RGCB

DISCOVERIES FOR A BETTER TOMORROW

Azadi Ka Amrit Mahotsav

ANNUAL REPORT 2022-23

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Rajiv Gandhi Centre For Biotechnology

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FROM THE DIRECTOR

"The best way to predict the future is to create it"
Peter Drucker

As climate change and the constantly evolving demands in every aspect of society significantly impact the well-being of biological entities and the global economic order, the only viable path forward in the current scenario is to harness the inherent potential of the pool of scientific knowledge and technology to shape the future. Upholding the motto 'Discoveries for a Better Tomorrow' and armed with the audacity to create a brighter future, RGCB embarked on another fruitful journey in the past year. We are living in an era characterized by dwindling resources and a rapidly growing global population. Therefore, it is imperative that we must think differently and redefine what is considered 'normal' to produce novel. The recent innovative "Bio-manufacturing" initiatives launched by the Department of Biotechnology across six thematic sectors, which envision transforming the ordinary into the extraordinary, are poised to revolutionize the biotechnology sector in our country. RGCB is committed to enhance the impact of its research endeavors by embracing such innovative ideas and refining its ongoing research dimensions.

We have consistently maintained our commitment to research and knowledge dissemination in the past year also and further enriched the scientific content within our six thematic scientific domains through the publication of high-quality research articles. RGCB continues to lead innovation and discovery, and our dedicated efforts are reflected in the publication of over 170 research papers resulting from our studies. The continuous enhancement of our research infrastructure with state-of-the-art equipment is of paramount importance for conducting cutting-edge research. In the current year, we have introduced several valuable additions to our equipment pool, including a 200 KV transmission electron microscope, motorized upright fluorescence microscope with metaphase finder karyotyping capabilities, a fully motorized fluorescence microscope, and a 10x genomics single cell analysis (chromium X) system. Furthermore, to reinforce our pathogen biology research component, we have established a fully-fledged BSL-2+ facility at our main campus in Jagathy.

The shedding of fulfilled leaves and the emergence of new ones are undoubtedly the symbols of growth. Farewell is always accompanied by a tinge of sorrow, but it becomes inevitable as RGCB enters its fourth decade of existence.

We bid farewell to seven of our founding members in the scientific community. The institute deeply cherishes and values their significant contributions, which have played a pivotal role in shaping RGCB into its current standing. To address the void created by their departure, we have welcomed seven new scientists into our fold. Among them are six Scientist Cs and one Scientist F, each holding an excellent career record and outstanding expertise within RGCB's mandate field. We extend a warm welcome to our new colleagues, and we look forward to witness their contributions as they embark on a productive and fruitful journey here at RGCB.

RGCB is committed to build a high-quality workforce in our country to serve the educational and industrial sectors in biotechnology. Our M.Sc Biotechnology program, which was initiated in 2019, is progressing excellently. The second batch consisting of 31 students has successfully completed the program and is currently engaged in research at premier institutes across India. In the past year, RGCB produced 23 doctoral degrees, with a focus on topics related to disease biology. Additionally, RGCB conducted regular hands-on technical training sessions in confocal microscopy, mass spectrometry, imaging, and bioinformatics and several aspirants were benefitted from these training sessions. As part of our commitment to skill development, the instrumentation wing of our Centre organizes 10-day hands-on training program on "Biotechnology Instrumentation Systems" and this year 63 undergraduate and postgraduate students from various arts and science colleges, and engineering colleges utilized this training program for their career improvement. Furthermore, the Molecular Forensics & DNA Technologies division provided three weeks training to 14 individuals in DNA fingerprinting and barcoding. Over 50 postgraduate students from medical colleges and arts and science institutions completed their dissertation work as part of their academic curriculum at the Laboratory of Medicine and Molecular Diagnostics division by employing various molecular diagnostic methods. Similar to previous years, this year also many postgraduate students from universities and colleges across India chose RGCB to undertake their dissertation work in various laboratories.

RGCB is committed to fulfill its social responsibilities and translating research findings for the benefit of the public. Our three major service arms, namely Medical Laboratory

Services, Laboratory Medicine & Molecular Diagnostics, and Molecular Forensics & DNA Technologies, have continued to provide invaluable services to the public, as well as to the judiciary and law enforcement agencies. Laboratory Medicine and Molecular Diagnostics made a significant contribution by providing over 18,000 sequences to the 'Indian SARS-CoV-2 Genetics Consortium' (INSACOG). In addition, our Centre has inaugurated a Science Museum at the Government Higher Secondary School in Meppadi, near Kalpetta, Wayanad district. This initiative aligns with the Union government's objective to promote a scientific approach among students and local communities. The state-of-the-art facility has been handed over to the school authorities and is one of the 75 such museums that the Center has planned as part of the nationwide 'Azadi Ka Amrit Mahotsav'.

We wish to express our heartfelt appreciation and gratitude for the unwavering support and encouragement we have received from the esteemed Chairman and Members of the Governing Council and Scientific Advisory Committee, who have played a pivotal role in propelling the institute forward. We are deeply grateful for the invaluable guidance and encouragement provided by the Secretary of DBT. The help and support from the Nodal Officer have been commendable.

I extend my sincere thanks to each and every member of our fraternity for their dedicated efforts in elevating RGCB to a prominent position among global research institutions.

With great pride, I present the Annual Report for the year 2022-23.

Jai Hind

Professor Chandrabhas Narayana, FASc, FRSC, FNASc
Director

Abdul Jaleel K. A, PhD
Scientist G
Cardiovascular Diseases & Diabetes Biology Program

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.

LABORATORY STRENGTH

JRF: 1 | SRF: 1 | Project Assistant: 1 | Technical Assistant: 1
Lab Assistant: 1 | Post Doctoral Fellows: 2 | PhD Students: 1

Front row (from left): Akhila Suresh, Gopika Satheesh, Susmi T R
Back row (from left): Reshmi P B, Dr Nandini R J,
Dr Kalaivani V, Sreebharathi R

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

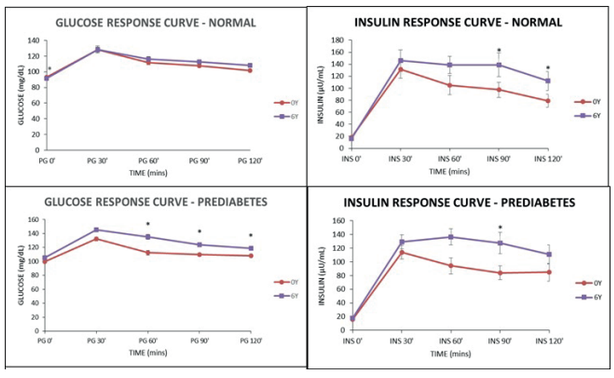
The previous DBT-funded project conducted in our laboratory entitled "Metabolomics Profiling of Normal Healthy People in Kerala: Impact of Family History of Diabetes" revealed impaired postprandial metabolism and early metabolic changes associated with insulin resistance in normoglycemic young adults who have risk of diabetes such as overweight (not obese), family history of diabetes etc . A higher degree of insulin resistance was observed in young men when compared to women. Out of the 94 participants, 9 developed T2DM, 51 had prediabetes and 34 remained normal. Glucose and Insulin response (Figure-1) to a mixed-meal challenge showed alteration during the follow-up when compared based on their glucose status and gender-wise. All the participants exhibited elevation in Body Mass Index, Waist Hip Ratio, Total Cholesterol, Triglyceride, and VLDL levels during the follow-up. Metabolic profiling revealed alteration in the metabolites associated with insulin resistance.

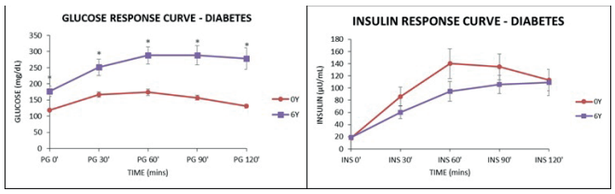
COMPREHENSIVE MASS SPECTROMETRY-BASED CLINICAL LIPIDOMICS PLATFORMS FOR PROMOTING BIOMEDICAL RESEARCH AND ADVANCED TRAINING FOR INDIAN RESEARCHERS

A) The installation of HRMS from Thermo Scientific (Orbitrap Eclipse Tribrid Fusion MS) has been completed. Application-based training was provided for the facility staff in lieu of developing a high-throughput, global and non-targeted in metabolomics, proteomics and lipidomics and services for the same started.

B) High Sensitive LC/MS/MS System (QqQ) Altis plus from Thermo Scientific has been installed for performing absolute quantification of biological compounds. Application training was provided to the facility staff. Assay for homocysteine and methylmalonic acid has been established both by standard curve as well as stable isotope dilution methods.

C) To meet the SAHAJ goals of education and research; the expansion and renovation plans for the core facility, website renovation to improve its accessibility and service reach, recruitment of sufficient staff members, hands-on training for the staff are currently being executed.





Repeated measures ANOVA were performed to explore difference in Guloce and insulin response to a mixed-meal challenge during the follow-up in the participants who remained normal. A p-value of <0.05 was considered significant.

PUBLICATIONS

- Kalaivani V, Krishna MS, Kumar AA, Satheesh G, Jaleel A. O-glycan structures in apo(a) subunit of human lipoprotein(a) suppresses the pro-angiogenic activity of galectin-1 on human umbilical vein endothelial cells. FASEB J. 2023;37(3):e22813.
- Chandran M, Sudhina S, Abhirami, Chandran A, Jaleel A, Plakkal Ayyappan J. Defining atherosclerotic plaque biology by mass spectrometry-based omics approaches. Mol Omics. 2023 16;19(1):6-26.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Comprehensive Mass Spectrometry-based Clinical Lipidomics platforms for promoting biomedical research and advanced training for Indian researchers	Department of Biotechnology	2021	4 years	PI
2.	Genome India: cataloguing the genetic variations in Indians	Department of Biotechnology	2020	4 years	CO-PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Vinitha A	Role of Cyclophilin A on the Efferocytosis of Apoptotic Cells and its Association with Atherosclerosis	MAHE	Awarded	2023

Ananda Mukherjee, PhD
DBT-Ramalingaswami Faculty Fellow
Cancer Research Program

BRIEF THEME OF LABORATORY

DNA damage response in cancer

LABORATORY STRENGTH

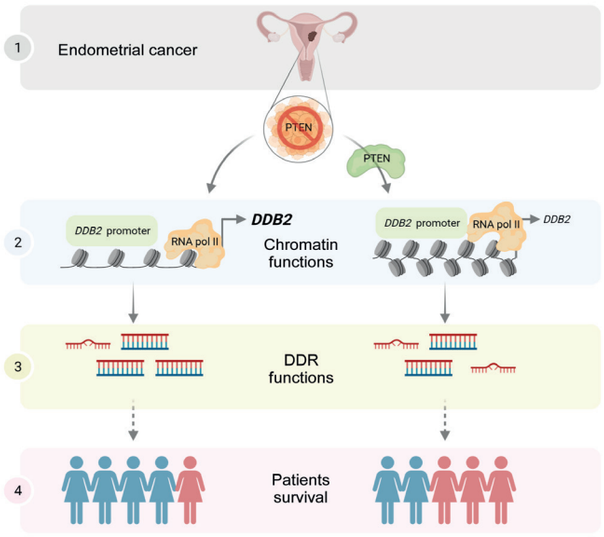
Laboratory Strength: | Project Assistant: 1
Technical Assistant: 1 | PhD Students: 1

TARGETING TRANSCRIPTIONAL ADDICTION IN ENDOMETRIAL CANCER THAT HACKS DNA REPAIR FUNCTION

Endometrial cancer (EC) arises from uterine endometrium tissue and is the most prevalent cancer of the female reproductive tract in developed nations. It has been predicted that EC's global prevalence will increase partly because of its positive association with economic growth and lifestyle. The majority of EC presented with endometrioid histology and mutations in the tumor suppressor gene PTEN, resulting in its loss of function. PTEN negatively regulates the PI3K/Akt/mTOR axis of cell proliferation, thus serving as a tumorigenesis gatekeeper. Through its chromatin functions, PTEN is also implicated in DNA repair procedures. However, our comprehension of how DNA repair occurs in the absence of PTEN function in EC is insufficient.

We used The Cancer Genome Atlas (TCGA) data analysis to establish a correlation between PTEN and DNA damage response genes in EC. This was followed by a series of cellular and biochemical assays to elucidate a molecular mechanism utilizing the AN3CA cell line model for EC.
The TCGA demonstrated an inverse correlation between the expression of the nucleotide excision repair (NER) damage sensor protein, DDB2, and PTEN in EC. The transcriptional activation of DDB2 is mediated by the recruitment of RNA polymerase II to the DDB2 promoter in the PTEN-null EC cells, revealing a correlation between increased DDB2 expression and increased NER activity that is inhibited by transcriptional inhibitors in the absence of PTEN.

Our study indicated a causal relationship between NER and EC that may be exploited in disease management.



A model depicted a probable mechanism and consequence of high DDB2 expression in PTEN-null endometrial cancer. Without PTEN, endometrial cancer cells exploit nucleotide excision repair, a versatile DNA repair mechanism, to protect their genome by overproducing the DNA damage sensor protein DDB2 which might contribute to their survival. Women in blue represented live patients; women in red represented deceased patients.

From left: Ahel Bhattacharya, Fathima Hameed J S

CONFERENCE PRESENTATION

- Fathima Hameed J S, Poster presentation: "A flowcytometric approach to quantify nucleotide excision repair (NER) activity in highly proliferative endometrial carcinoma cells" at Chromosome stability meeting 2022, held between 14-18 December 2022 at IISER, Thiruvananthapuram, India.

- Fathima Hameed J S, Poster presentation: "Targeting transcriptional addiction in endometrial cancer that hacks DNA repair function" at National symposium organized by Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram on 12th April 2023.

PUBLICATIONS

- 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 J S F, 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.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Studies on noncanonical role of tumor suppressor PTEN in endometrial adenocarcinoma.	DBT-RFF	2018	7 years	PI

Ananthalakshmy Sundararaman, PhD
DBT-Ramalingaswami Faculty Fellow
Cardiovascular Diseases & Diabetes Biology Program

BRIEF THEME OF LABORATORY

Mitochondria-derived Vesicle Trafficking and Mitochondrial Quality Control in Cardiovascular Health and Disease- Focus on mito-nuclear transit of proteins and retrograde signalling

LABORATORY STRENGTH

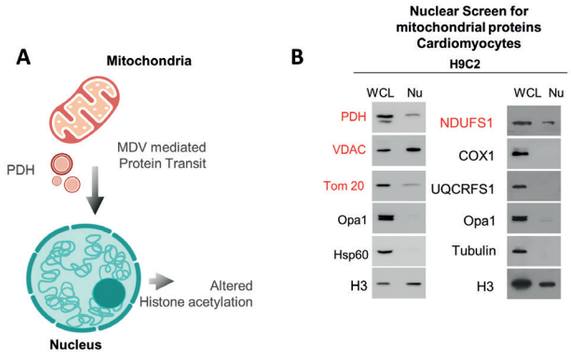
JRF:1 | Project Assistant: 1 | PhD Students: 1

From left: Keerthi Jomy, Mrithula Jaykumar, Lariza Ramesh, Thejaswitha Rajeev, Ananthu Chandran

MITOCHONDRIA-DERIVED VESICLE TRAFFICKING IN CARDIOVASCULAR HEALTH AND DISEASE

The burden of cardiovascular diseases is increasing in India. Cardiovascular diseases contribute to 28.1% of total deaths in India1. Mitochondrial dysfunction characterizes several cardiovascular diseases like atherosclerosis, ischemia-reperfusion injury, diabetic microvascular disease, and heart failure. Cardiomyocytes are crucially dependent on healthy mitochondria for their bioenergetics. Dysfunctional mitochondria are a center of ROS production and oxidative damage leading to cell death. Mitochondrial quality is maintained through several mechanisms like mitophagy, unfolded protein response mechanisms leading to proteasomal degradation of proteins within the mitochondrial matrix and the cytosol, and regulated fission and fusion. In adult cardiomyocytes, however, mitochondria embedded within the myofibrils rarely undergo fission and fusion, suggesting that these cells are likely to be crucially dependent on other forms of mitochondrial quality control. Recently, two novel quality control mechanisms, the formation of actin cages around mitochondria and the release of a novel class of vesicles called mitochondria-derived vesicles (MDVs), were described. We hope to derive new insights into these quality control processes in cardiovascular pathophysiology, where the key players and pathways remain unexplored.

Our group hopes to develop an understanding of endothelial and cardiomyocyte mitochondrial quality control to discover clinically relevant stimulators of mitochondrial network quality and function to delay cardiovascular pathology.



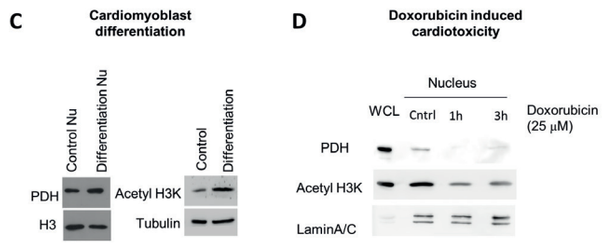


Figure A- Working model for MDV mediated nuclear transit of mitochondrial proteins like PDH that produce acetyl CoA in the nucleus to alter histone acetylation and transcriptional response.

Figure B- Nuclear screen to reveal mitochondrial protein targets that transit to the nucleus.

Figure C- Cardiomyoblast differentiation model showing increased nuclear PDH and acetyl histone levels following retinoic acid induced differentiation of cardiomyoblasts.

Figure D- The DNA intercalator doxorubicin reduces global histone acetylation in cardiomyoblasts and this parallels reduction in nuclear PDH levels.

PUBLICATIONS

- Samuel V, Rajeev T, Ramesh L, Sundararaman A. Book chapter: Integrin Trafficking in Health and Disease; Book entitled Receptor Endocytosis and Signalling in Health and Disease, Academic Press, Vol 196, (2023), 271-302.
- Nair NY, Samuel V, Ramesh L, Marib A, David DT, Sundararaman A. Actin cytoskeleton in angiogenesis. Biol Open. 2022;11(12):bio058899.
- Gan X, Ramesh L, Nair N, Sundararaman A. Book chapter: Fibronectin fibrillogenesis during angiogenesis Book entitled: Matrix Pathobiology and Angiogenesis, Springer Publishing, vol 12, 1-27 ISBN: 978-3-031-19615-7.
- Andugulapati SB, Sundararaman A, Lahiry M, Rangarajan A. AMP-activated protein kinase promotes breast cancer stemness and drug resistance. Dis Model Mech. 2022;15(6):dmm049203.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Characterising Mitochondria-derived Vesicle Trafficking through a Proximity Labelling Approach- A possible Novel Mito-nuclear Communication Pathway	Science and Engineering Research Board	2022	3 years	PI
2.	Role of RhoGTPases in the Intracellular Trafficking of Mitochondria Derived Vesicles (MDVs) and Angiogenesis	Department of Biotechnology	2020	5 years	PI

Ani V Das, PhD
Senior Program Scientist
Cancer Research Program

BRIEF THEME OF LABORATORY

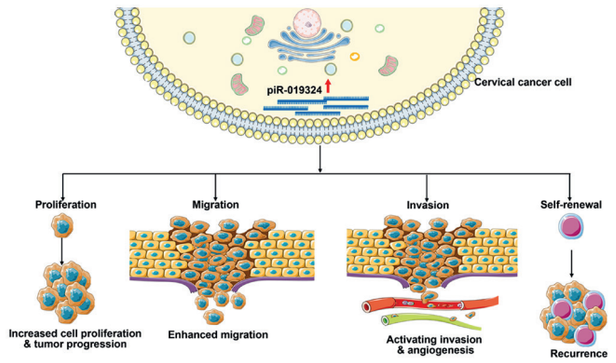
Laboratory focuses on two aspects of cancer stem cells (CSCs): how they are maintained and how they can be targeted. On one hand, we investigate the involvement of Piwi proteins and associated non-coding RNAs called piRNAs in the cancer stem cell maintenance. On the other hand, we are looking at the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

DECIPHERING THE ROLE AND REGULATION OF PIWIL1 IN HPV-MEDIATED CERVICAL CANCER

Piwi proteins play major role in germ stem cell maintenance. Recent studies suggest their aberrant expression in various cancers. Among the homologues, over-expression of PiwiL1 has been reported in many cancers including cervical cancer. However, the molecular mechanism by which these proteins contribute to tumorigenesis and their regulation in cancer cells is still unclear. Here, we display evidence that PiwiL1 is not only over-expressed, but also play a major role in tumor induction and progression. Abolition of PiwiL1 in CaSki cells led to a dramatic decrease in cell proliferation, viability and metastatic abilities, whereas, it's up-regulation conferred malignant transformation of normal HaCaT cells. We also found that PiwiL1 play a major role in the regulation of stemness-associated properties of cervical cancer cells.

More importantly, our study delineates a novel interaction and link between HPV oncogenes, E6 and E7 with PiwiL1. HPV-E6 and E7 induced PiwiL1 expression in normal HaCaT cells. Further, an in silico analysis of the promoter regions of PiwiL1 suggested a possible binding of p53 and E2F1, the two targets of E6/E7. We found that both p53 and E2F1 bind and differentially regulate PiwiL1 promoter in a context-dependent manner. Our data suggested that HPV E6 and E7 accentuated the expression of PiwiL1 via degradation of p53 and stabilization of E2F1, respectively. Interestingly, PiwiL1 is found to be physically interacting with both E6 and E7 in cervical cells as indicated from co-immunoprecipitation assays.

It is well-known that Piwi proteins, in association with piRNAs, can act as guiding signals for many epigenetic factors. We thought to identify the piRNAs that are differentially expressed in cervical cancer cells. Also we wanted to isolate PiwiL1-specific piRNAs in cervical cancer cells. Through an in silico approach, from small RNA Sequencing Data (SRP119662) using PILFER, we identified a few differentially expressed piRNAs in cervical cancer. Validation of these selected piRNAs revealed one promising piRNA, piR-hsa-X which showed a ~12fold increase in CaSki cells, compared to controls. When analyzed, this piRNA was proved to be oncogenic and its inhibition resulted in reduced tumorigenic properties of CaSki cells in vitro (Figure). Also, PiwiL1-specific piRNAs in cervical cancer cells were identified by RNA-immunoprecipitation assay, from which we found three piRNAs (DQ571333, DQ596538, DQ570812) that are interacting with PiwiL1. Functional evaluation of their roles in cervical tumorigenesis is being carried out in the lab.



LABORATORY STRENGTH

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

From left: Vidya S, Pooja S R, Midhunaraj K

piR-019324 acts as a oncogene in cervical cancer cells. piR-019324 is highly expressed in cervical cancer cells. It is an oncogenic piRNA as its inhibition led to decreased proliferation, increased cell death with a significant reduction in all cancer-associated properties in CaSki cells. More importantly, the inhibition of piR-019324 affected the stemness properties of CaSki cells

CONFERENCE PRESENTATION

- Midhunaraj K: Flash talk, Solving the maze of PiwiL1 overexpression in cervical cancer cells, Virtual EMBO workshop on piRNAs and PIWI proteins from 6-9 April 2022.

- Midhunaraj K: Oral presentation, Unfolding the role of PiwiL1 in cervical cancer and its association and regulation by HPV in International virtual conference on evolving paradigms in biotechnology; combating contemporary challenges organized by the Department of Life Sciences, Kristu Jayanti College, Autonomous in association with KSCSTE-Jawaharlal Nehru Tropical Botanic Garden & Research Institute on 1- 2 April, 2022.

- Pooja SR: Oral presentation, Unravelling the regulation of RFX1 in cancer stem cells International Virtual Conference on Evolving Paradigms in Biotechnology: Combating Contemporary Challenges organized by the Department of Life Sciences, Kristu Jayanti College, Autonomous in collaboration with Jawaharlal Nehru Tropical Botanic Garden and Research Institute held from 1 to 2nd April 2022.

- Pooja SR: Poster presentation, Unfolding RFX1 mediated regulation of cancer stem cells, ASBMB Annual Meeting/Experimental Biology 2022 held at Pennsylvania Convention Center, Philadelphia, USA from 2nd to 5th April 2022.

- Midhunaraj K: Oral presentation, Association and regulation of PiwiL1 by HPV oncogenes in cervical cancer cells in National Seminar on Recent Approaches in Biochemical Research from 23-24 June 2022, Kerala University, Thiruvananthapuram.

- Pooja SR: Oral presentation, Understanding the regulation of RFX1 in cancer stem cells, National Seminar on Recent Approaches in Biochemical Research organized by Department of Biochemistry & Advanced Centre for Tissue Engineering, University of Kerala held from 23 to 24 June, 2022.

- Midhunaraj K: Poster presentation, Cross talk between PiwiL1 and HPV in cervical cancer cells: a reason or cause for tumorigenesis, National symposium on biotechnology for sustainable development-2022 on 27th July 2022, Rajiv Gandhi centre for biotechnology, Thiruvananthapuram.

- Pooja SR: Poster presentation, Understanding the role of RFX1 in cancer stemness 42nd International Annual Conference of the Indian Association for Cancer Research (IACR-2023) on Bringing basic and translational research to the clinic: Challenges and Opportunities Organized by ACTREC, TATA Memorial Centre, Kharghar, Navi Mumbai from 12th to 16th January 2023.

- Midhunaraj K: Poster presentation, PiwiL1 misregulation in cervical cancer: are HPV oncoproteins responsible? 42nd International Annual Conference of the Indian Association for Cancer Research (IACR-2023) on Bringing basic and translational research to the clinic: Challenges and Opportunities Organized by ACTREC, TATA Memorial Centre, Kharghar, Navi Mumbai from 12th to 16th January 2023.

PATENTS APPLIED/ GRANTED

- One application has been submitted for Provisional Patent.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Deciphering the regulation of PiwiL1 in Cancer Stem cells	Science and Engineering Research Board	2021	3 years	PI

DELINEATION OF MICRORNA-532 3P AS A TUMOR SUPPRESSOR AND ITS PLAUSIBLE ROLE IN COLORECTAL CANCER PROGRESSION VIA FOXM1 REGULATION

FOXM1 (forkhead-box M1) is a critical proliferation-associated transcription factor, that is widely, spatiotemporally expressed during the cell cycle. Therefore, it is essential to study how its expression can be regulated. A chromatin immunoprecipitation study conducted in lab revealed the ability of FOXM1 to bind to the promoters of a few microRNAs. MicroRNAs are one of the classes of post-transcriptional regulators of gene expression. A thorough database mining was carried out to list several candidate microRNAs that might regulate the

Asha Nair S, PhD
Scientist G
Cancer Research Program

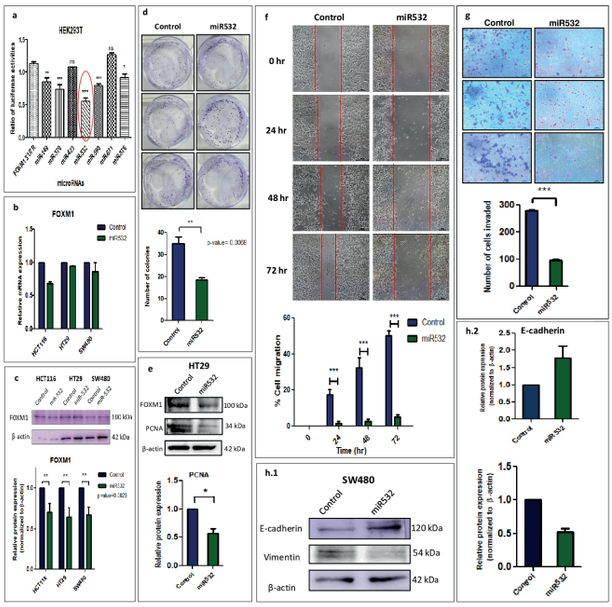
BRIEF THEME OF LABORATORY

The Clinical research program target stem cell markers in Colorectal Cancer (CRC) and their manifestation of probable molecular pathways for chemotherapeutic intervention as prognostic indicators of CRC and Early-Onset of CRC. Pertaining to basic research, molecular targets relevant to the cell cycle machinery are being studied. The program on photodynamic therapy is on screening of photosensitizers and evaluation of biological property of Graphene & Molybdenum Quantum Dots.

LABORATORY STRENGTH

JRF: 2 | SRF: 1 | Project Assistant: 1 | Technical Assistant: 1
Lab Assistant: 1 | PhD Students: 3

expression of FOXM1. The interaction between the microRNAs and FOXM1 3'UTR was quantitated by conducting a dual luciferase reporter assay. The selected candidate, microRNA-532 was overexpressed in three colorectal cancer cell lines. The effects of this overexpression were checked in various cancer-related phenomena. The effect on cell cycle progression and cell death by apoptosis was checked by flow cytometry. Clonogenic assay was conducted to check the effect on proliferation. Wound healing assay and matrigel invasion were carried out to investigate the role in regulation of cellular migration and invasion. Numerous hits were obtained from database mining, from which seven microRNAs were selected for validation of interaction. Dual luciferase reporter assay with selected hits revealed that miR-532 interacted with 3'UTR of FOXM1 mRNA transcript most efficiently. Its overexpression resulted in decreased protein levels of FOXM1 in colorectal cancer cells. No significant effect was seen in the progression of cell cycle or in the amount of cell death. Overexpression of miR-532 resulted in a significant reduction in cellular proliferation in HT29 cells. This was confirmed by the reduction in the protein levels of PCNA. Also, cellular migration and invasion in SW480 cells was found to be significantly reduced. The elevated levels of E-cadherin and the reduced levels of vimentin supported these experimental outcomes. Thus, microRNA-532 was found to be a regulator of cellular proliferation and migration in colorectal cancer cells.



Front row (from left): Anjana Soman, Krishna R, Meera R Nair, Shilpa R, Poornima S R
Back row (from left): Samu John, Evangeline Surya Hermon, Kumari Shobha, Divya J, Rajshree R Nair, Ketakee Mahajan

a) Dual luciferase reporter assay quantitating microRNA-FOXM1 3'UTR interaction
b&c) mRNA and protein levels of FOXM1 on ectopic overexpression of miR532
d) Clonogenic assay in HT29 cells and its quantitation
e) Protein levels of PCNA on ectopic overexpression of miR532
f) Wound healing assay in SW480 cells ectopic overexpression of miR532
g) Matrigel invasion assay in SW480 cells ectopic overexpression of miR532
h) 1 & h.2. Expression of EMT markers in SW480 cells ectopic overexpression of miR532

INVITED TALKS [PI]

- Colorectal cancer and surgical margins: is more less? Invitation as expert speaker for the scientific session in One day seminar on Standardisation and preclinical studies of Ayurvedic formulation- 6th march 2023 NARIP cheruthuruthy.

CONFERENCE PRESENTATION

- R. Rajashree Nair: Oral presentation, Molecular effectiviness of a photosensitizer, biotin conjugate Aza-BODIPY KERALA SCIENCE CONGRESS 2023, Feb13-15, Kuttikanam, Idukki.

PUBLICATIONS

- Soman A, Asha Nair S. Unfolding the cascade of SERPINA3: Inflammation to cancer. Biochim Biophys Acta Rev Cancer. 2022;1877(5):188760.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	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

Chandrabhas Narayana, **PhD FASc, FRSC, FNASc**
Director
Transdisciplinary Biology Program

BRIEF THEME OF LABORATORY

Our laboratory focuses on developing Raman spectroscopy-based techniques 1. to detect bio-molecules for diagnostic purposes; 2. for the characterisation of novel functional inorganic, organic, and organometallic materials. We have been also focusing on making Raman spectroscopes portable, and semi-automatic with the aim of integrating the Raman spectroscopy based in-situ detection and diagnostics as a part of regular pathology.

LABORATORY STRENGTH

JRF: 1 | Project Assistant: 1 | Technical Assistant: 1
Lab Assistant: 1 | PhD Student: 1 | Post Doctoral Fellow: 1

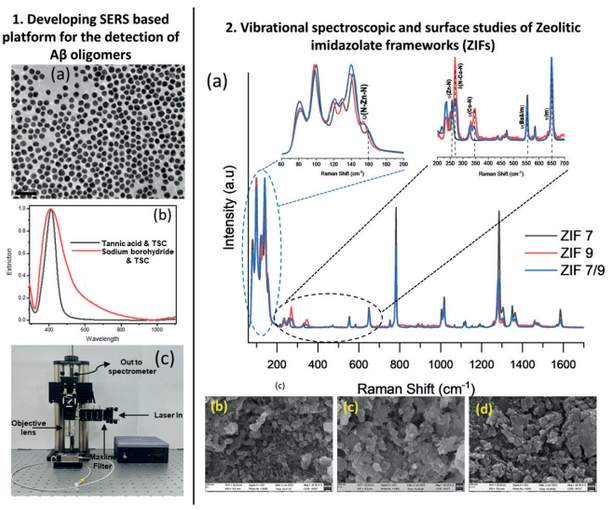
From left: Irfan Shafi Malik, Kanakangi Sukumar, Sudhanshu Mani Tripathi, Dr Debanjan Bhowmik, Harikrishnan KS, Kamallata Chakraborty, Shambhavi Mishra

BUILDING HAND HELD RAMAN SPECTROMETER AND SILVER NANOSTRUCTURES/FUNCTIONALIZED MXENE HYBRIDS FOR SERS BASED DETECTION OF EXOSOMES

For the detection of Aβ oligomers, Rose Bengal dye functionalized AgNPs were synthesized. Rose Bengal (RB) is a fluorescein derivative and its unique SERS spectrum of RB has been exploited for Raman imaging. Remarkably, RB also shows a strong affinity to Aβ peptides and can be used as inhibitors for Aβ aggregation. So far, we have achieved highly monodisperse silver nanoparticles via Tannic acid reduction method Figure 1 (a-b). We have already made the structural part of the portable Raman spectrometer and the image is shown below in Figure 1 (c). We have assembled the filters, objective lens and sample stage and optical alignment needs to optimize for further assembling laser and spectrometer.

The ambient Raman spectrum of the ZIF-7, ZIF-9, and hybrid of both the Zeolite imidazole framework is shown in Figure 2 (a) respectively. Raman analysis of hybrid ZIF 7/9 have similar peak position of individual ZIF 7 and ZIF 9 crystals which conclusively shows the hybrid nature and high uniformity of the mixed-linker materials. Figure 2 (b-d) shows surface morphological analysis of ZIF 7, ZIF 9 and its hybrid form. The crystallized ZIF 7/9 is a true hybrid and not just physical mixtures of equivalent ZIF's.

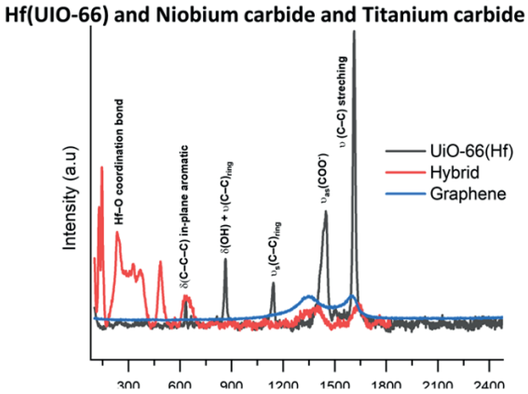
Raman spectra of Taurine functionalized Graphene on Hf (UIO-66) is shown in Figure 3 (a). Synthesis and delamination of 2D ultrathin Nb2C nanosheets were achieved by a liquid exfoliation method combining HF etching (delamination) and tetrapropylammonium hydroxide (TPAOH) intercalation (disintegration). Raman spectra of Nb2C nanosheets were analysed and shown in Figure 4. Delamination of 2D ultrathin Ti3C2 nanosheets were achieved by a liquid exfoliation method combining HF etching (delamination) and tetramethylammonium hydroxide (TMAOH) intercalation and Raman spectra is shown in Figure 4.



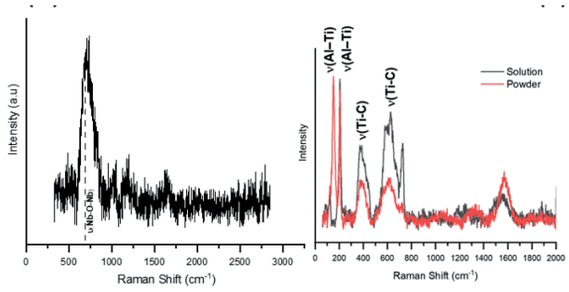
1) (a) TEM of AgNPs (b) Absorption spectra of AgNPs (c) Portable Raman Spectrometer

2) Comparison of ambient Raman spectra of ZIF 7 (black), ZIF 9 (red) and hybrid of ZIF 7/9 (blue) and SEM images of ZIF 7 (a), ZIF 9 (b) and (c) hybrid of ZIF 7/9

3. Raman studies of Taurine functionalized Graphene on Hf(UIO-66) and Niobium carbide and Titanium carbide MXenes



4. Raman spectral studies of MXenes



3) Comparison of ambient Raman spectra of UIO-66 (black), Hybrid (red) and taurine functionalized Graphene (blue)

4) Raman spectra of Nb2C nanosheets and Raman spectra of Ti3C2 nanosheets

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 22;62(20):7703-7715.

- 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.

- Bhowmik D, Narayana C. Far-Field Spectroscopy and Surface-Enhanced Raman Spectroscopy (SERS). In Nanoscopy and Nanospectroscopy edited by Dhara S, Jariwala D, Das S. CRC Press, 2023. pp 97-129.

AWARDS (PI)

- Dr. Raja Ramanna Award from the Indian Society of Analytical Scientists (ISAS) for his distinguished achievements and outstanding contributions in the advancement of Raman Spectroscopy and Brillouin spectroscopy for drug discovery and diagnostic applications. Indian Analytical Science Congress 2023, 23-25, March, 2023, Kochi.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Scientific reinvention of ethnic food and medicine from Kerala for functional food and drug development	Department of Science and Technology	2021	3 years	PI
2.	Development of portable Raman spectrometer for identifying amyloid deposition in the peripheral region of the body	Department of Science and Technology	2021	3 years	PI

Debanjan Bhowmik, PhD
Ramanujan Faculty Fellow
Transdisciplinary Biology Program

BRIEF THEME OF LABORATORY

Our lab focuses on developing fluorescence and Raman spectroscopy-based methods for the detection of various types of biomarkers. We design and test variety of gold and silver-based surface functionalized metal nanoconstructs for this purpose. We are also interested in developing new compact instrumental setups in the lab.

