules sequester specialized transcripts and promote the translation of protective proteins. Studies indicate that pathological aggregates in ALS, Alzheimer's disease, and other dementias co-localize with stress granules. TDP-43 is a significant component of tau-negative and ubiquitin-positive inclusions, characterizing ALS and FTLD linked to TDP-43 pathology (FTLD-TDP). TDP-43 aggregation and neuropathology span various neurodegenerative disorders, collectively termed TDP-43 proteinopathies, underscoring TDP-43's central role in disease pathogenesis. More than 35 missense mutations in the TARDBP gene suggest that abnormal TDP-43 function may cause, rather than result from, neurodegeneration in ALS and FTLD-TDP.

In normal tissues, TDP-43 is predominantly nuclear. However, in disease, TDP-43 mislocalizes to the cytoplasm, becomes ubiquitinylated, and hyperphosphorylated, epitomizing this pathological process. Other RNA binding proteins, such as FUS/TLS and heterogeneous nuclear ribonucleoproteins (hnRNPs), also mutate or aggregate in diseased brains. Like TDP-43, they regulate mRNA processing steps. Initially identified as a transcription factor, TDP-43's roles now include regulation of splicing, mRNA stability (including its own), microRNA processing, mRNA transport, and translation. Dysregulation of these processes may precede or follow TDP-43 aggregation and modification in affected cells. Discoveries regarding TDP-43's involvement in neurodegeneration, alongside findings for FUS/TLS and C9ORF72 (chromosome 9 open reading frame 72), have opened new avenues in the study of RNA binding proteins (RBPs) and RNA metabolism, a promising area in neuroscience. Our laboratory has developed a compartmental RNA isolation technique enabling the extraction of both RNA and protein from differentiated neuronal cells derived from induced pluripotent stem cells (iPSCs). Through the purification of axonal RNA and proteins from primary neurons utilizing compartmentalized cultures, our aim is to investigate the involvement of RNA and RNA-binding proteins (RBPs) in different disease states. This exploration promises to deepen our understanding of the factors influencing disease phenotypes, offering insights that could pave the way for novel therapeutic interventions targeting neurodegenerative conditions.

Graphical illustration of work plan





Technical Assistant: 1
Lab Assistant: 1
PhD Students: 1

Dr. Rajeevee Sivadasan, Udhaya Bharathy

AWARDS [STUDENTS]:

- One-day National Symposium on Biotechnology for Sustainable Development-2024. Best poster presentation award.

INVITED TALKS [PI ONLY]:

- Invited talk on the application of Gene analysis. - International workshop on Gene expression analysis 21-23 Feb 2024

PATENTS APPLIED/ GRANTED:

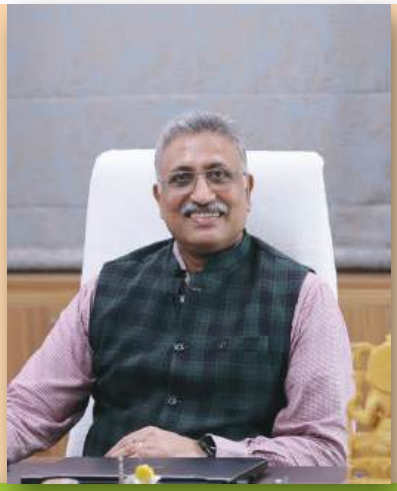
- Applied for a Provisional Patent application on- Discovery of a novel molecule that activates Wntsignaling.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	The study of small RNA in Neurodegenerative Diseases	Department of Biotechnology	2021	5 years	PI



TRANSDISCIPLINARY BIOLOGY PROGRAM



Chandrabhas Narayana, FASc, FRSC, FNASc

Director

Transdisciplinary Biology Program

BRIEF THEME OF LABORATORY

The exosomal contents are expected to reflect the nature of the cells they are originating from. We are in the process of identifying exosomes as potential biomarkers for early cancer diagnosis. We are isolating exosomes from various cancerous and non-cancerous cell lines and characterizing them using TEM, DLS, Raman spectroscopy, and SERS; to develop a database, which would help us in identifying and probing specific cancer-related exosomal biomarkers.

MAJOR RESEARCH AREA

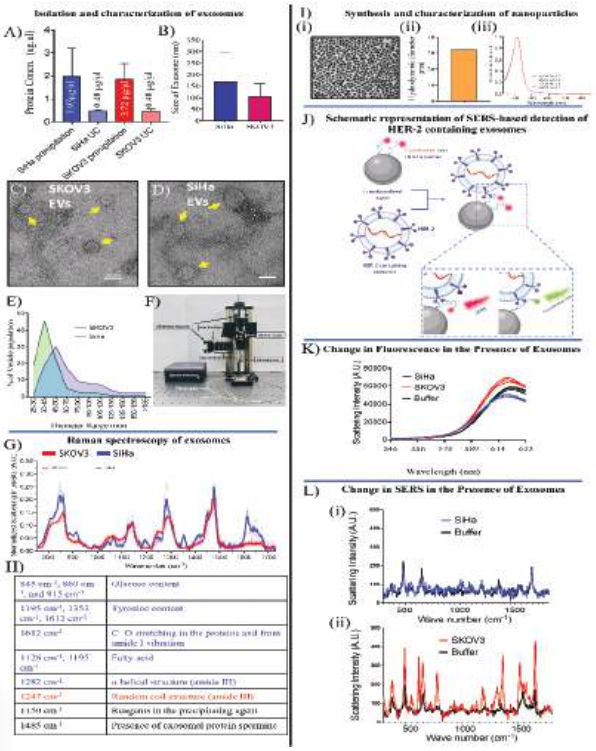
- Understanding differences in the exosomal constituents obtained from different cell lines using Raman spectroscopy.
- Surface-enhanced Raman spectroscopy (SERS) based probing of exosomal biomarkers.

- **APPLICATION OF RAMAN SPECTROSCOPY IN THE EARLY DIAGNOSIS OF CANCER.**

Cancer evolves silently and is often realized in advanced stages of metastasis. Also, the most practiced diagnostic tools, like Mammography, CT scan, MRI etc., often produce false positive results. We urgently need more sensitive, accurate, and cost-effective diagnosis techniques. In recent years, markers for various cancers have been identified in the contents of exosomes. Exosomes originating from cancer cells carry genetic materials, metabolites, and proteins that can significantly differ from the exosomal contents originating from healthy cells. Disease-specific exosomes, however, are expected to be present at very low concentrations along with several other types of exosomes in patient samples. Therefore, their detection method(s) must be highly sensitive and selective. We plan to establish Raman spectroscopy and SERS-based comparison for exosomes obtained from different types of cancer and non-cancer cell lines to identify and target potential biomarkers. Some of the cell lines of focus are- Estrogen Receptor and Progesterone Receptor positive BC cell line, MCF7, HER2 positive BC cell line, SKBR3, Triple negative BC cell line, SKBR7, HER2 positive ovarian cancer cell line, SKOV3, cervical cancer cell line, SiHa, and a noncancerous cell line, HEK293. The biophysical and biochemical characterization of exosomes are being done using TEM and DLS. We then obtain Raman spectra from these samples and then identify the differences (and similarities) of exosomal constituents by employing multivariate statistical techniques like Principal Component Analysis (PCA).

One of the major drawbacks of Raman Spectroscopy, however, is its inherently low signals. Tremendous enhancement of Raman signal intensities originating from the analytes on or near plasmonic surfaces have already been harnessed to probe biomarkers using Surface enhanced Raman spectroscopy (SERS). SERS is a distance-dependent phenomenon. Even a 1 nm change in the distance between the analyte and the SERS tag can drastically influence the SERS signal. This distance dependence in SERS intensities can be salvaged while selectively capturing and probing analytes near the plasmonic surfaces. For example, we have probed the presence of HER-2 (exosomal-biomarker) on the membrane of exosomes by targeting them with silver nanoparticles (AgNPs) carrying HER2-targeting aptamer (HApt) molecules on their surface. We used Alexa tags on H-Apt and probed the changes in their SERS and Fluorescence signals in the presence exosomes obtained from HER2 overexpressing cells and, as a control, from cells with normal level of HER2 expression. We observed a strong increase in the SERS signal originating from Alexa488 only in the presence of exosomes obtained from HER2 overexpressing SKOV3 cells. Exosomes obtained from SiHa cells did not result in any significant change in Alexa488 SERS. The interaction of HER2 with the H-Apt and the interaction of the Alexa488 dye with the AgNP surface play major roles in dictating the outcome of the SERS experiment. In the presence of exosomes obtained from SKOV3, the interaction between HER2 and HApt results in the reorientation of the Alexa488 tags on the AgNP surface, causing SERS enhancements. We are in the process of perfecting this techniques for diagnostic applications.

Estimation of exosomal protein concentration. UC is ultracentrifugation.
Size (diameter) distribution of the isolated exosomal samples measured via DLS.
and D) TEM images of exosomes isolated from SKOV-3 and SiHa cells.
Size distribution of exosomes as obtained from TEM.
Portable Raman spectrometer prototype developed in the lab.
Raman spectra of exosomes obtained from SKOV-3 (red) and SiHa (blue), and (H) assignments of their Raman spectral signatures.
(i) TEM image, (ii) diameter, and (iii) extinction spectra of AgNPs.
Schematic representation of SERS/Fluorescence-based detection of HER-2-containing exosomes.
Change in the fluorescence intensity of Alexa593 tags present on HApt molecules in the presence of exosome obtained from SKOV3 (red), and SiHa (blue) cells.
Change in the SERS intensity of Alexa488 tags present on HApt molecules in the presence of exosome obtained from SKOV3 (red), and SiHa (blue) cells.





PhD Students: 02
JRF : 01

From left to Right: Preetha R Nair, Harikrishnan KS, Athira B
Abhirami Ajith, Dr Debanjan Bhowmik
Prof Chandrabhas Narayana, Sudhanshu Mani Tripathi
Krishna Sawarn, Nithya K Nair, Shreya Unnithan

PUBLICATIONS:

- Jain P, Kumari G, Bhogra M, Yanda P, Joseph B, Waghmare UV, Narayana C. Raman evidence of multiple adsorption sites and structural transformation in ZIF-4. Inorg Chem. 2023;62(20):7703-7715.
- Biswas B, Rudra S, Rawat RS, Pandey N, Acharya S, Joseph A, Pillai AIK, Bansal M, de H-Óra M, Panda DP, Dey AB, Bertram F, Narayana C, MacManus-Driscoll J, Maity T, Garbrecht M, Saha B. Magnetic stress-driven metal-insulator transition in strongly correlated antiferromagnetic CrN. Phys Rev Lett. 2023;131(12):126302.
- Sunil J, Narayana C, Kumari G, Jayaramulu K. Raman spectroscopy, an ideal tool for studying the physical properties and applications of metal-organic frameworks (MOFs). Chem Soc Rev. 2023;52(10):3397-3437.

AWARDS [PI]:

- Kerala Academy of Sciences (https://kas.org.in/) conferred an honorary fellowship (honoris causa) to Professor Chandrabhas Narayana, Director RGCB. This was conferred by Shri. Arif Mohammad Khan, the honorable Governor of Kerala, in a function held at NIIST auditorium, Thiruvananthapuram, on 25th October 2023. KAS recognizes the scientists who have made outstanding contributions to science with the honorary fellowship.
- Professor Chandrabhas Narayana, Director RGCB, has been selected as the Chairman of the National Committee of International Science Council for the International Union of Crystallography (IUCr) for the term of January 1, 2024 – December 31. Indian National Science Academy (INSA) is the adhering organization of IUCr in India, and INSA made the recommendations.

INVITED TALKS [PI ONLY]:

- Delivered a lecture on the topic "Success in translational research from lab to land: Examples and learnings" at IISF 2023 at RCB&THSTI Faridabad, 18th January 2024

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Scientific reinvention of Ethinic Food and Medicine from Kerala for Functional Food and Drug Development	Departmet of Science & Technology - Science and Heritage Research Initiative	2021	3 years	PI
02	Development of Portable Raman Spectrometer for Identifying the Amyloid Deposition in the Peripheral Region of the Body	Departmet of Science & Technology- (DDP)	2021	3 years	PI
03	Creation of national network of existing and upcoming high risk pathogens laboratories (BSL - 3/4) labs across departments and keeping their 'interlinkages" functional for use during outbreak response across sectors" under NOHM component of PM-ABHIM	Indian Council of Medical Research	2024	3 years	PI



Dileep Vasudevan, PhD

Scientist F
Transdisciplinary Biology Program

BRIEF THEME OF LABORATORY

Our laboratory at RGCB focuses on chromatin and infectious disease structural biology. We work on nucleosome-interacting proteins and certain interesting proteins from pathogenic organisms. We make use of an integrative structural biology approach. In addition, structural biology projects are being taken up from time to time on a collaboration basis. As an experimental structural biology research group, our major tools are X-ray crystallography, small-angle X-ray scattering, and cryo-electron microscopy.

MAJOR RESEARCH AREA

- Bacterial expression protocols for recombinant human and Arabidopsis core histone proteins have been optimized.
- The strong positioning 145 bp '601' DNA has also been prepared, and the same, along with the recombinant histones, will be used to reconstitute nucleosome core particles in vitro.
- The cryo-EM structure of a 55 kDa nucleoplasmin domain has been completed to 2.0 Å resolution.
- The cryo-EM structure of a plant cytoplasmic ClpB protein revealing an unusual heptameric organization is being completed.

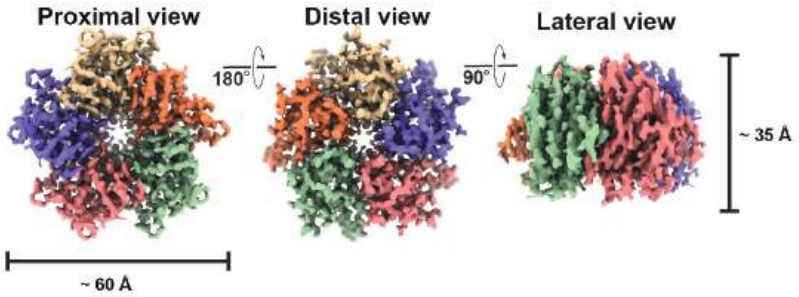
HIGH-RESOLUTION CRYO-EM STRUCTURE OF A 55 KDA PLANT NUCLEOPLASMIN

Obtaining atomic resolution structures of biological macromolecules is pertinent to having an in-depth understanding of their biological functions. In the past, conventional structural biology techniques, such as X-ray crystallography and NMR, have contributed significantly towards this end, whereas cryo-EM is an emerging technique in the field of structural biology. More recently, single-particle cryo-electron microscopy (cryo-EM) has become popular, particularly for studying the structure of large macromolecules and their complexes. The technique is particularly effective in obtaining atomic-resolution structures for molecules with sizes ranging from 200 kilodaltons to megadaltons. However, its effectiveness has been limited in achieving near-atomic resolution for macromolecules smaller than 100 kDa. Poor signal-to-noise ratio and low contrast are the major constraints in identifying particles from the background during particle picking. Therefore, particle alignment accuracy gets reduced, which further lowers the achievable resolution of 3D reconstruction.

Arabidopsis thaliana FKBP53 belongs to the nucleoplasmin family of histone chaperones. Studies have indicated that the N-terminal domain (NTD) of the protein tends to form a highly stable and compact pentamer comprised of five monomers, each weighing 11 kDa, as revealed by X-ray crystallography and small angle X-ray scattering. Recently, the cryo-EM structure of Nucleophosmin 1, a human nucleoplasmin, has been reported at 3.5 Å resolution. Consequently, we adopted a systematic strategy encompassing grid preparation, data collection, and processing to explore the possibility of enhancing resolution and determining the structure of small oligomeric proteins, using AtFKBP53 NTD as an example.

A pure and homogenous recombinant AtFKBP53 NTD pentamer preparation from the size-exclusion chromatography step was used for cryo-EM grid preparation. The best grids were chosen, and data was collected using a Titan Krios microscope equipped with a Falcon 4i detector and a Selectris X energy filter. To circumvent the issue of low contrast, we systematically curated data and optimized various stages, including particle picking, 2D classification, initial model generation, and 3D refinement, employing Bayesian polishing to enhance map quality and resolution. The neural network algorithm-based TOPAZ particle-picking program was employed to circumvent the preferred orientation issue during particle picking. Optimization of 3D refinement parameters, including refinement with masks and CTF refinement, led to the production of a high-resolution map. The 3D reconstruction was further improved by Bayesian polishing. The final 3D reconstruction from the selected 3D classes reached a resolution of 2.0 Å, as estimated using the gold standard FSC curve.

In summary, we have completed the cryo-EM structure of the 55 kDa pentameric AtFKBP53 nucleoplasmin domain at 2.0 Å resolution. This high-resolution structure was possible due to our strategies involving the selection of optimal grids for data collection, utilizing an advanced neural network for particle picking, and systematically aligning small particles to enhance the resolution of the final 3D reconstruction. Besides sample homogeneity and quality, we used an image processing workflow featuring more advanced artificial intelligence-based particle picking and Bayesian polishing approaches. This workflow could be adopted to achieve high-resolution structural details of small biological macromolecules using single-particle cryo-EM.



2.0 Å cryo-EM reconstruction of the 55 kDa AtFKBP53 nucleoplasmin domain. The final sharpened map of the AtFKBP53 pentamer is shown. The monomers are in different colors, and the proximal, distal, and lateral views are displayed. The bars illustrate the dimensions of the map.



PhD Students: 03
Research Associate: 01
SRF: 01

First Row: Dr Dharma Rao Tompa, Sonali Ghosal, Dr Dileep Vasudevan, Anagha Das, Neethu M

Second Row: Sovon Acharya, Bimal Jana

PUBLICATIONS:

- Jagdev MK, Tompa DR, Ling LL, Peoples AJ, Dandapat J, Mohapatra C, Lewis K, Vasudevan D. Crystal structure of the N-terminal domain of MtClpC1 in complex with the anti-mycobacterial natural peptide Lassomycin. Int J Biol Macromol. 2023;253(2):126771.
- Saharan K, Baral S, Shaikh NH, Vasudevan D. Structure-function analyses reveal Arabidopsis thaliana HDA7 to be an inactive histone deacetylase. Curr Res Struct Biol. 2024;7:100136.
- Kumar TP, Gireesh-Babu P, Vasudevan D, Pavan-Kumar A, Chaudhari A. Characterization of Kiss/Kissr system and expression profiling through developmental stages indicate kiss1 to be the active isotype in Clarias magur. Fish Physiol Biochem. 2024; 38647980.

INVITED TALKS [PI ONLY]:

- A structural journey with plant nucleoplasmins, School of Biology Seminar, 15 September 2023, IISER Thiruvananthapuram.
- Towards the structural basis of mycobacterial inhibition by Lassomycin, 50th National Seminar on Crystallography, 22-24 November 2023, CSIR-IMTECH, Chandigarh.
- Safari pentapus: A structure-function journey with plant nucleoplasmins, Invited talk, 29 November 2023, NIPGR, New Delhi.
- Strategies against WSSV: A structural perspective, International Fisheries Congress and Expo 2024, 12-14 January 2024, KUFOS, Kochi.
- Safari pentapus: A structure-function journey with plant nucleoplasmins, National Conference on Exploring Synergies: Insights in Life Science and Structural Biology (ESILS - 2024), 3-4 April 2024, Kannur University, Kannur.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Deciphering the structural and functional attributes of the putative nucleoplasmin from Plasmodium falciparum	Science and Engineering Research Board	2024	3 years	PI



Mahendran K.R, PhD

Scientist E-II
Transdisciplinary Biology

BRIEF THEME OF LABORATORY

The major interest of my laboratory is the engineering of transmembrane pores for applications in biotechnology and medicine. In particular, we focus on synthetic peptides-derived alpha-helical pores and biological transmembrane pores and explore their biophysical and electrical characterization using single-channel and single-molecule electrical sensing studies. Our research has important implications for developing stochastic sensors for biomolecules and chemical moieties and therapeutic development.