DEVELOPMENT OF A PORTABLE RAMAN SPECTROMETER AND VARIOUS TYPES OF GOLD (AUNP) AND SILVER (AGNP) BASED FUNCTIONALIZED NANOPARTICLES FOR SERS BASED DETECTION OF DISEASE SPECIFIC BIOMARKERS

The interaction of Rose Bengal Dye (RB) functionalized AuNPs has been previously utilized to detect Amyloid β oligomers in solution via SERS. As AgNP can provide much better sensitivity compared to AuNP, we aspired to make a RB functionalized AgNP platform. We have already been successful in synthesizing monodisperse AgNPs for that purpose (Figure 1 C, D). We are in the process of making a portable Raman system (Figure 1 B) for remote application of the technique, for this project.

We are developing gold nanostars (AuNS) and AgNP based nanoconstructs for SERS based detection of HER-2 containing exosomes. We have tethered HER-2 specific aptamers on these NPs for this purpose. NPs with control (with no HER-2 targeting) aptamers were also made. In the presence of exosome obtained from HER-2 overexpressing SKOV-3 cells, the SERS signals from the targeting nanoconstructs were greatly suppressed (Figure 2C and D), possibly due to the reorientation of the aptamer on the NP-surface upon HER-2 binding. The SERS signal from control aptamer labelled nanoconstructs remained almost unchanged (Figure 2E) even in the presence of exosomes. As a next step, we are using Alexa-488 labelled Aptamers to functionalize AgNPs (Figure 2F) to maximize the effect of the reorientation (upon HER-2 binding) of the aptamers on SERS.We had demonstrated that anisotropic AuNS (unlike spherical AuNPs) when functionalized to interact with specific receptors on plasma-membrane, can retain their targeting ability even in the presence of serum proteins. We are currently testing the effect of branch length and numbers on the retention of the targeting abilities. We have been successful in making AuNS having increasing branch lengths with the use of decreasing amount of 5 nm spherical AuNPs (Figure 1B), during seeded synthesis. We could also obtain longer branches at higher reaction temperatures with unchanged seed concentration (Figure 1C).

CONFERENCE PRESENTATION

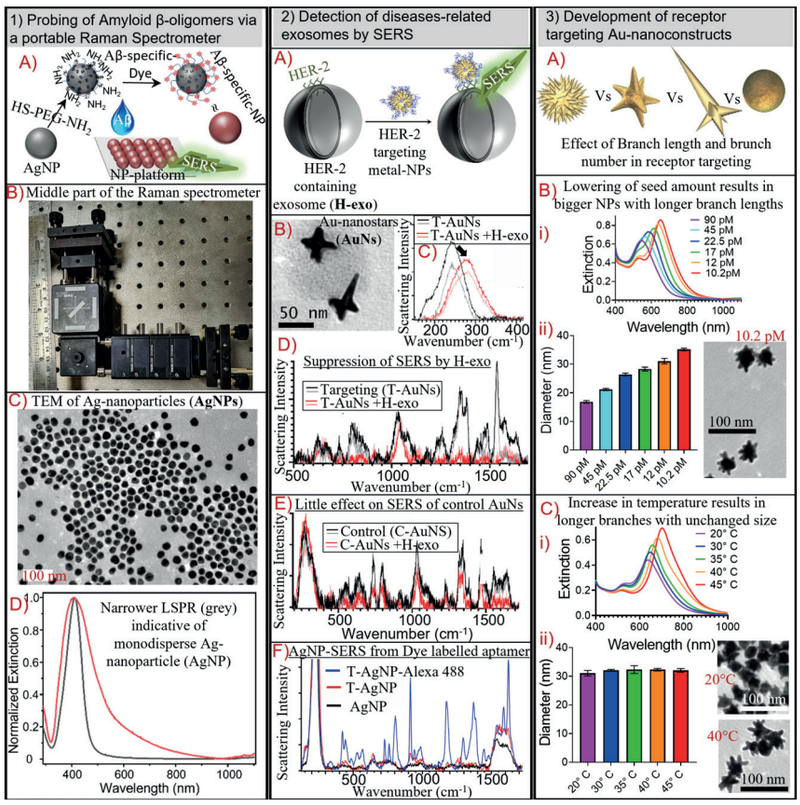
- Abhirami Ajith: Poster presentation, Early detection of diseases-related exosomes, FCS@50, 11.01.2023 and 12.01.2023, Kovalam, Kerala.
- Kanakangi Sukumar: Poster presentation, Probing of Amyloid β-Peptide deposits on skin, FCS@50, 11.01.2023 and 12.01.2023, Kovalam, Kerala.

INVITED TALKS [PI]

- Biotechnological application of surface-functionalized metal nanoconstructs: Diagnostics, Therapeutics, and Imaging,FCSXIII,10.01.2023, IISER Thiruvananthapuram.

PUBLICATIONS

- Bhowmik D, Narayana C. Nanoscopy and Nanospectroscopy, Chapter 5: Far-Field Spectroscopy and Surface-Enhanced Raman Spectroscopy (SERS). CRC Press, 2023, 97-129.
- Morck MM, Bhowmik D, Pathak D, Dawood A, Spudich J, Ruppel KM. Hypertrophic cardiomyopathy mutations in the pliant and light chain-binding regions of the lever arm of human β-cardiac myosin have divergent effects on myosin function. Elife. 2022; 11:e76805.



1. A: Detection of Amyloidβ via SERS; B: The spectrometer; C: Image of AgNPs; D: Extinction spectra of AgNPs.

2. A: Detection of HER-2 containing exosomes; B: Image of AuNS; C-D: SERS from targeting AuNS; E: SERS from nontargeting AuNS.

3. A: Synthesis of AuNS having different branch number and lengths; B: Extinction spectra (i) and size (ii) of AuNS synthesized using different seed concentration; C: Extinction spectra (i) and size (ii) of AuNS synthesized at different temperature.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	investigation of function-property relationship of various gold nanoparticle based nanoconstructs for biological applications	Science and Engineering Research Board	2021	5 years	PI

Debasree Dutta, PhD
Scientist E-II
Regenerative Biology Program

BRIEF THEME OF LABORATORY

The major focus of the laboratory is to identify cues in development that upon deregulation associate with diseased state.

LABORATORY STRENGTH

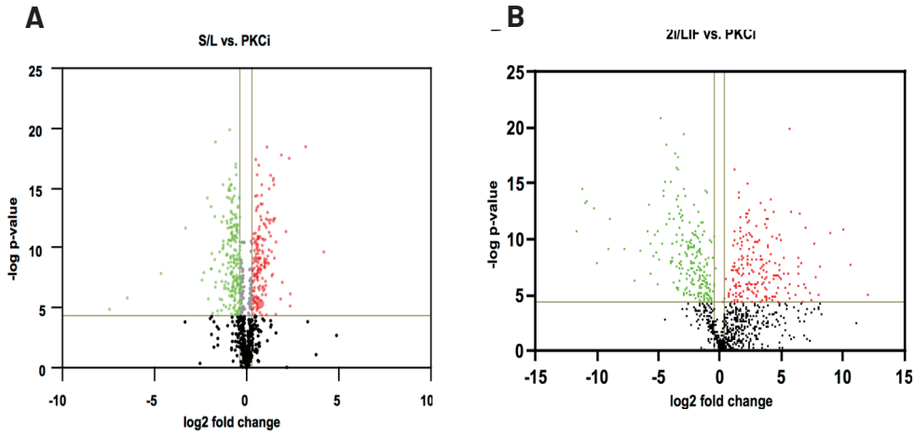
JRF: 2 | Technical Assistant: 1 | PhD Students: 5

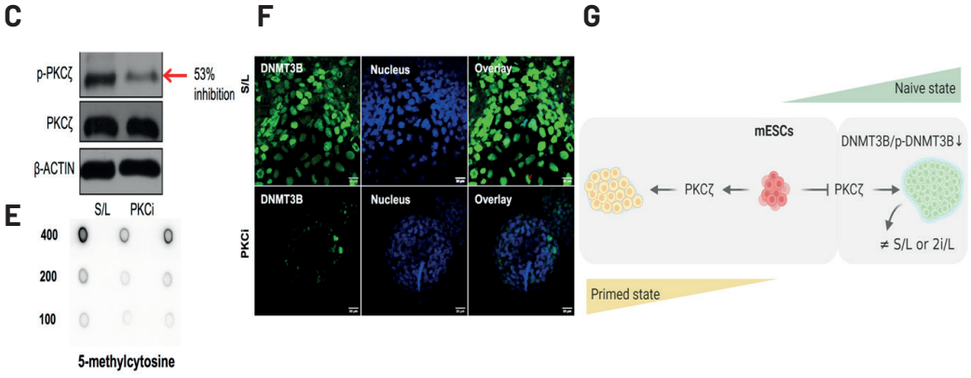
DISCOVERY OF A NOVEL STATE OF PLURIPOTENCY IN MURINE DEVELOPMENT UPON INHIBITION OF PKC SIGNALING

Inhibition of Protein Kinase C (PKCi) signaling maintains pluripotency of embryonic stem cells (ESCs) across different mammalian species. However, the position of PKCi maintained ESCs in the pluripotency continuum is largely unknown. Pluripoitency continuum comprises of ground, naive, poised, formative and finally primed states. Each of these states have been distinctively specified in murine embryonic development. However, the same lacks for human development. In the past year, we worked upon the leads detected earlier. Here we report that mouse ESCs when cultured continuously, with PKCi, for 75 days are retained in naïve state of pluripotency. Gene expression analysis and proteomics studies demonstrated enhanced naïve character of PKCi maintained ESCs in comparison to classical serum/LIF (S/L) supported ESCs or 2i/LIF supported ground state of pluripotent ESCs (Figure A & B). Molecular analysis revealed that activation of PKCζ isoform associate with primed state of pluripotency, present in epiblast-like stem cells generated in vitro while inhibition of PKCζ phosphorylation associated with naïve state of pluripotency in vitro and in vivo (Figure C). Phosphoproteomics, immunofluorescence and chromatin modification enzyme array based studies showed loss in DNA methyl transferase 3B (DNMT3B) and its phosphorylation level upon functional inhibition of PKCζ as one of the crucial components of this regulatory pathway

From left: Bindhu M S, Anjali Devarajan, Sruthy M R, Mayur Shirude, Debparna Nandy

(Figure D). Unlike ground state of pluripotency maintained by MEK/GSK3 inhibitor in addition to LIF (2i/LIF), loss in DNMT3B is a reversible phenomenon in PKCi maintained ESCs (Figure E). Absence of phosphorylation of c-MYC, RAF1, SPRY4 while presence of ERF, DUSP6, CIC and YAP1 phosphorylation underlined the phosphoproteomics signature of PKCi mediated maintenance of naïve pluripotency. States of pluripotency represent the developmental continuum and the existence of PKCi mediated mouse ESCs in a distinct state in the continuum of pluripotency (DiSCo) (Figure F) might contribute to the establishment of stages of murine embryonic development that were non-permissible till date.





A, B. Volcano plot demonstrate differential expression of proteins in S/L vs. PKCi and 2i/LIF vs. PKCi. C. Western blot analysis shows the significant loss in PKC zeta isoform upon inhibition of PKC signaling. D. Immunofluorescence analysis for the expression of DNMT3B in S/L vs PKCi maintained ESCs. E. Global DNA methylation level in ESCs under differnt conditions. F. Model for the existence of DiSCo pluripotency upon inhibition of PKC signaling.

AWARDS [PI]

- Best Teacher Award selected by RGCB MSc students 2020 batch.

AWARDS [STUDENTS]

- Pallavi Chinnu Varghese, ISSCR Zhongmei Chen Yong Award for Scientific Excellence, Role of histone chaperone APLF in cellular transition during embryonic development, ISSCR Annual Conference, June 12th to 15th, 2022, San Francisco, CA, USA.

- Pallavi Chinnu Varghese, Best PhD oral presentation, Epigenetic modifications in cellular transition during embryonic development, MAHE PhD Conclave 2022, May 27th-28th, 2022, Manipal Academy of Higher Education, Manipal.

- Ishita Baral, ASBMB Travel Award, Untangling the Web of Protein Kinase C Mediated Regulation of Naïve Vs Primed State of Pluripotency, Experimental Biology Annual Meet 2022 organized by American Society for Biochemistry and Molecular Biology, 2nd to 5th April at Philadelphia, USA.

- Debparna Nandy, Best Poster, Histone epigenetic classification of breast cancer: A pilot study., National Symposium on Biotechnology for sustainable development -2022, 27th July, 2022, Rajiv Gandhi Centre for Biotechnology, Kerala, India.

INVITED TALKS [PI]

- Invited talk at World Congress on Cancer held in February, 2023, Jaipur.

CONFERENCE PRESENTATION

- Pallavi Chinnu Varghese: Poster presentation, Role of histone haperone APLF in cellular transition during embryonic development, ISSCR Annual Conference, June 12th to 15th, 2022, San Francisco, CA, USA.

- Pallavi Chinnu Varghese: Oral presentation, Epigenetic modifications in cellular transition during embryonic development, MAHE PhD Conclave 2022, May 27th-28th, 2022, Manipal Academy of Higher Education, Manipal.

- Ishita Baral: Poster presentation, Untangling the Web of Protein Kinase C Mediated Regulation of Naïve Vs Primed State of Pluripotency, Experimental Biology Annual Meet 2022 organized by American Society for Biochemistry and Molecular Biology, 2nd to 5th April at Philadelphia, USA.

- Sruthy M R: Poster presentation, APLF as a regulator of centrosome integrity, RGCB- SEMINAR- JULY 27, 2022, DBT-RGCB, Thiruvananthapuram, Kerala.

- Sruthy M R: Poster presentation, Epigenetic control over centrosome duplication and spindle bipolarity?, All India Cell Biology Conference -2022, 2nd to 3rd September 2022, University of Kashmir, Srinagar.

- Sruthy M R: Poster presentation, A molecular link connecting DNA repair machinery and histone metabolism to centrosome duplication, Chromosome Stability 2022, 14th to 18th December 2022, IISER Thiruvananthapuram, Kerala.

- Sruthy M R: Poster presentation, Histone chaperone /DNA repair factor, APLF in surveillance of centrosome integrity - FCSXIII (National Workshop on Fluorescence and Raman Spectroscopy), 6th to 11th January 2022, IISER Thiruvananthapuram, Kerala.

- Debparna Nandy:Poster presentation, Histone epigenetic classification of breast cancer: A pilot study, National Symposium on Biotechnology for sustainable development -2022, 27th July, 2022, Rajiv Gandhi Centre for Biotechnology, Kerala, India.

- Debparna Nandy: Oral presentation, Role of histone epigenetic players in breast cancer, APOCP 11 (Asian Pacific Organization for Cancer Prevention 11) conference on cancer prevention, 8th-10th Dec 2022, Kolkata, West Bengal.

- Mayur Balkrishna Shirude: Poster presentation, Chromatin architecture regulation by histone chaperone HIRA dictates the cell fate in Chronic Myeloid Leukemia (CML) K562 cells, RGCB- SEMINAR- JULY 27, 2022, RGCB, Thiruvananthapuram, Kerala.

- Mayur Balkrishna Shirude: Poster presentation, Chromatin architecture regulation by histone chaperone HIRA dictates the cell fate in K562 cells, Chromosome Stability 2022, 14th to 18th December 2022, IISER Thiruvananthapuram, Kerala.

- Mayur Balkrishna Shirude: Poster presentation, Quantitative Fluorescence Lifetime Imaging (FLIM)-FRET to understand the role of histone chaperone HIRA in regulating chromatin architecture in Chronic Myeloid Leukemia (CML) cells, FCSXIII (National Workshop on Fluorescence and Raman Spectroscopy), 6th to 11th January 2022, IISER Thiruvananthapuram, Kerala.

PATENTS APPLIED/ GRANTED

- Title of the patent: MARKER PANEL AND METHOD THEREOF FOR IDENTIFYING THE PROGRESSION OF BREAST CANCER AND ITS SUBTYPE.

  Application # : 202241028767,Filed on May 19, 2022

PUBLICATIONS

- Rajam SM, Varghese PC, Dutta D. Histone Chaperones as Cardinal Players in Development. Front Cell Dev Biol. 2022;10:767773.

- Baral I, Varghese PC, Dutta D. Epigenetics as "conductor" in "orchestra" of pluripotent states. Cell Tissue Res. 2022;390(2):141-172.

- 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;19:1098-111.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Evaluation of histone chaperone APLF as a novel biomarker in triple negative breast cancer.	Department of Biotechnology	2018	5 years	PI
2.	Normal vs.abnormal hematopoiesis- result of a deregulated chromatin regulated by Histone chaperone HIRA?	Science and Engineering Research Board	2022	3 years	PI
3.	Epigenetic regulation of trophoblast stem cells involved in women reproductive health and disease: anobservational and functional study	Indian Council of Medical Research	2023	3 years	PI
4.	Dissecting domains of APLF chaperoning EMT in development	Science and Engineering Research Board	2023	3 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Pallavi Chinnu Varghese	Epigenetic modifications in cellular transitions during development	MAHE	Submitted	2023
2.	Ishita Baral	Role of PKC signaling in naive vs primed states of pluripotency	MAHE	Submitted	2023

IMMUNE RESPONSE TO SINGLE DOSE OF HPV VACCINE AFTER 10-YEARS OF VACCINATION

Devasena Anantharaman, PhD
Scientist F & Wellcome Trust-India Alliance Intermediate Fellow
Cancer Research Program

BRIEF THEME OF LABORATORY

Our lab investigates the contribution of HPV infections in several cancers with the aim of understanding cancer aetiology. Additional thrust areas include head and neck cancers where we focus on biomarker discovery for oral cancer risk prediction. These are achieved through epidemiologic studies integrated with high-throughput lab data (e.g. multiplex HPV testing methods and whole-exome sequencing).

LABORATORY STRENGTH

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

The present study aimed to determine whether recipients of a single-dose of quadrivalent HPV vaccine had sustained immune response against targeted HPV types (HPV 6, 11, 16, 18) at 10 years post-vaccination and whether this response was superior to the natural antibody titres observed in unvaccinated women. Blood samples were tested for type-specific antibodies using the Multiplex VLP-based IgG ELISA (M9ELISA) and type-specific antibodies specific to neutralizing epitopes using the high throughput pseudovirion-based neutralization assay in women who received single dose (n=324), two doses, (n=190) three doses (n=167) or were unvaccinated (n=350). In the M9ELISA assay, the amount of binding antibody present in the test serum relative to the standard/reference serum was calculated using the parallel line model (PLL). The binding and neutralization antibody titres were reported in International Units/ml (IU/ml) for HPV 16 and 18, and in arbitrary units/ml (AU/ml) for HPV 6 and 11. Geometric mean titre (GMT) of binding antibody against HPV 16 among the single dose recipients at 12-month post-vaccination was 9.72 IU/ml (95%CI 8.30 to 11.37). At 120 months the GMT was almost unchanged (9.90 IU/ml; 95%CI 8.76 to 11.19) with 96.0% of the single dose recipients having detectable antibody against HPV 16. The HPV 16 antibody titre in this group was 2.05 times (95%CI 1.34 to 3.16) higher compared to that observed in the unvaccinated participants, but significantly inferior compared to both two-dose and three-dose groups. The ratio of GMT in the single dose compared to three dose recipients was 0.28 (95%CI 0.24 to 0.33). The dynamics of binding antibody responses observed against HPV 18, HPV 6 and HPV 11 in the single-dose recipients over the 120 months closely mimicked the pattern observed for antibody against HPV 16. The neutralizing GMT against HPV 16 following a single dose administration at 18 months was 558 (95%CI 416 to 750) (Table 3). The same at 120 months was 819 (95%CI 701 to 957) with 97.8% of the participants having detectable neutralizing antibody. These finding demonstrate that although HPV type-specific (binding or neutralizing) antibody titres after a single-dose were significantly inferior to those after three doses of the quadrivalent HPV vaccine, they were all significantly higher than those observed in unvaccinated women following natural infections.

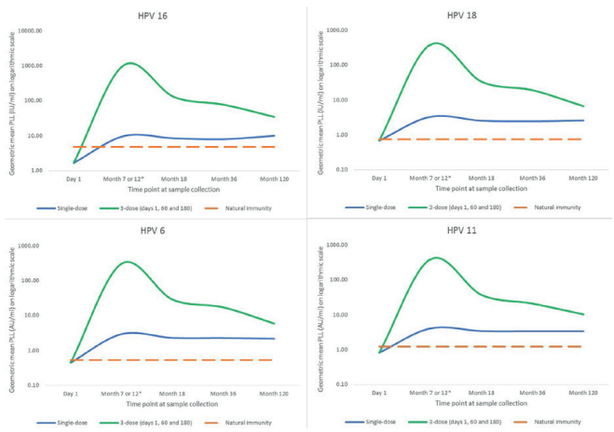


Figure represents the evolution of immune responses against HPV16, HPV18, HPV6 and HPV11 using M9ELISA assay across dose groups.

Front row (from left): Manju V, Kalyani Renjith, Kannan T R, Jinu Austin
Central row (from left): Anandi Shivaram, Sinumol George, Devika V A
Back row (from left): Lekshmy S R, Subha S

AWARDS [STUDENTS]

- Sinumol George: Best Poster Award, Characterization of the Cervicovaginal Microbiome: Data from the Indian Multicentre Study, International Conference on Antimicrobial Resistance & Microbiome under changing Climate (AMRMIC 2022), Pondicherry University, India (10-12th October 2022).

INVITED TALKS [PI]

- Oral, cancer prevention: Where the evidence lies for reduction of smokeless tobacco use and reversal of oral precancer, 19th Annual National KSOMP conference, Indira Gandhi Institute of Dental Sciences, Kochi, 3-5 March 2023.

CONFERENCE PRESENTATION

- Sinumol George: Poster presentation, Characterization of the Cervicovaginal Microbiome: Data from the Indian Multicentre Study, International Conference on Antimicrobial Resistance & Microbiome under changing Climate (AMRMIC 2022).

- Sinumol George: Poster presentation, Identification of core microbial signature in disease-free Indian women, 42nd Annual conference of the Indian Association of Cancer Research, Bringing Basic and Translational Research to the Clinic: Challenges and Opportunities, ACTREC-TMC, Navi Mumbai 12-16 January, 2023.

PUBLICATIONS

- 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 Jan 4;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 Jan;136:106244.

● Bouvard V, Nethan ST, Singh D, Warnakulasuriya S, Mehrotra R, Chaturvedi AK, Chen TH, Ayo-Yusuf OA, Gupta PC, Kerr AR, Tilakaratne WM, Anantharaman D, Conway DI, Gillenwater A, Johnson NW, Kowalski LP, Leon ME, Mandrik O, Nagao T, Prasad VM, Ramadas K, Roitberg F, Saintigny P, Sankaranarayanan R, Santos-Silva AR, Sinha DN, Vatanasapt P, Zain RB, Lauby-Secretan B. IARC Perspective on Oral Cancer Prevention. N Engl J Med. 2022 Nov 24;387(21):1999-2005.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Human papillomavirus (HPV)-related oropharyngeal cancer burden and the natural history of oral HPV infections: an Indian perspective	DBT/ Wellcome Trust	2019	5 years	PI
2.	Biomarkers of oral cancer risk prediction	DBT Glue Grant	2018	5 years	PI
3.	HPV genotyping for efficacy testing of generic qHPV vaccine development: Serum Institute of India study Serum Institute of India	Serum Institute of India	2019	3 years	PI
4.	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	IARC-WHO	2020	5 years	PI

MOLECULAR CHARACTERIZATION OF ZINGIBER-PYTHIUM PATHOSYSTEMS AND THE IDENTIFICATION OF HOST SUSCEPTIBILITY TARGETS

George Thomas, PhD
Scientist G
Plant Biotechnology & Disease Biology Program

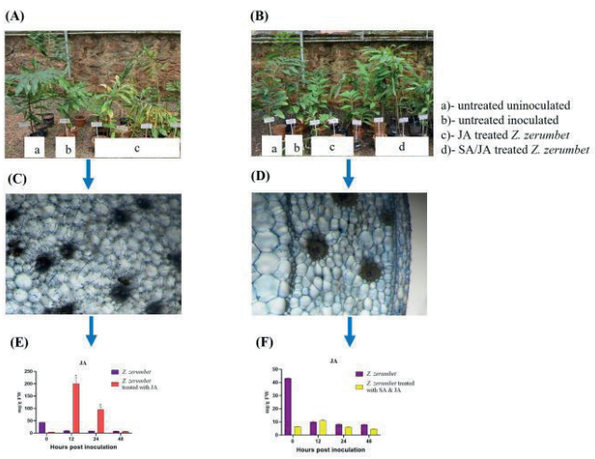
BRIEF THEME OF LABORATORY

We focus on molecular characterization of Zingiber-Pythium pathosystems. The pathogenicity factors produced by a pathogen decide the nature of host response to an invading pathogen. In compatible pathosystems, the pathogenicity factors secreted by the pathogen target certain genes in the host (susceptibility genes) and exploit host cellular machinery for its colonization. Our interest is to identify and characterize susceptibility genes in spice crop ginger targeted by Pythium myriotylum during soft rot disease development.

LABORATORY STRENGTH

Laboratory Strength: | Technical Assistant: 1
PhD Students: 2 | Post Doctoral Fellows: 1

The spice crop ginger (Zingiber officinale) is severely affected by the infection of soft rot disease-causing soil-borne necrotrophic oomycete pathogen Pythium myriotylum. The obligate asexuality and uniform susceptibility of all ginger cultivars to soft rot disease deter the application of conventional crop improvement techniques in ginger. Further, the pathogen stays long in the soil and the synthetic chemical fungicides and cultural practices are ineffective in controlling the pathogen. Earlier studies from the laboratory have identified P. myriotylum resistance in certain accessions of Z. zerumbet, a wild congener of ginger. The recent advances in gene editing methods have triggered an upsurge of interest among crop scientists to identify the susceptibility genes in the host and to evaluate the disease suppression efficiency of such genes by using gene silencing experiments. We explored the phytohormone-mediated defense against Pythium in the resistant Z. zerumbet. The external application of jasmonic acid (JA) evoked the susceptibility characteristics in the resistant Z. zerumbet. An additional treatment of salicylic acid (SA) before the JA treatment in Z. zerumbet reverted the susceptibility characters and rendered them resistant to Pythium. The pathogen completely colonised the aerial stem at 36hpi in JA treated Z. zerumbet whereas it was contained in the initial leaf whorl in the SA/JA treated Z. zerumbet. HPLC analysis revealed a tremendous increase in the JA content in JA-treated Z. zerumbet whereas in SA/JA-treated Z. zerumbet, the JA content was similar to the control. Downstream analysis of key defense signaling genes in the JA and SA/JA-treated Z. zerumbet showed a significant increase in the expression of JA biosynthetic and marker genes, a decrease in the phenylpropanoid genes and an increase in the cell death marker genes in JA-treated Z. zerumbet. Certain JA signaling genes were chosen from the transcriptome data and their expression signature was determined using RT-qPCR. Virus-induced gene silencing of some of these genes rendered tolerance against P. myriotylum to ginger.



Effect of jasmonic acid (JA) treatment in host defense of Zingiber zerumbet against the soft rot causing necrotrophic pathogen Pythium myriotylum. A-JA treated Z. zerumbet; B-SA/JA treated Z. zerumbet; C- Pythium colonization in aerial stem of JA treated Z. zerumbet at 36hpi; D-Pythium inhibited in the outer leaf whorl of SA/JA treated Z. zerumbet at 36hpi; E & F-JA content of JA treated Z. zerumbet and SA/JA treated Z. zerumbet respectively

From left: Vinitha M R, Lini Varghese

AWARDS [STUDENTS]

- Lesly Augustine: Best oral presentation, (Prof. M. Sabu award) to on the paper 'Contrasting pattern in the activation of phenylpropanoid biosynthesis and phytohormone cross talk contribute to the nature of host signaling in Zingiber-Pythium pathosystems', presented at International Seminar on Gingers, March 1-3, 2023, Malabar Botanical Garden and Institute of Plant Sciences, Calicut, Kerala. India.

- Lini Varghese: Best Oral Presentation award on the paper Evaluation and molecular characterization of salicylic acid induced priming efficacy in Zingiber officinale against soft rot pathogen Pythium myriotylum, presented at the International webinar on Biotechnology recent advances, challenges and opportunities (ICB-2022), December 14-16, 2022 Department of biotechnology, University of Kerala, Thiruvananthapuram, Kerala, India.

CONFERENCE PRESENTATION

- Gayathri R Satheesh: Presentation on Transcriptome resources for gene discovery: Dual RNA-seq of resistant and susceptible Zingiber spp. infected with soft rot pathogen Pythium myriotylum, presented at the International Conference of Plant Genetic Engineering and Genome Editing, October 27-29, 2022 Central University of Kerala, Kasaragod, Kerala, India.

- Lini Varghese: Presentation on Swing of phytohormone concentration equilibrium towards jasmonic acid produce susceptibility in Zingiber spp. to the necrotrophic oomycete Pythium myriotylum Drechsler, presented at International Conference on Current Trends and Future Prospects of Plant Biology, February 23-25, 2023, Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Telangana, India.

- Lini Varghese: Presentation on Elicitor-based defense priming against soft rot disease in ginger (Zingiber officinale Roscoe) caused by Pythium myriotylum Drechsler, presented at International Seminar on Gingers, March 1-3, 2023, Malabar Botanical Garden and Institute of Plant Sciences, Calicut, Kerala, India.

- Lesly Augustine: Presentation on Contrasting pattern in the activation of phenylpropanoid biosynthesis and phytohormone cross talk contribute to the nature of host

signaling in Zingiber-Pythium pathosystems. presented at International Seminar on Gingers, March 1-3, 2023, Malabar Botanical Garden and Institute of Plant Sciences, Calicut, Kerala, India.

- Smini Varghese: Presentation on Heterozygosity profiles and pathogen responsiveness in Zingiber species with contrasting breeding system. presented at International Seminar on Gingers, March 1-3, 2023, Malabar Botanical Garden and Institute of Plant Sciences, Calicut, Kerala, India.

- Geethu Elizabath Thomas: Presentation on Breeding behaviour, genetic diversity, and ecological correlations in Zingiber species, presented at International Seminar on Gingers, March 1-3, 2023, Malabar Botanical Garden and Institute of Plant Sciences, Calicut, Kerala, India.

PUBLICATIONS

- Varghese L, Thomas, G. Chitosan triggers tolerance to Pythium myriotylum infection in ginger (Zingiber officinale) by modulating multiple defense signaling pathways. Physiol. Mol. Plant Pathol; 125 (2023) 101983.

- Varghese L, Thomas G. Evaluation and molecular characterization of salicylic acid induced priming efficacy in Zingiber officinale against soft rot pathogen Pythium myriotylum. In A. Gangaprasad, A. Jayakumaran Nair, K. Shivakumar, Renu. M. Mohan, R. Supriya, Bindhumol Ismail, O. Venna, Neethu Hari, Geethu Chellapan, Mini. M. George (Eds.), Biotechnology recent advances, challenges and opportunities, Department of biotechnology, University of Kerala, Thiruvananthapuram, India, 2022, pp. 136- 162.

A MECHANISTIC EVALUATION OF CK-01 IN COLITIS ASSOCIATED COLORECTAL CANCER

CK-01 is an Ayurvedic formulation prepared as Sahasrayoga. The objective of the study to evaluate its effect in colitis and colitis associated cancer. The individual herbal components were authenticated at DNA fingerprinting facility of RGCB and drug was manufactured in a GMP certified facility as one batch. The Dextran sodium sulfate (DSS) induced colitis was done in mice model and drug was administered in two doses (10.8 and 16.2 ml/Kg. B wt) and treatment was started on the same day of DSS administration. The colitis associated colorectal cancer (CAC) was induced using AOM-DSS treatment. In DSS model, in the control group there is decrease in the body weight. A colonoscopy analysis was performed to analyse the severity of colitis and action of the drug (F1A). After sacrifice the colon length was analyzed. As compared to the untreated group there is a significant drop in colon length in vehicle treated group both the doses of CK-01 prevented the loss of colon length. In CAC model, we also calculated the Murine Endoscopic Index of Colitis Severity scores based on colonoscopy imaging and noticed that the scores were decreased in drug treated groups (F1B). Mice were sacrificed at day 80 and gross necropsy was performed. We observed that the large tumors were mostly present in the vehicle controls and numbers were significantly reduced in both treatment groups (F1C). The treatment groups showed very small tumors indicating that CK-01 treatment in both modalities were able to inhibit the tumor formation. We analyzed the expression of MDSCs in

Harikumar K.B, PhD
Scientist E-II
Cancer Research Program

BRIEF THEME OF LABORATORY

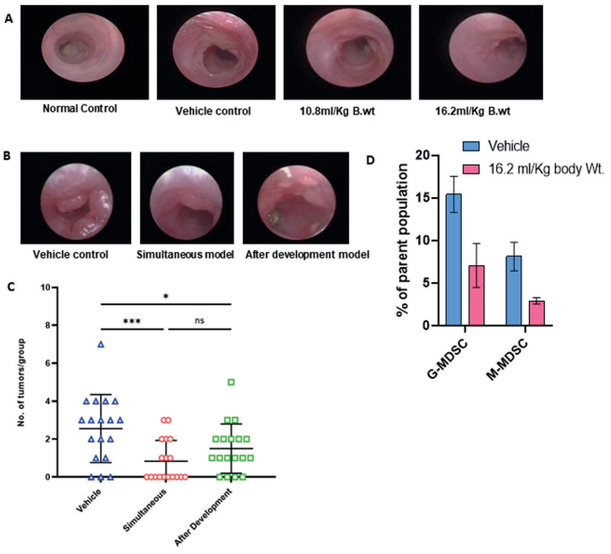
The laboratory's main focus is understanding the role of inflammation in physiology (innate immune response) and pathophysiology (cancer). We are particularly interested in the roles of Sphingosine 1-phosphate (S1P) in inflammation and carcinogenesis.

LABORATORY STRENGTH

PhD Students: 3 | JRF:1 | SRF:1 | Project Assistant:2
Lab Assistant:1 | Project Associate: 1

Front row (from left): Prameela Kumari T K, Parvathy G
Back row (from left): Aparna J S, Nashat Akhtar, Arun V, Rajeev J Thampi, Shirly James

colon and found that drug reduced the % of MDSCs in colon (F1D). To explore whether the CK-01 as modulated the gut microbiome, 16S rRNA sequencing was performed to identify gut microbiota profiles. To investigate bacterial diversity, we analysed alpha diversity and beta diversity of the samples. The principal component analysis with Bray-Curtis index showed significant differences among the groups. In conclusion, CK-01 is able to inhibit colonic inflammation and colorectal cancer in mice models through modulation of MDSCs and gut microbiome composition.



A-B: Colonoscopy images from colitis (A) and colitis associated cancer (B) models. C: Tumor number and tumor size of mice in different group. Data is expressed as mean ± SD. D: Circulating MDSCs in colon and data is expressed as mean ± SD

AWARDS [PI]

- RGCB Best Teacher Award in 2022.

CONFERENCE PRESENTATION

- Anu B: poster presentation, Cardamonin impedes colonic neoplasia through regulation of microRNA expression at the 5th international conference on nutraceuticals and chronic diseases (INCD 2022), October 7-9 2022 held at Delhi University , New Delhi.

PUBLICATIONS

- Suresh D, Srinivas AN, Prashant A, Harikumar KB, Kumar DP. Therapeutic options in hepatocellular carcinoma: a comprehensive review. Clin Exp Med. 2023 (Epub ahead of print).

- Beena TB, Jesil MA, Harikumar KB. Cross-talk between AMP-activated protein kinase and the sonic hedgehog pathway in the high-fat diet triggered colorectal cancer. Arch Biochem Biophys. 2023 ;735:109500.

- Baby K, Maity S, Mehta CH, Nayak UY, Shenoy GG, Pai KSR, Harikumar KB, Nayak Y. Computational drug repurposing of Akt-1 allosteric inhibitors for non-small cell lung cancer. Sci Rep. 2023 ;13(1):7947.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Understanding the role of sphingosine kinase isoforms in systemic lupus erythematosus (SLE)	Council of Scientific & Industrial Research	2018	5 years	PI
2.	DOT1L regulate the metabolic and epigenetic alterations in pancreatic cancer	Science and Engineering Research Board	2021	3 years	PI
3.	A lipid perspective on immune evasion mechanisms in pancreatic cancer metastasis	Indian Council of Medical Research	2023	3 years	PI
4.	Sensitizing of immune unresponsive colorectal cancers to checkpoint inhibitors through silencing of Acid Ceramidase expression	Science and Engineering Research Board	Approved	3 years	PI

Jackson James, PhD
Scientist G
Regenerative Biology Program

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.

LABORATORY STRENGTH

JRF: 1 | Technical Assistant: 1 | PhD Students: 6
Lab Assistant: 1

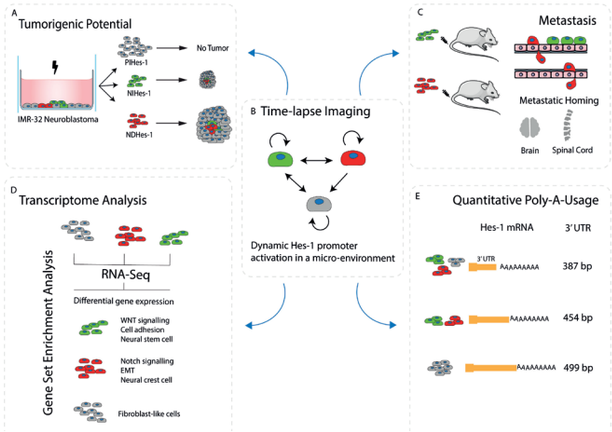
Front Row From Left: Akhila G N, Sreedevi L R, Sura Suresh, Riya Ann Paul, Aryasree R
Back Row From Left: Jyothi P Nair, Rahul Jose, Biju S Nair

HES1 PROMOTER ACTIVATION DYNAMICS REVEAL THE PLASTICITY, STEMNESS AND HETEROGENEITY IN NEUROBLASTOMA CANCER STEM CELLS

Notch signaling and its downstream target, HES1, play a critical role in regulating and maintaining cancer stem cells (CSCs), similar to embryonic development. We found a unique subclass of Notch Independent Hes-1 (NIHes-1) expressing Cancer Stem Cells (CSCs) in neuroblastoma along with Notch Dependent Hes-1 expressing (NDHes-1) CSCs. These CSCs maintain sustained HES1 expression by activation of HES1 promoter region upstream of classical CBF-1 binding sites thereby, completely bypassing Notch receptor activation. These stem cells have self-renewal ability and potential to restore entire tumor heterogeneity. Interestingly, we observed that NIHes-1 CSCs could transit to NDHes-1 expressing CSCs and vice versa and during this coordinated bidirectional transition, both CSCs gave rise to the majority of the bulk cancer cells which had HES1 promoter inactive (PIHes-1). A few of these PIHes-1 cells were capable of reverting to a CSC state. The varying modes of HES1 promoter activation and interplay between these modes of activation contribute significantly to cancer stem cell heterogeneity and plasticity (Riya et. al., J Cell Sci, 2022; 135)

Further, we found differential HES1 expression patterns between NI/NDHes-1 CSCs. However, a sustained low HES1 expression in NDPIHes-1 cells has intrigued us to examine the HES1 transcript stability regulated by its 3′ UTR region. Previous reports supported that longer UTR may enhance expression and transcript stability. Thus, the longer 3′ UTR isoform found in the PIHes-1 cells could contribute to its stability. Currently, we do not know the functional implication of differential 3′ UTR usage amongst these three cell types. To our knowledge, the first time, we identified alternate 3′ UTR usage of HES1 transcript in cancer stem cells. We assume that there could be a differential expression of alternative splice variants of many transcripts amongst these cell types that may define its characteristics and role in the tumor microenvironment.

In summary, there are two different subpopulations of cells within the cancer stem cells pool. In addition, the fibroblasts like PIHes-1 cells were also capable of reverting to a CSC state, thereby demonstrating robust plasticity between all the cell types within the neuroblastoma. This scenario also points towards the importance of eliminating the fibroblast-like cells in addition to NIHes-1 and NDHes-1 CSCs to reduce the chance of relapse and metastasis. Most importantly, this model can further be utilized for drug screening purposes, designing therapeutic targets and extrapolating to other cancer scenarios. It could also explain the frequent relapse generally observed with neuroblastoma and other neural-origin cancers and highlight the underlying challenges in designing therapeutic targets.



Regulation of stemness by pleiotropic HES1 expression in neuroblastoma. (A) IMR32 cell line has NIHes-1 expressing and NDHes-1 expressing cancer stem cells (CSCs) along with bulk cancer cells (PIHes-1). Both CSCs can generate tumor while PIHes-1 cells do not possess tumorigenic potential. (B) NIHes-1 CSCs transit to NDHes-1 expressing CSCs and vice versa and both CSCs could generate PIHes-1 cells that can revert back to CSC state. (C) NDHes-1 expressing CSCs possess higher metastatic potential than NIHes-1 CSCs. (D) Transcriptomic analysis of the three populations revealed neural stem cell signatures in NIHes-1 CSC with Wnt and cell adhesion related gene set enrichments. NDHes-1 had neural crest cell signatures, Notch signaling and EMT related gene set enrichments whereas, PIHes-1 cells fibroblast-like signatures. (E) PIHes-1 cells have a sustained low HES1 expression due to longer Hes-1 3'-UTR which provides higher mRNA stability. NIHes-1 and NDHes-1 CSCs have Hes1 mRNA with shorter 3'-UTR.

AWARDS [STUDENTS]

- Parvathy Surendran: Best oral paper presentation, Tlx3 is a crucial determinant for early cerebellar patterning, 34th Kerala science congress, February 10th -12th 2022 Organized by Kerala State Council for Science, Technology and Environment (Virtual Mode).

PUBLICATIONS

- Riya PA, Basu B, Surya S, Parvathy S, Lalitha S, Jyothi NP, Meera V, Jaikumar VS, Sunitha P, Shahina A, Sukumaran R, Nair AS, Dhanesh SB, Jiffy J, Nelson-Sati S, Maliekal TT, Das AV, James J. HES1 promoter activation dynamics reveal the plasticity, stemness and heterogeneity in neuroblastoma cancer stem cells. J Cell Sci. 2022;135(22):jcs260157.

INVITED TALKS [PI]

- A unique stem cell niche in SVZ maintains neural stem cells during early neurogenesis and in the adult corte, XL Annual meeting of Indian Academy of Neuroscience (IAN-2022), NEHU, Shillong, 7-10th December, 2022.

CONFERENCE PRESENTATION

- Meera Vadakkath: Virtual mode, Implications of differential HES-1 expression in adult sub-ventricular zone (SVZ) neurogenesis, 34th Kerala science congress, February 10th -12th 2022 Organized by Kerala State Council for Science, Technology and Environment .

- Budhaditya Basu: Presentation, Identification of the translating circular RNA molecule in retinal ganglion cells using viral TRAP, Neuroscience 2022 organized by Society for Neuroscience, November 12-16, 2022, San Diego Convention Center, San Diego, California, USA.

- Meera Vadakkath: Presentation, Derailed neurogenesis brings about deficits in olfactory fine tuning, Brain Conference organized by Federation of European Neuroscience Societies (FENS), 23-26 April 2023, Rungstedgaard, North Copenhagen, Denmark.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Functional relevance of a unique subclass of Notch independent Hes-1 (NIHes-1) expressing neural stem cells in developing/adult cortex	Science and Engineering Research Board	2022	3 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Riya Ann Paul	Differential Hes-1 promoter activation in regulating stemness in neocortical development and neuroblastoma	University of Kerala	Submitted	2023
2.	Parvathy Surendran	Functional Characterization of Tlx3 in Cerebellum	University of Kerala	Submitted	2023

John Bernet Johnson, PhD
Scientist E-I
Pathogen Biology Program

BRIEF THEME OF LABORATORY

The host's success in thwarting a pathogen or being usurped is determined early on in the infection. Employing Rhabdoviruses (vesicular stomatitis virus and Chandipura virus) and Alphaviruses (chikungunya virus), our laboratory primarily focuses on understanding the complex interaction of these viruses with the complement system a potent front line immune defense. This is pivotal for our larger goal of modifying these viruses into novel oncolytic and vaccine vectors that are both potent and safe.

LABORATORY STRENGTH

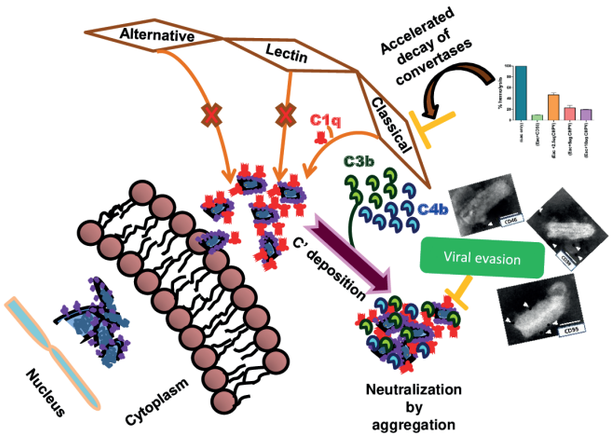
PhD Students: 4 | Lab Assistant: 1

Front row (from left): Sivaja M, Aranya Anunaya, Umerali K
Back row (from left): Devika S R, Reshma Balan, Karthika Rajeevan

INSIGHTS INTO CHANDIPURA VIRUS INTERACTION AND MODULATION OF THE HUMAN COMPLEMENT SYSTEM

The focus of the research activities in my laboratory is multifaceted, primarily involving RNA viruses and more specifically the members of the Rhabdoviridae and Togaviridae family. The goal is to understand host-pathogen interaction, using representative members like chikungunya virus (Togaviridae) and Chandipura virus (Rhabdoviridae). During the reporting period, extensive studies were carried out on the interaction of Chandipura virus (CHPV) with a front line immune defense barrier, the complement system. Chandipura virus, a potent human pathogen that has been predominantly reported in the Indian sub-continent, has been established to cause pediatric encephalitis resulting mostly in death. It has a characteristic bullet shaped morphology and is closely related to the prototypic Rhabdovirus, vesicular stomatitis virus (VSV). CHPV-complement interaction was never investigated prior to our studies, which revealed that the virus could activate the classical pathway of complement independent of antibodies. 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. Although the complement system is potent enough to neutralize viruses, the viruses have developed ingenious strategies to thwart complement.

Chandipura virus is a potent pathogen even though it is sensitive to human complement. Thus it was imperative to identify if CHPV also has mechanisms to evade the neutralizing effects of complement. Viruses employ a wide array of strategies including, utilization of soluble or membrane bound host RCAs, encode viral proteins that mimic RCAs, exploit RCAs as entry tools or decoys etc. We hypothesized that CHPV being an enveloped virus will incorporate the host membrane associated complement regulatory proteins (RCA). CHPV was cultured in 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 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. This was further confirmed by transmission electron microscopy and other biochemical assays. Functional assays specific for CD46 and CD55 revealed that the virion-associated RCAs were biologically active. While CHPV-CD46 could mediate the inactivation of C3b into iC3b which was dependent on human factor I, CHPV-CD55 could potentiate the rapid dissociation of active complement C3-convertases. Virus neutralization assays revealed that CHPV cultured in cells that lacked the RCAs were highly sensitive to complement while those grown in cells containing the regulators showed remarkable resistance. Our observations thus highlight the significance of host RCAs incorporated by CHPV during egress in limiting the neutralizing effects of complement. These findings gain significance as we embark upon exploiting CHPV as an oncolytic or vaccine vector and thus the choice of the cell line will be key in developing the vector of our choice.



Chandipura virus activates the classical pathway of human complement in a C1q dependent manner resulting in virus neutralization. The role of the alternative and the lectin pathways is negligible. Activation results in deposition of complement components like C3b and C4b resulting in virus aggregation. Although Chandipura virus is sensitive to human complement it also has adopted smart strategies to overcome complement-dependent neutralization, which includes hijacking of the host regulators of complement activation including CD46, CD59 and CD55. (Inset) Electron micrographs of CHPV showing the incorporation of CD59, CD55 and CD46. Scale bar equals 50 nm. Inset graph demonstrates the function of CHPV-CD55 in the accelerated decay of complement C3 convertase.

CONFERENCE PRESENTATION

- Reshma M. Balan: Poster presentation, "Resistance of chikungunya virus to human complement is cell-line independent." EMBO Lecture Course "Complement in kidney diseases", 31st January – 03 February 2023, National Centre for Cell Science, Pune, India.

- Umerali K: Poster presentation, "Complement evasion by Chandipura virus is RCA-dependent". EMBO Lecture Course "Complement in kidney diseases", 31st January – 03 February 2023, National Centre for Cell Science, Pune, India.

AWARDS [PI]

- Best Teacher Award (RGCB)

INVITED TALKS [PI]

- Oncolytic virotherapy – A next-generation tool to target cancer; Recent Trends in Omics, Regenerative and Precision Medicine: Interface between Infectious and Non-Infectious Diseases (6th Edition); July 22nd - July 24th, 2022; Dept. of Zoology, University of Kerala, Advanced Centre for Regenerative Medicine and Stem Cell Research in Cutaneous Biology (AcREM-Stem), Society for Advancements in Regenerative and Precision Medicine, Thiruvananthapuram, Kerala, India.

- An integrative approach to combat cancer: The oncolytic virotherapy approach; National Conference on Integrative Biology 2022; August 31st, 2022; iCEIB, University of Kerala, Thiruvananthapuram, Kerala.

- Targeting the pandemic of the century – An insight into novel approaches; National Conference on Post-COVID-19 complications – A biotechnological approach; September 7-8, 2022 Jamal Mohamed College, Tiruchirappalli, Tamilnadu, India.

PUBLICATIONS

- Vikraman D, Satheesan R, Rajendran M, Kumar NA, Johnson JB, R SK, Mahendran KR. Selective Translocation of Cyclic Sugars through Dynamic Bacterial Transporter. ACS Sens. 2022;7(6):1766-1776.

- Nag J., Kumar N.A., Mukesh R.K., Kunnakkadan U., Johnson J.B. Virome: Sentinels or Marauders in the Microbiome. In: Thomas S. (eds) Human Microbiome. Springer, Singapore. (2022).

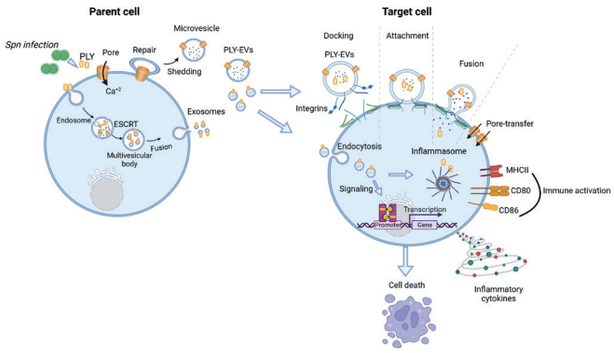
ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Understanding measles vaccine failure (and success) in Southern India.	NIH-USA	2017	More than 5 years	CO-PI

Karthik Subramanian, PhD
Scientist C & DBT-Ramalingaswami Faculty Fellow
Pathogen Biology Program

BACTERIAL PORE-FORMING TOXIN PNEUMOLYSIN DRIVES SYSTEMIC PATHOGENICITY THROIUGH SHEDDED TOXIN LOADED HOST EXTRACELLULAR VESICLES

Streptococcus pneumoniae is a global priority respiratory pathogen that kills over a million people annually and produces the pore-forming cytotoxin, pneumolysin (PLY). Host cells remove membrane assembled pore-forming toxins by shedding microvesicles, but the composition and consequences of the toxin-induced host extracellular vesicles (EVs) are unknown. Here, we found that EVs shed from PLY-challenged monocytes (PLY-EVs) harbor membrane-bound toxin that induced cytotoxicity upon fusion with recipient cells. We showed that membrane assembled PLY pores are expelled through EVs and the toxin pores are subsequently transferred to dendritic cells (DCs) upon EV fusion. Upon internalization, PLY-EVs induced DC maturation. EVs from monocytes challenged with recombinant PLY, as well as PLY-expressing pneumococcal strains evoked higher pro-inflammatory cytokines upon infection, compared to naïve EVs. Proteomic analysis of the PLY-EV cargo revealed an enrichment of key antimicrobial and inflammatory proteins such as IFI16, NLRC4, PTX3 and MMP9. In vivo, zebrafish administered with PLY-EVs showed mortality, pericardial edema and inflammation. Adoptive transfer of EVs from PLY-challenged mice into healthy recipient mice induced severe inflammation and toxicity. Our findings represent a paradigm shift from the current understanding that pore-forming toxins are completely neutralized through EV shedding and have major implications in pneumococcal disease pathogenesis.



BRIEF THEME OF LABORATORY

The global respiratory bacteria, Streptococcus pneumoniae causes above 1 million deaths annually due to life-threatening diseases such as pneumonia, septicemia, and meningitis worldwide and has been classified as a priority pathogen by the WHO. My laboratory focuses on studying the molecular pathogenesis of pneumococcal infections and mechanisms underlying pneumococcal interaction with the host immune cells and immunoevasion. Our pathogen of interest is S. pneumoniae and we use primary human cells and in vivo mouse models.

LABORATORY STRENGTH

JRF:1 | Project Assistant:1

Left to right: Shaheena Aziz, Chinmayi V Bhat, Aswathi CS

Figure. Pneumolysin challenged cells shed toxin-bearing EVs that induce systemic toxicity and inflammation. During infection, S. pneumoniae secretes the pore-forming toxin, PLY that oligomerizes and assembles to form pores on the plasma membrane of eukaryotic host cells. At sublytic toxin doses, Ca2+-dependent intrinsic cell membrane repair mechanisms 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. Together, these toxin-bearing extracellular vesicles (EVs) bind to target cells through docking receptors such as integrins. Upon fusion, the EV membrane gets integrated into the recipient cells, thereby transferring the PLY-pores and inducing cytotoxicity. The PLY-EVs can also be endocytosed, releasing the PLY into the cytosol, which activates immune receptors and inflammatory signalling pathways resulting in pro-inflammatory cytokines.

AWARDS [PI]

- Invited member of the Indian Society for Extracellular Vesicles.
- Associate Editor, Frontiers in Cellular and Infection Microbiology.
- Review Editor for Parasite and Host- Frontiers in Cellular and Infection Microbiology.
- Reviewer for SERB core research grants.
- Invited Reviewer for Nature Communications Biology and NPJ Vaccines.

INVITED TALKS [PI]

- Invited speaker at National Conference on Current Advances in Life Sciences, SRM University India.

PUBLICATIONS

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

PATENTS APPLIED/ GRANTED

- Patent filed- 1 PCT.
- MANNOSE RECEPTOR-DERIVED PEPTIDES FOR NEUTRALIZING PORE-FORMING TOXINS FOR THERAPEUTIC USES.
- United States application or PCT international application number 18/013,679 filed on December 29, 2022.
- Inventors: Birgitta Henriques Normark, Karthik Subramanian, Georgios Sotiriou.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	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
2.	Disarming bacterial pathogens using novel peptides that target pore-forming toxins: from in silico to in vivo	DST-INSPIRE	2020	5 years	PI
3.	Unravelling bacterial immunoevasion and host immune reprogramming strategies in invasive pneumococcal diseases.	Science and Engineering Research Board	2021	2 years	PI

Karthika Rajeeve , PhD
Scientist E-I
Pathogen Biology Program

BRIEF THEME OF LABORATORY

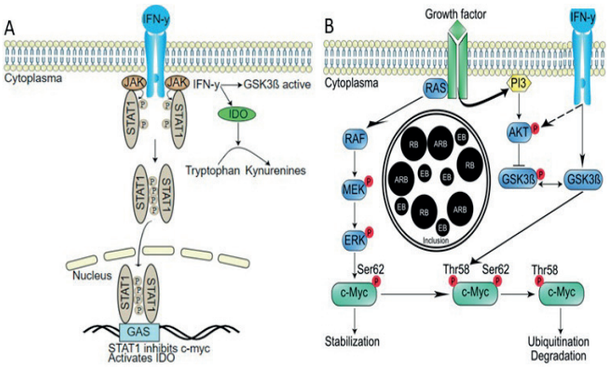
We study the pathogenesis of Chlamydia infections. As an obligate intracellular parasite, Chlamydia metabolically reprograms host cells. My group focuses on the infection-cancer interface and investigates how the pathogen evades the immune system. Our laboratory is also focused on the world's top infectious killer, Mycobacterium tuberculosis, the causative agent of tuberculosis. We investigate the fundamental mechanisms by which the bacteria evade the host immune system and create a protective niche in the human body.

LABORATORY STRENGTH

JRF: 1 | Technical Assistant: 1 | PhD Students:2

c-MYC PLAYS A KEY ROLE IN IFN-γ-INDUCED PERSISTENCE OF CHLAMYDIA TRACHOMATIS

Chlamydia trachomatis (Ctr) can persist over extended times within their host cell and thereby establish chronic infections. One of the major inducers of chlamydial persistence is interferon-gamma (IFN-γ) released by immune cells as a mechanism of immune defence. IFN-γ activates the catabolic depletion of L-tryptophan (Trp) via indoleamine-2,3-dioxygenase (IDO), resulting in persistent Ctr. Here, we show that IFN-γ induces the downregulation of c-Myc, the key regulator of host cell metabolism, in a STAT1-dependent manner. Expression of c-Myc rescued Ctr from IFN-γ-induced persistence in cell lines and human fallopian tube organoids. Trp concentrations control c-Myc levels most likely via the PI3K-GSK3β axis. Unbiased metabolic analysis revealed that Ctr infection reprograms the host cell tricarboxylic acid (TCA) cycle to support pyrimidine biosynthesis. Addition of TCA cycle intermediates or pyrimidine/purine nucleosides to infected cells rescued Ctr from IFN-γ-induced persistence. Thus, our results challenge the longstanding hypothesis of Trp depletion through IDO as the major mechanism of IFN-γ-induced metabolic immune defence and significantly extends the understanding of the role of IFN-γ as a broad modulator of host cell metabolism.



A. Cartoon depicting IFN-γ signalling. IFN-γ binds to the IFN-γ receptor which results in the phosphorylation of STAT1. pSTAT1 binds to the IFN-γ-activated sequence (GAS) sequence and thus blocks c-Myc transcription. IFN-γ can also induce indoleamine-2,3-dioxygenase (IDO) and thereby the degradation of L-tryptophan.

From left: Surya P R, Vineetha B R, Smitha R P, Paridhi Agarwal

B. Cartoon: Active phosphatidylinositol-3-kinase (PI3K) with inactive glycogen synthase kinase-3 (GSK3β) leads to c-Myc stabilization. IFN-γ binding to its receptor, activates PI3K and serine-threonine protein kinase (AKT) and induces the dephosphorylation and activation of GSK3β, leading to c-Myc depletion. Chlamydia infection activates the PI3K and MEK/ ERK pathway.

AWARDS [PI]

- Ben Barres Spotlight Award.

INVITED TALKS [PI]

- Metabolic reprogramming of host cells for the intracellular survival of Chlamydia trachomatis. International symposium on human diseases. 12. November 2022, Department of Biological Sciences, BITS Pilani Hyderabad Campus - 500078, Hyderabad, India.
- c Myc plays a key role in IFN gamma induced persistence of Chlamydia trachomatis, October 2022, IIN webinar (online).

PUBLICATIONS

- Vollmuth N, Schlicker L, Guo Y, Hovhannisyan P, Janaki-Raman S, Kurmasheva N, Schmitz W, Schulze A, Stelzner K, Rajeeve K*, Rudel T*. c-Myc plays a key role in IFN-γ-induced persistence of Chlamydia trachomatis. Elife. 2022;11:e76721. * Equal Contribution.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Unravelling the role of Chlamydial deubiquitinase in evading the host innate immune system.	Science and Engineering Research Board	2022	2 years	PI
2.	Deciphering the novel role of immune cells in the spread and dissemination of Chlamydia trachomatis	Science and Engineering Research Board	2021	3 years	PI
3.	Creation of patient derived endometrial organoids for understanding the underlying causes of RIF	Department of Biotechnology	2022	3 years	CO-PI

Kathiresan Natarajan, PhD
Scientist C
Transdisciplinary Biology Program

PORE-PERIPHERAL SALT BRIDGE IN MUSCLE ACHR: VOLTAGE AND DIVALENT SENSITIVE CONTRIBUTIONS TO OPEN CHANNEL NOISE

The mechanisms behind changes in ionic current through single acetylcholine receptor (AChR) channels have remained a mystery. In a recent research on muscle AChR, we discovered that changing a conserved intramembranous salt bridge in the beta- and delta-subunits significantly enhanced fluctuations in the open channel current that ranged from low to high frequency. Extracellular divalent cations minimize high-frequency oscillations while increasing low-frequency fluctuations. The low-frequency oscillations are shown to be caused by steps between two current levels, with the ratio of time spent at each level increasing e-fold for a 70 mV increase in membrane potential, suggesting regulation by a charged element inside the membrane field. Increasing the charge on the ion selectivity filter biases the ratio of current levels to the equivalent of a 50 mV rise in membrane potential but has no effect on the ratio's voltage dependency. Estimates of the distance between the ion selectivity filter and the voltage-sensing element may be made using the magnitudes of the voltage dependence and voltage bias. Studies using calcium or magnesium reveal that the two divalent cations work together to raise low-frequency fluctuations while acting separately to diminish high-frequency fluctuations, demonstrating the presence of numerous divalent cation binding sites. Molecular dynamics simulations of the Torpedo AChR structure show that changing the salt bridge affects the equilibrium locations and dynamics of residues around the mutation site and inside the AChR. A single ion channel activates a step increase in transmembrane ionic current, but the structural grounds for the amplitude and uniformity of the step rise remain unknown. In this research of the AChR channel from skeletal muscle, we combine knowledge of its atomic structure with observations of ionic fluctuations.

A single channel of current is used. Mutating a conserved intramembranous salt bridge changes the magnitude and uniformity of ion transport through the AChR channel, reducing the average rate of ion transport by 30% and increasing open channel noise by nearly 30-fold when compared to salt bridge intact receptors. On the ion selectivity filter, these effects are affected by voltage, divalent ions, and charge. Quantitative analyses of these findings provide a structural picture of the interaction between the salt bridge and the ion selectivity filter that contributes to the magnitude and uniformity of ion transport through the AChR channel.

BRIEF THEME OF LABORATORY

Our laboratory is interested in nicotinic acetylcholine receptors that play important role in fast signaling throughout the body. It is critical to understand the structure-function connection of nicotinic acetylcholine receptors to comprehend their function in health and disease. Numerous nicotinic receptor subtypes may be specifically targeted, and various biological entities can regulate receptor function. Our research focuses on drugs and physiological modulators that act differently on these receptors and are potentially therapeutic.

LABORATORY STRENGTH

JRF:2 | PhD Students: 2 | Post Doctoral Fellow: 2

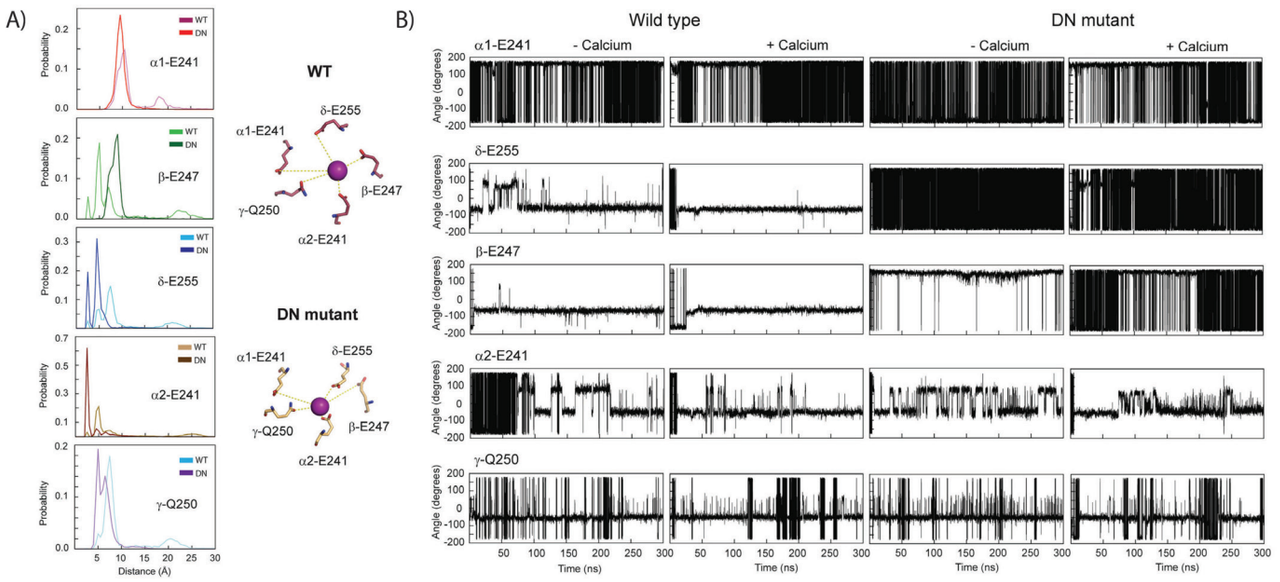
Front row (from left): Remya C P, Dr Sharanya Suresh
Second Row (from left): Thasni Fazil, Dr Wilbee D S, Anju Krishnan

AWARDS [STUDENTS]

- Sharanya C Suresh: Best Poster in GNR Centenary Symposium for Structural Biology organized by CUSAT Cochin on February 21-22, 2023.

INVITED TALKS [PI]

- Computational approaches in the fronteirs of the fight against SARS-CoV2, National level seminar on next generation therapeutics - a novel approach, Sep 23, 2022, Holy Cross College, Nagercoil, Tamil Nadu.
- Computational approaches for Drug Discovery, International Conference on Innovation in Applied Sciences, Feb 3, 2023, Department of Biochemistry, Mahalakshmi College of Arts and Science, Avadi, Chennai, Tamil Nadu.



A) Mode of calcium interaction with the -1 prime residues that form the ion selectivity filter during MD simulations. Probability distributions of the distance between calcium and the indicated carboxylate oxygen of the -1 prime residue were generated from the simulations in the presence of calcium. Average structures of the selectivity filter residues (stick representation) from each subunit and a calcium ion (purple ball) for WT and DN mutant receptors.

B) Time evolution of the side-chain dihedral angles of the glutamate or glutamine residues at the -1 prime position as a function of simulation time in the absence or presence of calcium.

PUBLICATIONS

- Francis N, Behera MR, Natarajan K, Laishram RS. Tyrosine phosphorylation controlled poly(A) polymerase I activity regulates general stress response in bacteria. Life Sci Alliance. 2022;6(3):e202101148.

- Strikwerda JR, Natarajan K, Sine SM. Impact on AChR open channel noise by pore-peripheral salt bridge depends on voltage and divalent cations. Biophysical Journal. 2023; 122:1-15.

CONFERENCE PRESENTATION

- Thasni Fazil: Poster Presentation, Impact of TUBB4B tubulin isotype mutations on microtubule dynamics, GNR Centenary Symposium for Structural Biology organized by CUSAT Cochin on February 21-22, 2023.

- Sharanya C Suresh: Poster Presentation, E-Pharmacophore based drug repurposing against SARS-CoV2 and insight into interaction with TMPRSS2, GNR Centenary Symposium for Structural Biology organized by CUSAT Cochin on February 21-22, 2023.

- Thasni Fazil: Poster Presentation, Impact of TUBB4B tubulin isotype mutations on microtubule dynamics, XV Triennial & III International Conference organized by Indian Women Scientist Association, Mumbai on 11-13 June 2023.

- Sharanya C Suresh: Poster Presentation, E-Pharmacophore based drug repurposing against SARS-CoV2 and insight into interaction with TMPRSS2, XV Triennial & III International Conference organized by Indian Women Scientist Association, Mumbai on 11-13 June 2023.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Impact of disease-associated tubulin isotype mutations on microtubule dynamics	Science and Engineering Research Board	2022	2 years	PI
2.	Alpha7 nicotinic acetylcholine receptor as a pharmacological target for treating neuronal disorders	Science and Engineering Research Board	2023	3 years	PI

Krishna Kurthkoti, PhD
DBT-Ramalingaswami Faculty Fellow
Pathogen Biology Program

BRIEF THEME OF LABORATORY

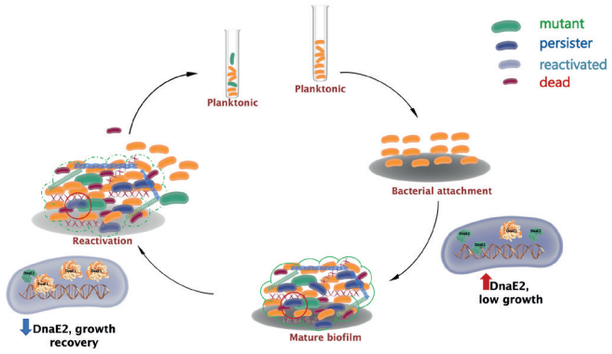
Mycobacterial persistence and evolution of antimicrobial resistance

DNAE2 EXPRESSION PROMOTES GENETIC DIVERSITY AND IMPEDES BACTERIAL GROWTH IN MYCOBACTERIAL BIOFILMS

The complex cellular architecture and the microenvironments within the biofilm give rise to a population that is both physiologically and genetically heterogeneous. Transcriptome analysis of Mycobacterium smegmatis in biofilm culture and its transition phase into planktonic growth was performed to identify the genetic basis of heterogeneity in the biofilm. Biofilms displayed redox imbalance resulting in increased levels of reactive oxygen species and activation of mycobacterial mutasome consisting of dnaE2, imuA, and imuB in the biofilm in comparison to planktonic and biofilm to planktonic transition. The whole genome sequence of the biofilm and planktonic cultures revealed a modest increase in the allelic variants in the biofilm culture compared to the planktonic culture. Deletion of dnaE2 causes lower mutation frequency and bacterial fitness compared to the parental strain in biofilm culture and reduces the allelic variation in the culture. The expression of dnaE2 contributes to a slower bacterial growth rate, potentially promoting persister formation. Our study uncovers the multiple benefits of dnaE2 expression in biofilm such as increasing genetic diversity and reducing growth rate; both of which are necessary for mycobacterial survival and adaptation.

INVITED TALKS [PI]

- Scientific talk at IBAB Bangalore, Role of DnaE2 in the emergence of antimicrobial resistance and genetic heterogeneity in Mycobacteria (December 2022).
- Scientific talk at IIT Pallakad, Mycobacterial strategies for developing antimicrobial resistance and survival in a non-replicative persistent state (March 2023).
- Scientific talk at Manipal School of Life Sciences, MAHE, Mycobacterial strategies for developing antimicrobial resistance and survival in a non-replicative persistent state (April 2023).



Mutagenesis and bacterial growth arrest by dnaE2 expression in biofilm. Biofilm formation initiates with the attachment of mycobacteria to a substratum. During maturation, an extracellular matrix encloses the heterogenous population with dead bacteria (red), growth-arrested bacteria (blue), and mutant bacteria (green) generated by reduced DNA repair and induction of error-prone polymerase DnaE2. The growth arrest within the sub-population emerges from the conflict between DnaE2 and the housekeeping DnaE1. During reactivation of biofilm to planktonic growth, expression of DnaE2 is repressed, and balance of polymerases shifts in favour of DnaE1 resulting in active multiplication.

CONFERENCE PRESENTATION

- Poster presentation at BTMO International Conference, Bangalore, Targeted protein turnover is required for mycobacterial growth and survival during iron starvation (January 2023).
- Poster presentation at the 15th Young Investigators meeting at IIT Gandhinagar, Evolution of antibiotic Poster presentation at BTMO International Conference, Bangalore, Targeted protein turnover is required for mycobacterial growth and survival during iron starvation (January 2023).
- Poster presentation at the 15th Young Investigators meeting at IIT Gandhinagar, Evolution of antibiotic resistance in mycobacterial persister cells is mediated by error-prone polymerase DnaE2 (February 2023).
- Poster presentation at the TB conference THSTI, The error-prone polymerase DnaE2 mediates the evolution of antibiotic resistance in persister mycobacterial cells (March 2023).

PUBLICATIONS

- Rao RSP, Ghate SD, Shastry RP, Kurthkoti K, Suravajhala P, Patil P, Shetty P. Prevalence and heterogeneity of antibiotic resistance genes in Orientia tsutsugamushi and other rickettsial genomes. Microb Pathog. 2023;174:105953.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Characterization of iron starvation induced dormancy in mycobacteria and its application in drug discovery	Department of Biotechnology	2017	6 years	PI

STUDY OF FUNCTIONAL PROPERTIES OF CARBON-BASED NANOMATERIALS FOR BIOMEDICAL APPLICATIONS

Advancement of nanosciences have led to the development of high throughput diagnostics and efficient therapeutics such as biosensors for point-of-care diagnostics and nanocarrier drug delivery systems respectively. Though the application of the science has been established, thorough understanding and the translational applications of such technology is still in the infant stage. One of the important areas is to study the interaction between nanomaterials and biomolecules at the interface, and deeper understanding is required that could be modulated further for robust technology.

An important components of biosensor is the biorecognition element that has direct interaction with the target analytes. Conventionally, biorecognition elements such as antibodies, enzymes, proteins, etc. are employed due to specific and selective binding sites for the target analytes. However, these biomolecules have low shelf-life, some are expensive, easily denatured, etc. that leads to the question - can we design an efficient enzyme-mimicking biomaterials? Metal based

Lightson N.G, PhD
Scientist C
Transdisciplinary Biology Program

BRIEF THEME OF LABORATORY

The lab focusses on studying the next generation biosensing techniques for biomedical and environmental relevant biomarkers employing biorecognition elements such as aptamer oligonucleotides, synthetic peptides, biomimetic materials, nanomaterials on micro/nanofluidics platforms. Our interest extends to a wide range of fusion of nanotechnology and biotechnology, understanding the phenomena in micro & nano scale, and practical applications such as developing portable and cost-effective biosensors or bioanalytical devices.

LABORATORY STRENGTH

JRF: 1 | PhD Student: 1

From left: Mariyam Razana, Aswathy Prasad, Anagha Das

nanomaterials have shown the enzyme-like catalytic effect as per the reports. However, these nanoparticles have certain limitations such as cytotoxicity, less biocompatible, challenging to prepare <20nm size, no fluorescence, etc., so one of the objectives is to focus on carbon-based nanodots for both diagnostics and therapeutics.

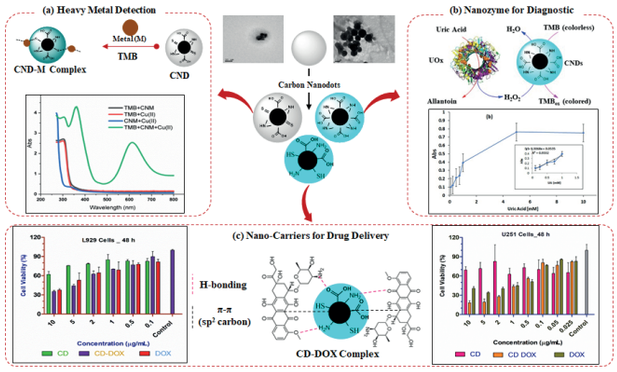
Carbon nanomaterials such as carbon dots (CDs) that mimic enzymes are also known as 'carbon nanozymes'. Initially, we design and synthesised peroxidase-mimic CDs following simple and green methods such as pyrolysis and hydrothermal methodologies. We successfully synthesised novel CDs that show high peroxidase mimicking and catalytic properties. These CDs can replace the redox enzymes such as peroxidase in developing biosensors to detect biomarkers of clinical and environmental relevant biomarkers. These novel CDs are explored and used for optical detection of important markers/biomarkers such as heavy metals (such as Fe, Cu, etc.), and uric acid/lactic acid as shown in Figure 1(a)-(b). In the study, we explored two optical properties, i.e., colorimetric and fluorescence. Another objective is to study the efficient delivery of drug molecule using CDs since carbon nanomaterials are more biocompatible, rich surface chemistry, etc. as compare to metallic nanoparticles. Currently, we study the interaction between CDs and doxorubicin (DOX), and the complex (CD-DOX) as shown in Figure 1(c). Through the optimized reaction conditions, we also studied the interaction such as cytotoxicity, biocompatibility, etc., of CD alone and CD-DOX complex with normal cell lines and cancer cell lines. Since we have found a multi-functional CDs, we are focusing on to understand the surface properties and the mechanism of interaction with different target analytes.

In the next phase, we are exploring further to mimic other important biological enzymes such as oxidase, catalase, etc. for diagnostics applications. Additionally, we are trying to understand the mechanism of the CD-drug molecule interaction before and after delivery. With this information, it's our aim to validate the interesting findings with the animal model in the near future.

CONFERENCE PRESENTATION

- Aman Grewal: Poster Presentation, Synthesis and characterisation of novel nitrogen-doped carbon nanodots as peroxidase-mimic for biosensing applications, National Conference on Biotechnology for Sustainable Development and Human Welfare, 23-24 Nov. 2022, Jamia Hamdard, New Delhi.