MAJOR RESEARCH AREA

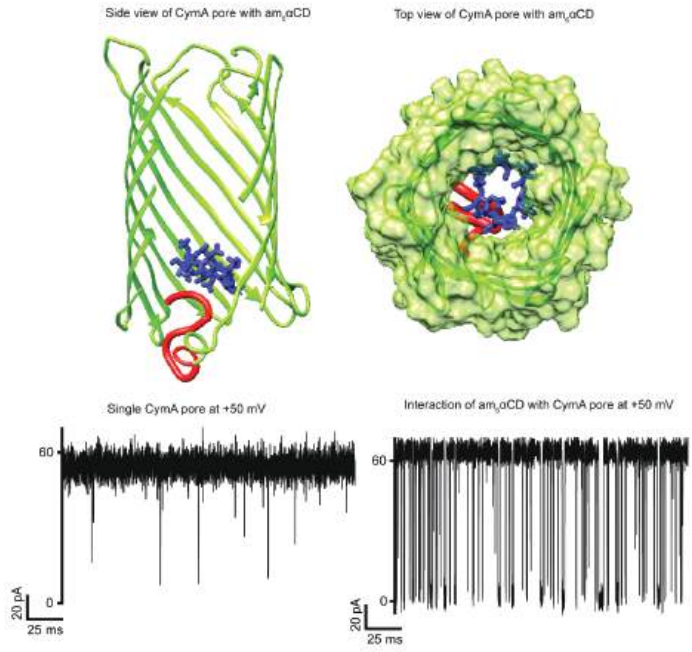
- Building unnatural peptide pores targeting bacterial membranes against antibiotic resistance.
- Assembly of synthetic alpha-helical transmembrane pores for nanobiotechnology and medicine
- Molecular transport across bacterial channels
- Membrane pores as single-molecule biosensors

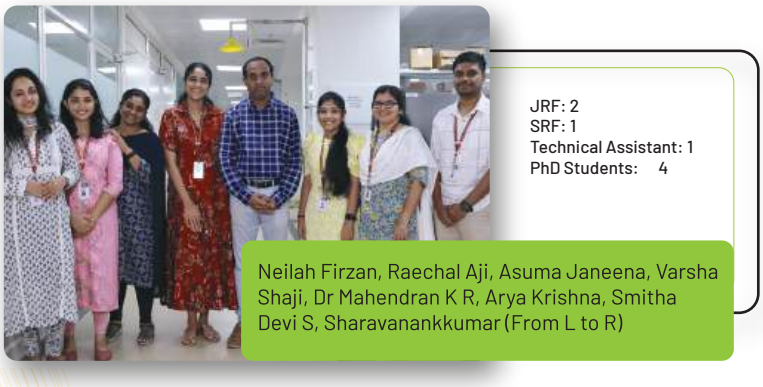
◆ MOLECULAR TRANSPORT ACROSS MEMBRANE PORES.

Bacterial outer membrane porins facilitate the translocation of small molecules while restricting large molecules and this mechanism at the molecular level remains elusive. Here, we investigate the selective uptake of large cyclic sugars across an unusual passive bacterial membrane transporter CymA, comprising a charged zone and a constricting N terminus segment. Using a combination of electrical recordings, protein mutagenesis and molecular dynamics simulations, we establish substrate translocation across CymA governed by the electrostatic pore properties and conformational dynamics of the constriction segment. Notably, we show that the variation in pH of the environment resulted in reversible modulation of the substrate binding site in the pore, thereby regulating charge-selective transport of cationic and anionic cyclic sugars. The quantitative kinetics of cyclic sugar translocation across CymA obtained in electrical recordings at different pHs are comparable to the free energetics revealed by molecular dynamics simulations. Furthermore, we show that the constriction segment is flexible and can reside inside or outside the pore lumen during the translocation of cyclic sugars. Importantly, we establish the molecular basis of substrate translocation where the constriction segment is not fully expelled from the pore, distinct from the ligand-gated transport mechanism. Our study contributes novel insights into energy-independent large molecular membrane transport for targeted drug design strategies against bacterial pathogens.

Membrane pores are exploited for the stochastic sensing of various analytes, and here, we used electrical recordings to explore the interaction of PEGylated peptides of different sizes with a protein pore, CymA. This wide-diameter natural pore comprises densely filled charged residues, facilitating electrophoretic binding of polyethylene glycol (PEG) tagged with nonaarginine peptide. The small-sized PEG 200 peptide conjugates produced monodisperse blockages and exhibited voltage-dependent translocation across the pores. Notably, the larger PEG 1000 and 2000 peptide conjugates yielded heterogeneous blockages, indicating a multitude of PEG conformations hindering its translocation through the pore. Furthermore, a much larger PEG 5000-peptide occludes the pore entrance, resulting in the complete closure. The competitive binding of different PEGylated peptides with the same pore produced specific blockage signals reflecting their identity, size and conformation. Our proposed model of sensing distinct polypeptide conformations corresponds to disordered protein unfolding, suggesting that this pore can find applications in proteomics.

Top panel: Structural properties of CymA highlighting the unique pore geometry due to the presence of the N terminus segment and cyclic sugar translocation. Bottom panel: Single-channel electrical properties of CymA porin reconstituted into the planar lipid bilayers indicating the interaction of cationic cyclodextrin with the pore.





JRF: 2
SRF: 1
Technical Assistant: 1
PhD Students: 4

Neilah Firzan, Raechal Aji, Asuma Janeena, Varsha Shaji, Dr Mahendran K R, Arya Krishna, Smitha Devi S, Sharavanankkumar (From L to R)

PUBLICATIONS:

- Vikraman D, Majumdar BB, Sk S, Weichbrodt C, Fertig N, Winterhalter M, Mondal J, Mahendran KR. Conformational flexibility driving charge-selective substrate translocation across a bacterial transporter. Chem Sci. 2024 May 13;15(24):9333-9344.
- Satheesan R, Vikraman D, Jayan P, Vijayan V, Chimerel C, Mahendran KR*. Sensing PEGylated Peptide Conformations Using a Protein Nanopore. Nano Letters, 2024; Mar 27;24 , 3566-3574.
- Das, A. D., K, V., & Mahendran, K. R*. Synthetic α-Helical Nanopore Reactor for Chemical Sensing. JACS Au, 2023; 3, 9, 2467-2477.
- Vijayanathan M, Abhinav KV, Bandyopadhyay D, Mahendran KR, Sharaf A, Pillai MR, Soniya EV. Structural analysis unravels the functional promiscuity of Quinolone synthase-mediated polyketide biosynthesis in Aegle marmelos Correa. 2024. Communications Biology (In press).

AWARDS [PI]:

- Mahendran KR: Selected as a member of the National Committee for the International Union of Pure & Applied Biophysics by the Indian National Science Academy.

AWARDS [STUDENTS]:

- Neilah Firzan CA: Best poster award at the 46th Annual Meeting of the Indian Biophysical Society, Hyderabad conducted at the University of Hyderabad and TIFR, Hyderabad on March 15th- March 17th 2024.

INVITED TALKS [PI ONLY]:

- Mahendran KR: 46th Annual Meeting of the Indian Biophysical Society, Hyderabad conducted at the University of Hyderabad and TIFR, Hyderabad on March 15th- March 17th 2024. Title of the talk: Molecular transport across bacterial porins

CONFERENCE PRESENTATION:

- Neilah Firzan CA: Poster presentation at 46th Annual Meeting of the Indian Biophysical Society, Hyderabad conducted at the University of Hyderabad and TIFR, Hyderabad on March 15th-March 17th 2024. Title: Fabricating mirror image pores at molecular and cellular level.
- Sharavanakumar SK: Poster presentation at 46th Annual Meeting of the Indian Biophysical Society, Hyderabad conducted at the University of Hyderabad and TIFR, Hyderabad on March 15th-March 17th 2024. Title: Channeling antibiotics across bacterial porins

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Structural Assembly of Functional Transmembrane peptide nanopores: From Synthesis to Sensing.	Department of Biotechnology, India	2021	3.5 years	PI
02	Engineered alpha-helical pores for single-molecule sensing of amyloid Structures	Science and Engineering Research Board, India	2022	3 years	PI
03	The porin passport control for antibiotic translocation: From single-molecule detection to biological relevance	Department of Biotechnology, India	2022	3 years	PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Smrithi Krishnan R	Assembly of Synthetic alpha-helical pores for nanobiotechnology	Manipal Academy of Higher Education	Awarded	2023
02	Devika Vikraman	Substrate translocation across bacterial membrane pores: From single-molecule detection to biological relevance	Manipal Academy of Higher Education	Submitted	2024
03	Remya Satheesan	Membrane pores as single-molecule sensors	Manipal Academy of Higher Education	Submitted	2024



Shijulal Nelson Sathi, PhD

Scientist C & Co-ordinator, Bioinformatics facility
Transdisciplinary Biology

BRIEF THEME OF LABORATORY

The Bioinformatics laboratory is mainly focusing on two research themes

a. Development and usage of integrated evolutionary bioinformatics techniques to better understanding of origin, adaptation, transmission and evolution of pathogenicity and antibiotic-resistance among bacterial priority pathogens in a wide variety of environments.

b. Discovery of new drug targets and syngergies to combat multi-drug resistance in pathogenic bacteria using structural bioinformatics techniques.

MAJOR RESEARCH AREA

- Discovery of small molecule entry inhibitors targeting the linoleic acid binding pocket of SARS-CoV-2 spike protein.
  We identified potential SARS-CoV-2 entry inhibitors using virtual screening and MD simulations from three chemical libraries comprising 6971 small molecules. Our in silico analysis suggests that these compounds may impede viral entry by interacting with linoleic acid binding site.
- Epidemiological monitoring of SARS-CoV-2 and its variants in wastewater systems in the major cities of Kerala.
  Total of 908 sewage samples were collected and processed from 31st Oct 2022 to 20th Oct 2023. Using bioinformatics approaches, specific lineages of SARS-CoV-2 were identified.

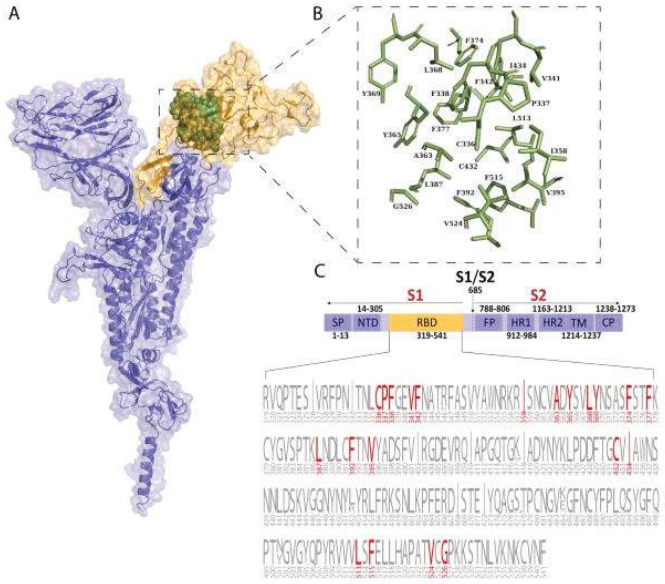
- **DISCOVERY OF SMALL MOLECULE ENTRY INHIBITORS TARGETING THE LINOLEIC ACID BINDING POCKET OF SARS-COV-2 SPIKE PROTEIN EPIDEMIOLOGICAL MONITORING OF SARS-COV-2 AND ITS VARIANTS IN WASTEWATER SYSTEMS IN THE MAJOR CITIES OF KERALA (THIRUVANANTHAPURAM), INDIA**

1. Discovery of small molecule entry inhibitors targeting the linoleic acid binding pocket of SARS-CoV-2 spike protein

Spike glycoprotein has a significant role in the entry of SARS-CoV-2 to host cells, which makes it a potential drug target. Continued accumulation of non-synonymous mutations in the receptor binding domain of spike protein poses great challenges in identifying antiviral drugs targeting this protein. This study aims to identify potential entry inhibitors of SARS-CoV-2 using virtual screening and molecular dynamics (MD) simulations from three distinct chemical libraries including Pandemic Response Box, Drugbank and DrugCentral, comprising 6971 small molecules. The molecules were screened against a binding pocket identified in the receptor-binding domain (RBD) region of the spike protein which is known as the linoleic acid binding pocket, a highly conserved motif among several SARS-CoV-2 variants. Through virtual screening and binding free energy calculations, we identified four top scoring compounds, MMV1579787 ([2-Oxo-2-[2-(3-phenoxyphenyl)ethylamino]ethyl]phosphonic acid), Tretinoin, MMV1633963 ((2E,4E)-5-[3-(3,5-dichlorophenoxy)phenyl]penta-2,4-dienoic acid) and Polydatin, which were previously reported to have antibacterial, antifungal or antiviral properties. These molecules showed stable binding on MD simulations over 100 ns and maintained stable interactions with TYR365, PHE338, PHE342, PHE377, TYR369, PHE374 and LEU368 of the spike protein RBD that are found to be conserved among SARS-CoV-2 variants. Our findings were further validated with free energy landscape, principal component analysis and dynamic cross-correlation analysis. Our in silico analysis of binding mode and MD simulation analyses suggest that the identified compounds may impede viral entrance by interacting with the linoleic acid binding site of the spike protein of SARS-CoV-2 regardless of its variants, and they thus demand for further in vitro and in vivo research.

2. Epidemiological monitoring of SARS-CoV-2 and its variants in wastewater systems in the major cities of Kerala (Thiruvananthapuram), India

The global SARS-CoV-2 pandemic has adversely affected human health, economy and environment. Due to widespread transmission, monitoring the virus in the human population, especially in asymptomatic individuals who pose a significant risk of disease recurrence, is extremely challenging. Wastewater surveillance for SARS-CoV-2 is a holistic approach to understanding the epidemiology of SARS CoV-2. Kerela is one of the severely affected states in India due to the SARS-CoV-2 virus. As part of SARS-CoV-2 sewage surveillance, during a period of one year from October 2022, wastewater samples from 2 STP's and 10 pumping stations in Thiruvananthapuram city were collected and processed for the detection of SARS CoV-2. A total of 908 samples were collected and processed from 31st Oct 2022 to 20th Oct 2023. Among these samples, 134 samples were found to be positive for SARS-CoV-2 with Ct value < 35 from qRT-PCR. All the positive samples were sequenced in Nanopore platform to detect the emerging variants of SARS-CoV-2. Using bioinformatics and computational approaches, the samples were identified with specific lineages of SARS-CoV-2, including B, B.1.1, B.1.1.529, XBB.1, XBB.1.16, and XBB.2.3.



Binding site region of SARS-CoV-2 spike glycoprotein:
(A) Three-dimensional SARS-CoV-2 spike monomeric subunit structure. The RBD region is shown in orange, and the predicted binding pocket is represented as green spheres.
(B) The binding pocket region's amino acid residues are represented as sticks.
(C) Logo plot shows non-synonymous mutations in the RBD region of major variants of SARS-CoV-2. The binding site region's amino acid residues are indicated in red.



Project Assistant: 1
Project Associate: 1
Postdoctoral Fellows: 1
PhD Students: 1

Kommu Vijay Kumar, Subakamakshi K.R, Dr. Shijulal NS, Bincy Varghese, H Kiran, Sreejith R.P (From L to R)

PUBLICATIONS:

- Prasad R, Ajith H, Kumar Chandrakumaran N, Dnyaneshwar Khangar P, Mohan A, Nelson-Sathi S. In silico study identifies peptide inhibitors that negate the effect of non-synonymous mutations in major drug targets of SARS-CoV-2 variants. J Biomol Struct Dyn. 2023;41(19):9551-9561.
- John J, Narendrakumar L, Thomas S, Nelson-Sathi S. Hybrid genome assembly and annotation of multidrug-resistant Staphylococcus aureus genotype ST672-SCCmec type IVd (2B). J Glob Antimicrob Resist. 2023;32:74-77.

INVITED TALKS [PI ONLY]:

- Structural and functional implications of nonsynonymous mutations in SARS-CoV-2 at Kerala University Researchers Fest 19th June 2023 Kerala University, Thiruvananthapuram.
- International Conclave on AMR and Future of Antibiotics on November 8-9, 2023, at SRM University, Andhra Pradesh.
- Evolutionary routes of antibiotic resistance in S. aureus at RGCB Research conference, September 20-23, 2023,Kollam.
- Recent trends in bioinformatics at International Conference & workshop on current trends in biotechnology, 17th-19th January 2024, University of Calicut.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Epidemiological Monitoring of SARS CoV-2, and its variants in wastewater systems in the major cities of Kerela, India. (2022-2024)	Science and Engineering Research Board	2022	2 years	PI

02	Genomic and evolutionary characteristics of multi-drug resistant S. aureus circulating in Kerala	Kerala State Council for Science - Technology and Environment	2023	3 years	PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Jiffy John	Evolutionary genomics of Staphylococcus aureus: insight into the resistance trait.	Manipal Academy of Higher Education	Awarded	2023



Lightson N.G, PhD

Scientist C

Transdisciplinary Biology

BRIEF THEME OF LABORATORY

The lab focuses on studying two aspects of bionanotechnology: (a) developing next-generation advanced portable bioanalytical platforms for biomedical and environmentally relevant biomarkers using aptamer oligonucleotides, synthetic peptides, biomimetic materials, nanomaterials, etc.; (b) developing novel, safe, and effective therapeutics based on nanoscale components and their combinations in the field of nanomedicine. We aim to contribute to cutting-edge technologies and emerging fields by bringing advanced biomaterials, functional materials, and nanoscale platforms to diagnostics and therapeutics.

- **STUDY THE CARBON QUANTUM DOTS - DOXORUBICIN CONJUGATES FOR CANCER THERAPEUTICS**

Cancer has become one of the leading causes of mortality worldwide. Chemotherapy, the most extensively employed technique, entails potent medicines that kill cancer cells. However, chemotherapy possesses many disadvantages, such as poor specificity, low therapeutic efficiency, and severe side effects. In contrast, reducing the potentially fatal adverse effects of chemotherapy is critical for making the treatment more efficient, safer, and tolerable. Conventional drug delivery systems require frequent administration and higher drug doses to attain therapeutic efficiency due to the poor bioavailability and non-specificity of many therapeutic agents.

To address these critical challenges, innovative drug delivery systems with superior features for the loading, release, tracking, and targeting of chemotherapeutic medicines are indispensable in cancer detection, and therapy-targeted drug delivery systems depend on the site-specific release of therapeutics predominantly at the cancerous tissues while minimizing the effect on normal tissues. Therefore, researchers must find a carrier for efficient and precise delivery to specific tumour sites. Nanoparticles as smart drug delivery vehicles have become a very strategic endeavor. Nano drug carriers possess the advantages of improved efficacy of tumor chemotherapy, enhanced biodistribution, selective cancer targeting, reduced toxicity, and fewer side effects.

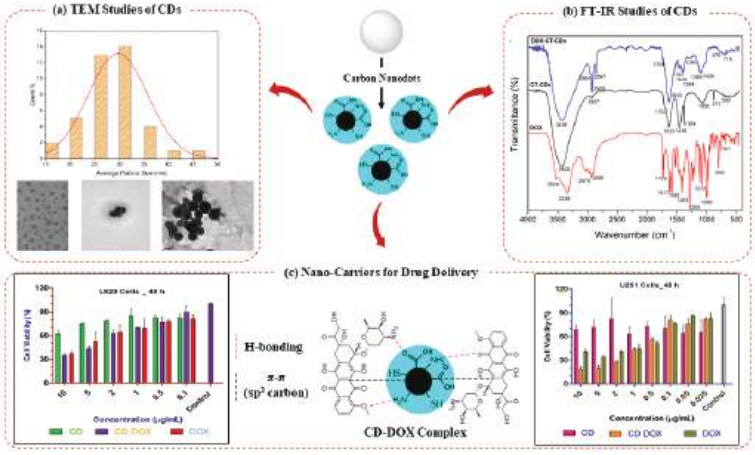
Carbon dots (CDs) are recently developed carbon nanomaterials that have drawn considerable interest in bionanotechnology. CDs have become potential nanomaterials due to their intriguing features, such as high solubility, low toxicity, and resistance to photobleaching, especially their easily tuneable fluorescent properties and flexibility in surface modifications. The excellent physical and chemical properties of CDs with low toxicity, rich surface groups, and biocompatibility can make them a potential nanocarrier for drug delivery. However, obtaining highly efficient loading and sustained release of DOX using CDs as nanocarriers is still challenging, which would influence therapeutic efficacy tremendously. We proposed to use CDs with multiple functional groups on the surface, developed novel functional carbon dots (CDs) and investigated the therapeutic efficacy by studying the loading efficiency and release behavior of the anticancer drug doxorubicin (DOX). Functional groups, morphology, particle size, and zeta potential of synthesized CDs and CDs loaded DOX were investigated by UV-visible, Fluorescence, DLS, Zeta Potential measurements, FTIR, and TEM. In vitro, drug release studies confirmed pH-dependent biphasic drug release, with an initial burst effect and sustained release of DOX. The CDs were non-toxic and biocompatible with L929 fibroblast cells. The cytotoxic effect of DOX-CDs showed a concentration-dependent effect after 48 h with Glioblastoma U251 cells. In the future, this work will be further assessed by in-vivo studies. In the meantime, the synthesized CDs will be studied on different cancer cell lines. Drug loading profile and in-depth mechanism of interaction between drug-carbon dots can also be investigated to provide further insight into efficient nanocarrier systems for better cancer therapeutics and outcomes. Additionally, the properties of the CDs may be modulated by incorporating different chemical functional linkers and molecular modification.