- Smita Das: Oral Presentation, Paper-based selective detection of methanol and formaldehyde, North-East Research Conclave, 20-22 May 2022, IIT Guwahati, Assam



Novel Carbon nanodots have been synthesized following pyrolysis and hydrothermal approach. (a) Synthesized carbon nanodots can catalyze and detect Cu(II) ions in an aqueous sample: blue color is obtained when CDs react with Cu(II) and 3,3',5,5'-Tetramethylbenzidine (TMB); (b) Peroxidase-mimic carbon dots as nanozyme: colorimetric determination of hydrogen peroxide (H2O2) reacting with chromogen TMB.

INVITED TALKS [PI]

- Selective and sensitive point-of-care testing kit for methanol and formaldehyde, 3rd Edition of Global Biotechnology Conference, 16-17 Sept. 2022, Plaza Hotel, Begumpet, Hyderabad, India.

- Carbon nanomaterials as nanozyme, Short Term Program (STP) on Bio privileged and Sustainable Chemistry, 17-21 Jan, 2022, Manav Rachna University, Faridabad India.

PATENTS APPLIED/ GRANTED

- Patent granted on 19th May 2022-
  Lightson Ngashangva, Pranab Goswami, 'Paper based portable kit for onsite determination of formaldehyde in aqueous sample', Patent No.: 397154.

PUBLICATIONS

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

- Ngashangva L, Hemdan BA, El-Liethy MA, Bachu V, Minteer SD, Goswami P. Emerging Bioanalytical Devices and Platforms for Rapid Detection of Pathogens in Environmental Samples. Micromachines (Basel). 2022;13(7):1083.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Studies of non-enzymatic paper-based bioanalytical devices for point-of-care diagnostic applications	Science and Engineering Research Board	2022	3 years	PI
1.	Paper-based Kit for Methanol and Formaldehyde Detection	BIRAC	2021	2 years	PI

Mahendran K.R, PhD
Scientist E-I
Transdisciplinary Biology Program

BRIEF THEME OF LABORATORY

Our laboratory uses the techniques of protein biochemistry, molecular biophysics and cell biology. Our work is centered on membrane proteins, in particular channels and pores. We investigate both the fundamental properties of these proteins and their applications in biotechnology.

LABORATORY STRENGTH

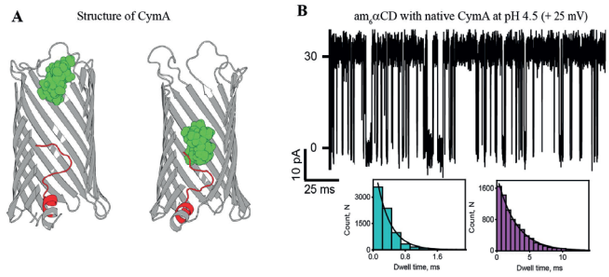
JRF: 1 | Project Assistant: 1 | Technical Assistant: 1
PhD Students: 4 | Post Doctoral Fellows: 1

From left: Smitha Devi S, Remya Satheeshan, NeilahFirzan C A, Smrithi Krishnan R, Varsha Shaji, Devika Vikraman

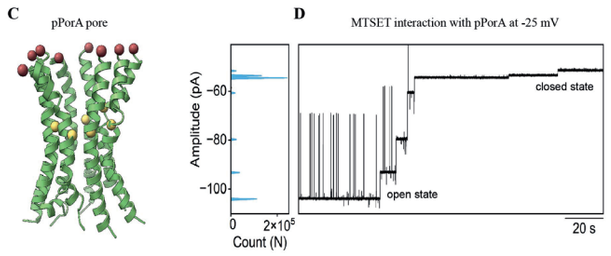
BUILDING MEMBRANE PROTEINS FOR NANOTECHNOLOGY AND MEDICINE

Bacterial outer membrane pores usually restrict the translocation of large molecules and this mechanism at the molecular level is not well-defined. Here we discuss an unusual passive bacterial membrane transporter CymA comprising charged zone and a constricting N terminus segment involved in the selective uptake of unusually large cyclic sugars. Previous studies have suggested the complete movement of the constriction segment out of the pore during substrate translocation, comparable to the active transporters, although no evidence confirms its exact location. Using a combination of electrical recordings, pore mutations and molecular dynamics simulations, we establish the charge-selective substrate translocation across CymA governed by the electrostatic pore properties and conformational dynamics of the constriction segment. Importantly, we show that the variation in pH of the environment resulted in reversible modulation of the substrate binding site in the pore regulating the transport of charged sugars. We further 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 provides new insights related to energy-independent large molecule membrane transport and will aid the development of nanopore sensors for complex biopolymer characterization.

The use of nanopores for the single-molecule sensing of folded proteins and biomacromolecules has recently gained attention. Here, we introduce a simplified synthetic alpha-helical transmembrane pore, pPorA, as a nanoreactor and sensor that exhibits functional versatility comparable to engineered protein and DNA nanopores. The pore, built from the assembly of synthetic 40-amino-acid-long peptides, is designed to contain cysteine residues within the lumen and at the pore terminus for site-specific chemical modification probed using single-channel electrical recordings. The reaction of the pore with differently charged activated thiol reagents was studied, wherein positively charged reagents electrophoretically driven into the pore resulted in pore blocking in discrete steps upon covalent bond formation. The asymmetric blockage patterns resulting from the cis and trans-side addition of reagents reveal the pore orientation in the lipid membrane. Furthermore, activated PEG thiols covalently blocked the pores over a longer duration in a charge-independent manner establishing the large diameter and orientation of the formed pores. While the covalent binding of thiol reagents caused a drop in the pore conductance, cationic cyclic octasaccharides produced time-resolved translocation events confirming the structural flexibility and tunability of the pores. The ability of the pore to accommodate large analytes and the considerable current amplitude variation following bond formation events is promising for developing platforms to resolve multistep chemical reactions at the single-molecule level for applications in synthetic nanobiotechnology.



Structural and electrical properties: A) Structure of CymA showing cyclodextrin translocation. B) Single-channel electrical recordings showing cyclodextrin-induced blockages. C) Structure model of pPorA pores. D) Electrical recordings showing the interaction of MTSET with the peptide pores.



INVITED TALKS [PI]

- Membrane protein pores for nanotechnology. TIFR, Hyderabad. 7-9 th September 2022.

- FCSXIII, the 13th National Workshop on Fluorescence and Raman Spectroscopy. Assembly of membrane protein pores. IISER Trivandrum and RGCB, Trivandrum. January 6th- January 11th 2023.

- 9th Indian Peptide Society meeting. Building transmembrane peptide pores. BITS Pilani, Goa. Feb 22-25th 2023.

PUBLICATIONS

- Krishnan R S, Jana K, Shaji AH, Nair KS, Das AD, Vikraman D, Bajaj H, Kleinekathöfer U, Mahendran KR. Assembly of transmembrane pores from mirror-image peptides. Nat Commun. 2022;13(1):5377.

- Vikraman D, Krishnan R S, Satheesan R, Das AD, Mahendran KR. Electrostatic Filtering of Polypeptides Through Membrane Protein Pores. Chem Asian J. 2022;17(24):e202200891.

- Vikraman D, Satheesan R, Rajendran M, Kumar NA, Johnson JB, R SK, Mahendran KR. Selective Translocation of Cyclic Sugars through Dynamic Bacterial Transporter. ACS Sens. 2022;7(6):1766-1776.

- Puthumadathil N, Krishnan R S, Nair GS, Mahendran KR. Assembly of alpha-helical transmembrane pores through an intermediate state. Nanoscale. 2022;14(17):6507-6517.

- Ajayakumar N, Narayanan P, Anitha AK, Mahendran KR, Kumar KS. Membrane Disruptive Action of Cationic Antibacterial Peptide B1CTcu3. Chembiochem. 2022;23(16):e202200239.

AWARDS [STUDENTS]

- Smrithi Krishnan R:
  Finalist, Inspiring Science Award (Among the eight finalists over 400 entries.) for the best-published Life Sciences paper by a student from India.

  Student Merit Award for the best research presentation at Rajiv Gandhi Centre for Biotechnology.

  DST-SERB International Travel Grant to attend Biointerface Science GRC from June 12 - 17, 2022 at Lucca (Barga), Italy.

  Best poster presentation award in 9th Indian Peptide Society Meeting' organized by BITS Pilani Goa- India.

- Devika Vikraman:
  Best poster presentation award in 9th Indian Peptide Society Meeting' organized by BITS Pilani Goa- India.

  Student Merit Award for the best research presentation at Rajiv Gandhi Centre for Biotechnology.

  Awarded "Indo-German Grant for Science and Technology" 6 months fellowship for research training at Nanion Technologies GmbH in Munich, Germany.

CONFERENCE PRESENTATION

- Smrithi Krishnan:
  Gordon Research Conference and Seminar, June 12 -17, 2022, Lucca (Barga), LU, Italy.

  9th Indian Peptide Society Meeting. Feb 22nd – Feb 25th. BITS Pilani Goa- India.

- Devika Vikraman:
  Physical and Quantitative Approaches to Overcome Antibiotic Resistance – Biophysical Society meeting. Karolinska Institute in Stockholm, Sweden, August 14-18, 2022.

  Single Molecule Peptide Sensing, SMPS3 conference in Delft, Netherlands. 31st October to 3rd November 2022.

  9th Indian Peptide Society Meeting. Feb 22nd – Feb 25th. BITS Pilani Goa- India.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Structure determination and targeting of ubiquitously expressed membrane integrated form of chloride intracellular channels for discovery of small anti-cancer therapeutics.	Department of Biotechnology	2019	4 years	PI
2.	Structural Assembly of Functional Transmembrane peptide nanopores: From Synthesis to Sensing.	Department of Biotechnology	2021	3 years	PI

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
3.	Engineered alpha-helical pores for single-molecule sensing of amyloid structures	Science and Engineering Research Board	2022	3 years	PI
4.	The porin passport control for antibiotic translocation: From single-molecule detection to biological relevance	Department of Biotechnology	2022	3 years	PI

CRACKING THE POLYCYSTIC OVARIAN SYNDROME (PCOS) PATHOGENESIS CODE

PCOS- a multiorgan endocrinopathy, characterized by hyperandrogenemia, oligo/anovulation, is one of the fastest-growing metabolic disorders with an incidence of 22% in the Indian population. Our work on this includes the study of the immune landscape and circadian derailment.

Immune landscape in PCOS: PCOS has low-grade inflammation, autoantibodies, altered immune profile, and reduced immune tolerance which is advocated to be responsible for sub-fertility due to implantation failure. Despite extensive research, there is an underlying uncertainty in its inflammatory/immune etiology. We had reported reduced Tregs frequency (Krishna et al 2015) thereby, explaining recurrent miscarriages in PCOS. We show this year altered AIRE-HIF1A-FOXP3 axis(Padmanabhan et al 2022) leading to Treg insufficiency. Our earlier finding that defective STAT5B phosphorylation is central to IL2- hypo-responsiveness (Krishna et al 2015), has led us to focus on deciphering STAT5 dephosphorylation causes by targeting specific phosphatases and negative regulatory molecules involved in STAT5 signaling. Elevated phosphatases gene expression (DUSP4,PTP1B,TCPTP) and negative regulators(SOCS1/2,PD1,SHP1/2) is seen in PBMC of PCOS women along with increased phosphatase activity. Moreover, Tregs of PCOS women exhibit elevated phosphatase gene expression. Our results reveal improved Treg differentiation upon DUSP4/PTP1B silencing. Thus, overexpressed phosphatases hinder STAT5 phosphorylation culminating in Treg down-regulation in PCOS. Our recent EPIMUNE analysis found higher PD1+ve expressing cell populations in PCOS subjects which have been validated through FACS analysis. Further work is ongoing to characterize Treg (DBT-IMF/ICMR-EMF) in the

Malini Laloraya, PhD, FNASc
Scientist G
Reproductive Biology Program

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 the 'black-box of assisted reproduction'. Polycystic Ovarian Syndrome 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.

LABORATORY STRENGTH

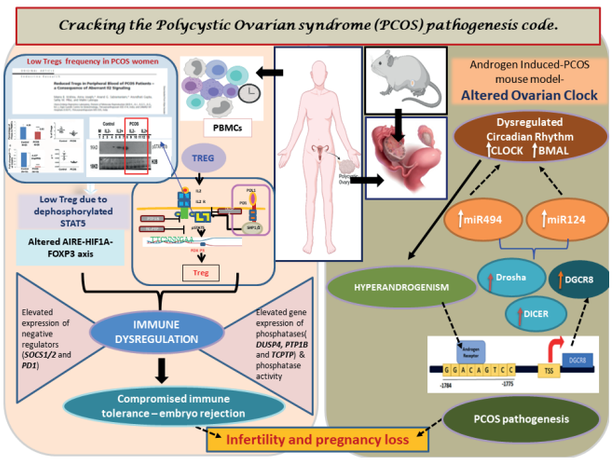
JRF: 5 | SRF: 2 | Lab Assistant: 1 | PhD Students: 2
Post Doctoral Fellows: 2

Front row (from left): Deepthi Prakash, Barsha Bharathi, Anjana M J, Lipika P Patra, Atheena Menon G
Back row (from left): Jeeva S E, Adithian M S, Anshad A, Vysakh G, Mahita Sahadevan, Shabith Raj

remaining 6 months at RGCB.

Circadian Rhythm Defect in PCOS: In the last year, we reported an altered circadian rhythm due to differential core clock gene expression changes. (Johnson et al 2022). CLOCK/BMAL1 knockdown in PBMCs (mimicking low CLOCK/BMAL1 levels in PCOS) led to reduced estradiol levels, but elevated dihydrotestosterone (DHT) synthesis via upregulated SRD5A1 and SRD5A2 in PBMCs. Altered ovarian core clock genes (Clock, Bmal1 and Per2) expression with loss of periodicity in DHEA-treated PCOS mice ovaries corroborated our human data in blood suggested the use of blood as a liquid biopsy for mirroring tissue-specific changes in blood.

The core clock gene alterations are predicted to be a consequence of differential circadian gene targeting by miRNA(Krishna et al 2023)(DBT-IMF). Our current data reveals overexpression of miRNA biogenesis assassins and down-regulation of miRNA degradation assassins in PCOS to be responsible for increased miRNA levels in PCOS. To understand what regulates miRNA changes, we assessed if miRNA Assassins are under regulation by testosterone and its metabolites. We report that DGCR8 gene is a direct target for androgen receptor by Chromatin Immunoprecipitation (ChIP). Hormone (DHEA, testosterone and DHT) treatment of PBMCs revealed upregulated expression of genes, DROSHA, DGCR8, DICER and TRBP2 and downregulated expression of XRN2 when compared with the vehicle control. The results collectively implicate an altered miRNA biogenesis and miRNA degradation mechanism in PCOS condition (DBT-EMF).



Cartoon showing Impaired mechanisms responsible for Polycystic Ovary Syndrome Pathogenesis.

CONFERENCE PRESENTATION

- Lipika Priyadarsini Patra: Oral Presentation, Defective phosphorylation mechanisms of STAT5 - a plausible cause of Treg downregulation in PCOS presented at International Conference on Reproductive Health with Emphasis on Innovations in Reproductive Sciences and Technologies: Hope, Risk and Responsibilities & 33rd Annual Meeting of the Indian Society for the Study of Reproduction and Fertility (ISSRF-2023) organized at Ravenshaw University, Cuttack during 24th–26th February, 2023.

INVITED TALKS [PI]

- Invited Speaker - Circadian rhythm misalignment in Polycystic Ovary Syndrome - Sleep Management and Women's Health at 8th International Conference on Molecular Signaling & 4th CeSin Symposium CSIR-Indian Institute of Chemical Biology, Kolkata during 16-18 March 2023.

- Invited talk entitled Science towards Healthy woman: a critical step to reproductive empowerment for sustainable development at 108th Indian Science Congress in the Plenary session on "ADVANCES IN ENDOCRINE AND CANCER BIOLOGY" during 3-8 January 2023.

- Chief Guest and Invited talk on Molecular insights in embryo development and implantation" in workshop In vitro fertilization as a part of KAUSHALYA (Knowledge Advancement Using Skills on High-end Applied Life technologY for Aspirants) at Gujarat Biotechnology Research Centre,Gandhinagar on 28.11.2022 (Monday).

- Invited Speaker – Peripheral circadian genes impairment contributes to hyperandrogenism in PCOS at ICRBCED and the 39th annual meeting of SRBCE at CSIR-CCMB on September 14-16, 2022.

AWARDS [PI]

- Expert, WHO Global Health Foresight Horizon Scan exercise 2022-2023.

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

AWARDS [STUDENTS]

- Betcy Susan Johnson: Prof. N. R. Moudgal Young Scientist Award-2023. Derailment of peripheral circadian genes- a pathogenic factor for hyperandrogenism in PCOS. International Conference on Reproductive Health with Emphasis on Innovations in Reproductive Sciences and Technologies: Hope, Risk and Responsibilities & 33rd Annual Meeting of the Indian Society for the Study of Reproduction and Fertility (ISSRF-2023) organized at Ravenshaw University, Cuttack during 24th–26th February 2023.

- Lipika Priyadarsini Patra: Best poster, Defective phosphorylation mechanisms of STAT5 - a plausible cause of Treg downregulation in PCOS . International Conference on Reproductive Health with Emphasis on Innovations in Reproductive Sciences and Technologies: Hope, Risk and Responsibilities & 33rd Annual Meeting of the Indian Society for the Study of Reproduction and Fertility (ISSRF-2023) organized at Ravenshaw University, Cuttack during 24th–26th February, 2023.

- Lipika Priyadarsini Patra's abstract entitled PCOS is associated with Treg heterogeneity with presence of PD1 high T-bet low Tregs was selected as one of the TOP FINALISTS in the TRAINEE POSTER COMPETITION 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. She was also selected for the Travel Award.

PUBLICATIONS

- Johnson BS, Krishna MB, Padmanabhan RA, Pillai SM, Jayakrishnan K, Laloraya M. Derailed peripheral circadian genes in polycystic ovary syndrome patients alters peripheral conversion of androgens synthesis. Hum Reprod. 2022;37(8):1835-1855.
- Padmanabhan RA, Zyju DP, Subramaniam AG, Nautiyal J, Laloraya M. Son of sevenless 1 (SOS1), the RasGEF, interacts with ERα and STAT3 during embryo implantation. J Mol Endocrinol. 2022;70(1):e220089.
- Padmanabhan RA, Johnson BS, Dhyani AK, Pillai SM, Jayakrishnan K, Laloraya M. Autoimmune regulator (AIRE): Takes a hypoxia-inducing factor 1A (HIF1A) route to regulate FOXP3 expression in PCOS. Am J Reprod Immunol. 2023;89(2):e13637.
- Krishna MB, Johnson BS, Vasudevan M, Pillai SM, Laloraya M. miRNA-mRNA Network in PBMCs of PCOS Women Identifies Overactivated Stress-Activated Kinases. Cell Physiol Biochem. 2023;57(2):137-156.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Investigating the role of superoxide in regulating the major events during embryo implantation.	Science and Engineering Research Board	2020	3 years 7 months 16 days	PI
2.	Molecular Analysis of Circadian Rhythm in Polycystic Ovarian Syndrome Patients.	Department of Biotechnology	2020	3 Years 4 months	PI
3.	Ascertaining the causes of low tregs in polycystic ovarian syndrome patients	Indian Council of Medical Research	2021	3 years	PI
4.	Creation of patient derived endometrial organoids for understanding the underlying causes of RIF	Department of Biotechnology	2022	3 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Betcy Susan Johnson	Evaluation of Circadian Rhythm in Pathophysiology of PCOS	University of Kerala	Awarded	2022

Manjula S, PhD
Scientist F
Plant Biotechnology & Disease Biology Program

BRIEF THEME OF LABORATORY

Our lab focuses on gaining 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'.

LABORATORY STRENGTH

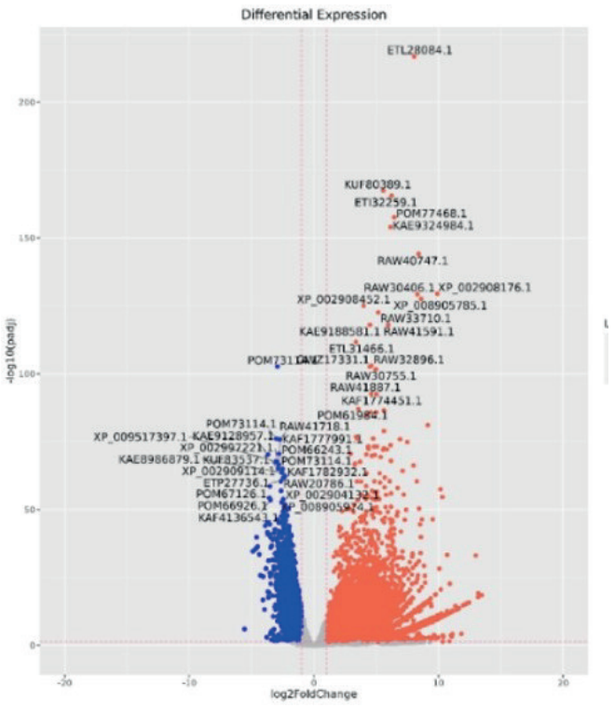
Technical Assistant: 1 | PhD Students: 4

IDENTIFICATION AND FUNCTIONAL CHARACTERISATION OF PATHOGENIC EFFECTORS FROM THE OOMYCETE PHYTOPHTHORA CAPSICI LEONIAN

Quick wilt in Piper nigrum L. (black pepper) is a major disease caused by the filamentous oomycete, Phytophthora capsici. P. capsici infects the host cell through a hemi-biotrophic interaction where the oomycete first germinates their zoospores on the cell surface, and further interacts with the cell membrane in the apoplastic space producing infection structures called haustoria inside the cell. Recent studies in other Phytophthora species have shown that the early interaction of Phytophthora is facilitated by specific pathogenic determinants called effector proteins which when secreted into the host cells modulate plant defence circuitry and enable parasitic colonization. Identification and functional characterization of these effectors are necessary to unravel their virulence and pathogenicity and to understand how they manipulate the plant defence pathways. Identification of these effectors will help in adopting strategies including priming, for durable crop protection.

An initial study to understand the expression pattern of P.capsici genes while infecting P.nigrum was carried out using mRNA seq approach. The sequence data of P.capsici mycelia from the axenic culture and two RNA pools of mycelia belonging to early infection hours (1.5hpi,3hpi, 6hpi) and late infection hours (12hpi,24hpi,48hpi) were generated using Illumina Novaseq 6000. On an average more than 93% of reads were mapped to reference genome of P.capsici and about 90% of the genes were uniquely mapped. A comparative differential expression analysis carried out between the libraries showed significant upregulation of 533 genes and downregulation of 482 genes as early as six hours post inoculation. On a closer look at the transcriptome data, we could observe a carefully co-ordinated expression of pathogenicity related genes, especially those belonging to effectors like RxLRs, CRNs, NLPs and cell wall degrading enzymes like pectate lyase and glycoside hydrolases. Stability of seven candidate reference genes: actin (act), α-tubulin (atub), β-tubulin (btub), translation elongation factor 1-α (ef1), elongation factor 2 (ef2), ubiquitin-conjugating enzyme (ubc) and 40S ribosomal protein S3A (ws21) in Phytophthora capsici was validated before carrying out gene expression analysis of selected candidate effector genes by Real Time PCR. Four algorithms: geNorm, NormFinder, BestKeeper, and the ΔCt method were compared, and a comprehensive ranking order was produced using RefFinder. The overall analysis revealed that ef1, ws21, and ubc are the three most stable genes in the combined dataset, ef1, ws21, and act were the most stable at the infection stages, and, ef1, btub, and ubc were most stable during the developmental stages. These findings were further corroborated by validating the P.capsici pathogenesis gene NPP1 expression. The findings are significant as this is the first study addressing the stability of reference genes for P.capsici-P.nigrum interaction studies. The reproducibility of the mRNA seq data we produced was validated by performing quantitative real time PCR of selected genes with functional assignments.

Front Row (from left): Saranya V, Mookul Samader
Back row (from left): Liya Kurien, Gayathri S S, Indu M



Phytophthora capsici transcriptome analysis. Volcano plot of differentially expressed genes in control group vs late infection group. Up and down regulated genes are reported as red and blue dots respectively, with p value<= 0.05.

PATENT APPLIED/GRANTED

- Name of patent: An Antifungal Synthetic Peptide Derived from Osmotin Protein
  Inventor: S.Manjula
  Patent Application No. 202041015632.

PUBLICATIONS

- Indu M, Meera B, Sivakumar K, Mahadevan C, Shafi K, Nagarathnam B, Sowdhamini R and Sakuntala M. 'Priming' protects Piper nigrum L. from Phytophthora capsici through reinforcement of phenylpropanoid pathway and possible enhancement of Piperine biosynthesis. Front. Plant Sci. 2022; 13:5016.

CONFERENCE PRESENTATION

- Saranya V: Oral Presentation, Time course transcriptomic analysis reveals orchestrated expression of pathogenicity related genes in Phytophthora capsici during its interaction with Piper nigrum L. at the International Conference on Recent Advances in Biological Science 2023, conducted by Inter University Centre for Evolutionary and Integrative Biology, University of Kerala, Thiruvananthapuram, from 17-19 January 2023.

- Indu M: Oral Presentation, Elucidating the molecular basis of 'Priming' in Piper nigrum L. for exploring its potential in crop protection at the International Conference on Recent Trends in Biological Sciences 2023, 17-19 January 2023.

- Indu M: Poster Presentation, Molecular investigation reveals the critical roles of lignin and Piperine biosynthetic pathways during Priming-mediated enhancement of innate immunity in Piper nigrum L., in response to Phytophthora capsici infection at the International conference on Current Trends and Future Prospects in Plant Biology organized by Department of Plant sciences, School of Life Sciences, University of Hyderabad, from 23rd to 25th February 2023.

- 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 at the International conference on Current Trends and Future Prospects in Plant Biology organized by Department of Plant sciences, School of Life Sciences, University of Hyderabad, from 23rd to 25th February 2023.

- Saranya V: Poster Presentation, Molecular dynamics of Piper nigrum- Phytophthora capsici interaction: the pathogen's perspective at the International Conference on Emerging Trends in Plant Science Research, conducted by Postgraduate & Research Department of Botany, Catholicate College, Pathanamthitta, Kerala, from 6-7 March, 2023.

GENETICS OF COMPLEX DISORDERS OF BRAIN

Moinak Banerjee, PhD
Scientist G
Neurobiology Program

BRIEF THEME OF LABORATORY

Human Molecular Genetics team works on complex disorders of brain with specific interest on genetics and epigenetics and developing tools for personalised medicine.

LABORATORY STRENGTH

JRF: 1 | SRF: 1 | Project Associate: 1 | PhD Students: 6
Post Doctoral Fellows: 1

Front row (from left): Ardra M, Alfiya F, Neethu Mohan, Binithamol K P
Back row (from left): Anil Prakash, Samyukta Bhass, Rashmi Sukumaran, Sreelekshmi

Artificial intelligence in identifying regulatory interactions: A.I models are rapidly transforming biological interaction. Use of attention layers have given rise to large language models (LLMs) and AI chatbots like ChatGPT. Attention models show lack of transparency and memory constraints when used on sequence datasets. To overcome these constraints, we developed a deep learning model named ISANREG that combines self-attention layers and attention-attribution mechanisms for making the model interpretable. ISANREG predicts transcription factor bound motif instances and DNA mediated TF-TF interactions using self-attention attribution scores derived from the network.

Epigenetic profiling of antipsychotic response in schizophrenia: Genome wide methylation variations associated with schizophrenia strongly colocalized with schizophrenia risk loci, strengthening the intertwined genetic and epigenetic regulation of mutifactorial disorders. Multitude of evidences suggest the epigenome modulatory effect of antipsychotic drug. The study has identified significant methylomic variation between

different categories. Promoter associated differentially methylated probes between responders and nonresponders were highly enriched for neuro-related pathways like neurodegeneration, neuroactive ligand -receptor interaction, axon guidance etc suggesting the disregulation of multiple pathways. Increased level of overlapping was observed between schizophrenia associated and treatment response associated differentially methylated CpG sites.

Genetics of Developmental and Epileptic Encephalopathies (DEE): DEE describes rare neurodevelopmental disorders with early infantile onset, characterized by the co-occurrence of epilepsy, intellectual disability, autism spectrum disorder and developmental delay. WES analysis was performed in 114 trios with probands being diagnosed to have well defined or unclassified phenotypes. Trio-WES revealed a diagnostic yield of 27.19% which includes 24.5% de novo variants and 5.26% of variants with homozygous recessive and compound heterozygous pattern of inheritance. This study substantiates the utility of trio-WES as a useful diagnostic tool in determining the etiology of patients with unknown causes of DEE.

Pharmaco-genetic of Methotrexate Response in Rheumatoid Arthritis (RA). The pathogenesis of RA is unclear resulting in ineffective treatment and unpredictable side effects. MTX is folic acid analog used for treatment of RA. The folate pathway genes for MTX responses in RA patients were screened. The drug transporter ABCB1 rs1045642 and drug metabolizer MTRR rs1532268 shows significant association with MTX responses in RA.

Pharmacoepigenetics of antihypertensive drugs: The "trial and error" strategy of antihypertensives results in adverse effects and treatment-resistant hypertension. Pharmacoepigenetics investigates the role of epigenetics in intra- and inter-individual variability in drug response, pharmacological effects on gene-expression profiles, and the mechanism of drug action and adverse drug reactions. It is challenging to demonstrate the effect of each drug and its impact on a patient due to usage of multiple medications. We examined how antihypertensives, Telmisartan Angiotensin-Receptor-Blocker and Enalapril, an Ace-inhibitor, affect global DNA methylation (GDM) and if they regulated by epigenetic genes in in-vitro model. We find Telmisartan and Enalapril do influence DNA methylation by influencing DNMTs and TETs expression.

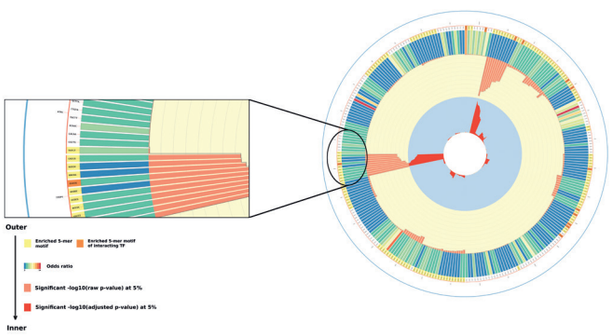


Figure1: Top enriched TF binding motifs identified by the model represented as circos plot.

AWARDS [STUDENTS]

- Binithamol K Polakkattil: Young Scientist Award ISHG2022 of Indian Society of Human Genetics, April 8-9. 2022.
- Anil Prakash: Special mention Young Scientist award ISHG2023- Indian Society of Human Genetics (ISHG), 23-25 Jan. 2023.

INVITED TALKS [PI]

- Dissecting complexities in common disorders. SCTIMST, 11th Aug. 2022.
- India 2047. A brainstorming meeting on security challenges. Habitat Centre, New Delhi 13-14 Dec. 2022.
- DBT Brainstorming session on Autism Spectrum Disorders. DBT New Delhi. 6th January 2023.
- Genetics and epigenetics dissection of neuropsychiatric disorders: Hypes and hopes. Conference on Recent Advances in Biological Sciences. IUCEIB, Kerala University, Thiruvananthapuram. 17-19 Jan. 2023.
- Genomics in AYUSH: an interface model for testing and validation. International summit on AYUSH, Kanyakumari. 27-29 Jan. 2023.
- Defining Commonalities in Epigenetics of Neurodevelopmental Disorders from a South Asian Perspective. INTERNATIONAL CONFERENCE ON OPEN AUTISM, Center for Advanced Research & Excellence in Autism & Developmental Disorders (CAREADD), St. John's National Academy of Health Sciences, Bengaluru, 4-5 March 2023.
- Dilemma in Epigenetics of Neuropsychiatric Diseases and the way to resolve. 23rd ADNAT Convention, Translating Human Evolutionary History to Precision Medicine. Banaras Hindu University Varanasi, 10-12 March, 2023.
- Emerging domains in forensic science. National University of Juridical Sciences, Kolkata. 14th March 2023.
- Integrating creatitivity and critical thinking in the learning process. Two Week National 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 on 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 2022.

CONFERENCE PRESENTATION

- Alfiya F: Oral Presentataion: Denovo mutations in Developmental and Epileptic Encephalopathies (DEE) in an Indian cohort, ECON Conference, 5-7, August 2022.

- Binithamol K Polakkattil: Oral Presentation. Epigenetic profiling of antipsychotic treatment response in schizophrenia patients, International Conference on Human Genetics and 46th Annual Meeting of the Indian Society of Human Genetics, School of Life Sciences, Manipal Academy of Higher Education. 8-9, April 2022.

- Anil Prakash: Oral Presentation. Resolving the role of regulatory regions and their crosstalk in different tissues using a deep learning model. 47th Annual Conference of ISHG, Department of Human Genetics, Andhra University, Vishakhapatnam, 23-25, January 2023.

- Rashmi Sukumaran: Oral Presentation : Understanding the burden of stroke in the last decade through its comorbid conditions: An ethnogenetic perspective. 47th Annual Conference of ISHG, Department of Human Genetics, Andhra University, Vishakhapatnam, on 23-25, January 2023.

- Samyukta Bhas: Oral Presentation Oral presentation for the National Seminar Epigenetics meets Meets Metabolomics, Institute for Communicative and Cognitive Neurosciences (ICCONS), Shoranur, 09-10th June 2023.

- Alfiya F: Poster presentation, Genetic Diagnostic Yield of Childhood Developmental Epileptic Encephalopathies: A Comparative Study of Whole Exome Sequencing and Global Screening Array, RMNC silver jubilee conference held on 3-4, August 2022.

- Alfiya F: Poster presentation, A novel ITPA variant associated with Developmental and Epileptic Encephalopathy (DEE), ECON Conference held on 5-7, August 2022.

- Alfiya F: Poster presentation, Potential variants identified in metabolic genes linked to developmental and epileptic encephalopathy. 47th Annual Conference of ISHG, Department of Human Genetics, Andhra University, Vishakhapatnam, on 23-25, January 2023.

- Krishnapriya: Poster presentation, Understanding the role of pharmaco-genetic markers for methotrexate response in rheumatoid arthritis patients. 47th Annual Conference of ISHG, Department of Human Genetics, Andhra University, Vishakhapatnam, 23-25, January 2023

- Samyukta Bhas: Poster presentation, Pharmacoepigenetics of antihypertensive drug: Impact on DNA demethylation. 47th Annual Conference of ISHG, Department of Human Genetics, Andhra University, Vishakhapatnam, on 23-25, January 2023.

PUBLICATIONS

- Sood A, Cherian LM, Heera R, Sathyan S, Banerjee M. Association between matrix metalloproteinases-2 and -9 gene polymorphism with basement membrane disruption in oral lichen planus: A case-control pilot study. J Oral Biol Craniofac Res. 2022 ;12(2):258-262.

- Srinivas L, Vellichirammal NN, Nair IV, Nair CM, Banerjee M. Contribution from MHC-Mediated Risk in Schizophrenia Can Reflect a More Ethnic-Specific Genetic and Comorbid Background. Cells. 2022;11(17):2695.

- Alfiya F, Jose M, Chandrasekharan SV, Sundaram S, Urulangodi M, Thomas B, Radhakrishnan A, Banerjee M, Menon RN. C12orf57 pathogenic variants: a unique cause of developmental encephalopathy in a south Indian child. J Genet. 2022;101:30.

- Fazal A, Jose M, Rudrabhatla PK, Chandrasekharan SV, Sundaram S, Radhakrishnan A, Banerjee M, Menon RN. Visual-sensitive epilepsy in GLUT-1 deficiency syndrome: Expanding the phenotype. Epileptic Disord. 2023;25(2):265-268.

- Pavuluri H, Jose M, Fasaludeen A, Sundaram S, Radhakrishnan A, Banerjee M, Menon RN. Arginase deficiency-An unheralded cause of developmental epileptic encephalopathy. Epileptic Disord. 2023;25(4):556-561.

- Anil Prakash, Moinak Banerjee, An interpretable block-attention network for identifying regulatory feature interactions. Briefings in Bioinformatics, 2023; 24(4):bbad250.

- Thrinath Mullapudi, Monojit Debnath, Ramajayam Govindaraj, Praveen Raj, Moinak Banerjee, Shivarama Varambally. Effects of a six-month yoga intervention on the immune-inflammatory pathway in antipsychotic-stabilized schizophrenia patients: A randomized controlled trial. Asian J Psychiatry (2023) 86:103636.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Genetics of complex pediatric epilepsy syndromes: electro-clinico-imaging based genotype-phenotype correlations in an Indian cohort.	Indian Council of Medical Research	2019	5 years	PI
2.	Mission mode program on genetics of rare diseases	Department of Biotechnology	2021	5 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Sindura KP	Genetic predisposition to non-syndromic deafness: An immunogenetic perspective	University of Kerala	Submitted	2022

NEURONAL CALCIUM SIGNALING IN HEALTH AND DISEASE

Calcium (Ca2+) signalling plays a fundamental role in the cellular and molecular processes involved in the physiology of learning and memory, as well as in pathological conditions such as excitotoxicity - a detrimental cellular phenomenon that leads to neuronal death in many neurodegenerative diseases. NMDAR mediated Ca2+ influx and the subsequent activation and downstream actions of CaMKII are essential events in learning and memory. CaMKII in association with NMDAR and protein phosphatase 1 (PP1) has been hypothesised to act as a bistable switch in maintaining molecular memory at synapses. Biochemical modulation of CaMKII by NMDR-GluN2B subunit is likely to play a crucial role in this mechanism. We are testing this possibility by AAV-mediated knock-in of mutants of CaMKII in neuronal cultures and in animal models.

R V Omkumar, PhD
Scientist G
Neurobiology Program

BRIEF THEME OF LABORATORY

The laboratory has been investigating the molecular basis of brain functions towards developing fundamental understanding as well as for inventing therapeutic strategies that target impaired pathways.Our studies have focused on calcium signaling in neurons that underlies several normal brain functions and the pathophysiology of several diseases. Proteins such as the calcium conducting NMDA receptor (NMDAR), voltage gated calcium channel (VGCC) and the calcium/calmodulin activated protein kinase II (CaMKII) have been of particular interest. We study these proteins in vitro and in vivo under normal and diseased conditions to understand their functional roles.