Carbon dots (CDs) – Doxorubicin (DOX) (CDs-DOX) conjugate as nanocarrier system.

(a) Physical characterization of synthesised CD with TEM,

(b) Functional group confirmation using FT-IR,

(c) Cell viability studies of the CDs-DOX conjugate





JRF: 1
PhD Students: 2

Mariyam Razana CA, Dr Lightson N G, Athulya VA (From L to R)

PUBLICATIONS:

- Ngashangva L, Chattopadhyay S. Biosensors for point-of-care testing and personalized monitoring of gastrointestinal microbiota. Front Microbiol. 2023;14:1114707.

INVITED TALKS [PI ONLY]:

- Microfluidic Biosensors for Point-of-Care Detection of Bio-analytes, Spectrum Seminar 2024, 30 Jan 2024, Kirori Mal College, Delhi University, New Delhi.

CONFERENCE PRESENTATION:

- Anagha Das, Aswathy Prasad, Aman Grewal, Lightson Ngashangva. Oral Presentation. Fluorescence-based Detection of Uric Acid and Iron using Novel Carbon Nanodots. 16th International Conference on Sensing Technology, BITS Hyderabad Campus, Hyderabad, 17-20 Dec 2023.
- Lightson Ngashangva. Poster. Studies of Carbon-based Nanozymes for Point-of-Care Biosensors Applications, IISF-Young Scientist Conference, DBT THSTI-RCB Campus, NCR Biotech Science Cluster, Faridabad, Haryana, 17-20 Jan 2024.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Paper-based kits for on-site detection of methanol and formaldehyde	Biotechnology Industry Research Assistance Council	2021	2 years	PI
02	Studies of non-enzymatic paper-based bioanalytical devices for point-of-care diagnostics	Science and Engineering Research Board	2022	3 years	PI
02	Analysis of tumor characteristics and therapeutic strategies for β-hCG positive breast cancer patients from Kerala using microfluidic organ-on-a-chip culture device	Indian Council of Medical Research	2024	3 years	CO-PI



Kathiresan Natarajan, PhD

Scientist C

Transdisciplinary Biology

BRIEF THEME OF LABORATORY

Tubulin isotype composition in neurons is crucial for neuronal development and functions like neuroblast migration and transport. Mutations in tubulin isotypes can cause neurological disorders called "tubulinopathies," causing developmental and neurodegenerative abnormalities. Understanding how tubulin isotypes and mutations affect microtubule dynamics is limited, but understanding these mutations could provide new insights into the structural regulation of microtubule dynamics and their relevance in neurodevelopmental diseases.

MAJOR RESEARCH AREA:

- We investigated the impact of TUBB4B mutations on microtubule dynamics and cellular functions. The TUBB4B wild-type incorporated filamentous microtubules, while the D161H mutant had a diffused appearance and less obvious network in 95% of mutant cells. The TUBB4B-D161H mutant showed a diffused appearance and higher intensity and number of EB3 proteins binding, indicating a higher repolymerization rate and more efficient interaction with EB3. The study revealed that TUBB4B mutant dynamics significantly differ from wild-type ones, indicating a significant role of TUBB4B isotype in microtubule dynamics.

IMPACT OF DISEASE-ASSOCIATED TUBB4B TUBULIN ISOTYPE MUTATIONS ON MICROTUBULE DYNAMICS

The study investigates the impact of TUBB4B mutations on microtubule dynamics and cellular functions. The TUBB4B wild-type incorporated filamentous microtubules, while the TUBB4B D161H mutant had a diffused appearance and less obvious network in 95% of mutant cells. Permeability removed unbound TUBB4B, making the network more visible in cells. End-binding proteins recognize the GTP/GDP-Pi cap at the plus end of microtubules, creating a stabilization zone for microtubules undergoing catastrophe. We analyzed repolymerization and depolymerization kinetics of TUBB4B wild-type and D161H mutants, finding that depolymerization completely aborted the microtubule network. The TUBB4B wild-type microtubule re-grew after 30 minutes of repolymerization, while the TUBB4B-D161H mutant showed a diffused appearance and higher intensity and number of EB3 proteins, indicating a higher repolymerization rate and more efficient interaction with EB3.

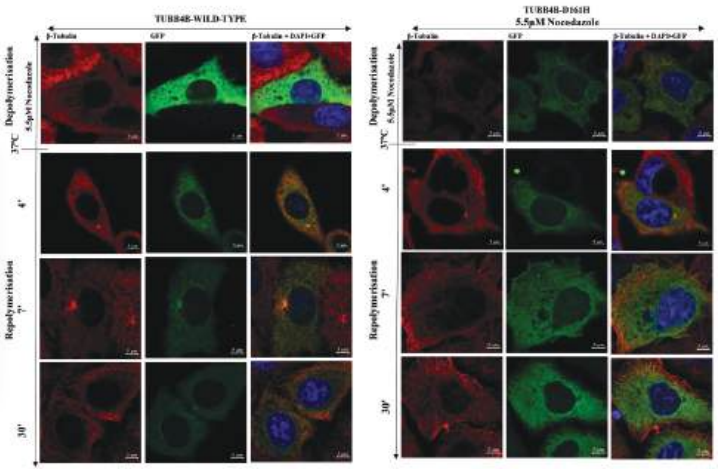
Nocodazole, a microtubule depolymerizing agent, completely abolished the microtubule network in A549 lung cancer cells. The D161H mutant had lower impact and higher repolymerization rate. The TUBB4B-D161H mutant did not alter the β-tubulin network in both wild-type and D161H cells. However, the regrowth of the microtubule 30' post-nocodazole treatment was higher in the mutant, but TUBB4B-GFP in the mutant maintained a diffused appearance (Figure 1). The TUBB4B-R391C mutant was created using site-directed mutagenesis to study the impact on microtubule dynamics. Transfection study using the N2A neuroblastoma cell line revealed that the expression of TUBB4B was lower in R391C-transfected cells, indicating structural changes in the microtubule. Confocal microscopy analysis revealed that R391C mutant cells have a less dense microtubule network compared to the wild type.

The impact of the β-D161H mutation in the TUBB4B dimer was explored using structural modelling and molecular dynamic simulations over 500 ns. Aspartic acid 161 is found in the H4 helix of β-tubulin and interacts with the S3 strand and H3 helix of α-tubulin through Glutamine 97 and Arginine 105 residues. In the D161H mutant, it only interacts with Arginine 105 of α-tubulin and forms a polar contact with Arginine 251, potentially stabilizing it and resulting in lower RMSD and RMSF profile compared to the WT.



JRF: 2
Postdoctoral Fellows: 2
PhD Students: 2

Dhanya Damodaran, Thasni Fazil, Kathiresan Natarajan Sharanya C Suresh, Wilbee D S, Anju Krishnan (From L to R)



Microtubule repolymerisation kinetics in the TUBB4B -WT and D161H mutant after nocodazole treatment. Scale bar 5 μm.

PUBLICATIONS:

- Strikwerda JR, Natarajan K, Sine SM. Impact on AChR open channel noise by pore-peripheral salt bridge depends on voltage and divalent cations. Biophys J. 2023;122;2430-44.
- Strikwerda JR, Natarajan K, Sine SM. Impact on AChR open channel noise by pore-peripheral salt bridge depends on voltage and divalent cations. Biophys J. 2023;122;2430-44.
- Mazzaferro S, Kang G, Natarajan K, Hibbs RE, Sine SM. Structural bases for stoichiometry-selective calcium potentiation of a neuronal nicotinic receptor. Br J Pharmacol. 2024 ;181(13):1973-1992.
- Prasad R, Kadam A, Padippurackal VV, Pulikuttymadom Balasubramanian A, Kumar Chandrakumaran N, Suresh Rangari K, Dnyaneshwar Khangar P, Ajith H, Natarajan K, Chandramohanadas R, Nelson-Sathi S. Discovery of small molecule entry inhibitors targeting the linoleic acid binding pocket of SARS-CoV-2 spike protein. J Biomol Struct Dyn. 2024 ; 23:1-15.
- Sharanya CS, Natarajan K. Nonsteroidal Anti-Inflammatory Drug Leads from Plant Sources. InDrugs from Nature: Targets, Assay Systems and Leads, Singapore: Springer Nature Singapore. 2024;179-203.

AWARDS [STUDENTS]:

- Remya C P, Best Poster Award, "impact of disease associated tuba1a tubulin isotype mutations on microtubule dynamics", 36th KERALA SCIENCE CONGRESS 8-11 February 2024, Kasaragod.

INVITED TALKS [PI ONLY]:

- "Mechanism of calcium potentiation in nicotinic acetylcholine receptors" International Conference on Transdisciplinary Research in Life Sciences: Perspectives and Prospects - 2024 (ICTRLS-2024), 22 - 23 February 2024, Kristu Jayanti College, Bengaluru, Karnataka, India

CONFERENCE PRESENTATION:

- Wilbee DS, "Screening of Allosteric Inhibitors of RdRp of SARS-CoV-2 Based on Drug Repurposing Strategy using Well Tempered Metadynamic Simulations" JNCASR-CECAM MD@60 conference, 26-29 February 2024, JNCASR, Bengaluru, Karnataka, India.
- Sharanya CS, "E-pharmacophore-based drug repurposing against SARS-CoV-2 and insight into interaction with TMPRSS2", 92nd ANNUAL MEET OF THE SOCIETY OF BIOLOGICAL CHEMISTS, 18-20th Dec 2023, BITS Pilani K K Birla Goa Campus, India.
- Thasni Fazil "Impact of TUBB4B tubulin isotype mutations on Microtubule dynamics" ", 92nd ANNUAL MEET OF THE SOCIETY OF BIOLOGICAL CHEMISTS, 18-20th Dec 2023, BITS Pilani K K Birla Goa Campus, India

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Impact of disease-associated tubulin isotype mutations on microtubule dynamics	Science and Engineering Research Board	2023	2 years	PI
02	Alpha7 nicotinic acetylcholine receptor as a pharmacological target for treating neuronal disorders	Science and Engineering Research Board	2022	3 years	PI



Nagarjun Narayanaswamy, PhD

Scientist C

Transdisciplinary Biology

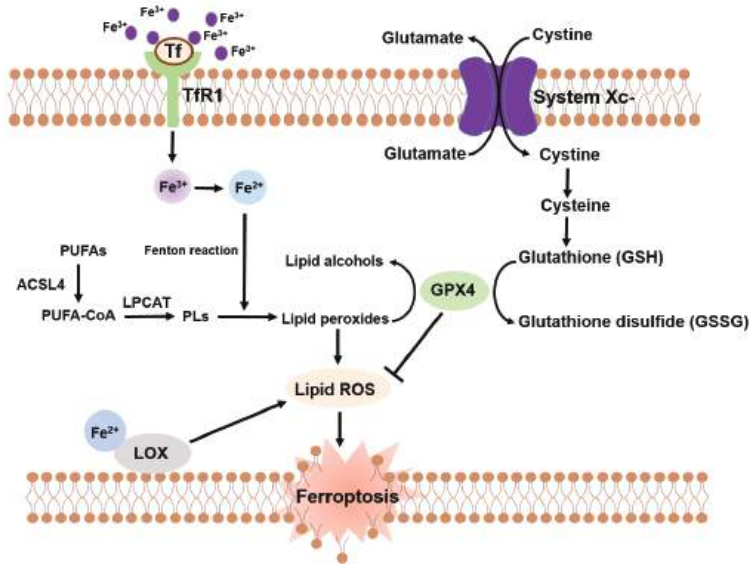
BRIEF THEME OF LABORATORY

Our lab at RGCB primarily focuses on developing new activity-based fluorescence probes/bioconjugation tools to uncover the biology of non-enzymatic protein-posttranslational modifications (PTMs), non-apoptotic cell death forms and intracellular organelle membrane contact sites (MCS). Further, we are also interested in developing DNA-based biosensors for detecting small molecular and protein biomarkers in liquid biopsy samples.

◆ ACTIVITY-BASED FLUORESCENCE PROBES TO UNRAVEL THE ROLE OF NON-APOPTOTIC CELL DEATHS IN CANCER

Apoptosis is a regulated cell death program mainly triggered by the activation of caspase family proteases. Resistance to apoptosis is a typical hallmark of cancer; this opens a new avenue to look for alternative non-apoptotic cell death forms to tackle resistance in cancer therapy. Ferroptosis is a new type of programmed cell death driven by iron-dependent lipid peroxidation-mediated membrane damage implicated in various pathological conditions like inflammation, ischemia-reperfusion injury, cancer, and neurodegeneration. Glutathione peroxidase 4 (GPX4) is a key regulator of ferroptosis by converting toxic lipid peroxides to corresponding non-toxic lipid hydroxides using glutathione as a cofactor. Typically, ferroptosis is determined by cell response to ferroptosis inducers or inhibitors by measuring oxidative stress levels. However, there are no potentially reliable biomarkers for predicting the ferroptosis process in cells. Hence, there is an unmet need to develop new activity-based fluorescence probes to understand the initiation and progression of ferroptosis physiologically and pathologically.

Herein, we will design a new class of activity-based fluorescence probes for GPX4 with masked nitrile-oxide chemical warheads. We will employ this new activity-based fluorescent probe to test the subcellular localization of GPX4 in a precise spatiotemporal manner, which further provides information about possible substrates and local cofactors in intracellular organelles. Additionally, we will use our new activity-based chemical tools to develop high-throughput screening methods for small-molecular pharmacological inducers of the ferroptosis process. Lipoxygenase 15 (15-LOX) and Acyl-CoA synthetase long-chain family member 4 (ACSL4) are two other key regulators of the ferroptosis process. We are also interested in developing new activity-based fluorescent probes for LOX-15 and ACSL4, which will help us obtain mechanistic insights into the ferroptosis process in living systems.



The key pathways involving the iron-mediated lipid peroxidation in ferroptosis cell death pathways. Glutathione peroxidase 4 (Gpx4) is the master regulator of ferroptosis in cells; it uses glutathione as a cofactor to reduce the lipid peroxides to lipid alcohols. ACSL4, acyl-CoA synthetase long-chain family member-4; GPX4, glutathione peroxidase-4; GSH, reduced glutathione; GSSG, oxidized glutathione; LOX, lipoxygenase; LPCAT, lysophosphatidylcholine acyltransferase; PUFA, polyunsaturated fatty acid; TfR1 transferrin receptor 1; System Xc- Cystine-glutamate antiporter.



PhD Students: 1

Shaima S, Dr Nagarjun Narayanaswamy

INVITED TALKS [PI ONLY]:

- "DNA-based fluorescent reporters for in vivo imaging of second messengers", conference on "Recent advances in Chemical Biology", organized by A.S.D Govt. Degree College for Women (A), Kakinada, Andhra Pradesh, on December 6th, 2023.
- "DNA-based fluorescent reporters for in vivo imaging of second messengers", theme: "Frontiers in Life Sciences, Chemical, Physical, and Medical Sciences" at IISF Young Scientist Conference YSC-2023 from 17-20 Jan, 2024 at DBT-THSTI, Faridabad.



Debanjan Bhowmik, PhD

Scientist C
Transdisciplinary Biology

BRIEF THEME OF LABORATORY

Our lab focuses on developing fluorescence and Raman spectroscopy-based diagnostics. We employ techniques including fluorescence cross-correlation spectroscopy (FCCS), FRET, SERS, etc. for probing.

We also have expertise in designing surface-functionalized gold nanoconstructs for theranostic applications. We are currently studying the efficiencies of such nanoconstructs by confocal imaging, in-situ probing of single nanoparticle dynamics in live cells, and measuring cytotoxicity at the cellular level prior to testing their efficiencies in animals.

MAJOR RESEARCH AREA

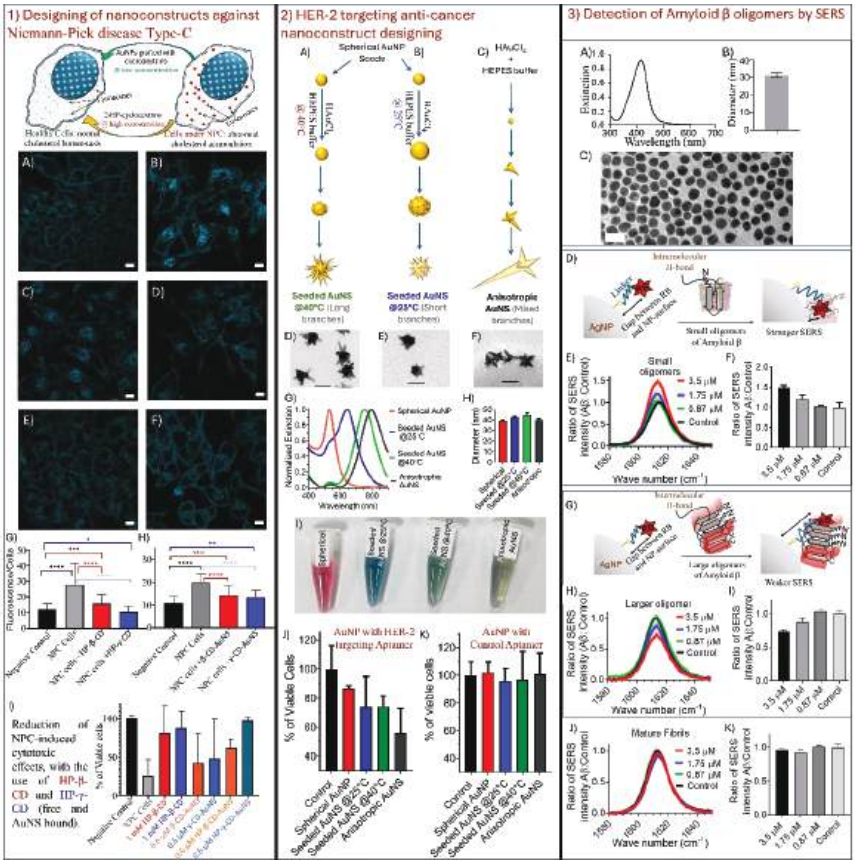
- Designing of metal nanoconstructs for drug delivery, receptor targeting, and bioimaging applications.
- The development of surface enhanced Raman spectroscopy (SERS)-based platforms for disease-diagnosis and for probing the presence of trace amounts of pesticides, carcinogens, pathogens, etc. in samples.
- Detection of disease-related exosomes (as biomarkers) by FCCS.
- Instrumentation: We are currently working on building a portable Raman spectrometer in the lab. In the future, we have plans to build an instrument capable of measuring fluorescence lifetime (by time-correlated single photon counting), FCCS, and fluorescence anisotropy.

◆ **DESIGN AND TEST OF SURFACE-FUNCTIONALIZED METAL NANOCONSTRUCTS FOR THERAPEUTIC APPLICATIONS AND FOR DEVELOPING SERS-BASED ULTRA-SENSITIVE SENSING PLATFORMS.**

The Niemann Pick disease type C (NPC) results in abnormal cholesterol accumulation in late endosomes (LE) and lysosomes (LS). 2-hydroxypropyl beta and gamma cyclodextrins (CDs) have been tried against NPC. Despite showing early promise, these efforts have failed during clinical trials due to poor pharmacokinetic profiles of the drugs. We propose to deliver the CDs by loading them on top of gold nanostars (AuNS) to achieve improved pharmacokinetics. Imaging after Filipin staining showed that our nanoconstructs were able to reduce the amount of cholesterol in LE and LS in NPC-induced cells (Figure 1B) with just 3 μM AuNS-loaded CDs (Figure 1C, 1D, and 1H). Comparable outcomes were also achieved with 1 mM of the free drugs (Figure 1E, 1F, and 1G). Also, AuNS-grafted-CDs were able to reduce the cytotoxicity in NPC-induced cells efficiently, as just 0.6 μM of the grafted-CDs were able to exert results comparable with what was achieved with 1 mM of the free drugs (Figure 1I).