LABORATORY STRENGTH

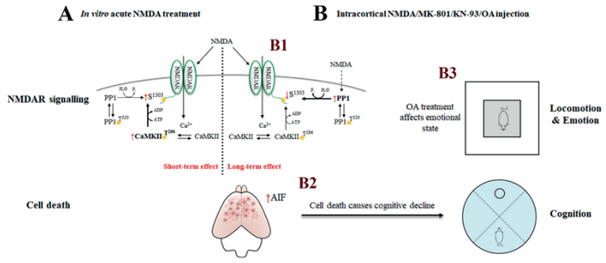
JRF: 1 | SRF: 1

We have also been investigating the regulation of NMDARs by microRNAs (miRNAs). Several such miRNAs were found to be dysregulated in schizophrenia leading to downregulation of NMDAR subunits. These miRNAs appear to act by causing translational repression. Antagonizing the action of these miRNAs emerge as a possible strategy to reduce the symptoms of schizophrenia in animal models.

Excessive activation of NMDAR leads to excitotoxicity that can cause neurodegeneration. Selective phosphorylation of the NMDAR-GluN2B subunit on Ser1303 by CaMKII is associated with intraneuronal Ca2+ overload during excitotoxicity (Farinelli et al., 2012, PLoS One, Vol. 7, e34047). Conversely, dephosphorylation of GluN2B-Ser1303 by PP1 (Ramya et al., 2012, Neurochem Int, Vol. 61, p981), occurs during neuroprotection. Under physiological conditions, the phosphorylation and dephosphorylation of GluN2B-Ser1303are dynamically regulated by kinases and phosphatases. Aberrations in this balance can dysregulate NMDAR function and consequently affect both physiology and behaviour. We studied the short- and long-term effects of NMDAR stimulation on GluN2B-Ser1303 phosphorylation and the regulation of PP1 expression in cortical neurons. Building on the previous observations on immediate molecular-level changes consequent tointracerebroventricular (ICV) injection of NMDA (Kumar et al., 2019, Neurosci Lett, Vol. 709, p134343), we further investigated the alterations in behaviour and associated biochemical changes induced by NMDA injection in the long-run. Additionally, we studied the role of Ca2+/CaM kinases and phosphatases in mediating the molecular and behavioural consequences of NMDAR activation.

Acute NMDA receptor activation in cortical neurons resulted in a significant increase in phospho-GluN2B-Ser1303 levels, which can be correlated with the concomitant activation of CaMKIIα. Inhibition of phosphatases with okadaic acid (OA) pre-treatment also resulted in increased levels of GluN2B phosphorylation. In our in vivo model of excitotoxicity, a single dose of intracortical injection of NMDA, MK-801 or KN-93 led to cognitive decline in adult rats when observed 4-5 weeks later. Interestingly, OA injection at the prefrontal cortex led to an increase in locomotor activity and reduced anxiety-like behaviour after 7-9 days, while the same were unaltered with memory-impairing MK-801 and KN-93 treatments. Cognitive decline in the MK-801 and KN-93 treated animals could be attributed to altered function of the NMDA receptor as seen by the dysregulation of NMDAR subunits and its downstream signalling molecules. NMDA treatment-induced increase in PP1α observed after 4-5 weeks could be causing the reduction of GluN2B phosphorylation at Ser1303. Furthermore, NMDA treatment seems to induce sustained cell death as seen by an increase in apoptosis-inducing factor (AIF).

Altogether, our results show that aberrations in the activities of different components of the NMDAR signalling pathway can lead to diverse behavioural and cellular consequences. The acute and chronic effects of transient fluctuations in the activities of NMDAR signaling components could differ significantly. This highlights the importance of a fine-tuned balance between the activities of phosphatases and kinases, in regulating signalling processes and thereby the emotional state. These findings offer valuable insights into the intricate mechanisms underlying excitotoxicity and may have implications for the development of targeted therapeutic interventions in neurodegenerative diseases and related disorders.



Modulation of GluN2B-Ser1303 phosphorylation and physiological consequences of aberrations in the NMDAR signalling pathway. (A) Primary cultures of cortical neurons were treated with NMDA for a brief period. Acute NMDA treatment resulted in increased GluN2B-Ser1303 phosphorylation, which was mediated through the activation of CaMKII. (B) Adult rats were subjected to a single dose of NMDA, MK-801, KN-93 or OA by intracortical injection. (B1) NMDA treatment increased PP1α levels when measured after ~4 weeks, leading to a decrease in GluN2B phosphorylation at Ser1303. (B2) Sustained cell death indicated by the increase in cell death proteins (AIF) observed with NMDA treatment may contribute to cognitive deficits observed in the MWM test. (B3) Intracortical OA injection induced hyperlocomotion and reduced anxiety in vivo. The thick arrows in the NMDAR signalling pathway represent the predominant activities that targetphosphor-GluN2B-Ser1303 at specific time frames, while the thin arrows indicate reactions that regulate the kinase-phosphatase balance within the cell. The dashed arrow represents an indirect effect.

PUBLICATIONS

- Gunasekaran S, Omkumar RV. miR-146a and miR-200b alter cognition by targeting NMDA receptor subunits. iScience. 2022;25(12):105515.
- Archana GM, Arunkumar RC, Omkumar RV. Assays for L-type voltage gated calcium channels. Anal Biochem. 2022;656:114827.
- Mohanan AG, Gunasekaran S, Jacob RS, Omkumar RV. Role of Ca2+/Calmodulin-Dependent Protein Kinase Type II in Mediating Function and Dysfunction at Glutamatergic Synapses. Front Mol Neurosci. 2022;15:855752.
- Gunasekaran S, Jacob RS, Omkumar RV. Differential expression of miR-148b, miR-129-2 and miR-296 in animal models of schizophrenia-Relevance to NMDA receptor hypofunction. Neuropharmacology. 2022 ;210:109024.

INVITED TALKS [PI]

- Plenary lecture entitled Strategies for neuroprotection by targeting NMDA receptor at the 64th International Symposium of the Korean Society of Life Science,during August, 2022, held at Gyeongju, Republic of Korea.

- Invited lecture entitled Novel approaches to discovery of calcium channel blockers on August 10, 2022, at the Dongguk Medical Research Institute, Dongguk University,Gyeonju, Republic of Korea.

- Invited lecture entitled NMDA receptor regulatory mechanisms – Avenues for neuroprotection at the Annual Meeting of the Society for Neurochemistry, India during November, 2022, held at Central Institute of Medical Sciences (CIMS) Nagpur, India.

CONFERENCE PRESENTATION

- Sowmya Gunasekaran: Poster presentation, "MicroRNAs targeting neuronal NMDA receptors" at the IBRO-RIKEN CBS Summer program-Architecture of the Brain, Japan (Virtual) in July 2022.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Novel derivatives of Tacrine, a cholinesterase inhibitor, with added pharmacological actions –A preclinical experimental study	Indian Council of Medical Research	2021	3 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Sowmya Gunasekaran	MicroRNAs targeting NMDA receptors in the brain	MAHE, Manipal	Awarded	2022

β-HCG MEDIATES IMMUNE SUPPRESSION THROUGH UPREGULATION OF CD11B+GR1+ MYELOID DERIVED SUPPRESSOR CELLS, CD206+M2 MACROPHAGES, AND CD4+ FOXP3+ REGULATORY T-CELLS IN BRCA1 DEFICIENT BREAST CANCERS

BRCA1 mutation is reported in about 70% of all triple negative breast cancers (TNBC), while BRCA1 defect due to promoter hypermethylation is seen in about 30%–60% of sporadic breast cancers. Although PARP inhibitors and platinum-based chemotherapy are used to treat these

Priya Srinivas, PhD
Scientist G
Cancer Research Program

BRIEF THEME OF LABORATORY

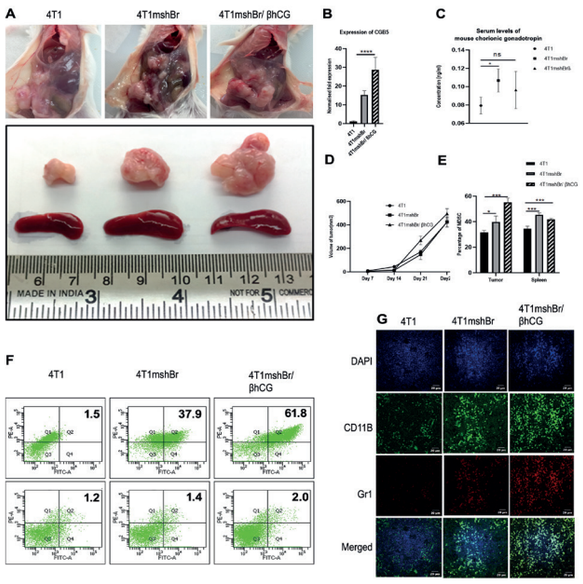
The major mandate of my laboratory is to understand the molecular mechanism of tumorigenesis in BRCA1 defective cancers and to identify diagnostic and therapeutic options. Other than this, my second major mandate is to develop non-invasive techniques for cancer diagnosis using body fluids.

LABORATORY STRENGTH

Project Assistant: 1 | PhD Students: 5 | Post Doctoral Fellows: 1

Front row (from left): Gayathri L T, Aswathy Ashokkumar, Arathy V Warrier
Back row (from left): Aishwaraya M A, Kavya S, Neetha R L, Shreya Sara Ittycheria, Prianka Kumari,

cancers, more efficient therapeutic approaches are required to overcome the resistance to treatment. Our previous findings have reported elevated βhCG expression but not αhCG in BRCA1 deficient breast cancers. As βhCG causes immune suppression in pregnancy, this study explored the immunomodulatory effect of βhCG in BRCA1mutated/deficient TNBC. We observed that Th1, Th2, and Th17 cytokines are upregulated in the presence of β hCG in BRCA1 defective cancers. In NOD-SCID and syngeneic mouse models, βhCG increases the frequency of Myeloid-derived suppressor cells in tumour tissues and contributes to macrophage reprogramming from antitumor M1 to pro-tumour M2 phenotype. βhCG reduces the CD4+T-cell infiltration while increasing the density of CD4+CD25+FOXP3+regulatory T-cell in BRCA1 deficient tumour tissues. In contrast, xenograft tumours with βhCG knocked down TNBC cells did not show these immune suppressive effects. We have also shown that βhCG upregulates pro-tumorigenic markers arginase 1 (Arg1), inducible nitric oxide synthase, PD-L1/PD-1, and NFκB in BRCA1 defective tumours. Thus, for the first time, this study proves that βhCG suppresses the host antitumor immune response and contributes to tumour progression in BRCA1 deficient tumours. This study will help develop new immunotherapeutic approaches for treating BRCA1 defective TNBC by regulating βhCG.



MDSC frequency is increased in BRCA1 deficient and β-hCG overexpressed BRCA1 deficient immunocompetent mice models

a) Immunocompetent BALB/c mice injected with 4T1, 4T1mshBr and 4T1mshBr/ β-hCG tumor cells, 4T1, 4T1mshBr and 4T1mshBr/ β-hCG tumors and spleens harvested respectively. b) qRT PCR of CGB5 expression in the mice tumors. c) Measured levels of mouse chorionic gonadotropin in the serum of 4T1, 4T1mshBr and 4T1mshBr/ β-hCG xenograft after the development of tumor were 20.325IU/L, 43.537IU/L and 25.414IU/L respectively. d) Tumor growth in 4T1, 4T1mshBr and 4T1mshBr/ β-hCG mice. e) Percentage of CD11b+Gr1+ MDSC in 4T1, 4T1mshBr and 4T1mshBr/ β-hCG spleens and tumors. f) Percentage of CD11b+Gr1+ MDSC in 4T1, 4T1mshBr and 4T1mshBr/ β-hCG tumors and spleens. g) Immunofluorescence analysis of colocalization of CD11b+Gr1+ MDSC on 4T1, 4T1mshBr and 4T1mshBr/ β-hCG tumors.

AWARDS [PI]

- Visiting Scientist Fellowship, Mayo Clinic, MN, USA (with ICMR-DHR International Fellowship for Senior Indian Biomedical Scientists, 2023.

AWARDS [STUDENTS]

- Dipyaman Patra: Best oral presentation award. NBR2, acts as key regulator of BRCA1 Hypermethylation mediated Breast Tumorigenesis . 41st Annual Conference of Indian Association for Cancer Research (IACR), Amity Institute of Molecular Medicine and Stem Cell Research, Amity University, Noida, 4th March 2022.

- Dipyaman Patra: ASBMB travel award for Spotlight Oral Talk. Mechanistic Insights into the hypermethylation of BRCA1 evinces a novel pathway to breast tumorigenesis. Experimental Biology, 2022 Philadelphia, PA, USA, 5th March 2022.

- Neetha R.L, Best Poster Presentation Award, BRCA1: One of the key player in transition of cancer associated fibroblasts (CAF) to metastasis associated fibroblasts (MAF). one-day national symposium on biotechnology for sustainable development-2023, 12th April, 2023 at RGCB.

INVITED TALKS [PI]

- Invited Lecture on the Joint Meeting of the International Ovarian Cancer Research Consortium and The International Society of Precision Cancer Medicine held on June 12-13, 2023 at the Oklahoma City, Oklahoma, USA.

PUBLICATIONS

- Patra D, Varghese G R, Kuppusamy K, Neethu K, Srinivas P. Defective Signallingof the BRCA1 Neighbouringgene, NBR2, leads to ER-α Negative tumours in breast cancer xenograft. mouse models. J Vet Anim. Sci. 2022; 53(4): 772-776.

- Rajan A, Varghese G R, Yadev I, Anandan J, Latha NR, Patra D, Krishnan N, Kuppusamy K, Warrier AV, Bhushan S, Nadhan R, Ram Kumar RM, Srinivas P. Modulation of BRCA1 mediated DNA damage repair by deregulated ER-α signaling in breast cancers. Am J Cancer Res. 2022;12(1):17-47.

- Varghese G R, Patra D, Jaikumar V S, Rajan A, Latha N R, Srinivas P. β-hCG mediates immune suppression through upregulation of CD11b+ Gr1+ myeloid derived suppressor cells, CD206+ M2 macrophages, and CD4+ FOXP3+ regulatory T-cells in BRCA1 deficient breast cancers. Immunology. 2023;170(2):270-285.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Identification of xenoestrogen induced spontaneous mutations and changes in promoter methylation status in the cancer causing genes BRCA1, BRCA2 and p53 in breast, ovarian, prostrate and pancreatic cancers.	Council of Scientific and Industrial Research	2020	3 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Geetu Rose Varghese	β-hCG, the pregnancy hormone mediated immune modulation in BRCA1 defective breast cancer	University of Kerala	Awarded	2022

Radhika Nair, PhD
Program Scientist
Cancer Research Program

DECIPHERING THE COMPLEXITY OF BREAST CANCER METASTASIS

Approach 1- Investigating the role of known molecular mediators of metastasis in breast cancer.

I have focused on the Inhibitor of differentiation (or Id) family of bHLH transcriptional repressor proteins which play a critical role in the metastatic spread of breast cancer cells to distant organs especially the lung. I have gone onto identify two potential pathways by which Id proteins control key cancer phenotypes – via negative transcriptional regulation of the Robo1 pathway and cell

BRIEF THEME OF LABORATORY

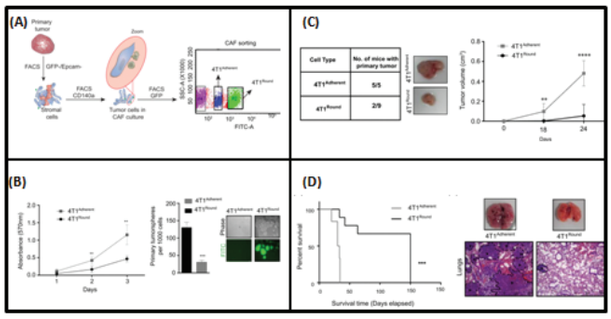
Comprehending the mechanisms that allow a tumour cell to survive and thrive in a hostile new environment of a distant organ is vital to deciphering the complexity underlying metastasis. The hypothesis underpinning our work is that breast cancer cells form metastases utilizing a combination of cell autonomous ('intrinsic') programs and microenvironmental ('extrinsic') changes. This has implications for understanding the critical molecules in metastasis and identifying its "Achilles' heel", which can be exploited for therapeutic purposes.

LABORATORY STRENGTH

PhD Students: 1

cycle pathway by impacting Kif11 and Aurka.

Approach 2: Identifying the molecular mediators involved in breast cancer metastasis using an unbiased approach.
I plan to expand this work to identify pathways which are critical for the metastatic phenotypes hardwired into the metastatic cells with the aim of discovering new potential therapeutic targets. I have isolated two phenotypically distinct tumor cell populations with differing metastatic potential. Resolving the molecular drivers behind the intratumoral heterogeneity revealed critical players which are druggable (manuscript under preparation). Using a validation cohort of 20 matched primary and metastatic tumors will allow me to further understand the genetics underlying the metastatic process and drive my work into more translational avenues in an Indian context.



Isolation of phenotypically distinct tumor cells from a heterogeneous population.

(A) Schematic showing the sorting of primary tumor. (B) Proliferation and tumorsphere assay. (C) The 4T1Adherent cells are highly aggressive. (D) Kaplan-Meier survival curves and representative H&E staining of lungs from two groups of mice. Magnification 10x. Data are expressed as mean ± standard deviation. n=3, p <0.05 are considered statistically significant with *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Archana PT	Understanding the role of phenotypic heterogeneity in breast cancer	MAHE	Awarded	2022

Radhakrishnan R Nair, PhD
Scientist F
Laboratory Medicine & Molecular Diagnostics

BRIEF THEME OF LABORATORY

To produce appropriate, reliable and timely patient test result by the process that combine, well trained competent personnel and the state-of-the-art technique that is efficient and fit for purpose to ethical practices and continual improvement

LABORATORY STRENGTH

Project Assistant: 3 | Technical Assistant: 8 |
Lab Assistant: 1 | Project Associate: 1

LABORATORY MEDICINE AND MOLECULAR DIAGNOSTICS

Laboratory Medicine and Molecular Diagnostics are serving the public by providing cost-effective molecular diagnostics with very less turnaround time. Even though the primary focus of the division is on molecular diagnostics, the division also participates in the co-development and validation of molecular diagnostic kits and instruments such as PCR machines, point-of-care testing kits, etc.

Molecular Diagnostics

Since there is a high demand for molecular diagnostics the division focus on diagnostics of SARS CoV 2, H1N1, HBV, HCV, HIV, Dengue, Genetic markers, etc. The lab offers 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. All the reports are generated through LIMS cloud-based reporting system. All the data are uploaded to the NIE database as well. Total revenue generated from January 2022 till June 2023 is: 10,54,4600INR.

Next Generation Sequencing

The division performs next-generation sequencing of SARS CoV 2 for the identification of new variants. 14046 samples have been sequenced from Jan 22 till June 23. The sequences generated in the division are uploaded on GISAID and IBDC. The division also does deep sequencing of the gut microbiome, P53 methylation studies for cancer genomics, etc.

Kit Validation

LMMD is validating diagnostic kits and instruments. The report generated by the division is approved by ICMR, CDSCO, DBT, and US FDA. A total of 128 validations are 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

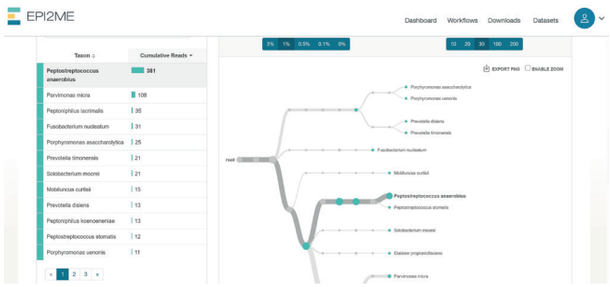
LMMD is an MCI approved rotation center for medical PG students; 241 Medical Postgraduate students (MD Biochemistry, MD Microbiology, MD Pharmacology, MD Pathology, and MD Transfusion Medicine) have completed their training in this Division so far. LMMD offers Biotechnology Skill development program for young budding researchers.

Front Row (from left): Midhula SU, Sreelekshmi. S, Sreya Sherin Biju, Vineetha PT, Sanughosh K, Deepak Mohan
Back row (from left): Ajo Jose, Rabeeha AR, Karthika V, Heera Pillai R, Chithra S, Soumya VK, Rahul JL

Skill development	10
Doctors	125
MSc Veterinary	29
MSc/Mtech	76
Total	240

Revenue generated

Molecular Diagnostics (2022 – June 2023)	10,54,4600
Validation	39,27,040
ICMR rapid kit validation fund allotted	4,50,000
INSACOG Phase II - 1 year (2022-2023)	13,74,400
Gut Microbiome RCC / NGS	75,000
Training (2022 -2023)	4,50,000



NGS Based analysis of Gut microbiome

PUBLICATIONS

- Seetha D, Nori SRC, Nair R R. 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.
- Nelson-Sathi S, Umasankar P K, Sreekumar E, Nair R R, Joseph I, Nori SRC, Philip JS, Prasad R, Navyasree K V, Ramesh S, Pillai H, Ghosh S, Santosh Kumar TR, Pillai MR. Mutational landscape and in silico structure models of SARS-CoV-2 spike receptor binding domain reveal key molecular determinants for virus-host interaction. BMC Mol Cell Biol. 2022;23(1):2.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	INSACOG Phase II : Genomic Surveillance for SARS CoV 2 in India	Department of Biotechnology	Jan 2023-Dec 2023	1 Year	PI
2.	INSACOG Phase I : Genomic Surveillance for SARS CoV 2 in India,	Department of Biotechnology	August 2021 – Dec 2022	1 Year	PI

Rajeeve Sivadasan, PhD
DBT- Ramalingaswami Faculty Fellow
Neurobiology Program

BRIEF THEME OF LABORATORY

Molecular Neurobiology of neuronal degeneration

LABORATORY STRENGTH

JRF: 1 | Project Assistant: 1 | Technical Assistant:1
Lab Assistant: 1 | PhD Student: 1

Udaya Bharathy S

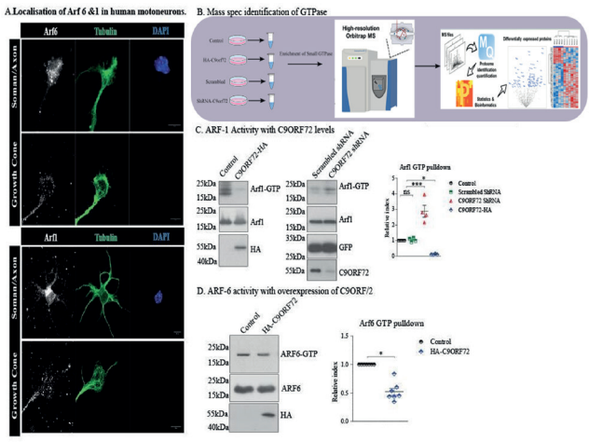
TO STUDY THE ROLE OF SMALL GTPASE IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

The nervous system is a highly evolved and sophisticated biological system that plays a critical role in living organisms. It comprises a complex network of neurons and glial cells, each with diverse subtypes. Among neurological disorders, Amyotrophic Lateral Sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) share clinical and pathological characteristics, suggesting a connection between these conditions. Both sporadic and familial forms of ALS and FTLD exist, with multiple genes implicated in their development. Protein aggregation within cells is a hallmark of both diseases.

The discovery of C9ORF72 repeat expansion is particularly significant as it establishes a direct link between ALS and FTLD. It also serves as a potential marker system observable in sporadic cases. This finding has provided valuable insights into the underlying mechanisms of these diseases and holds promise for further advancements in research and diagnostics. Current investigations are focused on understanding the role of small GT Pase activity regulation in the function of the C9ORF72 protein. Understanding the specific roles of small GTPase activity in the pathogenesis of these ALS forms could provide insights for developing therapeutic interventions.

Another crucial aspect to explore is the impact on small GTPase status when there is dysregulated expression of the C9ORF72 protein and hexanucleotide repeats in the axonal region. Investigating the relationship between C9ORF72 protein expression, hexanucleotide repeats, and small GTPase status in the axonal region may shed light on molecular interactions and potential dysfunctions that contribute to ALS pathology which could reveal novel targets for therapeutic interventions. Determining whether modulating small GTPase activity can be utilized as a pathogenesis.

targeted therapeutic approach or a diagnostic marker holds significant implications for managing and treating ALS. Addressing these questions and gaining a deeper understanding of the intricate relationship between small GTPase activity, C9ORF72 protein expression, and ALS



(A) Localisation of Arf6 and 1 in human neuronal culture. Where it clearly shows the localisation of the protein in both cell body and axonal shaft. (B) experimental setup for the identification and expression pattern of GTPase with C9ORF72 expression in human neuronal culture. (C) ARF1 and ARF 6 activity with the expression of C9ORF72

INVITED TALKS [PI]

- Invited talk at the 36th Annual Meeting of Society for Neurochemistry India (SNCI), &: International Conference on One Health and Translation Research in Neurosciences (SNCI-CON 2022) from 10th -12th November 2022. organised at Dr. G. M. Taori, Central India Institute of Medical Sciences, (CIIMS), Nagpur, India.

- Invited talk to International Symposium on Human Diseases (ISHD 2022)" on Nov. 12, 2022 , Organised by the Department of Biological Sciences, BITS-Pilani, Hyderabad Campus, India.

PATENTS APPLIED/ GRANTED

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

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	The study of small RNA in Neurodegenerative Diseases	Department of Biotechnology	2021	5 years	PI

Ram Mohan Ram Kumar, PhD
DBT- Ramalingaswami Faculty Fellow
Cancer Research Program

BRIEF THEME OF LABORATORY

The lab focuses on small extracellular vesicles to develop new treatment and diagnosis strategies. We hope to use the vesicles for packaging small-molecule, protein and RNA drugs or maybe use them as therapies against cancer. We hope to establish a multifunctional nano-platform for engineering and reprogramming vesicles and prove their potential to reach cancer cells

LABORATORY STRENGTH

JRF: 1 | Project Assistant: 1 | PhD Students: 1

From left: Gopika A G, Kavitha U K, Arya Devi U R

CANCER CELL-SPECIFIC PEPTIDE EQUIPPED SMALL EXTRACELLULAR VESICLES FOR SPECIFIC DELIVERY OF THERAPEUTIC MIRNA IN CERVICAL CANCER CELLS

The following are the proposed objectives: i., isolation of small extracellular vesicles (sEVs) and their characterisation. ii. Synthesis of cervical cancer-specific peptide (CCps) substrates. iii. Evaluating the CC-specific peptide conjugation on the surface of sEVs by chemical conjugation strategy and characterization. iv. Evaluating the loading of tumour suppressor miRNA in the sEVs conjugated targeting peptide. v. Assessing biosafety, tumour accumulation and antitumor efficiency of the sEVs-CCps-miRNA in vivo. Most tumour cells produce large quantities of sEVs that promote tumour progression, survival, invasion and angiogenesis and have potential safety issues [9]. Therefore, we generated sEVs from a non-tumorigenic human cell line, HEK293. The cell culture medium was collected for further processing of sEVs using an exosome isolation kit or ultracentrifugation method. Transmission electron microscopy (TEM) determined the purity of sEVs, and dynamic light scattering (DLS) analysis confirmed the size distribution and concentration of sEVs In TEM analysis, the sEVs were homogeneous round-like vesicles with an average diameter of 100.4 nm, while DLS confirmed the accepted size range of sEVs. Using sEVs markers such as Alix and CD63 further established the purity of isolated sEVs by western blots. Calnexin was used as the negative control (Figure 1). Cell uptake of sEVs: To elucidate the cell uptake of sEVs, 150 ug of PKH67 labeledsEVs were incubated in SiHa cells for 16 hours. After fixation and confocal microscopic analysis, we could visualize the presence of labeledsEVs in the cytoplasm and cell membrane of SiHa cells. The cytotoxicity of sEVs was evaluated in three cell lines (SiHa, HEK293 and HepG2). The CCK-8 assay demonstrated no significant difference in the cytotoxicity of cells incubated with 150 ug of sEVs for 48 hours. Cytotoxicity levels for miR-22 loaded sEVs in SiHa cells were assessed by MTT assay. The percentage of cell viability was significantly decreased from 48 to 72 hours on the treatment of sEVs-miR22 in SiHa cells. The expression of target genes of miR-22 was also analyzed in SiHa cells on treatment with sEVs-miR22 and downregulation of HDAC6 and Vimentin and an upregulation of E-cadherin was observed. The binding affinity of cancer specific peptides, FITC-KQNLAEG (CSP1), and FITC-QQLPSSSTSTYP (CSP2) in HeLa, SiHa and HEK293 cells were confirmed by confocal microscopy. As shown in figure 2, the peptide, CSP1, bound to the CC cell lines while no signal was seen in HEK293 cells confirming their specificity towards the cancer cells. CSP1 was conjugated on the surface of sEVs by EDC-Sulfo NHS coupling reaction. CSP1 conjugated sEVs was purified from the unbound CSP1 peptide by filtering through 100kDa MWCO columns. The absorbance spectrums of sEVs, sEVs-CSP1, and unbound CSP1 were taken. The absorbance Spectrum analysis showed that the spectrums of purified sEVs-CSP1 were in line with that of the pure population of sEVs but with a characteristic peak at 500nm, indicating bound CSP1. DLS analysis of sEVs and sEVs-CSP1 was taken, and no significant size difference was observed, indicating that the EDC-Sulfo NHS coupling reaction didn't affect the size of the vesicles.

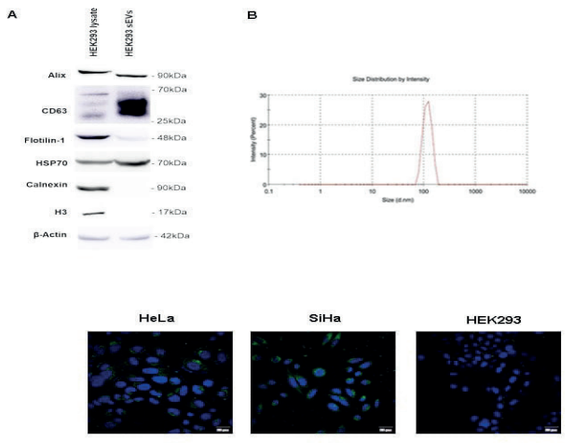


Figure 1: A. Western blot analysis of sEVs markers. B. DLS analysis of sEVs.

Figure 2: Confocal microscopy of cervical cancer cells and HEK293 on incubation with cancer peptides

PUBLICATIONS

- Rajan A, Varghese GR, Yadev I, Anandan J, Latha N R, Patra D, Krishnan N, Kuppusamy K, Warrier A V, Bhushan S, Nadhan R, Ram Kumar R M, Srinivas P. Modulation of BRCA1 mediated DNA damage repair by deregulated ER-α signaling in breast cancers. Am J Cancer Res. 2022;12(1):17-47.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Development of a low cost diagnostic kit based on miRNA detection for hepatocellular carcinoma	Indian Council of Medical Research	2023	3 years	PI
2.	Therapeutic microRNA delivery mediated by exosomes targeting cervical cancermetastases in the 3D and in vivo environments"	Department of Biotechnology	2020	5 years	PI

STUDYING RED CELL DEFORMATIONS IN DISEASE STATES TO UNRAVEL DRUGGABLE SCENARIOS

One major direction of the lab involves the identification of molecular mediators involved in host tropism and host-dependent adaptations. Plasmodia are host-specific at the organism and cellular levels, although emerging trends of zoonoses indicate progressive changes in such preferences. While P. falciparum (Pf) is able to infect all stages of RBCs (but with a preference to young blood cells), it's benign counterparts, P. vivax (Pv) and P. ovale display a predilection for young reticulocytes. Our laboratory is interested in identifying molecular determinants of host tropism and explore their viability as drug or vaccine targets. Another dimesion of our work involves identification of novel drug targets through omics approaches.

Rajesh Chandramohanadas, PhD
Scientist E-II
Pathogen Biology Program

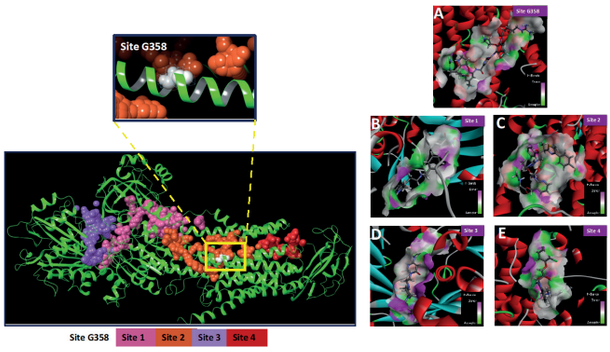
Our laboratry studies blood cell pathologies arising from systemic diseases, oxidative/chemical damage and microbial infectious agents such as Plasmodium spp, causative agent of Malaria. An exciting new direction of our lab aims to understand molecular determinants of red blood cell tropism exhibited by Plasmodium spp, with over-arching implications in parasitic adaptations, zoonosis and progressive drug resistance.

LABORATORY STRENGTH

Project Assistant: 1 | Technical Assistant: 1 | PhD Students: 3

From left: Lakshmi V S, Darsana K M

Chemically induced phenotypes serve as reliable indicators of the molecule of interest. P. falciparum and T. gondii, two well studied parasites, offer a good choice to undertake such phenotypic screens, since complementary experimental tools and reagents are available to dissect cellular phenotypes in these organisms. For instance, phenotypic features associated with impaired growth kinetics (i.e., fast vs delayed killing), host cytolysis (the end point of an intracellular replicative cycle), and host invasion (first step to establish a new infectious cycle), are well characterized in both parasites by us and others, and their therapeutic potential has been validated. Our laboratory studies these processes using a combination of small molecule screening, activity-based protein profiling and quantitative mass spectrometry to unravel novel small molecule inhibitors acting via hitherto un-explored mechanisms and their cellular targets for translational interventions against protozoan parasites and pathogenic viruses.



Identification of new smalll molecules belonging to the MMV Pandemic Response Box Library with binding affinity to PfATP4 protein, a novel drug target for antimalarial drug development.

AWARDS [STUDENTS]

- Keerthy Reghunandanan (Best Poster Award, Kerala Science Congress 2023).
- Christeen Davis (Best Poster Award, Kerala Science Congress 2023).

INVITED TALKS [PI]

- Rajesh Chandramohanadas, 15th Conference on Vectors and Vectors Borne Disease held in Goa from 15-17th February 2023.
- Rajesh Chandramohanadas, FS-Bio 2023, IISER TVM, March 18-20th 2023.
- Rajesh Chandramohanadas, Refresher course for Faculty, Kannur University, 14-16 september 2022.
- Rajesh Chandramohanadas, Recent trends in disease prevention and health management, NIIST TRivandrum, 14-15th December 2022.

CONFERENCE PRESENTATION

- Keerthy Reghunandanan: Poster Presentation, Plasmodial Egress inhibitors and mode of action, Kerala Science Congress 2023.
- Christeen Davis : Poster Presentation, Optical Diffraction Tomography and Image Reconstruction to measure host cell alterations caused by divergent Plasmodium species, Kerala Science Congress 2023.
- Akhila TP : Poster Presentation, Identification of Novel PfATP4 inhibitors from MMV Pandemic Response Box, Kerala Science Congress 2023.

PUBLICATIONS

- Reghunandanan K, T P A, 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. 2023 9:1-12.
- Ong JJY, Oh J, Yong Ang X, Naidu R, Chu TTT, Hyoung Im J, Manzoor U, Kha Nguyen T, Na SW, Han ET, Davis C, Sun Park W, Chun W, Jun H, Jin Lee S, Na S, Chan JKY, Park Y, Russell B, Chandramohanadas R, Han JH. Optical diffraction tomography and image reconstruction to measure host cell alterations caused by divergent Plasmodium species. Spectrochim Acta A Mol BiomolSpectrosc. 2023;286:122026.
- Reghunandanan K and Chandramohanadas R. Chemically induced phenotypes during the blood stage development of Plasmodium falciparum as indicators of the drug mode of action. Front. Drug. Discov. 2022; 2:920850.

Rakesh S. Laishram, PhD
Scientist F & Swarna Jayanti Fellow
Cardiovascular Diseases & Diabetes Biology Program

NON-CANONICAL CLEAVAGE AT THE PRE-MRNA 3'-UNTRANSLATED REGION REGULATES GENE EXPRESSION IN OXIDATIVE STRESS RESPONSE AND MYOCYTE HYPERTROPHY

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 NQO1 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. Our study indicates a signaling regulation of cleavage heterogeneity that controls gene expression in oxidative stress response and myocyte hypertrophy.

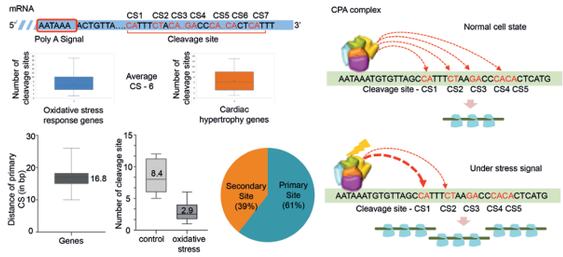
BRIEF THEME OF LABORATORY

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

LABORATORY STRENGTH

SRF: 1 | Technical Assistant: 1 | PhD Students: 5 | Post Doctoral Fellows: 1

Front row (from left): Unnimaya Sajeev, Feba Shaji, Dr Sumayya Shahzad, Diksha Singh
Back row (from left): Ciji Varghese, Malaya Ranjan Behera, Beauty Rani Koch, Neeraja K M, Sneha Sandra P S



Model showing how cleavge heterogeniety regulates gene expression in oxidative stress response.

AWARDS [STUDENTS]

- RGCB Merit Award (Feba Shaji).

INVITED TALKS [PI]

- 11th RNA Meeting, NCCS Pune, December 2022.
- One day conference on Exploring New Frontiers in Genetics and Genomics, mLAC-Bangalore – 2022.
- EMBO meeting on RNA Binding proteins, Pune (hybrid mode), 2022.
- IBSD-Imphal, Webinar, Communication and popularization of Science and Technology – Promote Scientific Thinking, 2022.
- Heart failure Conflux (Virtual Platform), India, 2022.

PUBLICATIONS

- Francis N, Behera MR, Natarajan K, Laishram RS. Tyrosine phosphorylation controlled poly(A) polymerase I activity regulates general stress response in bacteria. Life Sci Alliance. 2022 ;6(3):e202101148.

- Mohanan NK, Shaji F, Koshre GR, Laishram RS. Alternative polyadenylation: An enigma of transcript length variation in health and disease. Wiley Interdiscip Rev RNA. 2022;13(1):e1692.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Alternative polyadenylation in gene expression – implications in cardiovascular diseases	DST-SERB (Swarnajayenti Fellowship)	2020	5 years	PI

MECHANOBIOLOGY OF CANCER-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

High glucose (HG), a hallmark of the tumour microenvironment, is also a biomechanical stressor, as it exerts hyper-osmotic stress (HG-HO), but not much is known regarding how tumour cells mechanoadapt to HG-HO. Therefore, this study aimed to delineate the novel molecular mechanisms by which tumour cells mechanoadapt to HG/HG-HO and whether phytochemical-based interference in these mechanisms

Rashmi Mishra, PhD
Scientist E-II
Neurobiology Program

BRIEF THEME OF LABORATORY

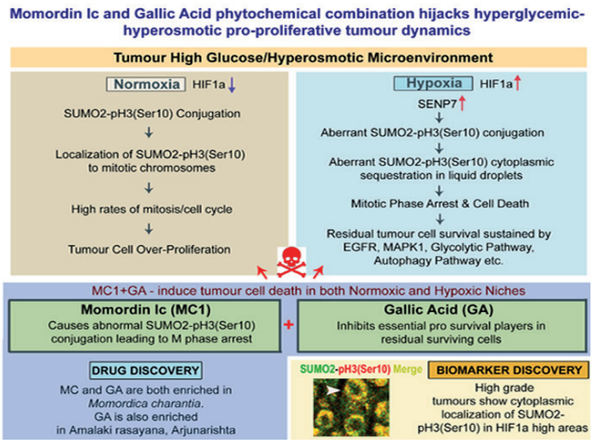
The goal of the translational mechanobiology lab is to understand 'how cells sense and respond to the mechanical microenvironment' with a therapeutic implications to cancers, neuro-cardio-degenerative diseases and abnormal ageing. The workflow involves examining the mechanical stresses at the cellular 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.

LABORATORY STRENGTH

JRF:1 | SRF: 1 | PhD Students: 2

From left: Puja Shinde, Sushobhan Dash

can generate tumour-cell-selective vulnerability to death. The results revealed that the tumour cells can efficiently mechanoadapt to HG-HO only in the normoxic microenvironment. Under normoxic HG/HG-HO stress, tumour cells polySUMOylate a higher pool of mitotic driver pH3(Ser10), which translocates to the nucleus and promotes faster cell divisions. On the contrary, acute hypoxia dampens HG/HG-HO-associated excessive proliferation by upregulating sentrin protease SENP7. SENP7 promotes abnormal SUMOylation of pH3(Ser10) which is phase separated into LLPS phenotype, thereby its nuclear entry is restricted and M-phase arrest as well as cell loss is triggered. However, the acute hypoxia-arrested cells that managed to survive, showed relapse upon reversal to normoxia as well as upregulation of prosurvival-associated SENP1, and players in tumour growth signalling, autophagy, glycolytic pathways etc. Depletion of SENP1 in both normoxia and hypoxia caused significant loss of tumour cells vs undepleted controls. SENP1 was ascertained to restrict the abnormal SUMOylation of pH3(Ser10) in both normoxia and hypoxia, although not so efficiently in hypoxia, due to the opposing activity of SENP7. Co-treatment with Momordin Ic (MC), a natural SENP1 inhibitor, and Gallic Acid (GA), an inhibitor of identified major pro-tumourigenic signalling (both enriched in Momordica charantia),eliminated surviving tumour cells in normal glucose, HG and HG-HO normoxic and hypoxic microenvironments, suggesting that appropriate and enhanced polySUMOylation of pH3(Ser10) in response to HG/HG-HO stress was attenuated by this treatment along with further dampening of other key tumourigenic signalling, due to which tumour cells could no longer proliferate and grow. Therefore, a combination of MomordinIc with Gallic Acid (GA), both enriched in the extract of Momordica charantia (under Phase II clinical trials, available for sale under traditional medicine systems)



represents a promising lead in anti-cancer drug development. pH3(Ser10) is identified as a novel target of SUMO2 conjugation. As high-grade patient tissues show the cytoplasmic accumulation of SUMO2-pH3(Ser10) in the HIF1a high areas, we propose SUMO2, pH3(Ser10) and HIF1a co-immunodetection as multiplexed panel biomarkers for prognosis and for predicting the probability of developing metastasis and relapse.

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.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Identification of the Role of Redox Signaling Pathways in the Mechanobiology of Glioblastoma multiforme.	Department of Biotechnology	2018	4.5 years	PI
2.	How Galectin-3 Drives Pressure Overload Mediated Cardiac Hypertrophy and Heart Failure	Indian Council of Medical Research	2021	3 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Gayathri K G	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 (MAHE)	Submitted	2023

Ruby John Anto, PhD, FNASc
Scientist G
Cancer Research Program

BIOPROSPECTING FOR CHEMOPREVENTIVES, CHEMOTHERAPEUTICS AND CHEMOSENSITIZERS

The invention on the anti-HCC effect of Utt-B has been granted multinational patents, the compound received 'Orphan drug' designation against liver cancer by the US FDA, and the technology has been transferred to the multinational Pharma company, Q Biomed, for clinical trials. Utt-B also promotes pro-survival autophagy in hepatic cancer cells and inhibition of autophagy significantly enhances Utt-B-induced apoptosis of HCC. We have validated the superior anti-HCC efficacy of Utt-B over sorafenib, the first-line treatment option against HCC usingin vitro and in vivo models of HCC. Our data indicate that apart from the superior therapeutic benefit over sorafenib, Utt-B is a pharmacologically safer molecule, and the drug-induced undesirable effects can, thus, be substantially alleviated in the context of HCC chemotherapy (Swetha et al, 2022, Pharmaceuticals).

Tpn was found to be very effective against melanoma and non-melanoma skin cancers. Microarray analysis of Tpn treated-melanoma cells followed by a STRING protein association network analysis revealed that differential expression of genes in melanoma converges at MITF-M, a melanocyte lineage-specific transcription factor. Key findings indicate that the anti-melanoma activity of Tpn is decisively contingent on its efficacy in down-regulating MITF-M expression and the study reveals Tpn as a promising anti-melanoma drug, by virtue of its attributes to impede melanoma invasion and metastasis through the attenuation of MITF-M (Shabna et al, 2022, Cellular and Molecular Life Sciences).

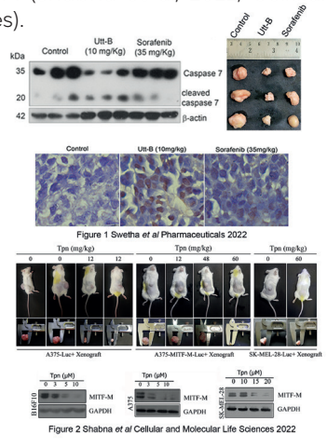


Figure 1 Swetha *et al* Pharmaceuticals 2022

Figure 2 Shabna *et al* Cellular and Molecular Life Sciences 2022

BRIEF THEME OF LABORATORY

My lab focuses on bioprospecting for anticancer products. We have evaluated several natural products as chemotherapeutics, chemosensitzers, and chemopreventives and have identified three plant-derived anti-cancer principles, uttroside B (Utt-B) from Solanum nigrum, tryptanthrin (Tpn) from Wrightia tinctoria, and kaempferide from Chromolaena odorata. Utt-B is exceptionally active against hepatocellular carcinoma and the invention has received multi-national patents. Tryptanthrin shows remarkable potency against melanoma and non-melanoma skin cancers and kaempferide exhibits therapeutic efficacy against cervical cancer.

LABORATORY STRENGTH

JRF: 2 | SRF: 2 | Post Doctoral Fellows: 1 | Project Assistant: 1
Project Associate (MK Bhan Fellow): 1

From left: Maria Joy P, Rayginia P Tennyson, Keerthana C K, Aiswarya U S, Dr. Kalimuthu Kalishwaralal

Figure 1: Utt-B inhibits the development of subcutaneous xenografts and induces apoptosis in tumor tissues, exhibiting better therapeutic efficacy compared to sorafenib.

Figure 2:Tpn inhibits the development of orthotopic tumors in mice by down-regulating MITF-M expression.

PUBLICATIONS

- Keerthana CK, Rayginia TP, Shifana SC, Anto NP, Kalimuthu K, Isakov N, Anto RJ. The role of AMPK in cancer metabolism and its impact on the immunomodulation of the tumor microenvironment. Front Immunol. 2023 ;14:1114582.

- Shabna A, Antony J, Vijayakurup V, Saikia M, Liju VB, Retnakumari AP, Amrutha NA, Alex VV, Swetha M, Aiswarya SU, Jannet S, Unni US, Sundaram S, Sherin DR, Anto NP, Bava SV, Chittalakkottu S, Ran S, Anto RJ. Pharmacological attenuation of melanoma by tryptanthrin pertains to the suppression of MITF-M through MEK/ERK signaling axis. Cell Mol Life Sci. 2022;79(9):478.

- Aiswarya SUD, Vikas G, Haritha NH, Liju VB, Shabna A, Swetha M, Rayginia TP, Keerthana CK, Nath LR, Reshma MV, Sundaram S, Anto NP, Lankalapalli RS, Anto RJ, Bava SV. Cucurbitacin B, Purified and Characterized From the Rhizome of Corallocarpus epigaeus Exhibits Anti-Melanoma Potential. Front Oncol. 2022;12:903832.

- Swetha M, Keerthana CK, Rayginia TP, Nath LR, Haritha NH, Shabna A, Kalimuthu K, Thangarasu AK, Aiswarya SU, Jannet S, Pillai S, Harikumar KB, Sundaram S, Anto NP, Wu DH, Lankalapalli RS, Towner R, Isakov N, Deepa SS, Anto RJ. Augmented Efficacy of Uttroside B over Sorafenib in a Murine Model of Human Hepatocellular Carcinoma. Pharmaceuticals (Basel). 2022;15(5):636.

AWARDS [PI]

- Selected for the TRIALECT - sponsored 'Clinical Trials Traineeship Program' at INSERM, Paris, France and successfully completed the training from 22nd August – 16th September, 2022 at Pitié-Salpêtrière Hospital, Paris.

AWARDS [STUDENTS]

- Rayginia P Tennyson, Best Oral Presentation Award Evaluating the phytosaponin, Uttroside B, as a candidate drug molecule against Non-alcoholic Steatohepatitis at 5th International Conference on Nutraceuticals and Chronic Diseases: On Pharmaceuticals and Nutraceuticals for Cancer and Other Chronic Diseases, held at University of Delhi, October 7-9, 2022.

- Keerthana C.K, Best Poster Presentation Award, Evaluation of the pharmacodynamics of Uttroside B against hepatocellular carcinoma at 5th International Conference on Nutraceuticals and Chronic Diseases: On Pharmaceuticals and Nutraceuticals for Cancer and Other Chronic Diseases, held at University of Delhi. October 7-9, 2022.

- Keerthana C.K, Best Oral Presentation Award, Deciphering Uttroside B–mediated modulation of critical signaling pathways in hepatocellular carcinoma at 4th Indo Oncology Summit, held at Bhubaneswar, November 11-13, 2022.

INVITED TALKS [PI]

- A receptor- independent combinatorial therapeutic regimen for breast cancer treatment, at Indian Science Congress held at Nagpur. January 3-7, 2023.

- Pharmacological targeting of CK2α/MEK/ERK attenuates melanoma invasion and metastasis through suppression of MITF-M, at 5th International Conference on Nutraceuticals and Chronic Diseases: On Pharmaceuticals and Nutraceuticals for Cancer and Other Chronic Diseases, held at University of Delhi. October 7-9, 2022.

- Bioprospecting for novel innovations to improve cancer therapeutics, Impact Lecture Series by IIC at St Thomas College, Palai on 07 July 2022.

- Mother Nature as a Potential source of Anticancer Lead Molecules: From in vitro studies to pre-clinical models at Amala Cancer research Centre, Thrissur on 23 April 2022.

- Impedance of autophagy improves the potency of uttroside B, a US FDA-approved orphan drug against hepatocellular carcinoma, Innovative Cancer Science-Translating Biology to Medicine: Organized by European Association for Cancer Research, from 20/06/2022 to 23/06/2022 at Seville, Spain.

CONFERENCE PRESENTATION

- Rayginia P Tennyson : Poster Presentation, The phyto-saponin, Uttroside B, displays an augmented therapeutic index over sorafenib in a pre-clinical model of hepatocellular carcinoma, Innovative Cancer Science-Translating Biology to Medicine: Organized by European Association for Cancer Research, from 20/06/2022 to 23/06/2022 at Seville, Spain.

- Rayginia P Tennyson: Oral Presentation, Evaluating the phytosaponin, uttroside b, as a candidate drug molecule against non-alcoholic steatohepatitis, 35th Kerala science congress, nanoscience and nanotechnology for humanwelfare, at Mar Baselios College of Engineering and Technology, Kuttikanam, Idukki, from 10-14February 2023.

- Rayginia P Tennyson: Poster Presentation, The phytosaponin, Uttroside B, is a candidate drug molecule against Non-alcoholic Steatohepatitis-Induced Hepatocellular carcinoma, at the 42nd IACR Conference, Organized by ACTREC, Mumbai, January 12-16, 2023.

- Keerthana C.K : Oral Presentation, Deciphering the pharmacodynamics and evaluating the immunomodulatory and therapeutic efficacy of Uttroside B against hepatocellular carcinoma using pre-clinical models,at the World Congress on Rare Diseases, Organized by Biogenesis Health Cluster, Bangalore, January 22, 2023.

- Keerthana C.K : Poster Presentation, Pre-clinical studies on the pharmacodynamics, immunomodulatory and therapeutic efficacy of Uttroside B against hepatocellular carcinoma,at the 42nd IACR Conference, Organized by ACTREC, Mumbai, January 12-16, 2023.

- Keerthana C.K : Poster Presentation Evaluation of the pharmacodynamics of Uttroside B against hepatocellular carcinoma,at 5th International Conference on Nutraceuticals and Chronic Diseases: On Pharmaceuticals and Nutraceuticals for Cancer and Other Chronic Diseases, held at University of Delhi. October 7-9, 2022.

- Keerthana C.K : Oral Presentation, Deciphering Uttroside B–mediated modulation of critical signaling pathways in hepatocellular carcinoma, at 4th Indo Oncology Summit, held at Bhubaneswar, November 11-13, 2022.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Evaluation of uttroside B, a furanosyl saponin fromSolanum nigrum Linn as a candidate drug moleculeagainstAflatoxin-inducedliver carcinogenesisand Non-alcoholicsteatohepatitis(NASH)	Science and Engineering Research Board	2022	3 years	PI
2.	Evaluation and invivovalidation of tryptanthrinanalogues aspotentlead moleculesformalignantmelanomachemotherapy	Council of Scientific and Industrial Research	2021	3 years	PI
3.	In vitro and in vivo validation of the efficacy of thesynergistic combination of curcuminand 5-FUinexterminating breast cancer stem-cell like	The Spices Board of India	2021	3 years	PI
4.	Pre-clinical evaluation of the mechanistic and immunological pharmacodynamics of a novelsaponin, Uttroside-B againstHCC	Department of Biotechnology	2021	3 years	Mentor

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Dr. Swetha M	Evaluation of Uttroside B as a candidate drug against Hepatocellular Carcinoma and comparison of its therapeutic potential with that of Solanum nigrum Linn. leaf extract	University of Kerala	Awarded	2023
2.	Dr. Aiswarya U. S. (Co-guide)	Evaluation of Cucurbitacin-B isolated from Corallocarpus epigaeus, as a candidate drug against malignant melanoma	University of Calicut	Awarded	2023

Sabu Thomas, PhD
Scientist F
Pathogen Biology Program

FUTURISTIC APPROACHES TO COMBAT MULTI-DRUG RESISTANT BACTERIAL PATHOGENS

Emergence of antibiotic resistance is a global threat that challenges the efficient control of bacterial infections. Antimicrobial susceptibility pattern of clinically relevant bacterial pathogens revealed high prevalence of Gram-negative bacteria resistant to last line antibiotics like colistin. Alternative strategies including probiotics and phytochemical-antibiotic combinations remain as promising tools to tackle the MDR pathogens. We have isolated and characterized a probiotic strain from infant gut microbiome against colistin resistant Klebsiella pneumoniae, Vibrio cholerae and MRSA. The prophylactic potential of Lacticaseibacillus analysed in Balb/c mice model showed enhanced survival, reduction in histopathological and microbiological alterations in pathogen challenged group. Genome sequencing showed that it is devoid of antibiotic resistance genes or virulence markers and predicted putative bacteriocin using antiSMASH analysis. This probiotic strain can be considered as a promising candidate as prophylactics to manage drug resistant bacterial infections.

Another major finding is the development of a synergistic combination of natural stilbenoid polyphenol (PC-9) and antibiotics against colistin resistant K. pneumoniae and MRSA. The treated pathogen exhibited >2 log reduction of viable bacterial count compared to control (Fig1). The promising combinations were evaluated in Galleria mellonella larvae infection model and exhibited increased survival rate, immunomodulatory effect and pathogen reduction compared to the infection control groups.

The rampant use of prophylactic antibiotics in the postpartum period leads to intestinal microbiota dysbiosis in infants and exacerbates the AMR threat. The team conducted a shotgun metagenomics study on mother-infant dyads with and without prophylactic antibiotics to analyze the variation in infant gut microbiomes and resistomes. The characteristic presence of Citrobacter werkmanii, an emerging MDR uropathogen, and a higher relative abundance of resistance genes encoding specific antibiotic group were noted in Ab (antibiotic) group when compared with non-Ab group.

BRIEF THEME OF LABORATORY

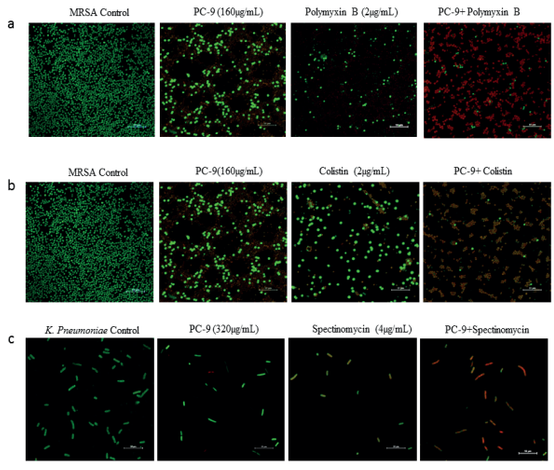
The lab is focused on bacterial pathogenesis and addressing the issue of antimicrobial resistance by alternative strategies like combinatorial therapies, biofilm/antivirulent strategies and human gut derived probiotics. Another major focus area is on studying the effect of interventions on shaping the gut microbiota and tapping human microbiome for probiotics producing beneficial multifaceted molecules.

LABORATORY STRENGTH

Project Assistant: 2 | Technical Assistant: 2 | Lab Assistant: 2
Post Doctoral Fellows: 1 | PhD Students: 2

From left to right: Dr. Karthika S, Divya R, Merin Paul, Deepa Mathew P, Geetha S L, Devika Das J

Total abundance of genes encoding resistance to aminoglycoside, tetracycline, fluoroquinolone, cephalosporin, and peptide antibiotics were higher in antibiotic group. The study highlights adverse effects of prophylactic antibiotic administration and emphasizes the need to strengthen policies regarding antibiotic prescription across the health sectors.



CLSM image of Live-Dead assay showed the effects of PC-9-antibiotics combinations on selected pathogens at 6hr. Effect of (a) PC-9-polymyxin B against MRSA; (b) PC-9-colistin against MRSA (c) PC-9-spectinomycin against colistin resistant K. pneumoniae. From left to right panel - non-treated control group, PC-9 alone, antibiotic alone and PC-9-antibiotic combination treated group respectively

AWARDS [STUDENTS]

- Merin Paul, Sabu Thomas. Best Oral. In vitro evaluation of the synergistic effect of a natural polyphenol antibiotic combination against multidrug resistant bacterial pathogens. International Conference on Biotechnological Advances towards Sustainable Developments - BIOSPECTRUM 2022 organized by (MACFAST), Tiruvalla. June 27-29, 2022.

INVITED TALKS [PI]

- Sabu Thomas, Emerging paradigms in microbiome research at 'one day workshop 'Microbes - A fascinating world of myriads'- organized by St. Mary's College for Women, Tiruvalla- 24th sep 2022.

- Sabu Thomas, 'Emerging Paradigms in Microbiome Research' organized by CSIR NIIST & Kerala academy of sciences, Trivandrum-17th Dec 2022.

- Sabu Thomas, Impact of Climate change on the Biodiversity in Arctic, All India Radio- 28th Jan 2023.

- Sabu Thomas, Antimicrobial Resistance: Impact of Superbugs, Lecture Series organized by Department of Zoology,University College ,Thiruvananthapuram,15th Feb 2023.

- Sabu Thomas, Genomics to track Antimicrobial Resistance determinants in pathogenic bacteria of clinical relevance- organized by Department of Biochemistry & Molecular Biology,Central University of Kerala,Kasaragod-22 nd Feb 2023.

CONFERENCE PRESENTATION

- Karthika S, Akhila VS, Sreeja S, Sabu Thomas: Oral presentation, Insights on the potential urolithin producing bacteria from human gut microbiome at 'Targeting Microbiota-2022' organized by International Society of Microbiota, 19-21 Oct 2022 at Paris, France.

- Merin Paul, Sabu Thomas: Oral presentation, In vitro evaluation of the synergistic effect of a natural polyphenol antibiotic combination against multidrug resistant bacterial pathogens. International Conference on Biotechnological Advances towards Sustainable Developments - BIOSPECTRUM 2022 organized by (MACFAST), Tiruvalla. June 27-29, 2022.

PUBLICATIONS

- Shankar A, Das DJ, Nayar S, Thomas S. Deciphering the effect of maternal postpartum antibiotic prophylaxis on the infant gut microbiome: a whole metagenomic analysis. Future Microbiol. 2023;18:427-441.

- Suryaletha K, Savithri AV, Nayar SA, Asokan S, Rajeswary D, Thomas S. Demystifying Bacteriocins of Human Microbiota by Genome Guided Prospects: An Impetus to Rekindle the Antimicrobial Research. Curr Protein Pept Sci. 2022;23(12):811-822.

- 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.

- Keerthi TR, Akhila VS, Honey C, Thomas S Deciphering Beneficial Health Promoting Properties of Lactic Acid Bacteria Isolated From Infant Gut Microbiome. Saudi J Life Sci. 2022; 7(9): 261-274.

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Devika Das J	Characterization of potential probiotic strain of human origin and study of its anti-infectious property against selected multidrug resistant bacterial pathogens	University of Kerala	Thesis submitted	2022

Santanu Chattopadhyay, PhD
GN Ramachandran Fellow
Pathogen Biology Program

MULTIPLE UNIQUE TRAITS IN THE GENOMES OF GASTRIC PATHOGEN HELICOBACTER PYLORI STRAINS FROM NORTH-EAST INDIAN STATE, SIKKIM

BRIEF THEME OF LABORATORY

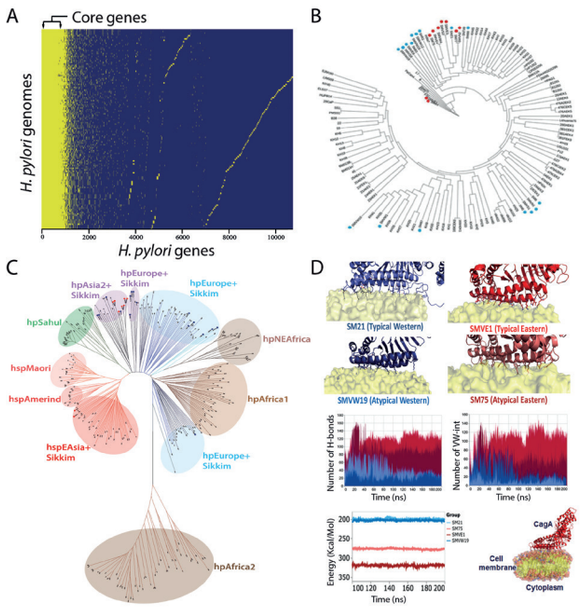
We work on genetic geography, virulence and antibiotic resistance of the gastric pathogen Helicobacter pylori and its interaction with various microbes in gastrointestinal microbiota in the context of severe gastric diseases. We use next-generation sequencing (NGS) based approaches to study microbial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

LABORATORY STRENGTH

PhD Students: 1

Retnakumar P J

Helicobacter pylori infection in human stomach is linked to the developments of peptic ulcer and gastric cancer. H. pylori is one of the oldest human pathogens known and it does not have any environmental reservoir. The infection is typically acquired during the initial years of life by an intrafamilial manner through oral/fecal-oral route and may remain colonized in the stomach for decades before causing severe diseases. Due to the lack of any other host and due to the long term colonization in the human stomach, the bacterium has co-evolved with human. Human migrations and settlements for at least 60,000 years and remarkably faster mutation rate in the evolving H. pylori genomes helped to form different phylogenetic clades in different geographical regions. In each geographical region, however, the H. pylori strains show a panmictic population structure. In India, different geographical regions have distinct human population, but the whole genome sequence (WGS) of Indian H. pylori strains remained understudied. There is no data on the phylogeny and virulence of the H. pylori strains that colonize the residents of North-East Indian state Sikkim, which shares borders with West Bengal, Nepal, Bhutan and China. We have isolated 25 H. pylori strains from all 4 districts (North, South, East and West) of Sikkim. Genomic DNA extracted from each isolated H. pylori strain was subjected to WGS analysis using Illumina NovaSeq6000 and Oxford NanoporeMinION. The WGS data of the 25 Sikkim H. pylori strains were compared with the H. pylori genomes from different parts of the world and 756 core genes were selected (Figure 1A). Phylogenetic analysis based on the 756 core genes of 133 H. pylori strains from different parts of the world including 25 strains from Sikkim showed that the H. pylori strains from Sikkim belong to multiple phylogeographical lineages (Figure 1B). Further phylogenetic analysis using multilocus sequence typing (MLST) of seven housekeeping genes showed that the Sikkim residents carry the strains that have either Eastern or Western traits, but no African traits (Figure 1C). Detailed analysis of the entire 40 kb cag-pathogenicity island (cag-PAI) was done for each Sikkim H. pylori strain. It was found that the cag-PAI of several of the H. pylori strains isolated from Sikkim have unique insertions and deletions of a particular set of genes. For Sikkim, the principal virulence gene, the oncogene cagA, which resides at the extreme 3'-end of the cag-PAI, are of two major types, the Western and the Eastern. Both Eastern and Western types of cagA showed several unique point mutations. Moreover, the frequency of nonsynonymous mutations in the cagA gene is higher than any other housekeeping and virulence genes of H. pylori. Our data showed that the nonsynonymous mutations in the H. pylori cagA led to the expression of few Sikkim-specific Eastern CagA types, which are apparently less virulent due to their relatively weaker membrane interactions than the typical Eastern CagA counterpart (Figure 1D).



A: Roary analysis of the whole genome of 25 Sikkim H. pylori strains and 109 reference strains from different geographical areas; B: The phylogenetic tree based on 756 core genes of the 134 whole genomes from different geographical regions (Eastern Sikkim: red and Western Sikkim: blue); C: MLST analysis using 7 housekeeping genes of 340 H. pylori strains from different parts of the world; D: In silico molecular dynamic simulation: CagA-membrane Phosphatidylserine interaction.

AWARDS [STUDENTS]

- Retnakumar R J, received best poster award in National Symposium on Biotechnology for Sustainable Development-2023 held at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram on April 12, 2023.
- Retnakumar RJ, 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]

- 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.
- Chattopadhyay S. 'My Helicobacter-Gut microbiome story' at the main auditorium of the Government Medical College, Kollam in Annual Conference of Association of Clinical Physiologists of Kerala (ACPK CON, September 1-2, 2022), which was followed by a panel discussion.

CONFERENCE PRESENTATION

- Retnakumar R J: Oral presentation (First Prize) , International Conference on 'Innovations in the Sphere of Biological Sciences' at AIHSHE, Coimbatore, on February 15 and 16, 2023.
- Retnakumar R J: Poster Presentation, work at the 'Frontier Symposium in Biology' at IISER-TVM on 17 to 19, March, 2023.
- Retnakumar R J: Poster Presentation, The work at the National symposium at RGCB, 12 April 2023 and secured best poster prize.
- Retnakumar R J: Poster Presentation, Work at the 2023-Manipal Research Colloquium, Manipal.

PUBLICATIONS

- Ngashangva L, Chattopadhyay S. Biosensors for point-of-care testing and personalized monitoring of gastrointestinal microbiota. Front Microbiol. 2023;14:1114707.
- Nath AN, Retnakumar RJ, Francis A, Chhetri P, Thapa N, Chattopadhyay S. Peptic Ulcer and Gastric Cancer: Is It All in the Complex Host-Microbiome Interplay That Is Encoded in the Genomes of "Us" and "Them"? Front Microbiol. 2022 25;13:835313.
- Nath A N, Shaji A M, R J Retnakumar, Nair G B, 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.
- Retnakumar RJ, Nath AN, Nair GB, Chattopadhyay S. Gastrointestinal microbiome in the context of Helicobacter pylori infection in stomach and gastroduodenal diseases. "Human Microbiome in Health and Disease-Part B" in Progress in Molecular Biology and Translational Science. Elsevier. Ed. Das B and Singn V, Vol. 192. 2022.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Genomic and metagenomics insights on Helicobacter pylori virulence and antimicrobial resistance	Science and Engineering Research Board	2023	2 years	PI

Santhosh Kumar T . R, PhD
Scientist G
Cancer Research Program

BRIEF THEME OF LABORATORY

The major focus of the lab is understanding the diverse mechanisms of Drug resistance and tumor recurrence in cancer using preclinical and clinical model system.

LABORATORY STRENGTH

Project Assistant: 2 | Technical Assistant: 1 | Lab Assistant: 1
Project Associate: 1 | Post Doctoral Fellows: 3 |
PhD Students: 5

Front row (from left): Aman Halikar, Jain Tiffee, Prakash V, Aijaz Ahmad Rather
Back row (from left): Dr Kiran S Kumar, Dr Shine V J, Dr Aneesh C, Shivanshu Kumar Tiwari, Dr Unnikrishnan P S

REAL-TIME SIMULTANEOUS IMAGING OF TEMPORAL ALTERATIONS IN CYTOPLASMIC AND MITOCHONDRIAL REDOX IN SINGLE CELLS DURING CELL DIVISION AND CELL DEATH

Cytosolic and organelle redox are highly interrelated, and their alterations play critical roles in both physiological and pathological cell states. This highly regulated process is crucial in life-death decisions of cells. Among organelles, the mitochondrion is the major source of intracellular-ROS and contributes to oxidation damage-induced cell death. Increase in cytosolic-redox and mitochondrial-redox is evident in cells undergoing diverse forms of cell death, such as apoptosis, necrosis, and necroptosis. The hierarchical profiling of redox signalling at the cytosol and mitochondria in a single cell is important to understand the relative contribution of each species in the initiation and shaping of cell death. Researchers from RGCB developed a cellular system expressing two genetically encoded probes for redox with unique spectral property for real time tracking of redox alterations at cytoplasm and mitochondria simultaneously. The use of ratiometric redox GFP (roGFP) and intensity-based redox-sensitive RFP (rxRFP) targeted to mitochondria revealed both rapid and slow progressing changes in redox during cell division and in cells undergoing multiple modes of cell death. The study first time demonstrated that moderate increase in cytosolic ROS marks the division stage and distinct forms of cell death trigger unique and temporally regulated redox change at the cytosol and mitochondria. The approach is having potential utility to dissect the nature of cell death pathways induced by specific forms of stress or drugs

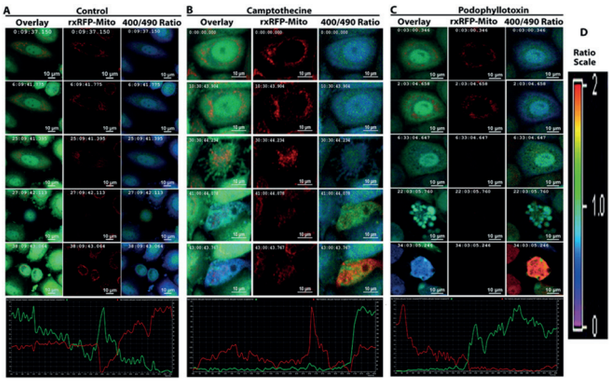


Figure 1: Real-time subcelluar redox alterations in untreated control and anti-cancer drugs. Real-time laser scanning confocal imaging for 47 hours with 30 minutes interval of (A) untreated control showing differential subcellular redox oscillation during cell division. (B) real-time imaging and quantification of Camptothecin (20 μM) (C) Podophyllotoxin (10 μM) induced redox alterations at cytosol and mitochondria.

PUBLICATIONS

- Chandrasekharan A, Varadarajan SN, Lekshmi A, Santhoshkumar TR. Real-time simultaneous imaging of temporal alterations in cytoplasmic and mitochondrial redox in single cells during cell division and cell death. Free RadicBiol Med. 2023;194:33-41.
- Parambil ST, Santhoshkumar TR , 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.
- Ravindran R, Velikkakath AKG, Narendradev ND, Chandrasekharan A, Santhoshkumar TR, Srinivasula SM. Endosomal-associated RFFL facilitates mitochondrial clearance by enhancing PRKN/parkin recruitment to mitochondria. Autophagy. 2022;18(12):2851-2864.

- Mohan AK, Minsa M, Santhoshkumar TR, Kumar GSV. Multi-Layered PLGA-PEI Nanoparticles Functionalized with TKD Peptide for Targeted Delivery of Pep5 to Breast Tumor Cells and Spheroids. Int J Nanomedicine. 2022;17:5581-5600.

- Tiwari SK, Sivasailam A, Maliakkal RT, Pillai PR, Surabhi SV, Prasad T, Santhoshkumar TR. Quantitative Analysis of Apoptosis and Necrosis in Live Cells Using Flow Cytometry. Methods Mol Biol. 2022; 2543:57-69.

- 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, Santhoshkumar 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.

- Varadarajan SN, Mathew KA, Chandrasekharan A, Lupitha SS, Lekshmi A, Mini M, Darvin P, Santhoshkumar TR. Real-time visualization and quantitation of cell death and cell cycle progression in 2D and 3D cultures utilizing genetically encoded probes. J Cell Biochem. 2022;123(4):782-797.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Establishment of fast life time imaging facility at Rajiv Gandhi Centre for Biotechnology	Department of Biotechnology	2019	4 years	PI
2.	Development of genetically encoded fluorescent single cell sensor for cell death and ACE2 RBD protein binding inhibition	Department of Biotechnology	2020	16 months	PI

Shijulal Nelson Sathi, PhD
Scientist C
Transdisciplinary Biology Program

BRIEF THEME OF LABORATORY

The bioinformatics laboratory is interested in studying the pangenome evolution of bacterial pathogens with a particular focus on a better understanding of the origin, adaptation, transmission, and evolution of antibiotic-resistant genes from an one-health perspective. We utilize sewage surveillance approaches coupled with NGS techniques and bioinformatics to identify novel strains/variants. Structural bioinformatics techniques are used to unravel both structural and functional implications of non-synonymous mutations occurring at the key targets

LABORATORY STRENGTH

Post Doctoral Fellows:1 | PhD Students:1 | JRF: 1
Technical Assistant: 1 | Project Associate: 1

HYBRID GENOME ASSEMBLY AND ANNOTATION OF MULTIDRUG-RESISTANT STAPHYLOCOCCUS AUREUS GENOTYPE ST672-SCCMEC TYPE IVD(2B)

We report complete genome sequence of multidrug-resistant S. aureus SCCmectypeIVd(2B) isolate, S. aureus S145. The whole-genome sequencing of S145 was performed using hybrid-genome approach and AR genes were detected and compared with publicly available S. aureus genomes. We obtained complete genome of S145 with 2.7 Mbp length, 32.8% GC content, and 2,548 protein-coding regions with 79 virulence factors and 90 AR genes. The S145 has 17-kb SCCmec, which encodes genes such as mecA, mecR1, ccrA2B2, and SCCmecIVd(2B) subtype gene CG002. We detected 30-kb multidrug-resistant plasmid with eight antibiotic-resistant genes forming three clusters. Cluster1 encoded for penicillin, Cluster2 for aminoglycoside-streptothricin, and Cluster3 for macrolides resistance genes. Comparative analysis of Cluster1-Cluster3 revealed that genetic organization of these clusters resembles resistance genes present in plasmids of USA300 S. aureus SCCmec type IVa strains (Figure 1). Our reported complete genome sequence of S. aureus SCCmecIVd(2B) can be used as a reference genome for further comparative genomic analysis.

EPIDEMIOLOGICAL MONITORING OF SARS-COV-2 AND ITS VARIANTS IN WASTEWATER SYSTEMS IN THE MAJOR CITIES OF KERALA, INDIA

Water samples were collected twice a week from ten pumping stations, two STPs and five open water sources in Thiruvananthapuram city. From October 31st, 2022 to March 23rd , 2023, a total of 303 samples were collected and RT-PCR has been done. 11 samples were found to be low positive and oxford nanopore sequencing has been done for all positive samples with CT value < 35 and found one Omicron variant and all other genomes were found as unclassified as purple; and the resistance genes (antibiotic and metal) are represented in blue. Inside the matrix, the presence of genes is represented by blue and their absence by green. The genes encoded within the plasmids of reported genomes are highlighted with an asterisk (*). Multidrug-resistance plasmid with eight antibiotic-resistance genes forming three distinct gene clusters, designated as Cluster1 (penicillin), Cluster2 (aminoglycoside-streptothricin), and Cluster3 (macrolides).