Anisotropic gold nanostars (AuNS)—characterized by their branches of varying lengths (Figure 2C, F, and I)—demonstrate exceptional capability in projecting targeting molecules (e.g. HER-2 targeting aptamer or HApt) for receptor-interactions, even in the presence of serum proteins. Under similar conditions, however, spherical AuNPs often lose their targeting abilities. In this project, we aimed to find out the effect of the branch lengths on the receptor targeting ability of AuNPs. Four types of AuNPs were selected: Spherical AuNPs (S-AuNPs), AuNS, and two varieties of star-shaped AuNPs (K-AuNPs) with similar sizes but one having significantly shorter (Figure 2E) branches than the other (Figure 2D). We functionalized these AuNPs with HApt and tested their efficiencies in executing targeted-killing of HER-2 overexpressing cancer cells (SKOV3) in the presence of serum proteins. In line with our anticipation, our study showed that both AuNS and K-AuNPs outperformed S-AuNPs (Figure 2J, H). This research sheds light on the intricate relationship between nanoparticle structure and their targeted drug delivery efficacy, offering insights into designing more efficient nanomedicines.

The diagnosis of Alzheimer's disease (AD) relies on detection of fibrillar plaques formed by the aggregates of Amyloid beta (Aβ) peptides, in the brain. Early detection of small oligomers of Aβ could aid in timely intervention. Surface Enhanced Raman Spectroscopy (SERS) presents a promising avenue for the detection of Aβ oligomers. In this study, we functionalized silver nanoparticles (AgNPs) with Rose Bengal (RB), a dye capable of exerting selective interaction with amyloid-oligomers. 6-amino-1-hexanethiol was used as a linker for the grafting of RBs on the AgNP-surface. This allowed the RB molecules to reorient while interacting with Aβ oligomers, which alters their SERS signal. As expected, we observed increase in the SERS intensities with increasing concentration of small oligomers in solution. In the presence of large oligomers, however, opposite trend was observed. The varied responses generated form the SERS-nanoconstruct while interacting with different aggregation intermediates of Aβ, can potentially revolutionize diagnostic practices for AD.



1) Restoration of cholesterol distribution in the NPC-induced cells. Cholesterol distribution in A) normal, B) NPC-induced, C) and D) nanoconstruct treated NPC-induced, E) and F) HP-β-CD and HP-γ-CD treated NPC-induced cells. G) and H) analysis of the imaging experiment. I) Effect on NPC-induced cytotoxicity.

2) Effect of nanoconstruct shapes on receptor targeting. A) to C) Protocols for obtaining AuNPs. D) to F) AuNPs with long, short, and variable branch lengths. G) to I) LSPR position, sizes, and colour of the AuNPs. J) and H) cytotoxicity of HER-2 targeting and non-targeting nanoconstructs.

3) Detection of Aβ. A) to C) characterization of the AgNPs. D) Nanoconstruct with small oligomers. E) and F) RB-SERS in the presence of small oligomers. G) Nanoconstruct with large oligomers. H) and I) RB-SERS in the presence of large oligomers. J) and K) RB-SERS in the presence of Aβ-fibrils.



JRF: 1
Project Assistant: 1
Lab Assistant: 1
Technical Officer: 1
PhD Students: 2

Sundhanshu Mani Tripathi, Nithya K Nair, Shreya B Unnithan, Dr Debanjan Bhowmik, Abhirami Ajith, Krishna Sawarn, Athira B, Preetha R Nair, Harikrishnan K S (From L to R)

INVITED TALKS [PI ONLY]:

- Designing of surface functionalized metal nanoconstructs for diagnostics, therapeutics, and imaging. Name of the Seminar: FCSXIV. Date: 9 to 14 December 2023. Venue: IISER-Mohali.

CONFERENCE PRESENTATION:

- Abhirami Ajith, Poster Presentation, SERS based probing of disease specific exosomes. At FCSXIV on Date: 9 to 14 December 2023. Venue: IISER-Mohali.
- Sudhanshu Mani Tripathi, Poster Presentation, Designing of SERS nanoconstructs for the detection of Amyloid beta oligomers at low concnetrations. At FCSXIV. Date: 9 to 14 December 2023. Venue: IISER-Mohali.



Umasankar P.K, PhD

Ramalingaswami Faculty Fellow
Transdisciplinary Biology

BRIEF THEME OF LABORATORY

The central theme of our lab is to understand the regulatory mechanisms of clathrin-mediated endocytosis (CME), a fundamental cellular process for the internalization of nutrients, signals and viruses in development and disease. Specifically, we seek to identify activators and inhibitors of the central clathrin-adaptor protein complex, AP-2 at cellular and organismal levels. We use cultured mammalian cells and zebrafish model for our investigations.

MAJOR RESEARCH AREA

- Identified and characterized a novel endocytic kinase, BMP2K.
- Delineated how the kinase switch operates during CME.
- Discovered a new role for cholesterol in the regulation of insulin-mTORC1 growth axis.
- Proposed cholesterol-induced Rag GTPase activation as a novel strategy to correct growth retardation in Smith-Lemli Opitz syndrome, a cholesterol-biosyntheis disorder.

REGULATORY MECHANISMS OF CLATHRIN-MEDIATED ENDOCYTOSIS IN DEVELOPMENT AND DISEASE

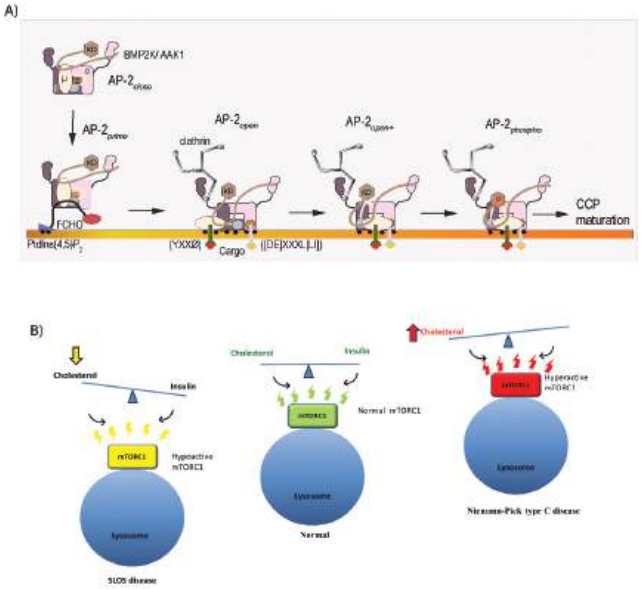
Clathrin-mediated endocytosis (CME) is a major cellular internalization pathway in eukaryotes. Aberrations in this process are hall marks of cancer, metabolic and neurological diseases. CME is also a favourite entry route for viruses and other intracellular pathogens. Yet how CME is regulated in vivo remains poorly understood. Our lab seeks to identify the regulatory mechanisms of CME in vertebrates with the hope of designing novel inhibitors and activators that could fine tune the process. We use cultured cells and zebrafish model for our investigations.

The heterotetrameric clathrin adaptor complex, AP-2 is the core organizer of CME. AP-2 recruits accessory proteins and clathrin to form clathrin-coated pits (CCP) into which cargo is sorted. Yet, how AP-2 is regulated remains poorly understood. Phosphorylation of a T156 residue in the µ subunit of AP-2 (T156µ) is considered a critical regulatory step in CME. Recent studies from our lab had identified a novel kinase, BMP2K that phosphorylates AP-2 and promotes CME at cellular and organismal level (Ramesh et al., 2019, Ramesh et al., 2021). Now we show that AP-2 priming by BMP2K and the redundant AAK1 act as a spatio-temporal regulatory mechanism for T156µ phosphorylation. the mechanism that regulates AP-2 phosphorylation. BMP2K has an N-terminal kinase domain, an extended region and a Q/H- rich prion-like domain which can possibly undergo liquid-liquid phase separation followed by a long unstructured C-terminus (CT). This priming event occurs when an extended region of BMP2K/AAK1 kinase domain interacts directly and specifically with membrane-bound AP-2. Disrupting this interaction by deletion of the binding region or depletion of BMP2K and AAK1 causes complete loss of AP-2 phosphorylation, delay in CCP maturation and reduction in the global rates of CME. Abolishing kinase-induced AP-2 priming in zebrafish leads to delay in integrin endocytosis causing aberrant cell migration defects during gastrulation (Ramesh et al, 2024). Our findings propose a novel layer of regulation and spatio-temporal fidelity to CME.

Another major project in the lab investigates role of CME in regulation of cell growth. CME is the main provider of the chief metabolite, cholesterol to all cells in our body. Along these lines, we discovered that cholesterol internalized via CME supports insulin-induced mTORC1 signaling on lysosomes for cell growth. We showed that cholesterol cooperates with autophagy to sustain mTORC1 reactivation and insulin responsiveness during prolonged starvation. We also noticed that insulin- mTORC1 axis is defective in Smith-Lemli-Opitz Syndrome (SLOS) patients deficient for cholesterol biosynthesis. These defects can be rescued by supplementing cholesterol and insulin or by expressing constitutively active Rag GTPase (Navyasree et al., 2023). On the contrary, we showed that the insulin-mTORC1 axis is hyperactive in Niemann-Pick type C (NPC) patients showing lysosomal cholesterol storage. Together, our findings propose novel therapeutic strategies for SLOS and NPC patients.

A) Schematic depiction of mechanism of BMP2K-mediated AP-2 phosphorylation during CME

B) Schematic showing role of cholesterol in the regulation of insulin-mTORC1 axis on lysosomes to drive normal cell growth and contrasting deregulation of the axis in SLOS and NPC patients





Project Assistant: 2
PhD Students: 2

Gopika B M,
Dr. Umasankar P K, Lekshmi K S

PUBLICATIONS:

- Navyasree KV, Ramesh ST, Umasankar PK. Cholesterol regulates insulin-induced mTORC1 signaling. J Cell Sci. 2023 ;136(22):jcs261402.

AWARDS [STUDENTS]:

- Shikha T. Ramesh got Best oral presentation award, phosphoregulation of clathrin-mediated endocytosis via BMP2K, a novel serine/threonine kinase at International Conference on Emerging Trends in Genomics and Biomedicine (ETGB), on 15th -17th November 2023, Inter-University Centre for Genomics and Gene Technology (IU-CGGT), University of Kerala, Thiruvananthapuram

INVITED TALKS (PI ONLY):

- Phosphoregulation of clathrin-mediated endocytosis Organellar Biology and Membrane Trafficking 2023 on 9-11 October 2023, NBRC, Manesar

CONFERENCE PRESENTATION:

- Shikha T Ramesh gave Oral presentation on Phosphoregulation Of Clathrin-Mediated Endocytosis Via Bmp2k, A Novel Serine/Threonine Kinase at International Conference on Emerging Trends in Genomics and Biomedicine (ETGB) on 15th -17th November 2023, Inter-University Centre for Genomics and Gene Technology (IU-CGGT), University of Kerala, Thiruvananthapuram
- Shikha T Ramesh, Poster Presentation on Understanding The Role Of Bmp-2 Inducible Protein Kinase In Clathrin-Mediated Endocytosis at Manipal Research Colloquium 2023 (MRC-2023), on 2th to 14th April 2023, MAHE, Manipal
- Navyasree K.V, Poster Presentation on Role of Endo-lysosomal Cholesterol transport in mTORC1 signaling at Manipal Research Colloquium 2023 (MRC-2023) on 12th to 14th April 2023, MAHE, Manipal

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Endocytic modulation of BMP signaling: deciphering mechanistic insights into health and disease	Department of Biotechnology (Ramalingaswami Fellowship), Government of India	2016	10 years	PI

PhD AWARDED:

SI.NO	NAME OF THE STUDENTS	TITLE OF THESIS	UNIVERSITY	AWARDED/ SUBMITTED	YEAR
01	Shikha T Ramesh	Understanding The Role Of Bmp-2 Inducible Protein Kinase (Bmp2k) In Clathrin-Mediated Endocytosis	Manipal Academy of Higher Education,	Submitted	2024
02	Navyasree K. V.	Understanding The Role Of Cholesterol In Insulin-Mtorc1 Growth Axis Regulation	Manipal Academy of Higher Education,	Submitted	2024



LABORATORY MEDICINE & MOLECULAR DIAGNOSTICS (LMMD)



Radhakrishnan R, PhD

Scientist F
Laboratory Medicine & Molecular Diagnostics

BRIEF THEME OF LABORATORY

LMMD is one division of its kind which performs molecular diagnostics, serology, and research on both infectious and non-infectious diseases. The division performs its duty as a social service arm of RGCB, helping the Government in disease control like outbreaks. The division also helps clinicians in planning treatment strategies for immunocompromised patients by doing Next Generation sequencing studies like whole genome sequencing, whole exome sequencing, and mutation-specific sequencing.

MAJOR RESEARCH AREA

- Co-developer of monoclonal antibodies against Nipah Virus.
- CDSCO, ICMR, DBT, and US FDA-approved kit validation center. Validation of all molecular Diagnostic kits, tools, and disinfectants (both instruments and reagents).
- WHO KARS NET Programme: Kerala Antimicrobial surveillance network.
- NGS based identification of mtDNA methylation levels.
- NGS based mutation analysis in POLE mutation in prognosis of endometrial cancer.
- Identification of Gut microbiome using NGS.
- Sequence-based analysis of Drug-resistant genotypes in HBV and HCV in planning treatment regime.
- Wastewater Genomic surveillance for emerging pathogens.
- Surveillance of mosquito-borne disease in Kerala in collaboration with Govt of Kerala, Dept of Health Service.

- **LABORATORY MEDICINE & MOLECULAR DIAGNOSTICS**

Laboratory Medicine and Molecular Diagnostics were initiated as a diagnostics arm of RGCB to help the public perform vital molecular diagnostics tests at low cost with less turnaround time. The lab has experience handling 1000 samples per day for different tests. Once the outbreak of Dengue, H1N1, and Chikungunya happened in the states of 2013, 2016, and 2020 the daily sample size was more than 1500 per day. The division has handled more than 5000 samples during the SARS CoV 2 pandemic situation. The major attraction of the division between the public and Government is the turnaround time. The division holds all the quality standards like NABL ISO 15189-2022, NABH, and ILAC, which make the reports generated from the division credible and accepted worldwide. As the division has developed from 4 parameters to 226 parameters, the division has improved its quality standards. The division has branched into multiple levels such as Sanger sequencing, Next Generation Sequencing, Collaborative research and Validation of kits.

Molecular Diagnostics

Molecular diagnostics has become a popular diagnostic platform for many diseases. The division focuses on diagnostics of molecular diagnostics for 250 infectious and non-infectious diseases. Excluding the 8 investigations in which viral culture on a BSL3 facility is required, every other parameter is in-house developed. Additional Director of Health Services (ADHS)Entomology and District Vector Control Unit (DVCU) submit patient and mosquito samples from different areas for monitoring the emergence of epidemics like Dengue, Chikungunya, Zika, Malaria, etc. The lab also conducts serology-based allergen tests and many other tests based on serology. All the reports are generated through the LIMS cloud-based reporting system. All the data are uploaded to the NIE database as well. Total revenue generated from January 2023 till June 2024 is: 3,28,83,716 INR. A total of 7431 samples have been screened from April 2023 to March 2024

Next Generation Sequencing

The division performs next-generation sequencing of SARS CoV 2 for the identification of new variants. A total of 19977 SARS CoV2 samples have been sequenced and the data has been uploaded in IHIP, IBDC and NCBI. The division also does deep sequencing of the gut microbiome, P53 methylation studies for cancer genomics, etc. The lab has introduced whole genome sequencing, whole exome sequencing and mutation specific sequencing for the prognosis of different kinds of cancer and cardiovascular diseases.

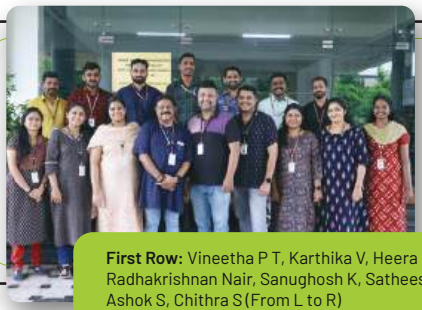
Kit Validation

LMMD is a CDSCO, ICMR, DBT, and US FDA-approved kit validation center. Validation of all molecular Diagnostic kits, tools, and disinfectants (both instruments and reagents). A total of 72 validations has been conducted so far. The revenue generated by the validation of kits and product development by translational research is also added to the total income generated by LMMD apart from molecular diagnostics.

Training

LMMD is an MCI-approved rotation center for medical PG students, (MD Biochemistry, MD Microbiology, MD Pharmacology, MD Pathology, and MD Transfusion Medicine) who have completed their training in this Division so far. LMMD offers a Biotechnology Skill development program for young budding researchers. A total of 355 students have completed the training from this division.





Project Assistant: 4
Technical Assistant: 5
Lab Assistant: 3
Project Officer: 1
Data Entry Operator: 1
Lab Technician: 6

First Row: Vineetha P T, Karthika V, Heera Pillai R, Dr R Radhakrishnan Nair, Sanughosh K, Satheesh D, Soumya V K, Athira Ashok S, Chithra S (From L to R)

Second Row: Rahul J L, Deepak M S, Ajo Jose, Shomu S, Surjith S B, Bibin B S, Sooraj Mohan C S (From L to R)

PUBLICATIONS:

- Seetha D, Nori SRC, Nair RR. Molecular-based study of scrub typhus in Kerala, South India from 2014 to 2021: a laboratory-based study. Comp Clin Path. 2023;32(3):347-356.

ONGOING GRANTS:

SI.NO	TITLE	FUNDING AGENCY	YEAR OF STARTING	DURATION	PI/CO-PI
01	Genome Surveillance for SARS CoV2 in India, INSACOG -Phase II	Department of Biotechnology	2022	3 years	PI
02	Expansion of INSACOG- water Genomics Surveillance for Emerging Pathogens and Antimicrobial Resistance through Genomics-Based Methods	Department of Biotechnology	2024	1 year	PI



GENERAL ADMINISTRATION

OFFICE OF THE DIRECTOR



First Row: Dr Chandrabhass Narayana, Mohanan Nair S
Second Row: Priya R, Jayalakshmi U.S, Venugopalan J, Anoop

The Office of the Director is responsible for successful leadership and management of the organization according to the strategic directions set by the institute management. This office develops the vision and strategic plan to guidethe organization, develop an operational plan which incorporates goals and objectives ensures that the operation of the organization meets expectations of its stakeholders and funding agencies. The Office of the Director also oversees efficient and effective day-to-day functions of the organization, draft policies for approval of the Governing Body; prepare procedures to implement organizational policies; review existing policies and recommend changes as appropriate; ensure that programs and services offered by the institute contribute to its mission; monitor day-to-day delivery of programs and services to maintain or improve quality, determine staffing requirements for organizational management and program delivery, recruit, interview and select staff that have the right technical and personal abilities to help further the organization's mission. The Office also is responsible to supervise preparation of a comprehensive budget and to secure adequate funding for the operation of the organization.

CHIEF CONTROLLER



Mr. Mohanan Nair S

Directs and coordinates the administrative, finance purchase & stores activities at the Institute, business service functions & procedures of the Institute and ensures compliance with all applicable regulations & policies. The Chief Controller is the primary link between the general administration groups and the Office of the Director. The Chief Controller also provides leadership & supervision of business services, administrative duties, all recruitment & promotions, compilation and monitoring of revenue, expenditures etc. The Chief Controller manages all procurements and provision of stores/stock. All legal matters are also supervised by the Chief Controller. The Security, Vigilance, Disciplinary matters are all dealt with by Chief controller.

GENERAL ADMINISTRATION

The management of research and development (R&D) and innovation has emerged as a specialized area within both research and higher education institutions. New modalities of research and innovation have evolved over the last 10 to 20 years against a backdrop of major changes in the tertiary research and education sector as a whole. The Administration Group is backbone of any such organization. An effective administrator is an asset to an organization. The Administration Group is the link between various units and sections of the organization and ensures the smooth flow of information from one part to the other. The Administration Group also provides administrative & technical support in the areas of human resources management, budgetary, strategic planning, legal affairs, pay and allowances, medical benefits, leave management, purchase procedures, management of stores and facilitates security.