From left: Kiran H, Anand Mohan, Suba Kamakshi, Roshny Prasad, Binvy Varghese

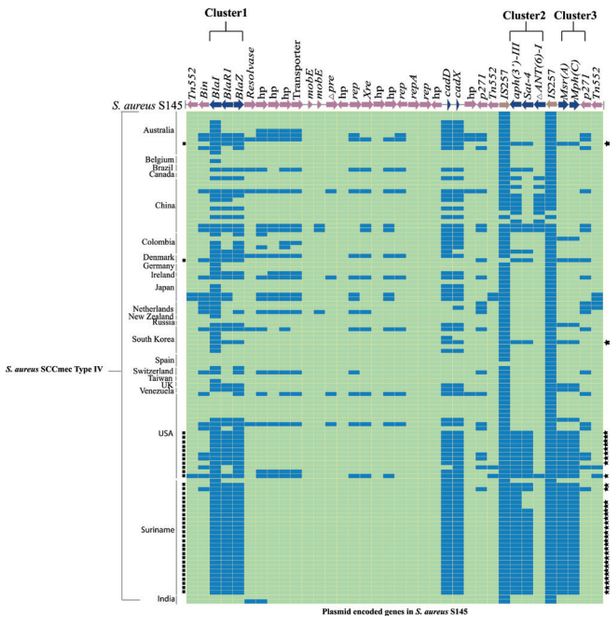


Figure 1. Distribution of 36 plasmid-encoded genes of Staphylococcus aureus S145 in 112 S. aureus SCCmec IV strains reported worldwide. X- axis represents the plasmid genes and Y- axis represents the 112 S. aureus strains sorted based on the respective countries. The first row represents the 36 annotated genes of S145 plasmid which are shown as arrows with direction representing the + and – strands. Insertion sequence elements are indicated in yellow; plasmid replication and its regulatory gene, transposons, and hypothetical genes are depicted

PUBLICATIONS

- 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.

- Nelson-Sathi S, Umasankar PK, Sreekumar E, Nair RR, Joseph I, Nori SRC, Philip JS, Prasad R, Navyasree KV, Ramesh S, Pillai H, Ghosh S, Santosh Kumar TR, Pillai MR. Mutational landscape and in silico structure models of SARS-CoV-2 spike receptor binding domain reveal key molecular determinants for virus-host interaction. BMC Mol Cell Biol. 2022;23(1):2.

- Prasad R, Ajith H, Kumar Chandrakumaran N, DnyaneshwarKhangar 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 BiomolStructDyn. 2022; 15:1-11.

- Lupitha SS, Darvin P, Chandrasekharan A, Varadarajan SN, Divakaran SJ, Easwaran S, Nelson-Sathi S, Umasankar PK, Jones S, Joseph I, Pillai MR, Santhoshkumar TR. A rapid bead-based assay for screening of SARS-CoV-2 neutralizing antibodies. AntibTher. 2022;5(2):100-110.

- Jesmina ARS, Induja DK, Drissya T, Sruthi CR, Raghu KG, Nelson-Sathi S, Kumar BNSAD, Lankalapalli RS. In vitro antibacterial effects of combination of ciprofloxacin with compounds isolated from Streptomyces luteireticuli NIIST-D75. J Antibiot (Tokyo). 2023;76(4):198-210.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Epidemiological Monitoring of SARS-CoV-2 and its variants in wastewater systems in the major cities of Kerala (Thiruvananthapuram), India	Science and Engineering Research Board	2022	1 year	PI
2.	Exploring the biomarkers and their pertinence to develop a biosensor for detecting antibiotic pollutant and their by-products in the marine organisms and aquaculture food products.	DBT MK BHAN Young Research Fellowship	2021	3 years	CO-PI
3.	Functional relevance of a unique subclass of Notch independent Hes-l (NIHes-l) expressing neural stem cells in developing/adult cortex	Science and Engineering Research Board	2022	3 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Jiffy John	Evolutionary genomics of Staphylococcus aureus: insights into the resistance trait	MAHE	Submitted	2023

Soniya E.V, PhD, FNASc
Scientist G
Transdisciplinary Biology Program

BRIEF THEME OF LABORATORY

Our lab focuses on understanding the molecular mechanisms working behind the interactions of plants with both biotic and abiotic factors, especially the plant-pathogen interactions and plant stress responses, and the molecular details of metabolic pathways for the production of secondary metabolites.

LABORATORY STRENGTH

Post Doctoral Fellows: 1 | PhD Students: 6 | JRF: 1
Technical Assistant: 1 | Lab Assistant: 2 | Project Associate: 1

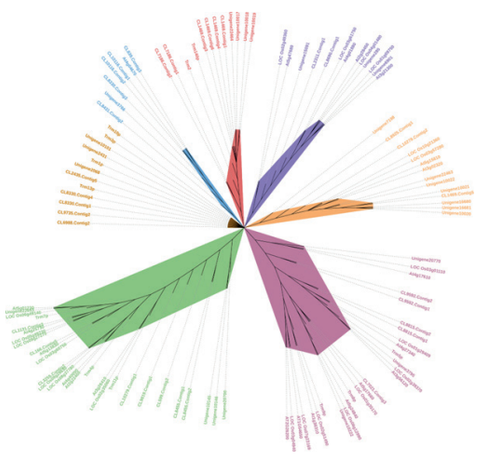
Front row (from left): Apsara I G, Sinsha Prakashan
Back row (from left): Akash P, Hima Parvathy A, Kalapriya V S, Amal T S

INTEGRATED OMICS APPROACH FOR REVEALING THE DEVELOPMENT AND DEFENCE REGULATION IN PIPER NIGRUM L. AND TO UNVEIL THE NOVEL TYPE III POLYKETIDE SYNTHASES INVOLVED IN THE PRODUCTION OF MEDICINALLY IMPORTANT NATURAL COMPOUNDS GENOME INDIA: CATALOGUING THE GENETIC VARIATION IN INDIANS

Piper nigrum, which is more often referred to as black pepper, is an medicinally and economically significant spice crop that is cultivated in most of the tropical regions. Phythophthoracapsici, an oomycete pathogen in black pepper causing severe yield loss is a major threat to this medicinally important crop. Our Laboratory focuses on developing Phythophthoracapsici resistant black pepper through various molecular approaches. We have identified and validated novel miRNA, lncRNAs and N6-methyladenosine modifications involved in fighting off the pathogens. A high throughput tRF-target cleavage validation was also done to understand the implication of tRFs in stress response. Concurrently, we are also exploring the function of small RNAs generated by Phythophthoracapsici during the encounter in an effort to uncover methods of reducing disease severity. We are also investigating the metagenomic profile of Piper spp. to understand its impact on disease resistance induction in resistant cultivars to susceptible cultivars to explore root rhizosphere and endospheric bacterial microbiome. Attempts were also made to manage the disease condition using green nanoparticles. The effect of colonisation by P. indica in Piper nigrum on growth advantages, floral induction, and evocation is also being carried out. Thus, our lab's extensive molecular discoveries will give unique strategies for combating this dreadful oomycete pathogen.

Our lab also investigates on identifying and modulating type III polyketide synthases that are involved in the production of therapeutically relevant secondary metabolites from medicinal plant Strobilanthesalternata and its endophytes. The genes involved in the synthesis of type III PKS will be studied using RNA sequencing methods, and the endophytic composition of microorganisms will be investigated using metagenomic approach. Novel Type III PKS identification from endophytes widen the area of research by opening a pavement for understanding the crosstalk and correlation of type III PKS in endophyte and its host.

Genome India is a National project with 20 participating Institutes to study the genetic variations that are present within the Indian population. The project aims at performing whole genome sequencing (WGS) of 10,000 individuals in order to create a detailed catalogue of genetic variations of representative population groups across the country with a goal to generate a reference genome for Indians. The data thus generated will be an all-encompassing national resource that will serve both needs of the scientific and medical community. RGCB collected the blood samples from 5 ethnic groups of Kerala.



The phylogenetic tree of MTase candidates from Black pepper

AWARDS [STUDENTS]

- Akash P, Best Oral Presentation, Genome mining: illuminating the antagonistic and plant growth-promoting potentials of Paenibacillussp., International Conference on Plant Biotechnology and Genome Editing, June 27 to 29, 2023, Jointly organized by Kakatiya University, Telangana, India and Aberystwyth University, Wales, UK.

- Akash P, Best Oral Presentation, Potential of Paenibacillus sp. as an antagonist in the biological control of quick wilt disease of black pepper, International Conference on Plant Genetic Engineering and Genome Editing , October 27 to 29, 2022, CUK, Kasaragod.

INVITED TALKS [PI]

- Fostering Resilience in Black Pepper by molecular omics approaches for combating quick wilt disease. International Conference on Plant Biotechnology and Genome Editing, June 27 to 29, 2023, Jointly organized by Kakatiya University, Telangana, India and Aberystwyth University, Wales, UK.

- Plant Biotechnology, Applications and Challenges, INTERNATIONAL WEBINAR ON BIOTECHNOLOGY - RECENT ADVANCES, CHALLENGES & OPPORTUNITIES (ICB'- 2022) December 14 – 16, 2022.

- Whole Genome and Transcriptome Data Analysis, Inter University Centre for Genomics and Gene Technology National Workshop on December 20-22, 2022 .

CONFERENCE PRESENTATION

- Apsara I G, Poster Presentation, Anendophytic fluorescent Pseudomonas sp. to enhance suppression of quick wilt disease, International Conference on Plant Genetic Engineering and Genome Editing , October 27 to 29, 2022, CUK, Kasaragod.

PUBLICATIONS

- Usha A, Kattupalli D, Viswam P, Bharathan S, Soniya EV. Phytophthoracapsici infection causes dynamic alterations in tRNA modifications and their associated gene candidates in black pepper. ComputStructBiotechnol J. 2022;20:6055-6066.

- Lekshmi RS, Sora S, Anith KN, Soniya EV. Root colonization by the endophytic fungus Piriformosporaindica shortens the juvenile phase of Piper nigrum L. by fine tuning the floral promotion pathways. Front Plant Sci. 2022 ;13:954693.

- Sreekumar S, Divya K, Joy N, Soniya EV. De novo transcriptome profiling unveils the regulation of phenylpropanoid biosynthesis in unripe Piper nigrum berries. BMC Plant Biol. 2022;22(1):501.

- Sruthi KB, Menon A, P A, Soniya EV. Pervasive translation of small open reading frames in plant long non-coding RNAs. Front Plant Sci. 2022;13:975938.

- Santhoshkumar R, AkashP, Viswam P, Soniya EV. Imprints of PGPB association on the metabolic dynamism of Piper nigrum. J Plant Interact 2022; 17(1), 967-979.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	DBT BUILDER: RGCB Interdisciplinary Life Science Program for Research Based Learning.	Department of Biotechnology	2020	3 years	PI
2.	Development of a disease management strategy against Phytophthoracapsici utilizing an effective biocontrol agent and green synthesized silver nanoparticles from black pepper	Department of Science & Technology	2019	3 years	PI
3.	GenomeIndia: Cataloguing the Genetic Variation in Indians	Department of Biotechnology	2019	3 years	PI

Sreeja S, Ph.D
Scientist F
Cancer Research Program

IDENTIFYING ROLE OF 14-3-3ζ IN ORAL CANCER AND IDENTIFYING UROLITHIN A AS A POTENT ANTICANCER AGENT IN ORAL SQUAMOUS CELL CARCINOMA

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer type among the head and neck cancer worldwide with a high frequency of recurrence and poor prognosis rate. In spite of significant development in multimodal approaches and treatment modalities, the overall survival rates remain a major concern, which is largely due to the lack of early detection markers. Hence, it is imperative to identify OSCC related biomarkers to improve the clinical outcomes in OSCC patients. Therefore, more insights into the underlying molecular mechanisms of OSCC progression and identifying potential therapeutic targets are crucial to improve the prognosis of OSCC patients.

14-3-3ζ has been demonstrated to be a promising biomarker to identify high-risk patients for more aggressive and alternative therapy at earlier stages of cancer progression. There are only very few reports demonstrating the significance of 14-3-3ζ over expression in oral cancer. Targeting pathways activated by 14-3-3ζ would accelerate the discovery of therapies that may be effective in patients with 14-3-3ζ overexpressing tumors. This is an area of research, which needs more focus and may bring the greatest benefit to patients with 14-3-3ζ overexpressing tumors. Additionally, 14-3-3ζ is known to modulate multiple downstream molecules of several survival signaling pathways. Therefore, we tried to explore 14-3-3ζ or the downstream pathways regulated by 14-3-3ζ that may sensitize cells to apoptosis and serve as effective anti-cancer strategies in patients whose tumors overexpress 14-3-3ζ. .The present study revealed that 14-3-3 ζ is highly expressed at the early stages of oral squamous cell carcinoma. Down-regulation of 14-3-3 ζ sensitized cells to stress induced apoptosis via p38-JNK pathway. Furthermore, 14-3-3 ζ promoted cytoskeletal remodeling of F-actin cytoskeleton and enhanced proliferation, migration and invasion of OSCC cell line via cytoplasmic p27 mediated Cdc42 activated Limkinase1. Our in vivo and ex vivo studies further confirmed that 14-3-3 ζ could serve as a novel target candidate for inhibiting OSCC proliferation and metastasis.

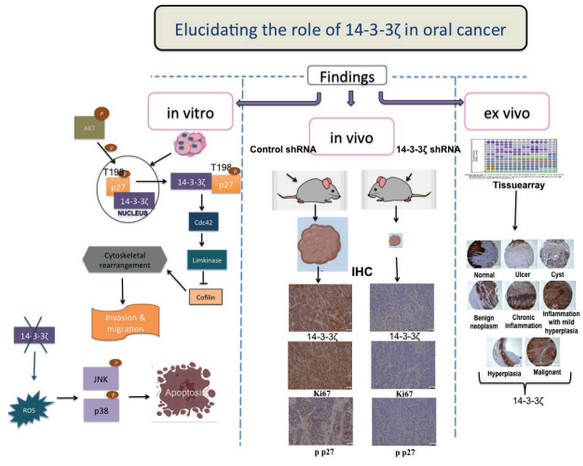
BRIEF THEME OF LABORATORY

Our laboratory investigated the mechanisms and pathways in estrogen and progesterone action in hormone driven cancers, mainly in breast and thyroid cancer. We also identified a potent estrogen modulator, urolithin A, which is a colon microbiota metabolite and established its potential to attenuate the endogenous Selective estrogen receptor modulator (SERM), 27-hydroxycholesterol. We have studied the effect of urolithin A in breast and oral cancer.

LABORATORY STRENGTH

JRF: 1 | SRF: 3 | Project Assistant: 2 | Project Associate:1

Front Row (from left): Juberiya M Azeez, Viji R, Sajitha T, Susmi T R
Back Row (From left): Anjana S S, Swathy Ravindran, Ayswarya R S, Vini Ravindran, Krishnendhu B



AWARDS [STUDENTS]

- Vini Ravindran, Best Oral Presentation, 27-hydroxycholesterol and its influence on epigenetic marks in breast cancer, 3rd World Congress on Translational Cancer Research and Immunotherapy [WCTCRI-2023], February 3-5, 2023 at Mahatma Gandhi Medical College and Hospital, Jaipur.

- Vini Ravindran, Best poster presentation, 27 Hydroxycholesterol represses G9a expression via Estrogen Receptor alpha in breast cancer, National symposium on Biotechnology organized by Rajiv Gandhi Centre for Biotechnology, June 27, 2022.

CONFERENCE PRESENTATION

- Vini Ravindran, Oral Presentation, 27-hydroxycholesterol and its influence on epigenetic marks in breast cancer, at 3rd World Congress on Translational Cancer Research and Immunotherapy [WCTCRI-2023] organized by Centre for Cancer Immunotherapy (CCI) held during February 3-5, 2023 at Mahatma Gandhi Medical College and Hospital, Jaipur.

PUBLICATIONS

- Vini R, Jaikumar V S, Remadevi V, Ravindran S, Azeez J M, Sasikumar A, Sundaram S, Sreeja S. Urolithin A: A promising selective estrogen receptor modulator and 27-hydroxycholesterol attenuator in breast cancer. Phytother Res. 2023 (Epub ahead of print).

- Vini R, Sreekumar S, Azeez J M, Sreeja S. Pomegranate Extract Protects Endothelial Cells from TNF-α Associated Damage. In conference BioSangam 2022: Emerging trends in Biotechnology (BIOSANGAM 2022) (pp. 276-289). Atlantis Press.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Elucidating the regulatory role of epigenetic players in 27 hydroxycholesterol (27HC) mediated breast cancer proliferation	Indian Council of Medical Research	2021	2 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Viji Remadevi	Elucidating the role of 14-3-3ζ in oral cancer and identifying urolithin A as potent anti-cancer agent for the treatment and prevention of oral squamous cell carcinoma	University of Kerala	Submitted	2023
2.	Juberiya M Azeez	Biphasic modulatory effect of progesterone signaling in breast cancer	University of Kerala	Submitted	2023
3.	Vini Ravindran	Aberrant epigenetic alterations induced by 27-hydroxycholesterol and identification of urolithin A as its attenuator in breast cancer	University of Kerala	Submitted	2023

Sreekumar E, PhD
Scientist F
Pathogen Biology Program

BRIEF THEME OF LABORATORY

Our laboratory focus on host-pathogen interaction studies on dengue and chikungunya, two major mosquito-borne virus diseases of the tropics. Our aim is to develop direct acting and host-targeted antivirals as well as disease modifiers

LABORATORY STRENGTH

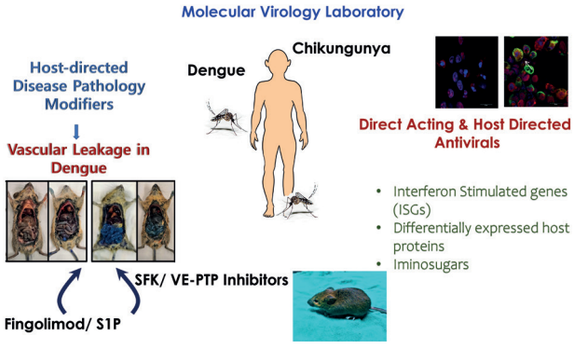
PhD Students: 6

From Left: Ayan Modak, Srishti Rajkumar Mishra, Parvanendhu Pradeep, Seethalakshmi S, Mansi Awasthi, Ariya Ajay, Guhan KS

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

With a focus to identify host targets for antiviral development, we studied Nucleophosmin (NPM1), which was a differential expressed cellular protein. We found that NPM1 played a role in restricting chikungunya virus infection. In our subsequent studies carried out last year, we further observed that this protein interacts with the CHIKV viral protein nsP3 and that the key residues in the macrodomain of the viral protein that are determinants of CHIKV virulence, interact with NPM1. Our observations have implications in developing antiviral molecules that will target either the NPM1 or its counterpart nsP3 macrodomain. In another approach to identify host viral restriction factors, we had developed assyas to evaluate Interferon-stimulated Gene modulators; and using these assays we screened a flavonoid library to identify a few promising hits. Recently, a family of antiviral cellular proteins named the Surface-Hinged, Rigidly-Extended Killer (SHREK) was identified. These proteins are also reported to demonstrate broad-spectrum host antivirals by blocking the infection of a diverse family of viruses. The mechanism underlying the action of the SHREK family of proteins is being explored.

Vascular leakage is a major complication of severe dengue; and our studies for the last several years identified key endothelial cell pathways that are involved in regulating the endothelial permeability upon dengue virus infection. We found that targeting these pathways are promising in alleviating the enhanced permeability in both cellular as well animal models of dengue infection. In a newly developed AG129 mice model of dengue, we found that treatment with sphingosine analogues such as FTY720 restored vascular integrity in DENV infected animals. These studies are expected to identify disease modifiers that addresses the key pathophysiological changes of the disease rather acting as antivirals.



AWARDS [STUDENTS]

- Srishti Rajkumar Mishra: Won best poster award presented in National Symposium on Biotechnology for Sustainable development-2022 conducted on July 27, 2022 at RGCB, Thiruvanathpuram.
- Parvanendhu Pradeep: Best poster in National Symposium on Biotechnology for Sustainable development- 2022 conducted at RGCB, Thiruvananthapuram.
- Parvanendhu Pradeep : First prize in the Turbo Talk competition at the MAB-2022 workshop held at the Institute of Advanced Virology, Thiruvananthapuram from December 8–9, 2022.

CONFERENCE PRESENTATION

- Srishti Rajkumar Mishra; Poster Presentation, in National Symposium on Biotechnology for Sustainable development-2022 conducted on July 27, 2022 at RGCB, Thiruvanathpuram.

- Parvanendhu Pradeep; Poster Presentation, in National Symposium on Biotechnology for Sustainable development-2022 conducted at RGCB, Thiruvananthapuram on 27 July 2022.

- Ayan Modak: Poster Presentation, FTY alleviates Dengue virus induced hyperpermeability in HMEC-1 Cells and provides better survivability in as AG129 mice model. Poster presented at National Symposium on Biotechnology for Sustainable Development – 2022 at RGCB, Thiruvananthapuram on 27 July 2022.

- Ayan Modak: Poster Presentation, Evaluation of the effect of Sphingosine receptor modulation in Dengue virus induced vascular permeability. Poster presented at IUBMB Focused Meeting on Biochemistry & Molecular Biology of RNA Viruses, from 15 – 18 November 2022, Regional Centre for Biotechnology, Faridabad.

- Paravenendhu Pradeep, Oral Presentation, Cytoplasmic role of Nucleophosmin (NPM1/B23) in restricting Chikungunya virus replication in human astrocytic cells. American Society for microbiology, ASM-Microbe 2022,Washington DC,US, June 9-13,2022.

PUBLICATIONS

- Nair SR, Abraham R, Sreekumar E. Generation of a Live-Attenuated Strain of Chikungunya Virus from an Indian Isolate for Vaccine Development. Vaccines (Basel). 2022; 10(11):1939.

- Chandran D, Sreekumar E, Prajitha KC, Sharahudeen A, Raveendran CL; Research team. Breakthrough infection with SARS-CoV-2 delta variant in old-age homes in a Southern District of Kerala, India. Indian J Public Health. 2022;66(Supplement):S36-S40.

ALTERED FLOW-INDUCED ENDOTHELIAL MESENCHYMAL PLASTICITY IN THE PATHOGENESIS OF CEREBRAL ARTERIOVENOUS MALFORMATIONS

Cerebral arteriovenous malformations (cAVM) are a significant cause of intracranial hemorrhagic stroke and brain damage. The arteriovenous junctions in AVM nidus are known to have hemodynamic disturbances such as altered shear stress, which could lead to endothelial dysfunction. The molecular mechanisms coupling shear stress and endothelial dysfunction in cAVMs are poorly understood. We speculated that disturbed blood flow in artery-vein junctions activates Notch receptors and promotes endothelial mesenchymal plasticity during cAVM

Sumi S, PhD
Program Scientist
Cardiovascular Diseases & Diabetes Biology Program

BRIEF THEME OF LABORATORY

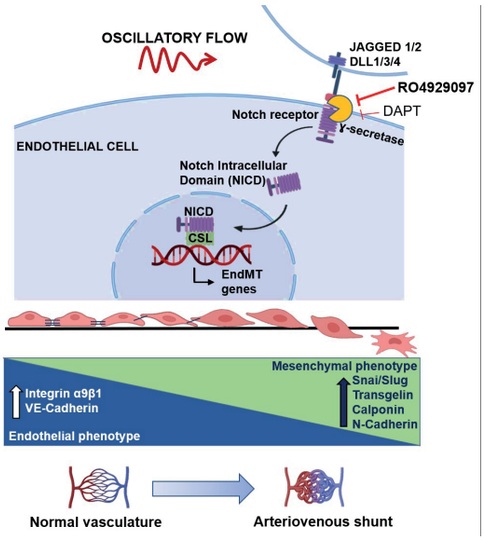
The unifying theme of our research program is to delineate the molecular mechanisms underlying endothelial dysfunction, and their impact on vascular disease pathogenesis. We aim to address the following aspects.

Vascular mechanobiology in arteriovenous malformations in brain and chronic venous diseases
Endothelial responses to altered biomechanical forces, e.g. fluid shear stress
Hemodynamic shear stress sensing and signal transduction, and their molecular mechanisms

LABORATORY STRENGTH

SRF: 2 | PhD Students: 1

formation. We found evidence for endothelial to mesenchymal transition (EndMT) and enhanced expression of activated Notch intracellular domain (NICD3 and NICD4) in human AVM nidus samples. The expression of transmembrane adhesion receptor integrin α9/β1 is significantly reduced in cAVMnidal vessels. Cell-cell adhesion proteins such as VE-cadherin and N-cadherin were differentially expressed in AVM nidus compared with control brain tissues. Using well-characterized hCMECs, we show that altered fluid shear stress steers Notch3 nuclear translocation and promotes SNAI1/2 expression and nuclear localization. Oscillatory flow downregulates integrin α9/β1 and VE-cadherin expression, while N-cadherin expression and endothelial cell invasiveness are augmented. Gamma-secretase inhibitor RO4929097, and to a lesser level DAPT, prevent the mesenchymal transition and invasiveness of cerebral microvascular endothelial cells exposed to oscillatory fluid flow. Our study provides, for the first time, evidence for the role of oscillatory shear stress in mediating the EndMT process and dysregulated expression of cell adhesion molecules, especially multifunctional integrin α9/β1 in human cAVM nidus. Concomitantly, our findings indicate the potential use of small-molecular inhibitors such as RO4929097 in the less-invasive therapeutic management of cAVMs.



Schematic diagram demonstrating the role of oscillatory shear stress in modulating Notch-mediated endothelial mesenchymal plasticity in cerebral arteriovenous malformations

From left: Jeeva Prasannan, Sreelakshmi B J, Jayalekshmi V S, Ahalya S, Karthika C L

AWARDS [PI]

- RGCB Best Teacher Award in 2022.

INVITED TALKS [PI]

- System Genetics Approaches to Comprehend Metabolic Reprogramming in Heart Failure. The Heart Failure Conflux, 2023, held virtually from 4th-5th Feb 2023.

CONFERENCE PRESENTATION

- Sreelakshmi B J, Poster Presentation, Disturbed hemodynamics activates aberrant endothelial notch signaling via mechanosensitive Ets-1 in varicose veins. Advances in Cardiovascular Medicine and Research (ACMR-2023) held at PGIMER, Chandigarh, 16-18 February, 2023.

PUBLICATIONS

- Karthika CL, Vani Venugopal, Sreelakshmi BJ, Krithika S, Jaya Mary Thomas, Mathew Abraham, Kartha CC, Rajavelu A, Sumi S. Oscillatory shear stress modulates Notch-mediated endothelial mesenchymal plasticity in cerebral arteriovenous malformations. Cell MolBiol Lett. 2023 28:22.

- Thomas JM, Sasankan D, Abraham M, Sumi S, Kartha CC, Rajavelu A. DNA methylation signatures on vascular differentiation genes are aberrant in vessels of human cerebral arteriovenous malformation nidus. ClinEpigen. 2022 13; 14(1):127.

- Sumi S and Kartha CC. Genetic and Epigenetic Regulation by Gut Microbe-Modulated Metabolites in Chronic Metabolic Diseases. In: Thomas, S. (eds) Human Microbiome. Springer, Singapore. 2022. 110-126.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Do epigenetic alterations in shear stress regulatory genes induce endothelial mesenchymal transition in patients with cerebral arteriovenous malformations?	Indian Council of Medical Research	2019	3 years	PI

2.	Molecular Pathogenesis of varicose veins	Dr N Radhakrishnan Foundation for Research on Venous Diseases	2018	6 years	PI
3.	Piezo-KLF2 axis in endothelial dysfunction and venous wall remodelling in varicose veins	Science and Engineering Research Board	2021	3 years	PI

Sunil Martin, PhD
Scientist E-II
Cancer Research Program

BUILDING IMMUNITY TO CANCER

We are generating synthetic receptors to study the biology of immune effector αβ and γδ T cells to boost the specific and durable immune response. The focus of the lab is currently on investigating the CARs targeting CD19 –a pan B cell malignancy marker- to augment the antigen specificity of T cells. CARs are synthetic receptors with an antigen-binding ectodomain, a co-stimulatory domain, and an endodomain derived from CD3ζ, which signals on behalf of the TCR. This modular design facilitates HLA-independent antigen recognition and robust T-cell effector functions due to the judicious integration of multiple signaling domains. Although promising, the utility of CAR therapy across Indian health systems is limited due to its untenable price tag. On the other hand, off-tumor effect and cytokine toxicity remain as major biological limitations that need to address at the level of receptor design. We have previously generated a workflow for the expansion of CD19 CAR T cells in serum-free media and characterized its antitumor function against B cell Acute lymphoblastic leukemia (B-ALL) cell systems. Because the hinge and transmembrane domains of CAR influence the efficacy and toxicity, we are now testing various configurations of CAR T cells. We have established a clean room and laboratory facilities for generating and testing CAR T cells in vitro. Since γδ T cells can recognize tumor cells in an HLA-independent manner, we are planning to develop 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

BRIEF THEME OF LABORATORY

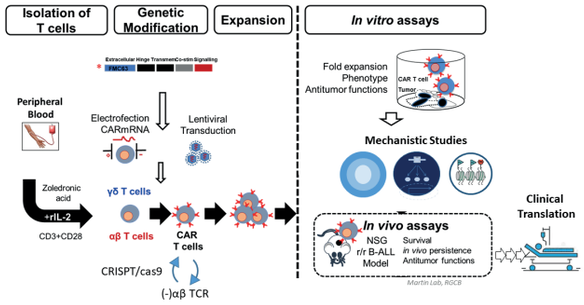
Our laboratory aims to apply synthetic immunology approaches to reprogram the antitumor immunity of T cells.

LABORATORY STRENGTH

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

Left to right: Abhijith K,Muhammad Shafi, Muthuvel Muthuganesh, Praseetha N G, Anjitha S

Work flow for T cell engineering



The workflow for the generation and charecterization of CAR T cells are illustrated

INVITED TALKS [PI]

- Cancer Immunity and immunotherapy. Advanced Workshop on Immunology Concepts & Techniques' on 31st March & 1st April, Department of Clinical Immunology & Rheumatology, Christian Medical College, Vellore, India.
- Immune Cell engineering and Therapy. Advanced Workshop on Immunology Concepts & Techniques' on 31st March & 1st April, Department of Clinical Immunology & Rheumatology, Christian Medical College, Vellore, India.

PUBLICATIONS

- Singh A, Alexander SG, Martin S. Gut microbiome homeostasis and the future of probiotics in cancer immunotherapy. Front Immunol. 2023;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;72(2):277-286.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Engineering Natural Killer Cells and γδ T cells with CD19 CAR for adoptive immunotherapy	Department of Biotechnology	2018	5 years	PI
2.	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
3.	Engineering of anti-CD37 CAR T cells to target B cell Non-Hodgkin's Lymphoma (B-NHL)	Science and Engineering Research Board	2022	2 years	PI

THE CYTOSKELETAL PROTEIN FODRIN IN MICROTUBULE NUCLEATION AND APOPTOSIS

α-fodrin as a modulator of γ-tubulin mediated microtubule nucleation

Fodrin is a protein mostly known for its role in the maintenance of structural integrity in eukaryotic cells. However, we have earlier shown that fodrin interacts with the microtubule nucleator γ-tubulin in neural cells.

Suparna Sengupta, PhD
Scientist G
Cancer Research Program

BRIEF THEME OF LABORATORY

Cytoskeleton research and the involvement of cytoskeletal elements in cancer

LABORATORY STRENGTH

Post Doctoral Fellows: 1 | PhD Students: 6 | JRF: 1
Technical Assistant: 1 | Lab Assistant: 2 | Project Associate:1

From left: Athira SS, Athira Jyothy, Julfequar Hussain

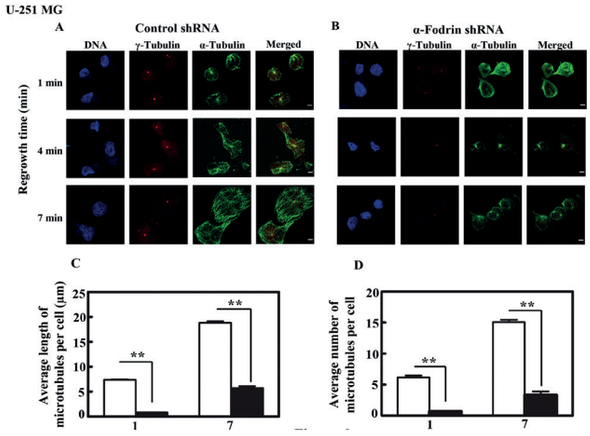
To understand the cellular significance of α-fodrin – γ-tubulin interaction, we show that depletion of α-fodrin brings in a significant reduction of γ-tubulin in neural cell centrosomes making it functionally under-efficient. This causes a loss of nucleation ability that cannot efficiently form microtubules in interphase cells and astral microtubules in mitosis. Fluorescence Recovery after Photobleaching (FRAP) experiment implies that α-fodrin is important in the recruitment of γ-tubulin to the centrosome resulting in the aforementioned effects. Further, our experiments indicate that the interaction of α-fodrin with certain pericentriolar matrix proteins such as Pericentrin and CDK5RAP2 are crucial for the recruitment of γ-tubulin to the centrosome. Earlier we reported that α-fodrin limits the nucleation potential of γ-TuRC. In that context, this study suggests that α-fodrin is a γ-tubulin recruiting protein to the centrosome thus preventing cytoplasmic microtubule nucleation and thereby compartmentalizing the process to the centrosome for maximum efficiency (Figure).

α-fodrin in cancer and apoptosis

The other function that we showed earlier was the role of α-fodrin in chromosome congression and mitotic organization through the mitotic kinesin CENP-E. Based on this role, we hypothesize that α-fodrin should have a role in cancer and apoptosis. A global proteomics approach also suggests that. From an early tissue array analysis, we find that in a pancreatic cancer tissue array, α-fodrin level increases with the progression of cancer. Further, depletion of α-fodrin causes an increase in the level of apoptosis induced by a known apoptosis inducer in pancreatic cancer cells. We are testing this result now in other pancreatic cancer cell lines. This is also being tested in xenograft tumours in SCID/NOD mice. We have also checked that α-fodrinand CENP-E interacts with each other in pancreatic cancer cells. We are now trying to prove the interaction by confocal microscopy. α-fodrin depletion, however, reduced CENP-E level in pancrearic cancer cells and tissues. By TCGA analysis, we have found that CENP-E and SPTAN1 together suffer from survival disadvantage, which means that cancer patients would survive the least when both CENP-E and α-fodrin are highly expressing. By bioinformatics analysis also, α-fodrin and CENP-E were found to interact. The interaction site has been docked.

Purification of small sized exosomes by low speed centrifugation in two and half hours

We have also purified small sized exosomes from human serum by conjugating exosome specific CD 63 antibody with novel gold nanoparticles. These exosomes, characterized by the presence of CD63, CD9 and CD81, and sized between 20 nm to 50 nm, was confirmed by western blot, dynamic light scattering (DLS), transmission electron microscopy (TEM) and nanoparticle tracking analyser (NTA). These exosomes will be used in liquid biopsy for diagnostic purposes.



Reduced regrowth of microtubules from the centrosomes in α-fodrin depleted cells. Confocal images of U-251 MG cells fixed after 1, 4 and 7 min of regrowth in (a) control shRNA and (b) α-fodrinshRNA treated cells. Bar graph showing the (c) average length of microtubules per cell and (d) average number of microtubules per cell in control shRNA (white bar) and α-fodrinshRNA (black bar) treated cells at 1 and 7 min

CONFERENCE PRESENTATION

- Athira Jyothy, Poster Presentation, Role of Fodrin in the Progression of Cancer and Apoptosis - National Symposium on Biotechnology for sustainable development on 27th July, 2022 at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695014 .
- Athira Jyothy, Role of Fodrin in the Progression of Cancer and Apoptosis - 42nd Annual Conference of the Indian Association of Cancer Research on 16-20 January 2023 at ACTREC, Navi Mumbai.

PUBLICATIONS

- Jamuna S. Sreeja, Athira Jyothy, Rohith Kumar Nellikka, SayanGhorai, Paul Ann Riya, Jackson James &SuparnaSengupta.* The centrosomal recruitment of γ-tubulin and its microtubule nucleation activity is α-fodrin guided. Cell Cycle 2023; 22(3):361-378.
- Maliekal TT, Dharmapal D and Sengupta S. Tubulin Isotypes: Emerging Roles in Defining Cancer Stem Cell Niche. Front. Immunol. 2022; 13:876278.
- Pammi Guru KT, Sreeja JS, Dharmapal D, Sengupta S, Basu PK. Novel Gold Nanoparticle-Based Quick Small-Exosome Isolation Technique from Serum Sample at a Low Centrifugal Force. Nanomaterials (Basel). 2022;12(10):1660.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Exploration of the role of fodrin, a protein required in functional microtubule organization, in cancer and apoptosis	Indian Council of Medical Research	2021	3 years	PI

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Dhrishya Dharmapal	Deciphering the Role of Tubulin β4B in Self Renewal of Cancer Stem Cells	University of Kerala	Awarded	2022

THE CANONICAL AND NON-CANONICAL TGF-β PATHWAYS REGULATE INTEGRIN α5 THAT BLOCKS THE DIFFERENTIATION OF ORAL CANCER STEM CELLS

Cancer stem cells (CSCs), a subset of cancer cells possessing self-renewal ability, are found to be the key players for the tumour relapse. Targeting the signalling pathways that play an important role in the regulation of CSCs would pave way to the development of potential drugs that helps in better prognosis. Phosphoproteomic analysis for the pathways specifically activated in self-renewal condition of oral cancer stem cells revealed the involvement of Integrin α5 and TGF β signalling pathway. It is also known that TGF β regulates Integrin signalling in different context. Here, we attempted to decipher how TGF β modulates Integrin α5 in the regulation of CSCs. We also explored the involvement of the canonical and non-canonical TGF β components in the process. The canonical and non-canonical component chosen for the study was SMAD4 and TIF1γ, respectively. Oral cancer cell line HSC-3, expressing ALDH1A1 DsRed2, reporting CSCs were used for the study. ShRNA –mediated

Tessy Thomas Maliekal, PhD
Scientist E-II
Cancer Research Program

BRIEF THEME OF LABORATORY

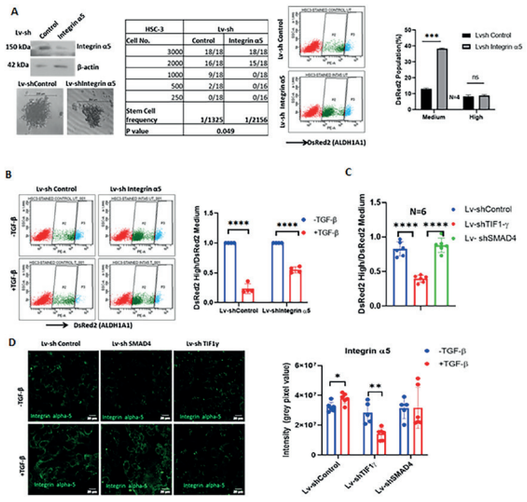
The focus of my lab is to understand the role of different signaling pathways in cancer. Primarily the lab focusses on a transcriptional regulator, TIF1γ, and explore its upstream and downstream regulator molecules in cancer stem cells (CSCs). We have seen that besides TIF1γ, Eph/Ephrin pathway is essential for the maintenance of oral cancer CSCs. Also, we attempt to decipher the molecular regulation of JNK/AP1 signaling induced by a novel apoptosis inducing peptide, SSTP1.