The main responsibility of general administration group is to ensure all requirements are implemented for the efficient performance of all research related services at RGCB. The General Administration Group serves as the connecting link between the senior management and employees. The major mandates of general administration include good coordination among all the departments ensuring attainment of organizational goals; optimum utilization of resources, minimization of cost, human resources and payroll, transportation, fulfilment of social and economic needs of the employees and organization as well as development and growth of the institute. The General Administration also implements work related to Estate Affairs, House Keeping & Welfare, Legal matters and implementation of various Acts (including RTI), Building Engineering & Construction, Security & Surveillance, Vigilance & Disciplinary matters and official language.

ADMINISTRATIVE OFFICE, AKKULAM CAMPUS



First Row: Dr Chandrabhass Narayana, Mohanan Nair S
Second Row: Bineesh, Aneez, Ananthu, Ananthu Ashok, R. Kumar, Anoop, Gowri, Karthika, Anjana, Neethu (L to R)

ADMINISTRATIVE OFFICE, MAIN CAMPUS



First Row: Mohanan Nair S, Dr. Chandrabhas Narayana, Shiby Benny (L to R)
Second Row: Nidheesh Raj R, Usha B, Lakshmidarsan S, Sujitha S, Sandhya S J, Priya R, Jayalakshmi U S, Jayachandran Nair R (L to R)
Third Row: Sreejith S, Ganesh Babu B, Vishnu P, Venugopalan J, Reena Prasad, Preetha J, Manukumar V M, Vishnu S (L to R)
Last Row: Dileep Kumar R, Dr. Vishnu T S, Vinod Kumar S R, Subash K, Wilson T, Praveen B, Ratheesh R, Vijayakumar S, Ashok Kumar S (L to R)

ADMINISTRATIVE OFFICE, IPP MAIN CAMPUS



First Row: Shiby Benny, Mohanan Nair S, Priya R, Jayalekshmi U S(from L to R)
Second Row: Aryasri, Priya, Jyothisree, Athirachandran (from L to R)
Third Row: Athira V L, Chitra G S, Smitha, Sreevidhya, Prathibha, Meena, Asha V S, Rahul, Abhilal (from L to R) **Fourth Row:** Anitha, Sajeesh, Vinu, Renjith Kurup, Rakesh, Arun, Sreejith G S, Pradeep, Krishnakumar, Byju, Vaisakh, Harikrishnan (from L to R) **Last Row:** Dinesh, Rahul, Sarath S N, Akshaykumar Nair, Deepu (from L to R)

FINANCE & IFC DIVISIONS

The Finance Division of RGCB has been inventive in budget planning and its real-time reporting, always in absolute synchronization with the scientific fraternity of the Institute. Preparation & Monitoring of Budget and Resource Generation are always aimed to acclimatize the available resources' utilization in achievement of its mandated science, thereby paving the way for productive application of all available resources. Prompt generation and submission of internal management information by the Finance Division always facilitates RGCB in taking accurate and apt decisions. Matters related to RGCB's Finance Committee, audit, processing of payments, TDS/GST and returns, accounting of receipts & disbursements, revenue refunds, reconciliation of bank accounts and rendition of utilization certificates and statements of expenditure are always promptly implemented by the Finance Division. The Final Accounts along with Audit Report are placed on the tables of both Houses of Parliament through the Department of Biotechnology. The dynamic contributions of Finance Division have always resulted in building organizational strength, enthusiastic and motivated personnel and hence a robust Institution.

A dedicated IFC enhances in all Financial as well as Administrative/Establishment works related to extra-mural funded projects of the Institute, all matters related to PhD, M.Sc, Summer training programs, Post-Doctoral Fellows etc. Accounting in respect of all service facilities of the Institute are exclusively done by this Group. This specialized Group plays an extremely important management role of all extramural and Institute generated funds. It is the connecting link between all funding agencies and RGCB. The vital duties of IFC includes implementation of procedures related to accounting, payment, preparation and rendition of Utilization Certificates and Statements of Expenditure in respect of all cases except the Core Grants, Ph.D Fellowships, Post Doctoral Fellowships, Program Scientist Fellowships and fund management of Extra Mural Projects. The IFC is also the internal link in respect of all matters pertaining to purchases in utilisation of such extra-mural funds & receipt and issue of stores.

OFFICE OF ACADEMIC AFFAIRS (OAA) & MANAGEMENT

The Rajiv Gandhi Centre for Biotechnology Academic Council (RGCB AC)is the highest academic body of the institute, chaired by the Director.AC supports professional development, continuous quality improvement, program assessment, tutoring services, educational initiatives and awards. To fulfill this mission, we research and evaluate best practices in teaching and learning to ensure optimal learning experiences for students within the institute. RGCB AC advises them an agementon all academic matters and is also responsible for structuring the course syllabus for the PhD course work and MSc Biotechnology course, admission procedures, program initiatives, faculty support, academic calendar, setting the timetables and conducting examinations.This committee also recommends suitable persons as faculty/adjunct faculty to run the programs,decideson times and duration of specialty training and internships and ensures coordination between academic affairs management and all other administrative section softhe institute.

RGCB ACADEMIC COUNSEL

Name of the Faculty	Position	Designation
Professor Chandrabhas Narayana	Director	Chairman
Dr. George Thomas	Scientist G & Dean (Research Administration)	Member
Dr. Priya Srinivas	Scientist G & Dean (Academic Affairs)	Member
Dr. Jackson Games	Scientist G	Member
Dr. Vinod Kumar G S	Scientist F (PhD coordinator)	Member
Dr. Hari Kumar K B	Scientist EII	Member
Dr. Debasree Dutta	Scientist EII	Member
Dr. Rajesh Chandramohanadas	Scientist EII (M.Sc Coordinator)	Member
Dr. Santhosh Kumar K	Senior Consultant (Academic Administration)	Member Secretory
Dr Sumi S	Program Scientist (M.Sc Coordinator)	Special Invitee



Briji S, Ajith Gopal, Harish G, Beena Nair L, Dr K Santhosh Kumar, Dr Priya Srinivas (From L to R)

Office of Academic Affairs(OAA) supports the management of academic programs at RGCB including PhD and MSc programs, short and long term training programs, Post-doctoral training, biotechnology skill development programs and other specialized training programs of the institute. The OAA assist the Academic Council to provide leadership roles for activities related to curriculum development such as orientation, residence life, and student activities, implementation, and policy in development of a strong academic program, policy formulation, program planning and student research progresse valuation.OAA keeps abreast of trends and changes in higher education; works for institutional vision, survival, stability, growth, and excellence; provides a connection between administration and faculty; serves as catalyst to createaclimateconducivetoscholarlyresearchinanatmospherecommitt edtothemandates of the institute and the Department of Biotechnology. OAA is also responsible for the maintenance of all official records related to academics, conducting examinations and publishing results in a time bound manner and distribution of all certificates related to academic activities. The Office of Academic Affairs ensure in stimulating best practices, supportive environments, continuous improvement to achieve best quality learning for students and the excellence measured by results

RGCB PHD PROGRAMME.

Each year RGCB invites applications twice from postgraduates in Life/Environmental/Veterinary/Pharmaceutical/Medical Sciences or allied subjects (Biochemistry/ Biotechnology/ Bioinformatics/ Biophysics/ Chemistry/ Microbiology, etc.) for the prestigious research programs leading to PhD award. To ensure the highest standard of research excellence, the applicants should have a master's degree in various domains of Disease Biology, Neuroscience, Plant Science and Bioinformatics with minimum of 60% marks and a Doctoral Fellowships award (UGC/CSIR/ICMR/DBT/DST-INSPIRE or any other nationally-competitive fellowship) offered by the Government of India, valid for a period of five years. RGCB PhD programme is currently affiliated to three esteemed Universities, The Regional Centre for Biotechnology (RCB),Faridabad, The Manipal Academy of Higher Education (MAHI), Manipal and The University of Kerala, Thiruvananthapuram.In the last academic year (April 2023 to March 2024)twenty-four students across India were selected for the Ph.D. program. These students have embarked on research in various domains, including Disease Biology and Neuroscience, Plant Science and Bioinformatics.

From April 2023 to March 2024, twenty-one students were awarded Ph.D degrees upon successful completion and open defense of their research work. Among the Ph.D awardees, one student was from RCB, Faridabad, nine students were from MAHE, Manipal, and eleven students were from the University of Kerala, Thiruvananthapuram.

STUDENT MERIT AND BEST THESIS AWARDS

RGCB constituted Student Merit Award and Best Thesis Award each year for the PhD students. The Student Merit Award is conferred upon PhD students who have completed a minimum of three years research to acknowledge their academic excellence and research accomplishments. The Best Thesis Award is presented to Ph.D. awarded students who have demonstrated exceptional research output and have made significant contributions to their field of research. This prestigious award serves as a testament to the quality and impact of the research work of the student conducted at RGCB.The selection of recipients for these prestigious awards is undertaken by a committee constituted by the Director, RGCB. This committee rigorously scrutinizes the applications ensuring that only the most deserving candidates, who meet all the stringent eligibility criteria, are chosen for these honors.These awards are presented annually on RGCB Day, observed on November 18th. In 2023 Mr.Mayur Balkrishna Shirude, who is working under the mentorship of Dr. Debasree Dutta received the Student merit award. Dr. Gayathri G, who worked under the mentorship of Dr. Arumugham Rajavelu, won the best thesis award (2021) for her thesis work titled "Exploration of Intraerythrocytic Epitranscriptome of Human Malaria Parasite Plasmodium falciparum"Dr. Pallavi Chinnu Varghese,who worked under the mentorship of Dr. Debasree Dutta won the best thesis award (2022) for her thesis titled "" Epigenetic Modifications In Cellular Transition During Development".



Mr.Mayur Balkrishna Shirude



Dr. Gayathri G



Dr. Pallavi Chinnu Varghese

RGCB MASTER'S DEGREE PROGRAMME IN BIOTECHNOLOGY:

In July 2019, RGCB launched a highly innovative in-house MSc Biotechnology Program affiliated to the Regional Centre for Biotechnology an "Institution of National Importance" for providing education, training and research established by the Department of Biotechnology, Government of India under the auspices of the United Nations Educational, Scientific and Cultural Organization or UNESCO, a specialized agency of the United Nations (UN) based in Paris. The RGCB MSc Biotechnology is a two years program inclusive of four semesterswith two unique specializations - Disease Biology (10 seats), and Genetic Engineering (10 seats). Students with valid GAT-B score and having an aggregate of 60% marks (or an equivalent grade point average) in their Bachelor's degree in any branch of Science, Engineering, or Medicine are eligible to apply.The final selection is based on the GAT-B score.

accomplishments. Ms. Parvathi K of the MSc 2021-23 batchown the Best Outgoing Student wards and Mr. Charanraj C A who worked under the mentorship of Dr.Debasree Dutta,own the best MSc Dissertation award for his thesis work titled "Role of histone chaperone HIRA in t ransition of normal hematopoietic stem cells to leukemia stem cells". Ms. Ritika Sachdeva of the 2022-24 batch student is selected for the prestegious Khorana Scholar-2023, an award jointly constituted by DBT, Indo-U.S. Science and Technology Forum (IUSSTF) and WINStep which enable her to carry outthree-monthinternship at the University of Alabama, Birmingham, USA. RGCB MSc Biotechnology students (2021-2023 batch) seventeen students cleared GATE examination and ten students cleared National Doctoral fellowship examination which include CSIR/UGC NET, DBT BET.



Ms. Ritika Sachdeva Khorana Scholar-2023

OFFICE OF TECHNOLOGY VENTURES
BRIC-RGCB



Vivek Hari S, Dr Asha Nair S, Dr Anish P, Jayakrishnan K S
(From L to R)

- **Patents:**During the reporting period, RGCB- OTV facilitated to achieve two more patents for the invention, "Uttroside B and derivatives thereof as therapeutics for hepatocellular carcinoma" (USPTO No. 11,738,036 dated 29-08-2023 and European Patent No. EP3463382 dated 30-o8-2023). Notably, Uttroside B, a molecule demonstrating therapeutic promise against liver cancer, was extracted from the medicinal plant Solanumnigrum and characterized. The compound is presently undergoing clinical trials and has obtained orphan drug designation from the FDA. The compound has been previously licenced to multinational company Q-Biomed for further development in a joint initiative with RGCB and Oklahoma Medical Research Foundation (OMRF), USA.

  Other patents granted includes the inventions "Polypeptides for Managing Viral Infections" (USPT No. 11666630 dated 06 June, 2023), and "A Process for the Preparation of Plant Extract that can Inhibit A Neuronal Ligand Gated Calcium Channel" (Indian PT No. 480483 dated 12 December, 2023). Additionally, a provisional patent application was filed for a novel stem cell activator protein (Indian Application No.202341056900 dated 24 August, 2023).

- **Major Agreements/Collaborations:** RGCB-OTV assisted in drafting several research collaboration agreements and memorandums of understanding (MoUs) with organizations and institutions such as CMET, K-DISC, Government Medical College Trivandrum, IIT-Madras, St. John's Medical College, PRS Hospitals, Manipal Academy of Higher Education, THSTI and more.During the reporting period RGCB collaborated with signed MoU with 22 organizations for collaborative research and development. These collaborations highlight the commitment to advancing research in various areas of biotechnology.

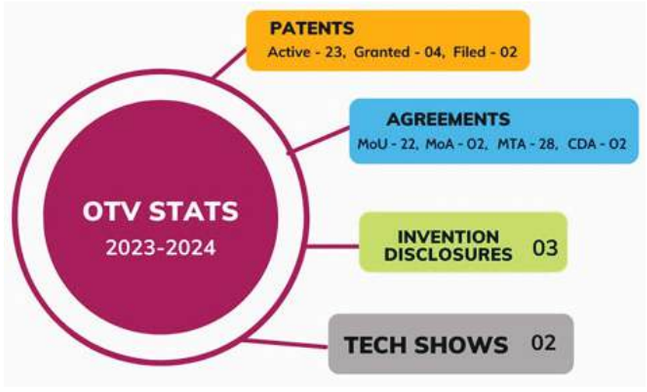
- RGCB-OTV has successfully facilitated the deposition of 16 plasmid constructs in Addgene, a non-profit plasmid repository (https://www.addgene.org/depositing/82137/).These plasmids were developed as part of the research conducted by Dr. R. V. Omkumar at RGCB and are now accessible to researchers worldwide.

- RGCB-OTV facilitated the process of securing membership for RGCB in the Indian Cancer Genome Atlas (ICGA) Foundation. This foundation is involved in processing and banking tissue/biospecimen samples, as well as conducting multi-omics analysis and data annotations sequencing. The curated data from the ICGA project will be accessible for research in the public domain, accompanied by appropriate safeguards, documentation, bioethics, and data privacy guidelines.

- Establishment of a Centre of Excellence in Microbiomes in Kerala has been formally made by the collaboration of Kerala Development and Innovation Strategic Council (K-DISC), Kerala State Council for Science, Technology and Environment (KSCSTE), and RGCB.

- **Participation in Tech Shows:** RGCB participated in various tech shows including the BioConnect Industrial Conclave Kerala-2023, National Technology Week, and Global Bio India 2023, showcasing RGCB expertise, technologies and contributions to the life sciences field.

The Office of Technology Ventures (OTV) at RGCB is aiming to manage the intellectual property (IP) resulting from the institution's research activities, with a focus on translating scientific output into societal impact through conscientious IP management and encouragement of innovative ideas. The office's mission is to enhance IP generation, manage IP, foster collaboration with the industry, and uphold the core values of the institution.The activities and achievements of RGCB-OTV during 2023-24 demonstrate significant progress in research, collaborations, and patent grants. Here are the key outcomes:



The research collaborations, agreements, and patent grants indicate RGCB's commitment to advancing healthcare solutions. The collaborations with academic institutions, research centers, and healthcare facilities signify a collective effort towards addressing complex research challenges. It can lead to the development of innovative approaches and sharing of knowledge, ultimately benefiting the broader scientific and medical community.Overall, the societal impact of RGCB's activities during 2023-24 is significant, with potential contributions to healthcare, economic development, knowledge sharing, and capacity building.

PURCHASE & STORES DIVISION

The Purchase & Stores Group occupies a vital and unique position in RGCB. This unit ensures procurement of the right material in right quantities and of appropriate quality. The section ensures procurement from right and reliable source or vendor as well as procurement of the material economically, i.e., at right or reasonable price. The RGCB Central Stores serves all four campuses of the institute. The most common yet major responsibilities that are carried by stores include receipt of incoming goods, inspection of all receipts, storage and preservation, identification of all materials stored, materials handling, packaging maintenance of stock records, inventory control and stock-taking.



FACILITIES & SERVICES

ANIMAL RESEARCH FACILITY (ARF)

The Animal Research Facility is a state-of-the-art facility dedicated to the maintenance, breeding, and supply of healthy and well-defined laboratory animals for research projects. Our primary focus is to ensure the health, quality, and ethical treatment of all animals under our care, adhering strictly to regulatory guidelines. Our facility houses various strain of mice, rat, rabbit and zebra fish.

The animals are maintained in a well-controlled micro and macro environment according to their species specific preferences. Continuous health monitoring and rigorous quality control measures are implemented to ensure the well-being of the laboratory animals. This includes regular health checks, veterinary care, and environmental monitoring to maintain optimal living conditions. We comply with all guidelines laid down by the Committee for the Control and Supervision of Experiments on Animals (CCSEA) and our registration number is 326/GO/ReBiBt/S/01/CCSEA.

The Animal Research Facility (ARF) operates under the stringent design, procedures, and practices of Biosafety Level II standards. This ensures a safe and controlled environment for both researchers and animals, minimizing the risk of contamination and exposure to hazardous agents. Our dedicated team of Veterinarians and technical staff has successfully supported numerous research projects within and outside the institute, contributing to significant scientific advancements. The income of ARF for FY 23-24 from in-house studies is Rs. 5,06,480 and from contract studies is Rs. 66,670. ARF offer comprehensive teaching and training programs in the area of laboratory animal science. These programs are designed to educate our in-house researchers, technicians, and students on best practices in animal care and use, promoting ethical and humane treatment of laboratory animals.

At ARF, we are dedicated to advancing the field of animal research through quality service, expertise, and a commitment to ethical practices.



First Row: Sumaja V, Dr Archana S, Dr Quienee, Govind
Second Row: Vinod, Rajeev R V, Anilkumar B



Dr Arya Aravind, Neethu S M

BIO-IMAGING, FLOW CYTOMETRY, AND HISTOLOGY CORE FACILITY

FLOW CYTOMETRY CORE

The FACS facility of RGCB is equipped with the following flow cytometer sorters and analyzer. The facility support RGCB research programs requiring all application of cell analysis and sorting and is made available to external academia and industries as per the DBT SAHAJ guidelines. The facility also provides regular training and workshop to students and faculty in basic and advanced flow cytometer applications.

High Speed Flowcytometer Sorter System: (FACSAria III)

FACSAria III is a Bench top fixed aligned high speed 4-way sorter system from Becton Dickinson, USA and is equipped with the laser lines, 488 nm, 355 UV, 405 Violet, 561 yellow green and 633 nm red. The machine allows plate sorting. Also equipped with aerosol management system. Location: RGCB Main campus, Central Instrumentation.

High Speed Flow Cytometer Sorter System: (FACSAria II)

FACS Aria II is a Bench top fixed aligned high speed 4-way sorter systems from Becton Dickinson, USA and is equipped with the laser lines 488 nm, 375 UV, 405 Violet lasers, and 633 nm. Also equipped with aerosol management system. Location: RGCB Main campus,Central Instrumentation.

High Speed Flow Cytometer Sorter System: (FACSAria III)

FACS Aria III, Bench top fixed aligned flow cytometer is a high speed



Indu Ramachandran, Dr S.Asha Nair, Arya V.S

4-way sorter system from Becton Dickinson, USA and is equipped with the following laser lines 488 nm Laser, 405 nm Violet lasers, 561nm laser and 633 nm laser. Location BIC, KINFRA, Central Instrumentation

High Speed Jet in Air Flow Cytometer Sorter System: (Astrios EQs)

This high-speed cell sorter is a six-way jet-in-air sorter from Beckman with 7 spatially separated lasers of 355 nm, 405 nm, 488 nm, 532 nm, 560 nm, 592 nm, 645 nm. Location: Akkulam Campus. The unit is housed in Custom Baker SterilGARD BSL2 cabinet to ensure sterile sorting applications. Location: Akkulam, Central Instrumentation

BIO IMAGING CORE

Spectral Confocal Microscope with Resonance Scanner for Fast Live Cell Imaging (A1Rsi)

A1R si, NIKON is the most advanced and fully automated spectral confocal microscope from Nikon, capable of capturing high quality confocal images with high speed and sensitivity. This machine includes a 32 array spectral detector and high speed resonance scanner that can achieve more than 25 frames per second at 512x512 pixel dimension apart from motorized xy stage for multipoint confocal imaging. Available laser lines are 488nm Solid state, Diode laser 561 nm, HeNe 633 nm, Blue diode Laser 405nm, all with AOTF control. Also supported by live cell incubation chamber from Okolab for live cell imaging.

Confocal Laser scanning Microscope with high sensitivity spectral detector (Olympus FV3000)

Olympus FV3000 is equipped with a high sensitivity spectral detector (HSD) with GsAsP PMTs which enables it to view samples having weak emission. The desired emission range can be selected using the spectral detectors. The Diode Laser lines available are 405nm, 488nm, 514nm, 561nm and 640nm with 4x, 10x, 20x, 40x and 60x objectives.



First Row: Ciji Varghese, Viji S (From L to R)
Second Row: Parvathy, Deepa Mathew, Athira, Saravana kumar, Sreelekshmi (From L to R)

Confocal Laser Scanning Microscope with GaAsP Detector for Multifluorescence and Live Cell Imaging (Leica SP8 WLL Confocal Microscope)

Leica SP8 Spectral Confocal with WLL is an advanced confocal microscope from Leica Microsystems, Germany. This equipment is configured with white light laser (WLL) that can support any laser lines between 470-670nm and AOBS for filter less emission tuning, in addition to the highly sensitive GaAsP detectors.

SP8 3X FLACON LIFETIME IMAGER WITH STED

This high end imaging device Leica SP8 3X FALCON is a fully configured Lifetime imaging unit with pulsed supercontinuum light source WLL that can support any laser lines between 470-670nm and AOBS for filter less emission tuning. Both spectral imaging and lifetime imaging is possible in thus system. The imager also supports super resolution STED imaging.

NIKON SPINNING DISC CONFOCAL IMAGER

The Nikon A1R confocal imager from Nikon, Japan is configured with a Yokogawa spinning Disc confocal unit with dual EMCCD camera from Andor to support ultrafast live cell confocal imaging. Most widely used laser lines such as 405, 488, 562 and 633 from Omicron is inbuilt in to the system to support most of the dyes and fluorescent proteins and simultaneous dual color imaging.

IVIS® Spectrum in vivo imaging system

The In vivo animal imaging system from Perkin Elmer is located in both campuses. This is an ideal platform for non-invasive monitoring of disease progression and cell trafficking and able to perform high-sensitivity in vivo imaging based on fluorescence and bioluminescence.

HISTOLOGY CORE

The histology core facility is equipped with necessary infrastructure to help the ongoing research at RGCB. The core has a microtome, cryostat and automatic tissue processor and tissue embedder

BIOSAFETY LEVEL 3 FACILITY (BSL3)



Dr. Sara Jones, Jayalekshmi D, Dr Rajesh Chandramohanadas, Sanjai D, Vishnu V M (From L to R)

BRIC-Rajiv Gandhi Centre for Biotechnology (RGCB) has established a modular BSL-3 laboratory at the new campus in Aakkulam. Upon getting certification from the Institutional Bio-Safety Committee (IBSC), the Kerala State Pollution Control Board (KPCB) and the Review Committee on Genetic Manipulation (RCGM), the facility became fully functional on January 15, 2024, and is dedicated to research on viral pathogens with high potential for causing life-threatening diseases.

The facility includes two fully equipped research suites, a procedure room, and an animal holding area. Essential equipment includes wall-mounted double-door autoclaves, pass-boxes, annually certified biosafety cabinets, and all the necessary personal protective equipment (PPE) to ensure personnel safety.

The RGCB BSL-3 facility is supported by a dedicated team ensuring effective management and operations. Currently, the core team consists of four trained staff members responsible for managing and executing essential research/service activities within the facility. In addition, RGC BSL-3 Facility has become a part of the ICMR project titled "Creation of a National Network of Existing and Upcoming High-Risk Pathogen Laboratories (BSL-3/4) Across Departments," to effectively respond to emerging infectious disease threats.

BIOINFORMATICS FACILITY



Dr. Sivakumar K C, Dr. jamshaid Ali, Dr. Shijulal Nelson Sathi, Dr. Kathiresan Natarajan, Harikrishnan G (From L to R)

The Bioinformatics Facility is located at the RGCB (Campus-II) of Rajiv Gandhi Centre for Biotechnology. Considering the growing demand for computational approaches to solve biological problems, the facility is offering various bioinformatics services and training programs to students and researchers from RGCB and academia. We provide (i) Computational infrastructure (Servers and Storages) for performing large scale biological data analysis and storage (ii) Short term (1 day) and long term (6-months/1 year) training programs (The facility is conducting the training programs in online format) (iii) Essential bioinformatics services to students and researchers from academics' (iv) Academic projects (Bioinformatics) to both internal and external students

1) Computational Infrastructure (Servers & storage)

The Bioinformatics facility provided 32 server accounts to trainees/researchers for high-performance computing and storage from April 2023 to March 2024.

2) Short-term and Long-term Training Program

(i) Short-term Training program (One-day virtual workshops)

A total of seven online workshops were held, covering a wide range of bioinformatics topics. These workshops attracted a diverse group of 385 participants, including both researchers and students, eager to expand their knowledge and skills in this rapidly evolving field

Name of the workshop	Date
Introduction to MD simulations	13-04-23
Python programming for beginners	25-05-23
Practises in molecular docking	24-06-23
Protein structure modelling & visualisation	27-07-23
Introduction to NGS data Analysis	23-08-23
Protein structure modelling and visualization	07-10-23
Introduction to RNA-Seq data analysis	28-12-23

(ii) Long-term Training Program (Certificate Program in Bioinformatics 2023-24)

The certificate program provides a solid base to the use of bioinformatics by providing theory and application training in methods and resources appropriate to all major fields of biological research. It includes best strategies for undertaking bioinformatics analysis, computer programming, statistical methods, data management and reproducibility. In the year 2023-2024, 17 students from different parts of India has been participated in the certificate program in bioinformatics conducted by the bioinformatics facility.

Part	No.of students
Part-A (Online)	17
Part-B (Offline)	04

3) Academic Projects

We provide academic project training for MSc/BSc/Btech/Mtech students with an exposure to an international quality bioinformatics research environment. Projects and dissertations for 2-3 months or 4-6 months. In the year 2023-2024, a total of 47students have participated in various academic projects offered by the bioinformatics facility.

Duration	No.of students
1 month	22
2-3 months	16
4-6 months	09
Total	**47**

4) Bioinformatics Services

We provide customized, accurate, and specific solutions to the researchers in the following areas:
Molecular Docking, Protein Mutation design and Structural analysis, Protein 3D Structure modelling, Molecular Dynamics Simulations, Functional annotations, NGS analysis and denovo assembly, RNA-Seq analysis, Phylogenetic analysis, Metagenomics, Heat maps/3D plots/Interactive charts.

Name of Service	No.of Service
Protein structure Analysis	09
MD simulation support	275

GENOMICS SERVICE FACILITY



Deepthi Mohan, Dr. P Manoj

Facility offers comprehensive support for DNA-based genomic research methodologies to RGCB and outside researchers across India. The facility is equipped to perform Sanger sequencing and genotyping on a variety of bacterial, viral, plant, and human samples using two Multicapillarry systems 3730 & 3730 XL DNA analyzers, for both internal and external samples. On a fee-for-service basis, research divisions provide internal samples. Major institutes and companies like SCTIMST, Rubber Board (Kottayam), NIIST, Calicut University, CVAS, KAU-Vellayani, EnfysPvt Ltd (Kochi), Genespec Kochi..etc are our regular genomic service users. They had utilized this facility in 2023-2023 for their research needs with a cost worth of Rs. 2, 64,100/-.

For genescan analysis, samples of plants / animals were processed for

Genomics core facility equipped with following instruments.

1. 3730 Xl Genetic analyzer
2. 3730 Genetic analyzer
3. Quant studio 5 (96 well RTPCR)
4. Quant studio 5 (384 well RTPCR)

SSR analysis, SNP genotyping and microsatellite analysis for various research laboratories. The facility is outfitted with Quantstudio 5 (both 96-well and 384-well), for Q-PCR applications. For various research labs of both internal and external investigators, gene expression studies with Real Time PCRs Quantstudio are used in SNP analysis, absolute quantitation, relative quantitation and Allelic discrimination applications using SYBR green/Taqman chemistry.

Genomics Team provided training in DNA Sanger sequencing RTPCR, genotyping and phylogenetic analysis to students and researchers all over India. Amrita University (Kollam), Terumopenpol, MIT, VIT, SRM and SCTIMST-Trichy utilized the service and generated revenue of Rs 1,20,000/-

LIBRARY AND INFORMATION SERVICES - CENTRAL LIBRARY RGCB



First Row: Meera N.V, Dr. Manjula S, Suma S Nair (From L to R)
Second Row: Shibin S.B, Balagopal S, Gopakumar G (From L to R)

The RGCB Central Library is a haven of immense knowledge and information, enriched by a vast collection of international and domestic books and journals in the field of life sciences. In alignment with the institute's culture, the library is well-equipped and continually exploring new technologies and frontiers. This aligns perfectly with the words of Shri S.R. Ranganathan, the father of Indian Library Science: "A library is a growing organism." The library is fully prepared to meet the current and future knowledge and information needs of researchers, academics, and other benefactors of the centre. All services are efficiently managed through fully automated operations supported by state-of-the-art open-source management software. The library is committed to providing comprehensive offline and online library and information services to its benefactors in alignment with the current and anticipated research and educational policies of the institute.

Library Resources

The library hosts a diverse collection of traditional and digital resources, including:

- Books, Journals, and Magazines: Over 8500 printed documents, featuring a significant number of internationally acclaimed books on life sciences, and numerous national and international journals and magazines.
- Standards, Manuals, and Protocols: A substantial reserve of standards, manuals, protocols, reports, reprints, and back volumes of periodicals.
- Theses and Dissertations: An extensive collection of theses and dissertations submitted by researchers and scholars affiliated with the institute.
- Online Public Access Catalogue (OPAC): Provides metadata for all resources and is accessible globally.
- E-Resources: Digital references and a variety of subscribed resources such as e-books, e-journals, e-databases, and research support software. Access to online resources from national and international publishers through the DBT e-Library Consortium (DeLCON) and DELNET (Developing Library Network).
- Digital Library: Equipped with several PCs integrated with multimedia platforms to optimize access to e-resources. Provisions are in place to fulfil subscribers' additional queries through resource sharing with other institutes.

LIBRARY SERVICES

The library operates on an open-access system, providing excellent service to all users, including:

- OPAC access
- Digital library resources
- New arrivals alerts
- Reference and consultation services
- User orientations
- Reprographic services
- Media clipping services
- Citation and bibliographic analysis
- Document delivery (both print and electronic)
- Current Awareness Services (CAS)
- Selective Dissemination of Information (SDI)

These offerings are designed to update and support the user community with the latest information in their respective fields.

Research Support Tools

The library has established itself as a cornerstone of professionalism with its advanced infrastructure, continuously updating its technology to meet the evolving demands of users. Essential tools for research activities within the RGCB community include:

- **Turnitin:** A comprehensive plagiarism detection and academic integrity tool that incorporates advanced AI technologies to identify text manipulations and ensure originality in scholarly writing.
- **FlowJo:** A data analysis software designed for single-cell flow cytometry, enabling researchers to analyze, visualize, and interpret complex cellular data with precision and flexibility.
- **GraphPad Prism:** A versatile biostatistics software used for analyzing and graphing scientific data, offering robust tools for data visualization, statistical analysis, and curve fitting, making it ideal for research documentation and presentations.
- **EndNote:** A comprehensive reference management software that facilitates efficient organization, formatting, and citation of bibliographic references, allowing researchers to streamline their writing and focus on their research activities.
- **Grammarly:** An AI-powered writing assistant that enhances writing quality by providing real-time grammar, spelling, punctuation, and style suggestions, ensuring clear and professional documentation.
- **BioRender:** A user-friendly software tool that allows researchers to create professional scientific illustrations and visualizations, enhancing the clarity and impact of scientific presentations and publications.
- **Knimbus Digital Library:** A comprehensive digital library platform that provides access to a vast collection of academic and research content and remote access to all the subscribed resources.

The library is always available, dedicated, and ready to assist its benefactors at all times.

MASS SPECTROMETRY & PROTEOMICS CORE FACILITY



First Row: Arun Surendran, Dr. Anish Kundu, Dr Abdul Jaleel, Sudha B Nair, Sudha B Nair (From L to R)
Second Row: Anoop, Reeba Parameswaran, Akhila Suresh (From L to R)

The Central Mass Spectrometry and Proteomic Core Facility at RGCB is also known as "DBT-SAHAJ National facility for Mass Spectrometry-based Proteomics, Metabolomics &Lipidomics Platforms". The facility has also been supported by the DBT-SAHAJ program awarded in August 2021 under the title "Comprehensive mass spectrometry-based Proteomics, Metabolomics, and Lipidomics platforms for promoting biomedical research and advanced training for Indian researchers".

Infrastructure

The facility houses four state-of-the-art systems namely;

- UltraFlextreme MALDI-TOF/TOF (BrukerDaltonics)
- Synapt G2 HDMS Q-TOF connected with nanoAquity UPLC system (Waters)
- Orbitrap Eclipse Fusion Tribrid MS connected with UltiMate 3000 RSLCnano UHPLC or vanquish UHPLC system (Thermo Scientific) and
- Altis Plus Triple Quadrupole MS connected with vanquish UHPLC system (Thermo Scientific).

Analytical Services

The facility offers MS based analyses services for; Molecular weight confirmation, Protein identification, De-novo sequencing, PTM analyses, Polymer analysis, Protein profiling, Label-free relative protein quantification, Quantitative proteomics using labeled methods (TMT or iTRAQ), Protein complexes identification, Phosphoproteomics, Untargeted Metabolomics andLipidomics and finally Targeted analysis of molecules or Absolute quantification.

Lipidomics

As part of the DBT-SAHAJ program, the facility is in the process of establishing a panel of more than 300 lipid molecules/species, which comes under 25 major lipid classes. At present the panels include 36 lipid molecules, which come under 13 lipid classes and 2 metabolites of one carbon metabolism. The list of the available panel is given in the link here (https://www.rgcb.res.in/Targeted-Metabolomics-Lipidomic.php). These panels are being established in the MS facility to meet the research needs of investigators on a case-by-case basis.

Users, Samples and Revenue

The analytical services provided by the MS facility are being utilized by researchers in academia and industries across the nation. In addition,trainings and workshops on various MS based methods are being conducted for the investigators and students.
The user details of the year 2023-24 are given in the table below.

Number of Users		Number of Samples	Revenue Generated in INR
Internal Users	75	498	6,53,800.00
External Users	98	606	35,52,390.00
Total	173	1104	42,06,190.00

Workshops

These workshops/courses were structured to provide training on various tools and protocols within the workflow of mass spectrometry-based proteomics.

1. Three day workshop (6th to 8th February 2024) on Mass spectrometry-based proteomics for 10 participants
2. Three day workshop (11th to 13th October 2023) on Mass spectrometry-based proteomics for 10 participants
3. One day (13th September 2023) workshop on "Introduction to Proteomics" for 20 participants of the 49th ACBICON 2023.

MEDICAL LABORATORY SERVICES



Padmavathy Amma B



Dr. Ashok R



Dr. Vishnu T.S



Dr. Bobby R.G

RGCB Medical Laboratory Services (MLS) comes into the forefront with its unique translational capability in advanced laboratory and theoretical skills in disease biology as well as having a highly qualified and trained man power was initiated in 13-09-2017. RGCB MLS is an indispensable clinical Laboratory professional partner that provides clinical lab information and services to the Public by use of optional but advanced levels of health care resources. Medical laboratory testing plays a crucial role in the detection, diagnosis and treatment of patients. While technology continues to improve the productivity of today's Laboratories, new technologies, new diseases and disease epidemics continue to drive for the need for more innovative tests and testing methods. Approximately 60 t0 70 percent of all decisions regarding a patient diagnosis, treatment, hospital admission and discharge are based on laboratory test results. This permits maximizing effective delivery of care in today's complex health care system by accurate test results that enable providers to make the right diagnostic and therapeutic decisions. RGCB MLS provides all standard hematological, Clinical Pathological Biochemical, Immunological, Microbiological and Serological analysis performed on the latest state of the art analyzers, fully automated and interfaced platforms to cope up with the productivity of today's new technologies new diseases and disease epidemic that continue to drive the need for more innovative tests and testing methods drive the need for rapid diagnosis with stringent Internal and External Quality Control program. We are providing accurate reports by involving in strict internal control program for all sections and 2 External Quality Control Program of CMC Vellore and BIORAD USA.

While technology continues to improve the productivity of two days laboratories, new technologies new diseases and disease epidemics continue to drive the need for more innovative tests and analyzing methods.

We have started many new generation post covid analysis round the clock and doing all types of analysis for almost all Govt. primary Health Centers, Community Health Centers, Homeo Clinics, Ayurveda Clinics etc. Using latest cloud based software, reports reach the persons immediately following the approval of reports.

RGCB MLS was awarded NABH accreditation for 5 main Centers separately on September 2018 and renewed up to September 2025. Labs are registered with Clinical Establishment Act 2018 also.

RGCB MLS provides investigations on Central Govt. Rates (CGHS) with special concessions to BPL patients.

Started many new parameters and installed new analyzers like

PERKIN ELMER DELFIA X PRESS 600 IMMUNOANALYZER USA WITH LIFE CYCLE SOFTWARE

LifeCycle™ software is the comprehensive informatics and risk calculation package for prenatal screening. It provides laboratory teams the support needed in managing an effective, high quality risk assessment program. Because the system has been thoroughly tested and all calculation methods, algorithms and values are supported by current published literature, LifeCycle™ will give us full confidence in our risk assessment program.

FULLY AUTOMATED CAPILLARY ELECTROPHORESIS SYSTEM

System is a capillary based electrophoresis system that is dedicated to Thalassemia and Hemoglobinopathy testing and Screening
The system has additional option to perform HbA1c, Hb, Serum protein, Immuno typing and CDT. (Carbohydrate Deficient Transferin) a marker of chronic or sustained alcohol abuse.

VAPOUR PRESSURE OSMOMETER – ELITECH USA

For Executing Serum and Urine Osmolality analysis by direct measurement.

MAGNUS MX 21i LED MICROSCOPE installed for doing ANA, ds DNA I C-ANCA, P-ANCA IFA and other autoimmune parameters.

Anti nuclear antibody- IFA test refers to antinuclear antibody (ANA) test through indirect fluorescent antibody (IFA) method to detect the presence of ANA in blood. ANAs are auto antibodies that attack body's healthy cells and cause damage to the joints, skin, muscles, and other body parts. This test is suggested to people at risk or having autoimmune disorders such as rheumatoid arthritis, autoimmune hepatitis, scleroderma and systemic lupus erythematosis (SLE).

RGCBMLS is currently functioning in various Govt. Major Hospitals namely

1. Medical College Hospital TVPM – 24x7
2. General Hospital TVPM – 24x7
3. General Hospital Neyyattinkara – 24x7
4. District Hospital Nedumangadu – 24x7
5.District Hospital Chirayinkeezhu – 24x7
6.General Hospital PALA – 24x7

And in Govt. Offices like

1. Govt. Secretariat TVPM
2. Govt. Legislative Secretariat TVPM.
3. Public Office,
4. Collectrate
5. P.S.C. Office
6. Corporation Office

Apart from this centers 250 collection and report Centers are functioning in various primary Health Centers and Family Health Centers, Homeo Dispensaries and Ayurveda Dispensaries in TVPM under Health Services with Govt. Order. It is also functioning in SreeCithiraThirunal Center(SCTIMST), Govt. Ayurveda College, Govt. Panchakarma Hospital, Govt. Homeo Hospital, Govt. Siddha Hospital etc and in Govt. Offices like Collect rate, P.S.C. Office, Public Office, ThozhilBhavan, B.S.N.L. etc

RGCB MLS is involved in investigating various types of analysis for many projects for RGCB Research program, For Cardiology, Ayurveda research Center and Homeo Research Division etc.

Medical camps are conducted for doing various analysis panel required in Offices and Flats on CGHS rate. Recently we have started Home collection facility for Clinical Investigations for all parameters for Palliative care patients, pregnant ladies, children, old age persons or for any one on request to provide rapid information needed to triage patients on CGHS rates seems to be very helpful to the public.

RGCB MLS is empaneled under State Govt. CGHS and ESI hence reimbursement available.

Now the GOVT. have encouraged us to provide advanced training in Medical Diagnostics and related areas to the staff and others engaged in the field of clinical investigations in Microbiology, Biochemistry, immunology and Haematolology. Short term training for two weeks and long term training for 3 months for DMLT, B.SC MLT, B.SC Biochemistry and Microbiology students as well as for M.SC Biochemistry and Microbiology students after completion of course.

Associated in doing Special Investigations for MD and PHD Thesis as well as research Projects under Medical, Homeo and Ayurveda Divisions.
RGCB Medical Laboratory Services, with Loyal, dedicated and talented personnel along with Energetic and Excellent Infrastructure provides service with commitment to Society round the clock.

NO. OF PATIENTS FROM ALL MLS CENTERS FOR THE PERIOD FROM 1ST APRIL 2023 TO 31ST MARCH 2024

Department	No. of Patients
BIOCHEMISTRY	290240
HAEMATOLOGY	125810
IMMUNOASSAY	178430
MICROBIOLOGY	46200
CLINICAL PATHOLOGY	22920
SEROLOGY	20770
PRENATAL SCREENING	1680
TOTAL	686050
BILLED AMOUNT	14,11,63,937 /-
FEES RECEIVED FROM TRAINEES	6,21.944 /-
GRAND TOTAL	14,17,85,881 /-

MOLECULAR FORENSICS & DNA TECHNOLOGIES (MFDT)



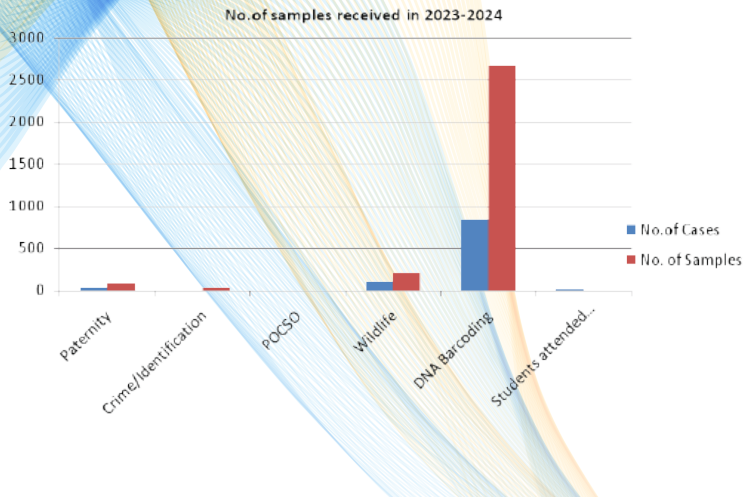
First Row: Parvathy, Remya R C, Preetha Rajan, Dr Radhakrishnan, Dr P Manoj (From L to R)
Second Row: Abhilash M K, Ratheesh R V, Johny G, Anilkumar P
Back Row: Vinod Kumar S, Anandhu A, Sureshkumar U (From L to R)

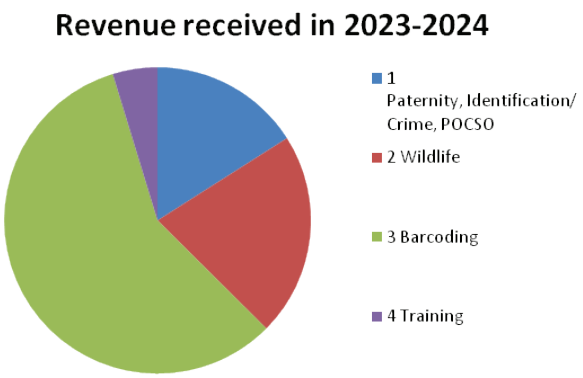
MFDT offers DNA fingerprinting services to legal bodies, crime investigating and law enforcing agencies. The samples analysed at MFDT relates to maternity/paternity disputes, crime, rape incidents and cases involving man missing. CO1-based molecular identification and DNA barcoding of fauna especially for species identification in wildlife forensics is yet another service offered by MFDT. Other services offered by this facility include DNA fingerprinting of plants and animals in case-by-case manner using RAPD, AFLP or microsatellite markers and DNA barcoding of animals using CO1 gene and plants using matK and rbcL. The facility also offers hands on training on DNA fingerprinting and DNA barcoding techniques. Details about various DNA fingerprinting/barcoding services and training programmes are provided in our website.

In 2023-2024 we analysed more than 32 cases related to identification, maternity/paternity and relationship disputes forwarded by Courts from different districts of Kerala, Child welfare Committee and Kerala Women's Commission. In addition we have analysed 6 forensic cases in which 3 cases from Vizhinjam Coastal Police Station, Parippally Police station and Malappuram Crime branch (Perinthalmanna Police Station) to identify the body recovered from the crime site, one man missing case from Thiruvananthapuram Crime branch (Aryanad Police Station), one child abuse case from Mannanthala Police Station, one crime case from Kattakkada Police Station to identify the species of snake used for attempt to murder.

We have also received more than 97 cases related to animal poaching forwarded from various forest range offices through court. Animal poaching is one of the major threats to the animals in wild. It is imperative to punish the offenders to prevent illegal poaching. Samples confiscated by forest officers in Kerala Forest Department are forwarded to our lab for identification of species, so as to enable them to charge the case and punish the offenders. DNA Barcoding helps to identify animals even from minute or cooked samples. But the exact identification of species from the Western Ghats region of Kerala, which is one of the hottest biodiversity hotspots, is often difficult or not possible due to the lack of reference sequences in databases.

Description of Case	No. of Cases	No. of Samples
Paternity	29	92
Crime/Identification	6	30
POCSO	3	9
Wildlife	97	214
DNA Barcoding	837	2936
Students attended training	23 (Applications)	23





Description of Case	Amount Received(Rs)
Paternity, Identification/Crime, POCSO	11,50,000.00
Wildlife	15,44,960.00
Barcoding	41,62,250.00
Training	3,40,000.00
Total	**71,97,210.00**

Major Clients for Paternity/ Crime/ POCSO/Wildlife

- Family Court's of Kerala
- High Court of Kerala
- Kerala Women's Commission
- Child Welfare Committee
- Judicial First Class Magistrate, Androth Island, Lakshadweep (POCSO)
- District & Sessions Court, Kavaratti, Lakshadweep (POCSO)
- Chief Judicial Magistrate, Amini Island, Lakshadweep (POCSO)
- All Judicial Court's of Kerala for Crime Cases
- All Judicial Court's of Kerala for Wildlife Cases
- Special Court for Forest Offences, Nagercoil, Tamil Nadu
- Additional Chief Judicial & Judicial Magistrate of First Class, Chintamani, Karnataka.

We have five training programs namely Human DNA Fingerprinting & Identification- Scheme I, DNA Barcoding techniques & analysis- Scheme II, DNA Fingerprinting following RAPD/ISSR techniques & Analysis- Scheme III, DNA Fingerprinting following Microsatellite technique & Analysis – Scheme IV &Bioinformatics Analysis of DNA Sequence data – Scheme V. Fourteen candidates were given training in DNA fingerprinting/barcoding during this period. We have received more than 837 cases for DNA Barcoding/Fingerprinting/Sequencing analysis from various research institutions, colleges and universities from all over India.

Major Clients for DNA barcoding

- Annamalai University, Tamil Nadu
- Assam Agricultural University, Jorhat
- Bharathidasan University, Trichy
- Bharathiyar University, Coimbatore
- Botanical Survey of India, West Bengal &Shillong
- Centre for DNA Fingerprinting & Diagnostics, Hyderabad
- Cochin University of Science & Technology, Kochi
- College of Agriculture, Vellanikkara, Thrissur
- College of Agriculture, Vellayani, Thiruvananthapuram
- College of Dairy & Food Technology, MaharanaPratap University of Agriculture and Technology, Rajasthan
- College of Dairy Science & Technology, Wayanad
- College of Science, Albaha University, Saudi Arabia
- CSIR-NIIST, Pappanamcode, Thiruvananthapuram
- ICAR Sugarcane Breeding Institute, Kannur
- Institute of Animal Health & Veterinary Biologicals, Thiruvananthapuram
- Integrated Farming System Research Station, Thiruvananthapuram
- JamiatAlkhaneel Tree Trading Palms, UAE
- Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Thiruvananthapuram
- Kerala Forest Research Institute, Thrissur
- Kerala University of Fisheries & Ocean Studies, Kochi
- Kerala Veterinary & Animal Science University, Thrissur
- Mormugao Zonal Base of Fishery Survey of India, Goa
- National Centre for Coastal Research, Chennai
- Pepper Research Station, Kannur
- Punjab University, Punjab
- Rajasthan Agriculture University, Udaipur
- Rice Research Station, Alappuzha
- University of Jammu & Kashmir
- University of Kerala.
- Vidyasagar University, West Bengal
- Zoological Survey of India, West Bengal & Calicut

RESEARCH ENGINEERING SERVICES



First Row: Hareesh V R, Vinod B, Sajin G S, Aseem A, T S Ancy Prince, Santhosh Kumar, Jaseel N, Shaji S (From L to R)
Second Row: Sajith Mohan, Mohammed Sanofar S, Jithu R, Ratheesh Kumar A, Akhilesh P.M, Ajay S Kumar, Arun R (From L to R)



First Row: Rahul C S Nair, Radhika U, Parvathy, Aswathy G Raj, Ancy Prince T S
Second Row: Amal V, Mohan, Sidharth Achthan, Ullas, Rajasekharan K, Akhil, Premkumar

CORE RESPONSIBILITIES

1. Equipment Installation and Maintenance:

- Sophisticated and General Research Equipment: The department is responsible for the installation, maintenance, and service of both sophisticated and general research equipment, including Central Instrumentation Facilities at the Main Campus and Bio Innovation Centres.
- Engineering Workshop: Maintains a well-equipped workshop for repairing sophisticated instrumentation systems, which reduces downtime and repair costs. The in-house engineers handle complex hardware issues, saving on annual maintenance contracts (AMC) and comprehensive annual maintenance contracts (CAMC).

2. Customization and Fabrication:

- Research Automation: Customizes, designs, and fabricates components of research automation systems to meet specific user requirements, enhancing convenience and functionality.

3. Procurement Support:

- Technical Specifications: Assists in the procurement process by analyzing user needs, evaluating current technology, and preparing technical specifications within budget constraints for purchases.

4. Calibration Facilities:

- In-house Calibration: Operates calibration facilities for instruments like pipettes, electronic balances, centrifuges, autoclaves, freezers, incubators, and PCR machines. These facilities include standards and measuring instruments with certifications from recognized national agencies, aiding laboratories in achieving NABH/NABL accreditation.

5. Training Programs:

- Engineering Students: Provides training on the operation, application, calibration, and maintenance of instrumentation systems used in biotechnology and life science research. In the 2023-25 period alone, 63 students benefited from these programs.

6. Maintenance of Infrastructure:

- Various Systems: Manages a broad array of infrastructure, including:
  - Computers and security surveillance systems
  - Biometric time attendance recorders
  - Online conferencing and communication systems
  - Liquid nitrogen plant
  - Auditoriums and convention center
  - Electrical substations and Central AC plants
  - Automation with PLC/DCS/SCADA control systems
  - Maintenance of various instruments available in variour MLS labs

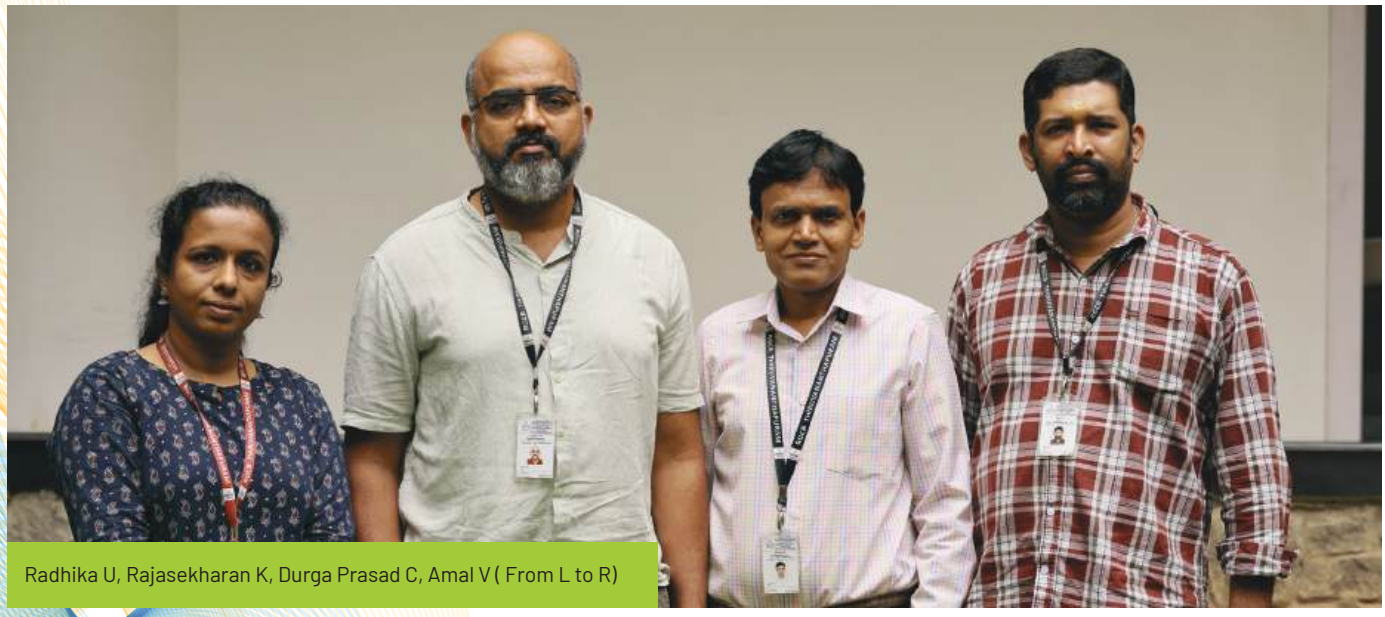
Impact and Innovation:

- Cutting-edge Technologies: The department is pivotal in integrating advanced technologies to minimize the man-machine interface, thus optimizing instrumentation systems.
- Benchmark Setting: The division has established new standards and benchmarks for similar departments nationwide, positioning itself as a model for excellence and innovation.

The Research Engineering Services Department continues to make significant, though often understated, contributions to the Institute's growth and success. Its commitment to quality and innovation positions it as a leader in supporting the Institute's mission and vision, paving the way for future advancements and achievements.



Lekshmi R, Anand Mohan, Remya Rajan (from L to R)



Radhika U, Rajasekharan K, Durga Prasad C, Amal V (From L to R)

The IT infrastructure of RGCB's main campus includes 13 Servers, more than 400 Desktops, Laptops, Network Printers, etc.. It houses one of the best computing networks, constantly upgrading to provide students and staff with state-of-the-art facilities. The Institute has been connected to the National Knowledge Network, which provides a 1Gbps leased line with multiple redundant backups.

The highly distributed computing environment at RGCB uses sophisticated computer simulation to solve the problems of staff and research scholars. It is managed and actively supported by experienced IT department engineers. The IT department is also responsible for maintaining and administrating Mail Servers. IT department provides technical support to staff and students within the Institute on LINUX and WINDOWS platforms and includes software development for research groups.

IT department designs, develops, updates, hosts, and supports the RGCB Website, online admission portal, leave management system for PhD students/ project staff, laboratory management system, online training portal, online portal for various positions at RGCB, conference websites, intranet applications for various administration and scientific activities, yearly portals for updating annual reports, SAC and integrating payment gateway for various web applications. The IT team supported the smooth functioning of online classes for MSc and PhD students via Moodle, hosted and fine-tuned by the IT Team.

Internet facilities are provided throughout the campus through 1 Gbps and 100 Mbps leased lines from NKN and BSNL. The Akkulam campus has a fully redundant smart row data center and Internet facility for the Akkulam campus research and hostel blocks.RGCB implemented Fibre Optic Backbone with high-end security for networking across the campuses. Wireless connectivity is provided at strategic locations to provide Internet access to the faculty. The Information Technology Division of the Bio-innovation Centre at KINFRA, Kazhakuttom, provides high-quality services and capabilities to different research groups. It includes a structured infrastructure and a secured network with authentication, and dual 100Mbps leased lines.

INNOVATIONS AND IMPACT AT KRIBS-BIONEST: FOSTERING BIOTECH STARTUPS AND TECHNOLOGIES



First Row: Antony KPI , Deepak BS, Dr. K. Ampady IIS, S. Aishakumari P P, Smitha S, Roshna R Nair, Resika G Nair (L to R)
Second Row: Manoj A, Dr.Divya Purushothaman, Charutha P.P,Dr. Anish N P

KRIBS-BioNest - Biotech Park - Kochi, the technology incubation centre of RGCB, operates in collaboration with Kerala Start-up Mission at Kerala Technology Innovation Zone, Kalamassery. KRIBS- BioNest offering incubator facilities and advanced biotechnology instrumentation to support emerging biotech and life science ventures in their journey towards commercializing innovative technologies. The center aims to accelerate the development and commercialization of new technologies, nurture emerging ventures, create networking opportunities with other biotech entities, academia, and government, and provide specialized training programs for students and employees. With a total floor area of over 44,000 square feet, it offers around 16,000 square feet of bio incubation space and a common laboratory measuring 10,000 square feet to the Start-ups housed within the facility. BioNest offers a variety of cutting-edge facilities such as the Pilot Spray Dryer for phytochemistry, Freeze Dryer for the bioprocess lab, DNA Sequencer, Gel Doc System, and more, catering to needs in bio-molecules quantification, chromatography tests, fermentation process development, DNA sequencing, and cell and molecular biology assays among others. BioNest also provides services and technical advices from RGCB faculties and the cutting edge facilities available in RGCB for entrepreneurs in the various spheres of life sciences.

Activities in 2023-2024 showcased the significant impact of KRIBS-BioNest in incubating startups and fostering innovation. The facility supported 22 startups physically and 8 virtually, while also facilitating external entrepreneurs and researchers in various R&D activities. During the reporting period, M/s Cirist Ecosystem Pvt. Ltd was welcomed as a new virtual incubatee in KRIBS-BioNest.

During the year KRIBS-BioNest participated in events like Global BioIndia 2023 and India International Science Festival and showcased the products of its incubatees and network with industry peers. The Authorities from KRIBS-BioNest also visited similar facilities such as the Atal Incubation Center-CCMB in Hyderabad and ASPIRE-BioNest at the University of Hyderabad, among others.

Notable interactions during the reporting period includes the visit of Miss Debjani Pal, the Trade Commissioner of the Consulate of Canada in Bangalore, who visited KRIBS-BioNeston December 18, 2023, to engage and network with startups aiming to foster collaborations. As a part of training activities, BioNest conducted an interaction session on patents and IP rights with the Technology Transfer Office at TiMed, SreeChitraTirunal Institute for Medical Sciences and Technology on 21st July 2023. During the year, over 200 visitors explored the facility and engaged with the startups under incubation. Students and faculty from SDV College of Arts & Applied Sciences, Alappuzha, SD College, Alappuzha, Christ College, Irinjalakkuda, School of BioScience MG University etc. visited the facility and interacted with the startups housed in KRIBS-BioNest.

Several startups achieved major milestones, such as M/s. Heka Medicals India Pvt Ltd developing innovative healthcare devices and receiving patents, and M/s. Greenovative Foods Pvt Ltd creating a plant-based meat substitute, "The Greenmeat." Moreover, the startups incubated at KRIBS-BioNest developed a range of cutting-edge products and technologies, further demonstrating the facility's impact on fostering industry innovation. During the reporting period the startups incubated at KRIBS-BioNest employed more than 35 personals in various capacities.

KRIBS-BioNestincubatees have made significant strides in the field of healthcare and biotechnology. M/s. Heka Medicals India Pvt Ltd stands out for innovating the "Heka Flow- High-flow Nasal Oxygen Therapy Device" and the "HekaNeo Bubble CPAP" for neonatal respiratory support. Their accolades include patents, manufacturing licenses, and recognition through various grants and awards, showcasing their commitment to advancing medical technology. Additionally, M/s. Avisa Biotech PvtLtd's development of "Melanin and Nanolignin based sunscreens" and participation in international programs like "Hello Tomorrow" in Paris, France, underscore their global impact and collaboration efforts in the biotech industry. M/s. Zygene Biotechnologies Pvt. Ltd's attainment of ISO 9001:2005 certification further reinforces the commitment to quality and excellence within the biotech sector.

On the innovative front, M/s. Greenovative Foods PvtLtd's "The Greenmeat" adds a new dimension to plant-based meat alternatives, tailored for Indian culinary preferences. M/s. Scopeful Bioresearch PvtLtd's patent filings for formulations like "Coconut sap based composition with high nutritional and therapeutic value" exemplify their focus on sustainable and nutritious product development. Together, these achievements demonstrate the diverse expertise and impactful contributions of KRIBS-BioNestincubatees in shaping the future of healthcare and biotechnology.

Major technologies/products that have been developed by the Startups incubated at KRIBS-BioNest in the year 2023-24 are given in the table below.

Product Name	Name of Incubate	Uses
Melanin	M/s. Avisa Biotech Pvt. Ltd.	UV Blocking
Melanin based sunscreen	M/s. Avisa Biotech Pvt. Ltd.	Protection from UV rays
Melanin based hair dye	M/s. Avisa Biotech Pvt. Ltd.	Hair dye
Green pepper oleoresin, granulated essential oils	M/s. Spiceor Bio Nutrilities Pvt. Ltd.	Used in food, pharmaceutical and cosmetics industries
ThermoCeler- BB70	M/s. Scire Sciences Pvt Ltd	Bead Dry Bath
Nutricon C and its derived products	M/s. Scopeful Bioresearch Pvt Ltd	Dietry supplement

Product Name	Name of Incubate	Uses
Sweetrue	M/s. Scopeful Bioresearch Pvt Ltd	Zero calorie natural sweetner
Tea 4 All	M/s. Scopeful Bioresearch Pvt Ltd	Health beverage
HEKAFLO HFNC	M/s. Heka medicals India Pvt Ltd	High flow nasal oxygen therapy device for the treatment of spontaneously breathing hypoxemic patients
HekaNeo Bubble CPAP	M/s. Heka medicals India Pvt Ltd	A non-invasive breathing support system for neonates and preterm babies
Meat-like Chunks	M/s.Greenovative Foods Pvt Ltd	Ready to cook product called "Meat-like Chunks" which can be marinated and cooked just like curry cuts from animal-based meats
Pepper Greenmeat and Greenmeat Roast	M/s. Greenovative Foods Pvt Ltd	Ready to eat products which are two curries using popular traditional South Indian recipes

Some of the products developed by the startups incubated at KRIBS- BioNEST



REVIVING TRIBAL TRADITIONS: A HOLISTIC APPROACH BY RGCB

Preserving tribal heritage in the contemporary era of modernization is crucial for maintaining cultural diversity, safeguarding environmental knowledge, and promoting sustainable development. Under the leadership of Prof. ChandrabhasNarayana, the team has made significant progress in documenting traditional knowledge, identifying technology gaps, and revitalizing tribal traditions in the targeted districts of Thiruvananthapuram, Idukki, and Wayanad with the support of the Department of Science and Technology's SHRI program.

The team has successfully achieved its objectives by conducting a survey of 56 tribal settlements in 12 blocks across 3 targeted districts in Kerala and interviewing 130 tribal informants. This extensive fieldwork has been the foundation for documenting indigenous knowledge and identifying technological gaps. Subsequently, the project team has leveraged this wisdom to empower the tribal communities by integrating targeted interventions for reviving traditional occupations and promoting sustainable practices.

One of the key achievements of the project is the comprehensive survey of landraces/varieties of wild tubers, yams, and fruits used by tribal communities, which resulting in the documentation of 70 ethnic food recipes. This exploration has provided an in-depth understanding of Kerala's tribal communities' traditional culinary practices, emphasizing ethnic food recipes, traditional cooking methods, the unique health benefits derived from indigenous ingredients, and the promotion and conservation of wild edibles, fruits, and indigenous varieties. Moreover, the project has explored into the native oil-yielding plants, such as Sarcostigmakleinii and Hydnocarpus species, revealing their potential for sustainable fixed-oil requirements and providing insights into their traditional uses and modern applications.

The initiative has also actively engaged in enhancing the value of locally available bioresources through various efforts. Studies have highlighted the nutritional richness of dioscorea tubers, jackfruits, and bananas, leading to reduced postharvest losses, increased income for tribes, and sustainable economic activities. Furthermore, the integration of millets, pulses, and mango flour into innovative food and beverage products not only diversifies dietary options but also promotes overall health and cultural preservation within tribal communities.

The project has also extended its support towards the establishment of community enterprises, reviving traditional skills, and wisdom, thereby providing livelihood opportunities. Efforts have been made to revitalize the lemongrass oil extraction industry, resulting in the establishment of advanced steam distillation facilities by tribal groups, significantly enhancing yields and reducing extraction time. Additionally, the project encompasses the conservation of traditional crop varieties and wild edibles, including the establishment of conservation plots and nurseries, contributing to the preservation of genetic wealth.

The research papers published during 2023-24 further showcased the comprehensive exploration of botanical resources and traditional

knowledge utilized by tribal communities in Kerala. These efforts have emphasized the importance of preserving biodiversity and promoting livelihood options for rural communities and have contributed to a deeper understanding of the unique flora and traditional practices that play a crucial role in the cultural and economic landscape of Kerala.

Ultimately, the project's holistic approach has empowered tribal communities and promoted economic growth while preserving traditional knowledge and practices. The interventions have led to the establishment of 18 community enterprises, benefiting around 315 tribal families directly.

In conclusion, the initiative not only contributes to the preservation of indigenous wisdom but also supports the sustainable well-being and cultural richness of tribal groups in Kerala by ensuring that their traditional knowledge remains accessible and protected for future generations. The project's impact has been significant in revitalizing tribal traditions, promoting sustainable development, and safeguarding the cultural heritage of Kerala's tribal communities.

List of Community enterprises established under RGCB-Tribal Heritage Project

Name of Community Enterprise	Activity	District/ Community	No. of direct beneficiaries
Samridhi Nursery	Nursery and sale of wild edibles	Idukki / Mala arayan	22
Nellara Nursery	Nursery and sale of wild edibles	Wayanad / Kuruma&Kurichya	18
Agasthya Nursery	Nursery and sale of wild edibles	Thiruvananthapuram / Kani	15
Aavani food processing unit	Ethnic food and value addition of wild edibles	Idukki/ Mannan	20
Viyana food processing unit	Ethnic food and value addition of wild edibles	Wayanad / Kurichya	22
Triphala Medicinal Plant Processing Unit	Medicinal plant processing	Thiruvananthapuram / Kani	18
Astra Tribal Society	NTFP Processing	Wayanad/Kurichya	25
Karuthaponnu Pepper Nursery	Nursery and sale of pepper	Idukki / Mala arayan	15
Fresh Grass oil Unit	Lemon Grass oil extraction	Idukki / Ulladan	25
Vanasree NTFP processing unit	Bamboo craft & NTFP Processing	Idukki/ Urali	30
UnarvuBamboocraft Unit	Bamboo craft heritage	Idukki/ Urali	45
Nellimala Farmers Club Unit	Lemon Grass oil extraction	Wayanad / Kurichya&Paniya	25
Harithasree ST Koottayima	Paddy Processing unit	Wayanad/ Kurichya	22
Agasthya Pickle Unit	Ethnic food and value addition of wild edibles	Thiruvananthapuram / Kani	22
Seed Bank cum Rice Museum	Traditional paddy varieties conservation	Wayanad/ Kurichya	35
GothraVanavasiSangham	Ethnic medical practice	Thiruvananthapuram / Kani	15
Agasthyalayam Microenterprise Unit	Ethnic medical practice	Thiruvananthapuram / Kani	12
BhaskaraRao SHG	Fixed oils from wild sources	Thiruvananthapuram / Kani	15
VanamitraAkshayasreePurusha SHG	Fixed oils from wild sources	Wayanad/ Kurichya	12

Figure Caption

Fig. A. Prof. ChandrabhasNarayana, Director of RGCB, inaugurated the wild edibles nursery and conservation plot established in collaboration with the Kani tribal community at Vlavila tribal settlement, Koottor, KuttichalGramaPanchayat, Thiruvananthapuram.

Fig. B.Prof. ChandrabhasNarayana, Director of RGCB, inaugurated the Wild Edibles' Demonstration plot and Nursery established with the participation of tribal SHG at Pattayakudi, KanjikuzhiGramapanchayath, Idukki.

Fig. C. Awareness program on ethnic food.

Fig. D. The Director of RGCB inaugurated the Triphala Society for Tribal Medicine and Development in Peringamala, Thiruvananthapuram, which focuses

on sustainable harvesting and value addition of medicinal plants, as well as the preservation of traditional tribal medicine practices.

Fig. E. Bamboo craft training program for the traditional tribal artisans of the Manali settlement, VidhuraPanchayath Thiruvananthapuram.
Fig. F. Prof. ChandrabhasNarayana, Director, inaugurated the Conservation plot and Nursery for wild edibles established with the active participation of the tribal group NellaraPattikaVargaKashakaSangam at Nellarachal, MuttilGramapanchayath, Wayanad.

Fig. G. Director RGCB, Prof. ChandrabhasNarayana, inaugurated the Agasthyalayam Traditional Medicine Unit in Kallar, Ponmudi, Thiruvananthapuram on February 22, 2024. The initiative focuses on the practice and promotion of tribal medicinal treatments, particularly traditional steam baths using medicinal herbs.

Wayanad Team: (Left to Right) Mr.SyamSankaren and Mr. A C. Sebastine.
Idukki Team: (Left to Right) Mr.JishnuJanardhanan, Mr.Rajesh K. T. and Mr.Hari M. K

Thiruvananthapuram Team (photo taken at RGCB)





















CAFETERIA

An exclusive well-maintained cafeteria is available in all our RGCB campuses offering tasty and hygienic food. In order to cater to all the students /staff and visitors from different parts of India and abroad, South Indian, North Indian and Chinese dishes are offered. Food quality and hygiene are the two most important factors in the cafeteria. There is regular quality control and quality checks at the cafeteria to ensure highest standards of hygiene. There is never a compromise on food quality, cleanliness, and overall hygiene at thecafeteria. Be it kitchen or raw materials used for preparation of food, everything goes through a stringent quality check. The "Onam" feast with more than 20 different dishes is just one example of the culinary skills of the cafeteria chefs.

We aim to minimize the impact of catering operations on the environment and promote sustainable practices and consumption. The RGCB cafeteria runs on a "no profit no loss basis"



First Row: Jayakrishnan N, Mohanan Nair S, Gopakumar M S (from L to R)
Second Row: Till Bahadhoor Bashya, Prasanna kumara t R,
Sandhya R, Rajani T, Shylakumari, Lisa I, Meena R P (From L to R)
Back Row: Najeeb, Nandhu, Thyagarajan, Rathesh, Manojkumar,
Byju, Akhil M K, Sureshkumar (From L to R)



RGCB EVENTS 2023-24













APRIL 2023

RGCB organized a one-day symposium on Biotechnology for sustainable development-2023 and felicitation of senior scientists on 12th April 2023. Five senior faculties (Dr S Sreeja, Dr Sabu Thomas, Dr RV Omkumar, Dr Ruby John Anto, Dr Suparna Sengupta) were felicitated for their valuable contribution to RGCB.


















MAY 2023

Dr Vinod Kumar Paul, Member NITI, Aayog, and Dr Rajesh S Gokhale, Secretary, Department of Biotechnology, visited RGCB on 6th May 2023 and interacted with students, staff and faculties. A central facility for Mass Spectrometry-based Proteomics, Metabolomics and Lipidomics platforms was inaugurated by Dr Paul, in presence of Dr Gokhale, The facility was supported by the DBT-SAHAJ program



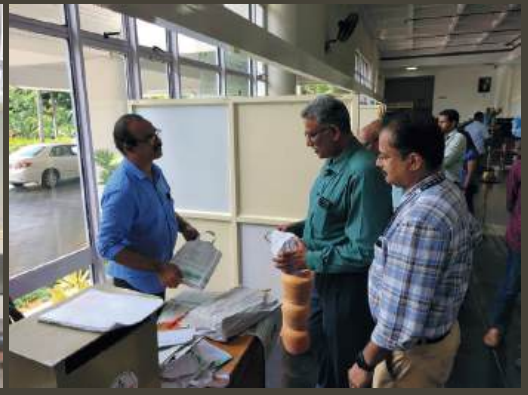


MAY 2023

National Technology Day 2023 invited lecture was delivered by Mr C.V. Muraleedharan, Scientist G, SCTIMST, Thiruvananthapuram on the topic "Technology development of high-risk medical devices – Experience fromSCTIMST on 11th May 2023











JUNE 2023

RGCB started a Gymnasium for staff and students supported by the GATI Project. The facility was formally inaugurated by Professor Chandrabhas Narayana, Director RGCB









JUNE 2023

RGCB celebrated International Yoga Day (IDY2023) on 21st June 2023. The theme for this year is 'Yoga for VasudhaivaKutumbakam'.





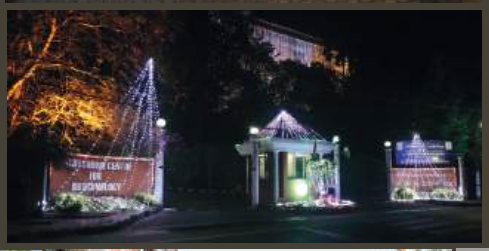




JULY 2023

The third batch of RGCB MSc Biotechnology (2021-2023) students was given an official send-off on 27th July 2023 by RGCB. The best outgoing student, Ms. Parvathi K, was awarded a citation and a medal. Mr Charanraj C A got the best dissertation award and received a citation.









AUGUST 2023

RGCB celebrated the 77th Independence day on 15th August 2023. Professor Chandrabhas Narayana, Director hoisted the National flag. He also distributed the prizes to winners of the various competitions organized as a part of AzadiKaAmritMahotsav










AUGUST 2023

Celebration of the harvest festival of Kerala "Onam" in a traditional style was celebrated on 18th August 2023







SEPTEMBER 2023

The self-contained mobile virology testing unit of RGCB is flagged off on 14th September 2023 by Mrs. Veena George, Hon'ble Minister for Health & Family Welfare, , in the presence of Mr.Mohammed Hanish IAS, Principal Secretary, Health and Family Welfare Department, Government of Kerala.






SEPTEMBER 2023

Professor Chandrabhas Narayana, inaugurated the Conservation plot and Nursery for wild edibles established at Nellarachal, MuttilGramapanchayath, Wayanad on 16th September 2023. This has been established with the active participation of the tribal group "NellaraPattikaVargaKashakaSangam" under the DST-Tribal Heritage Project.








SEPTEMBER 2023

1st RGCB Research Conference (RC-2023) was held from 20-23rd September 2023. Dr Umesh Varshney, IISc, Bengaluru was given the key note lecture







SEPTEMBER 2023

RGCB celebrated the Hindi Divas/Pakhwara from 14th September to 29thSeptember by conducting different competitions on Hind. ProfessorChandrabhas Narayana, Director, addressed the gathering and distributed theprizes to winners. Dr. Anish Kundu read the message from the DBT secretary, Government of India. Dr.Debasree Dutta proposed the vote of thanks













OCTOBER 2023

Six day (1-6th October 2023) workshop organized by RGCB in association with IUIS, IIS and FIMSA "Immuno-India 2023: Epigenetic Regulation of Immune Responses". The inaugural lecture was delivered by Dr Shekhar Mande, Former DG, CSIR.










OCTOBER 2023

Swachhta Awareness Program-
26 October 2023






NOVEMBER 2023

The vigilance awareness week was concluded at RGCB with an invited lecture on 6th November 2023 by Shri. Sudhakara Babu, Senior Administrative Officer, VSSC, Thiruvananthapuram, on the topic "Ethics and Governance." Dr S Manjula, Scientist and Chief Vigilance Officer (CVO, RGCB) Coordinated the event







NOVEMBER 2023

The 135th Birth Anniversary of Sir C V Raman was celebrated at the Govt GHSS, Meppadi Wayanad on 7th November 2023








NOVEMBER 2023

RGCB commemorated Sir C.V. Raman's 135th birthday with a popular lecture series on 9th November 2023. This year the speaker was Dr S Unnikrishnan Nair, Director, VSSC, Thiruvananthapuram and the title of the talk was " Challenges in human space flight mission"



NOVEMBER 2023 | RGCBFoundation Day was celebrated on 17th November 2023. The chief guest was Dr.Satyajit Mayor, NCBS, Bangalore and delivered the foundation day lecture on "The Edge of A Cell: A Living Fabric Where Physics Meets Biology".



DECEMBER 2023

RGCB, along with Vivertana Distributors Pvt. Ltd, Bangalore, and ICDD, USA, organized the RGCB Crystallography Workshop 2023 on the 12th and 13th of December 2023, in the Akkulam campus






JANUARY 2024

RGCB in association with Kerala State Council for Science, Technology and Environment (KSCSTE) organized Dr. GN Ramachandran Memorial Lecture on 12th January 2024. Dr.Shekhar C Mande, Former DG, CSIR delivered the memorial lecture








JANUARY 2024

The Biosafety level III (BSL III) facility at RGCB officially started functioning on 15th January 2024 on the auspicious occasion of Makar Sankranti/Pongal. Professor Chandrabhas Narayana, Director, Mr. S. Mohanan Nair, Chief Controller, Dr. T R Santhosh Kumar, Chairman IBSC, Dr. Rajesh Chandramohandas, Faculty-in-Charge, BSL III, other faculties, staff and students were joined in the program.





JANUARY 2024

On the occasion of the 75th Republic Day of Our Nation, Professor Chandrabhas Narayana, Director, RGCB unfurled the national flag at the main campus. The faculties, staff, and students attended the function.







FEBRUARY 2024

A two-day colloquium on "Yoga in Life Sciences; Beyond—Emerging Possibilities for a New World Order," organized by RGCB, Regional Cancer Centre (RCC) and the International Yoga Research Foundation on the RGCB campus here. Shri Pinrayai Vijayan, Hon'ble Chief Minister of Kerala inaugurated the function. Sri M , the Founder President, The Satsang Foundation, delivered the key-note address. Shri V P Joy, Former Chief Secretary, Govt of Kerala presided over the function.


















FEBRUARY 2024

National Science Day was celebrated on 28th February 2024. Dr N R Jagannathan, Former Professor. and Head Dept of NMR, AIIMS, New Delhi, was the keynote speaker and delivered a talk on "MRI in Pre-clinical and Clinical Research". A monograph was released on this occasion titled as " Revitalizing tribal traditions: Initiatives for sustainable vikasitBharath". A science exhibition was also conducted on the same day











MARCH 2024

On the occasion of International Women's Day (IWD) the keynote address was delivered by Dr Tessy Thomas, former Director General of Aeronautical Systems, DRDO and Vice Chancellor of Noorul Islam Centre for Higher Education,Kumarakovil, Tamil Nadu on 18th March 2024. Dr Abhishek Shankar (Consultant Oncologist, AIIMS, New Delhi) gave a talk on Prevention & Early Detection of Women Cancers and Dr.Reji Jose (Professor Community Medicine, Gokulam Medical College, Venjarammoodu) gave a talk on Clinical Breast Examination: A Tool for Early Detection on 1th June 2023





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DISCOVERIES FOR A BETTER TOMORROW

Rajiv Gandhi Centre For Biotechnology (RGCB)
An Autonomous Institute of the Department of Biotechnology, Ministry of Science & Technology, Government of India
Thycaud Post, Poojappura, Thiruvananthapuram 695 014, Kerala, India.
Ph: +91-471-2529400, 2347975, 2348753, Fax: +91 471 2348096
webmaster@rgcb.res.in, www.rgcb.res.in