LABORATORY STRENGTH

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

Front row (from left): Zubair Amad Mir, Jannet S, Reshma Raj R
Back row (from left): Padmaja K P, Gayathri Mohan

knock-down was employed to study the role of Integrin α5, SMAD4 and TIF1γ. Serial dilution sphere formation assay was employed to check the self-renewal ability of cancer cells whereas FACS analysis was performed to study the DsRed2 population of CSCs. Our results show that loss of TGF-β, TIF1γ or Integrin α5 reduces stem cell pool and induces their differentiation. Immunofluorescence assay revealed that the TGF-β-mediated surface localization of Integrin α5 is found to be more dependent on TIF1γ, a non-canonical component of TGF β signalling pathway while its total expression is dependent on the canonical mediator, SMAD4. The effect of TUBB4B which is found to have a role on transportation of membrane proteins to plasma membrane was also studied and the results suggests that TGF-β-mediated surface localization of Integrin α5 might be regulated through TUBB4B, which warrants further validations.



A) Loss of Integrin α5 leads to lo reduced sphere formation efficiency and CSC frequency due to the differentiation of CCS. B) Depletion of Integrin α5 attenuates the TGF-β-mediated arrest of CSC differentiation C) Knock-down of TIF1γ abolish the TGF-β-induced block of CSC differentiation D) The surface localization of Integrin α 5 depends on TIF1γ

INVITED TALKS [PI]

- Tumor markers in Squamous cell carcinoma with special reference to early invasive oral cancer, 19th KSOMP Annual conference, Indira Gandhi institute of Dental Sciences, Cochin, March,5th, 2023.

CONFERENCE PRESENTATION

- Padmaja K P, Poster Presentation, A dual reporter system to identify Cancer Stem Cell hierarchy,., 42nd Annual Conference of the Indian Association for Cancer Research,12-16th January 2023, ACTREC, Navi Mumbai.

- Gayathri Mohan, Poster Presentation, A novel host defence peptide, SSTP1, alters IL6 pathway to induce apoptosis in Triple Negative Breast Cancer (TNBC), 42nd Annual Conference of the Indian Association for Cancer Research,12-16th January 2023, ACTREC, Navi Mumbai.

- Reshma Raj R, Poster presentation, EphA2/Ephrin B1 signaling supports cancer stem cells leading to worse prognosis in oral carcinoma, 42nd Annual Conference of the Indian Association for Cancer Research,12-16th January 2023, ACTREC, Navi Mumbai.

PATENTS APPLIED/ GRANTED

- Apoptosis inducing peptide (SSTP1), Patent Application No. 17/919,130, USA 14th October 2022.

PUBLICATIONS

- Riya PA, Basu B, Surya S, Parvathy S, Lalitha S, Jyothi NP, Meera V, Jaikumar VS, Sunitha P, Shahina A, Sukumaran R, Nair AS, Dhanesh SB, Jiffy J, Nelson-Sati S, Maliekal TT, Das AV, James J. HES1 promoter activation dynamics reveal the plasticity, stemness and heterogeneity in neuroblastoma cancer stem cells. J Cell Sci. 2022;135(22):jcs260157.

- Maliekal TT, Dharmapal D, Sengupta S. Tubulin Isotypes: Emerging Roles in Defining Cancer Stem Cell Niche. Front Immunol. 2022;13:876278.

- Senapati P, Bhattacharya A, Das S, Dey S, Sudarshan D, G S, Vishwakarma J, Sudevan S, Ramachandran R, Maliekal TT, Kundu TK. Histone Chaperone Nucleophosmin Regulates Transcription of Key Genes Involved in Oral Tumorigenesis. Mol Cell Biol. 2022;42(2):e0066920.

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Amrutha Mohan	Evaluation of TIF1γ as a mediator of self-renewal of cancer stem cells in Oral Squamous Cell Carcinoma	MAHE, Manipal	Submitted	2023

Umasankar P.K , PhD
Ramalingaswami Faculty Fellow
Transdisciplinary Biology Program

REGULATORY MECHANISMS OF CLATHRIN-MEDIATED ENDOCYTOSIS IN VERTEBRATES

Clathrin-mediated endocytosis (CME) is vital for the internalization of nutrients, signals, lipids and pathogens 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 initiation mechanisms of CME in vertebrates with the hope of designing novel inhibitors and activators that could regulate the process. We use cultured cells and zebrafish model for our investigations.

The heterotetramericclathrin 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. Phosphorylation of a T156 residue in the µ subunit of AP-2 (T156µ) is considered a critical regulatory step in CME. Recently, we identified a novel kinase, BMP2K that phosphorylates T156µ AP-2 and promotes CME in cells and in zebrafish embryos (Ramesh et al., 2019, Ramesh et al., 2021). BMP2K has an N-terminal kinase domain, a Q/H- rich prion-like domain which can possibly undergo liquid-liquid phase separation followed by a long unstructured C-terminus (CT). Our biochemical experiments revealed a highly conserved and novel, 22-amino acid short linear motif (SLiM) within CT which interacts with the C-domain of AP-2 µ subunit. Currently, we are studying in vivo significance of this interaction using BMP2K gene-edited cells and zebrafish embryos (Ramesh et al, 2023).

Another major project in the lab investigates role of CME in regulation of cell growth. Along these lines, we discovered that LDL-cholesterol internalized via CME supports insulin-induced mTORC1 signaling on lysosomes for cell growth. We also 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. This defect can be rescued by supplementing cholesterol and insulin or by expressing constitutively active Rag GTPase suggesting novel therapeutic strategies for SLOS patients (Navyasree et al., 2023).

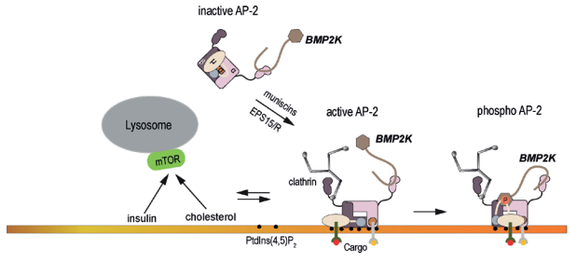
BRIEF THEME OF LABORATORY

The central theme of our Intracellular Trafficking lab is to understand the regulatory mechanisms of clathrin-mediated endocytosis (CME), a fundamental process for the internalization of macromolecules in eukaryotes. 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 cells and the zebrafish model for our investigations.

LABORATORY STRENGTH

Project Assistant: 1 | PhD Students: 2

From left: Navyasree K V, Shikha Ramesh T



Schematic depiction of various mechanisms of CME regulation and role of CME in the lysosomal integration of mTORC1 to drive cell growth

INVITED TALKS [PI]

- Phosphoregulation of clathrin-mediated endocytosis. Autophagy India Network (AIN) meeting, 17-19th February, 2023, CSIR-Institute of Microbial Technology (IMTECH), Chandigarh.

CONFERENCE PRESENTATION

- Shikha Ramesh, Poster Presentation, Understanding the role of BMP-2 inducible kinase in clathrin-mediated endocytosis. 45th All India Cell Biology Conference and International Symposium of Biology of Development and Disease.20-23rd Jan 2023, Banarus Hindu University, Varanasi.
- Navyasree K. V, Poster Presentation, Cholesterol regulates insulin-dependent mTORC1 growth signalling. International Conference on advances in molecular diagnosis and precision medicine. 15th-17th September 2022, Anna University, Chennai.
- Navyasree K. V, Poster Presentation: Cholesterol regulates insulin-dependent mTORC1 growth signalling. Frontier Symposium in Biology FS-BIO 2023.17th-19th March 2023. IISER, Thiruvananthapuram.

PUBLICATIONS

- Zaccai NR, Kadlecova Z, Dickson VK, Korobchevskaya K, Kamenicky J, Kovtun O, Umasankar PK, Wrobel AG, Kaufman JGG, Gray SR, Qu K, Evans PR, Fritzsche M, Sroubek F, Höning S, Briggs JAG, Kelly BT, Owen DJ, Traub LM. FCHO controls AP2's initiating role in endocytosis through a PtdIns(4,5)P2-dependent switch. Sci Adv. 2022;8(17):eabn2018.

ONGOING GRANTS

Sl No.	Title	Funding Agency	Year of Starting	Duration	PI/CO-PI
1.	Endocytic modulation of BMP signaling: deciphering mechanistic insights into health and disease	Department of Biotechnology	2016	10 years	PI

(A) DEVELOPMENT OF A NOVEL THREE DIMENSIONAL SELF AGGREGATING PEPTIDE FIBER AS AN IMPLANT FOR BRAIN TUMORS

(B) A STABLE PCL-PEG NANOMICELLES BRIDGED BY MALEIC ANHYDRIDE FOR DRUG RELEASE TO BREAST CANCER

G. S. Vinod Kumar, PhD
Scientist F
Cancer Research Program

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. 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.

LABORATORY STRENGTH

PhD Students: 6

From left: Vipin C L, Naresh Goud, Arsha U P, Jyothilakshmi V A

(A) Development of a novel three dimensional self aggregating peptide fiber as an implant for brain tumors

We have reported a novel self-aggregating peptide hydrogel with the combination of drug entrapped nanoparticles targeted to the neuropilin receptors on the cell surface to inhibit the post-surgical recurrence of GBM. The peptide sequence was designed from the logic that amphiphilicity can lead to secondary structure formation and results in hydrogel. The MD simulation studies showed self-aggregation with 10mer peptide fibers forms beta sheet confirmed with the RMSD graph showing lowest energy curves. The synthesized peptide was characterized and was checked gelation in different buffers. The designed peptide showed good gelation behavior and drug holding capacity being an excellent candidate for the post-surgical implant system. The rheological analysis showed the stability of the peptide hydrogel at shear stress and strain. The hydrogel can mimic the brain tissue since its having the similar rheological properties, hence the implant system can be used to reduce the shocks and pressure difference created in the brain at the resection site. The local application of the hydrogel with drug entrapped targeted nanoparticles gives much better results in both in vitro and in vivo experiments. The main advantage in the peptide hydrogel as an implant system when compared to the available hydrogels is it is biocompatible and reduces any post surgical shocks created at the site.

(B) A stable PCL-PEG nanomicelles bridged by maleic anhydride for drug release to breast cancer

Breast cancer is the primary cause of cancer related death in the feminine population across the globe. Polycaprolactone (PCL)-Polyethylene glycol (PEG) co-polymer micelles have been described for different drug delivery applications for breast cancer. Herein, to deliver paclitaxel (PTX), a stable nanomicelle was developed using a maleic anhydride bridged PCL-PEG co-polymer (PCGM) synthesised by polycondensation reaction. PTX entrapped nanomicelles (PTX-PCGM) were prepared by a thin film hydration method. The spherical PTX-PCGM nanomicelles had a diameter of 235 nm with a negative surface charge, which facilitated their long-term circulation in the blood stream. Additionally, the PTX-PCGM nanomicelles showed an excellent stability in PBS over a period of 30 days. In vitro characterization and cellular experiments showed that the nanomicelles entered MDA-MB-231 cells via passive targeting and cytotoxicity of PTX was significantly increased by an enhanced stabilization of tubulin. A steady and sustained release was observed in NOD-SCID mice bearing MDA-MB-231 breast cancer. Moreover, the administration of PTX-PCGM exhibited a superior anticncer activity in vivo with a tumor growth inhibition rate of 90.6% with no side effects in major organs. In conclusion, PTX-PCGM nanomicelles could serve as a potential tool for breast cancer therapy (Figure 1).

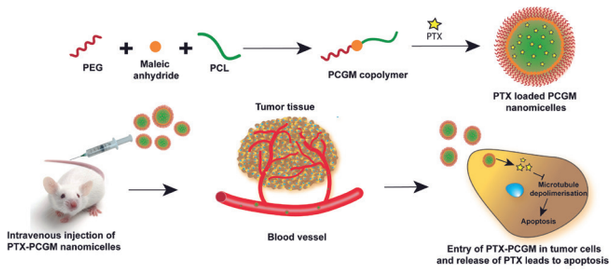


Figure 1: The schematic illustration of synthesis of PCGM and the model of PTX-PCGM nanomicelles in inhibiting the tumor growth in human breast cancer cells.

INVITED TALKS [PI]

- Modern Trends in Biotechnology, Two day International Conference on New Frontiers and Developments in Chemistry. Jan 9 2023, Department of chemistry, Catholicate College, Pathanamthitta.

PUBLICATIONS

- Wanjale MV, Sunil Jaikumar V, Sivakumar KC, Ann Paul R, James J, Kumar GSV. Supramolecular Hydrogel Based Post-Surgical Implant System for Hydrophobic Drug Delivery Against Glioma Recurrence. Int J Nanomedicine. 2022;17:2203-2224.

- Nanditha CK, Kumar GSV. Bioactive peptides laden nano and micro-sized particles enriched ECM inspired dressing for skin regeneration in diabetic wounds. Mater Today Bio. 2022;14:100235.

- Mohan AK, M M, Kumar TRS, Kumar GSV. Multi-Layered PLGA-PEI Nanoparticles Functionalized with TKD Peptide for Targeted Delivery of Pep5 to Breast Tumor Cells and Spheroids. Int J Nanomedicine. 2022;17:5581-5600.

- Chirayil TJ, Kumar GSV. Sorafenib-Entrapped, Self-Assembled Pullulan-Stearic Acid Biopolymer-Derived Drug Delivery System to PLC/PRF/5 Hepatocellular Carcinoma Model. Int J Nanomedicine. 2022;17:5099-5116.

PhD AWARDED

Sl No.	Name of the Students	Title of Thesis	University	Awarded/ Submitted	Year
1.	Wanjale Mrunal Vitthal	Development of drug releasing implant systems for post-surgical treatment of glioma	University of Kerala	Awarded	2022
2.	Nanditha C.K	Development of novel therapeutic approach for chronic skin wounds	University of Kerala	Awarded	2022
3.	Teena Jacob Chirayil	Development of novel prototype nanoparticle based systems for drug delivery to Hepatocellular Carcinoma	Kerala University	Submitted	2022
4.	Akhil K Mohan	Development of nanoparticle based drug releasing systems for breast cancer targeting	University of Kerala	Submitted	2022

OFFICE OF THE DIRECTOR

Back row (from left): Anoop Radhakrishnan, Jayalakshmy U S, Priya R, Venugopalan J

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 guide the 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

S Mohanan Nair

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.

ADMINISTRATION: MAIN CAMPUS

Front row (from left): Praveen B, Nidheesh Raj R, Sathichandran S, Jayakrishnan N, Mohanan Nair S, Nandkumar Nair, Jayachandran Nair R
Central row (from left): Vishnu P, Usha B, Asha R Nair, Priya R, Sandhya S J
Back row (from left): Anilkumar R, Sreejith S, Thankamany R, Sujitha S, Preetha J, Vinod Lal K A, Jayalakshmy U S, Dileep Kumar R, Reena Prasad, Subhash K, Wilson T, Vishnu S

ADMINISTRATIVE OFFICE, AKKULAM CAMPUS

Back row (from left): Anoop Radhakrishnan, Akhiljith S, Aneez S, R Kumar , Anand Mohan, Ananthu Ashok, Anandu

PROJECT ENGINEERING TEAM

Front Row (From Left): Ganesh Babu B, Shibu.S.Panicker, Narayanan K K, Ashtami M R, Gowri Sanker R
Back Row (From Left) : Anup R, Dawn K S, Vaisakh H, Anand N P

SUPPORTING STAFF

From Left: Praveen R, Sathya Das, Suresh, Deepu Nair R V, Harikrishnan, Arun, Suresh Kumar S K, Pradeep Kumar, Krishnan Kumar, Suresh

INSTITUTE PROJECT PERSONNEL

Front row (from left): Ambika P Kumar, Priya R, Arathy S, Athira Chandran, Aryasri P
Central row (from left): Kumari Geetha, Lekshmi Darshan, Anithakumari O, Meena H, Veluthai G, Asha V S, Athira V L, Smitha S, Sreevidya C, Jyothisree, Renjith Kurup
Back row (from left): Rahul S, Sreejith G S, Sajeesh, Dnesh, Vinu S Nair, Vysakh V R, Abilal G O, Harikrishnan S, Akshyakumar, Chitra G S, Sarath S N

PURCHASE & STORES DIVISIONS

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.

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 THE ACADEMIC AFFAIRS (OAA) & MANAGEMENT

From left: Ajith Gopal, Beena Nair L, Dr K Santhosh Kumar, Harish G, Briji .S

From left: Dr Lekshmy Srinivas, Dr Lekshmi R S, Dr Aparna Shankar, Dr Mahesh S Krishna

M.SC 2022-2024 BATCH

Front row (From left): Kajori Mahanta, Soma Banerjee, Tanaya Mukharjee, Krishna Sawarn, Nazmin Hussain, Pahil Sen, Sejal Sanjay Raskar, Soham Ghosh
Central row (From left): Tejas Meshram, Jyoti Ranjan Behera, Ajay Durve, Ritika Sachdeva, Zion Mercy M, AM Amrutha, Sachin Yadav
Back row (From left): Sreenath M, Shibam Dutta, Shreyansh Maurya, Sumit Singh, Kamal Narayan Chakraborty

M.SC 2023-2025 BATCH

Front row (from left) : Anwesha Dutta, Sindhu Rai, Payal Anand, Atasi Ghanti, Nishita Thakur, Meghna Das, Saloo Sahu, Sanchari Roy Chowdhury, Divya Aharwal
Back row (from left) : Kaushik Jith, Aritra Kundu, Deepak Bhongale, Soumitra Joshi, Shane Papang, Prateek Nainavat, Nishan Diengdoh, Parmar Mihir, Nirmalendu Mukherjee

The RGCB Academic Council is the highest academic body of the institute, chaired by the Director and is responsible for the policies on maintenance of standards of instruction, student learning and experiences, examination and awards within the institute. Academic Council advises the RGCB management on all academic matters. This committee is also responsible for structuring the course syllabus, admission procedures, program initiatives, faculty support, academic calendar, setting the timetables, the mode of valuation for the PhD course work and MSc programs. This committee also recommends suitable persons as faculty and adjunct faculty for these programs, decides on times and duration of specialty training and internships and ensures coordination between academic affairs management and all other administrative sections of the institute.

CONSTITUTION OF THE ACADEMIC COMMITTEE		
Name	**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 James	Scientist G	Member
Dr. Vinod Kumar G S	Scientist F (PhD Coordinator)	Member
Dr. Harikumar 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

Office of Academic Affairs (OAA) supports the management of academic programs at RGCB including PhD program, MSc program, short term and long term training programs, Post-doctoral training, other specialized training programs and biotechnology skill development programs. The OAA assist the Academic Council to provide leadership roles for collaboration activities in curricular areas such as orientation, residence life, and student activities, while academic affairs tends to assume leadership roles for activities related to curriculum

development, implementation, and policy.in development of a strong academic program, policy formulation, and program planning and student research progress evaluation. 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 create a climate conducive to scholarly research in an atmosphere committed to the mandates of the institute and the Department of Biotechnology. OAA's 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 OAA's ensures coordination and collaboration to ensure quality learning for students and excellence in academic administration.

RGCB PhD Program

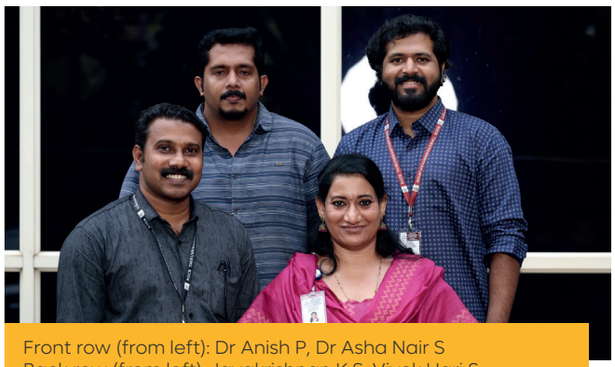
Each year RGCB invites application for admission to the PhD program in various domains of Disease Biology, Neuroscience, Plant Science and Bioinformatics from post-graduates in Life/ Environmental/ Veterinary/ Pharmaceutical/ Medical Sciences or allied subjects (Biochemistry/ Biotechnology/ Bioinformatics/ Biophysics/ Chemistry/ Microbiology, etc.) having a minimum of 60% marks and as per UGC guideline. The candidate should have a valid JRF (UGC/CSIR/ICMR/DBT/DST-INSPIRE or any other nationally-competitive fellowship) offered by the Government and should be valid for a period of 5 years. In the last year (April 2022 to March 2023) 22 students of RGCB awarded with PhD out of which 8 students from Manipal Academy of Higher Education (MAHE), Manipal and 14 from the University of Kerala, Thiruvananthapuram.

RGCB Master's Degree Program in Biotechnology

RGCB launched in July 2019, a highly innovative MSc Biotechnology Program with two unique specializations - Disease Biology, and Genetic Engineering. The MSc program of RGCB is affiliated to the Regional Centre for Biotechnology an "Institution of National Importance" 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 Master's Degree in Biotechnology will be for two years inclusive of four semesters. Students with valid GAT-B score having an aggregate of 60% marks (or an equivalent grade point average) in Bachelor's degree in any branch of Science, Engineering, or Medicine are eligible to apply.

The MSc program at RGCB is unique, since it covers the fundamental fields of theories in Biotechnology, while focusing on laboratory exercises and industrial as well as research applications. The students are also introduced to the concepts of "Enterprise and Entrepreneurship". This allows students who wish for a career beyond the laboratory in an existing biotechnology industry or for those who dream of starting a new biotechnology enterprise. Students get trained in a real business & technology development bio-incubator where start-up companies function. In the second batch (2020-2022) of 31 students, 24 students cleared GATE and seven of them cleared CSIR/UGC NET and 9 of them cleared DBT BET. In the last batch (2021-2023) 18 students cleared GATE examination and six of them cleared CSIR/UGC NET and four DBT BET examinations

OFFICE OF TECHNOLOGY VENTURES (OTV)



Front row (from left): Dr Anish P, Dr Asha Nair S
Back row (from left): Jayakrishnan K S, Vivek Hari S

Managing RGCB's intellectual property (IP) is the primary responsibility of the Office of Technology Ventures (OTV). The IP created by the institute's research community is evaluated, protected, and licenced as part of OTV's responsibilities. In addition, OTV manages the legal facets of the research work, which includes drafting, negotiating, reviewing, and administering agreements like Memorandums of Understandings, Memorandums of Association, Sponsored Research/Consultancy Service Agreements, and Technology Transfer Licencing Agreements to the in-house researchers. We are dedicated to disseminating the advantages of research to society at large. The following 4 patents were granted during the reporting period.

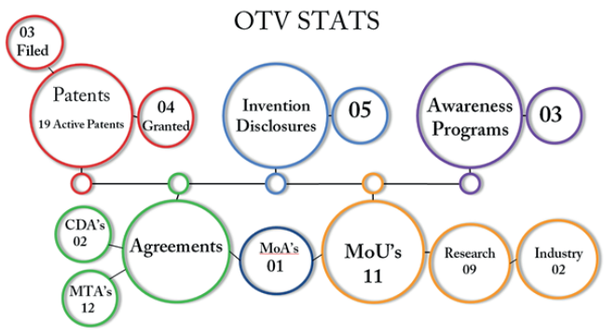
- Synthetic Transmembrane Peptide Pores for the Single-Molecule Sensing (Indian Patent No. 407575 dated 26 September, 2022)
- Methods and materials for identifying therapeutic response in chronic myeloid leukemia (Indian Patent No. 422187 dated 17 February, 2023)
- Uttroside B and derivatives thereof as therapeutics for hepatocellular carcinoma (USPTO No. 11607422 dated 21 March, 2023)
- Polypeptides for Managing Viral Infections (USPTO No. 11666630 dated 06th June, 2023)

During the reporting period, three Patent applications were filed with complete specifications and the details are given below:

- Marker Panel and Method Thereof for Identifying the Progression of Breast Cancer and its Subtype (Indian Patent Application No. 202241028767 dated May 19, 2022)
- Apoptosis Inducing Peptide (SSTP1) (USPTO Application No. 17/919,130 dated October 14, 2022)
- An Antifungal Synthetic Peptide Derived From Osmotin Protein (Brazil Patent Application No. BR 11 2022 020417 5 dated October 7, 2022

Overall, nineteen patent applications are now being processed by OTV. During the year, Five Preliminary Invention Disclosure Forms were received, and are under various stages of filing. Additionally, we were able to transfer Breast Cancer Cell Lines and Covid Cell Resources created by our various faculties to CancerTools, UK, and Applied Biological Material, Canada respectively.

During the reporting period, OTV held three brainstorming sessions with subject matter experts to promote the RGCB Entrepreneurship Ecosystem and participated in the National Technology Week Expo held at Pragati Maidan, Delhi on 11-14th May 2023 and Bio Connect Kerala 2023-Industrial Conclave on Life Sciences held on 25-26th May 2023 at Thiruvananthapuram and showcased RGCB technologies. The given pictogram depicts the status of activities during the reporting period.



ANIMAL RESEARCH FACILITY (ARF)

From left: Rajeev R V, Vinod K M, Anwar K Y, Dr Archana S, Anil Kumar S, Sumaja V, Dileep R K, Alex Anto A, Vinod G

From left: Neethu SM, Dr Arya Aravind, Anand B

Animal Research Facility (ARF) provides quality services and resources to accomplish the research objectives of our investigators with utmost diligence. ARF is operating from two campuses: the RGCB Main Campus, Jagathy, and the Bio-Innovation Centre, KINFRA. The Animal Research Facility has a laboratory rodent facility and a zebra fish facility. The facility is registered and is functioning, fulfilling the objectives of the "Committee for the Control and Supervision of Experiments on Animals". ARF currently houses three species of laboratory rodents (mouse, rat, and rabbit) and zebra fish. As part of advancing the facility to new horizons, work has been initiated to establish a guinea pig housing and experimental room. The animals are maintained in a species-specific controlled environment with individually ventilated caging system for mice and rats. The zebra fish are maintained in recirculating stand-alone housing system. All the animal caretakers are trained to provide proper care to the animal colonies assigned to them. All the activities in the facility are done under the supervision of veterinarians. The Animal House Management Committee takes decisions in matters associated with animal house policies, the addition of new animals, and the purchase.

The facility provides orientation and training for new users and assistance in generating in vivo models and experimental designs. In addition, it provides veterinary assistance in anesthesia, surgical procedures, drug administration, and the collection of biological samples.

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 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.

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.

From left: Dr Jackson James, Soumya S P, Jiji V, Ciji Varghese

From left: Laiza Paul, Anurup K G, Tilak Prasad, Sanjai D, Unnikrishnan V R, Ayswarya R S, Vishnu S Sanjeev, Surabhi S V

From left: Jayashree V, Arya V S, Dr S Asha Nair, Tanima Tomi, Indu Ramachandran

Viji S

Saravana Kumar M

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.

BIOINFORMATICS FACILITY

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

INFRASTRUCTURE

11 x Dell precision tower desktop servers
2 x DELL PowerEdge server with 128GB and
28TB 1 x NETGEAR storage device

a) Computational Infrastructure (Servers & storage)

The Bioinformatics facility provided 63 server accounts to trainees/researchers for high-performance computing and storage from April 2022 to March 2023.

b) Short-term program and Long-term program Training Program

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

(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

ONE DAY WORKSHOPS

11 online workshops were conducted in various topics of bioinformatics. A total of 349 students/researchers has participated in our workshops

Name of the workshop	Date	Revenue
Practises in molecular docking	27-05-2022	25500/-
Python programming for biologists	30-06-2022	13750/-
Introduction to molecular dynamic simulations	29-07-2022	19500/-
Protein structure modeling and visualization	26-08-2022	23250/-
Introduction to R Packages	27-09-2022	7500/-
Introduction to phylogenetics	29-10-2022	14250/-
Protein structure modeling and visualization	03-12-2022	25250/-
Introduction to next generation sequencing data analysis	31-12-2022	42250/-
Molecular modeling of proteins and 3-D visualization	28-01-2023	17500/-
Introduction to microbiome/metagenome data analysis	25-02-2023	25500/-
Introduction to molecular dynamic simulations	25-03-2023	15,000/-
Total		**2,29,250/-**

LONG-TERM PROGRAM

Certificate Program in Bioinformatics 2022-23

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 2022-2023, 35 students from different parts of India has been participated in the certificate program in bioinformatics conducted by the bioinformatics facility.

Front row (from left): Dr Sivakumar K C,
Dr Kathiresan Natarajan, Dr Shijulal Nelson Sathi,
Anand Mohan
Back row (from left): Harikrishnan G, Dr Jamshaid Ali

Part	No.of students	Fees	Revenue
Part -A (online)	35	30000/-	1050000/-
Part-B (offline)	05	20000/-	100000/-
Total			**1,150,000**

c) 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 2022-2023, a total of 28 students have participated in various academic projects offered by the bioinformatics facility.

Duration	Students	Fees	Revenue
1 month	12	15000	1,80,000/-
2-3 months	12	25000	2,75,000/-
4-6 months	04	35000	1,40,000/-
Total			**5,95,000/-**

d) Bioinformatics Services

Our bioinformaticians are proficient in computer programming, structural bioinformatics, and genomics enabling them for extensive and in-depth big data analysis. A combination of custom-built and open-source software is used in the analyses. Our bioinformatics services take your raw data and provide comprehensive analysis and figures customized to your research needs and data interpretation support.

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 modeling, 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	Revenue
Protein structure Analysis	08	1,29,820/-

GENOMICS SERVICE FACILITY

From left: Dr T R Barath Kumar, Dr P Manoj

RGCB Genomics service facility offers comprehensive support for DNA-based genomic research methodologies to RGCB and outside researchers across India. The RGCB Genomics Technical Team aspires to realize the vision of the RGCB's primary mandates (Technology Development, Translational Science, Training and Education).

The Genomics 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, in order to fulfill the mandate of Technology Development with Innovation and on to business.

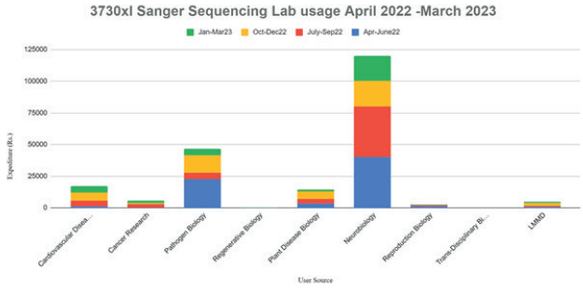
On a fee-for-service basis, research divisions provide internal samples (see pie charts below showing contributions for sequencing and qRTPCR separately).

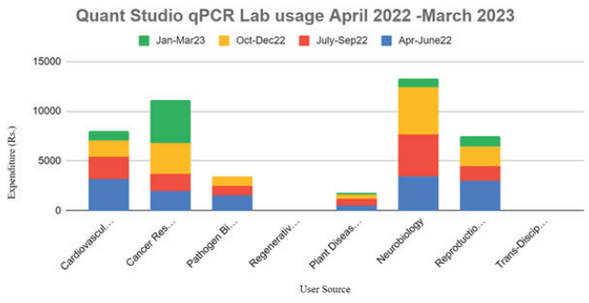
Major institutes and companies like SCTIMST, Rubber Board (Kottayam), NIIST, Calicut University, CVAS, KAU-Vellayani, Enfys Pvt Ltd (Kochi), Genespec, Kochi..etc are our regular genomic service users. They had utilised this facility in 2022-2023 for their research needs with a cost worth of Rs. 2,03,900/-.

- For genescan analysis, samples of plants / animals were processed for SSR analysis, SNP genotyping and microsatellite analysis for various research laboratories.
- The facility is outfitted with Quantstudio 5 (both 96-well and 384-well) and RTPCR 7500, 7900HT for Q-PCR applications.
- For various research labs of both internal and external investigators, gene expression studies with Real Time PCR 7500, 7900 HT systems, & Quantstudio are used in SNP analysis, absolute quantitation, relative quantitation, Allelic discrimination, & Taqman low Density (TLDA) array applications using SYBR green/Taqman chemistry..
- The affymetrix genechip technology microarray platform has been fully utilized with human and mouse arrays. The facility is also equipped with droplet digital PCR (BioRad) for copy number variation studies and genomic library quantitation.

Our lab is engaged in sequencing analyses for the Delta plus variant COVID mutation surveillance study to support the Molecular Infectious Virology Lab who were monitoring and suspecting double mutants in the Kerala population as part of the mandate of Translational Science to benefit individual, clinical, and public health decision. Our primary vision of developing a sustainable pipeline of biotechnology professionals (Training & Education), Genomics Team provided training in DNA Sanger sequencing RTPCR, genotyping and phylogenetic analysis and Microarrayanalysis to students and researchers all over India. Amrita University (Kollam), Terumopenpol and SCTIMST-Trichy utilized the service and generated revenue of Rs. 58,000.

Pie-charts shown below (above-3730xl sanger sequencing & Quant Studio 5 qPCR) represent proportion of usage fee given by corresponding RGCB Research Divisions. The total revenue generated between April-2022 and March-2023 to utilize sanger sequencing is Rs. 2,05,300/- and for qPCR is Rs. 45,100 (excluding external user fee).





LIBRARY AND INFORMATION SERVICES

Library is a place of immense knowledge and information bolstered by a treasure trove of international and domestic books and journals in the realm of life sciences. Benefited from the Institute culture, the library is well equipped and is adaptive in exploring new technologies and frontiers which realistically complies with the words "A library is a growing organism" by Shri.S.R Ranganathan, father of Indian Library Science. As of today, the library is all set to cater to the present and future knowledge/information appetite of research/academia/general benefactors of the centre. All services are well managed with fully automated operations backed by state-of-the-art open source management software. The library is always committed to provide offline and online library & information-associated services in all respects to its benefactors consistent with the present and anticipated research and educational policy of the institute.

Library Resources

The library hosts multifarious reserve of traditional and digital resources in terms of Books, journals, magazines, standards, manuals, protocols, multi-lingual newspapers etc. This includes an accumulation of more than 8500 printed documents, which contains a substantial number of internationally acclaimed books on life science, and a pile of national and international journals and magazines. Also, significant reserve of standards, manuals, protocols, reports, reprints, back volumes of periodicals, theses & dissertations from RGCB, etc. are available as extra resources. Online Public Access Catalogue (OPAC) streams the metadata of all these resources and can be approached globally too. Back volumes of more than a hundred international and national journals from 1995 onwards are on the stock and protected.

The e-resource amassment covers digital media references and various subscribed e-resources, including e-books, e-journals, e-databases, research support software, etc. Online resources in science and technology and related areas from national and international publishers are also available for the subscribers. Majority of the e-resources were effective through the DBT e-Library Consortium (DeLCON), which provides access to thousands of e-journals, e-books, databases, etc. The library is a member of DELNET (Developing Library Network), the network that promotes resource sharing among libraries. The library has an institutional repository, IR@RGCB, hosted on the Science Central platform, which collects, preserves, and disseminates the institutional research outputs in digital format. The library has subscribed to JoVE (Journal of Visualized Experiments)

From left: Balagopal S, Meera N V, Dr Manjula S, Shibin S B, Suma S Nair, Gopakumar G

Research Unlimited, a peer-reviewed scientific online video journal collection that publishes experimental methods in video formats. The digital library consists of a handful of PCs integrated with multimedia platforms for maximum use of e-resources in the library. Provisions are hooked up to fulfil additional requirements of subscribers' queries by resource sharing from other institutes.

Library Services

The library follows an open access system ensuring excellent service for all users. The library renders services such as OPAC services, digital library services, new arrivals alert, reference and consultation services, user orientations, reprographic services, media clipping service, citation and bibliographic analysis, document delivery services (print and electronic), CAS (Current Awareness Services) and SDI (Selective Dissemination of Information) services, etc., to update and support the user community with the latest information in their subject area.

Research Support Tools

The library has earned itself a key position with its professionalism and state-of-the-art infrastructural facilities. The library continuously updates technology to meet the demands of users. It facilitates access to the tools for the research activities of the RGCB Community. Some of the research supported tools are;

Turnitin - Software for plagiarism detection
FlowJo - Software for flow cytometry data analysis
GraphPad Prism - Software for statistical analysis
EndNote - Software for reference management
Grammarly - Academic writing tool
Quillbot – Academic writing tool

The library is on throughout, committed and tuned to hear from and assists its benefactors always and at all times.

MASS SPECTROMETRY & PROTEOMICS CORE FACILITY

The Central Mass Spectrometry and Proteomic Core Facility at RGCB is currently known as "DBT-SAHAJ National facility for Mass Spectrometry-based Proteomics, Metabolomics & Lipidomics Platforms". The DBT-SAHAJ infrastructure was inaugurated at RGCB on 6th May 2023 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 facility has also been supported by the DBT-SAHAJ program awarded in August 2021.

This DBT-SAHAJ National facility provides comprehensive mass spectrometry-based Proteomics, Metabolomics, and Lipidomics platforms for promoting biomedical research and advanced training for Indian researchers. The facility houses four state-of-the-art systems namely; 1) UltraFlextreme MALDI-TOF/TOF from Bruker Daltonics, 2) Synapt G2 HDMS Q-TOF from Waters, 3) Orbitrap Eclipse

Front row (from left): Sajitha T, Dr Abdul Jaleel, Sudha B Nair, Bindu Asokan
Back row (from left) Arun Surendran, Anoop Krishna P N

Fusion Tribrid MS from Thermo Fisher Scientific and 4) Altis Plus Triple Quadrupole MS from Thermo Fisher Scientific.
The facility have been offering MS based analyses services for; Molecular weight confirmation, Protein identification, De-novo sequencing, PTM analyses, Polymer analysis, Protein profiling, Relative protein quantification, Quantitative proteomics using labeled methods (TMT or iTRAQ), Protein complexes identification, Phosphoproteomics, Glycoproteomics, Metabolomics (non-targeted), Lipidomics (non-Targeted) and finally Targeted analysis of molecules or Absolute quantification. These analytical services are being utilized by researchers in academia and industries across the nation. Trainings and workshops on various MS based methods are being conducted for the investigators and students regularly.

MEDICAL LABORATORY SERVICES (MLS)

Dr. Ashok R

B Padmavathy Amma

Dr. Vishnu TS

Ambili S Nair

Dr. Bobby RG

Dr. Jenish Joseph

RGCB MLS is an indispensable clinical Laboratory professional partner that provides clinical lab information and services by use of optional but advanced levels of health care resources. This permits maximizing effective delivery of patient care in today's complex health care system by accurate test results that enable the clinicians to make the right diagnostic and therapeutic decisions. RGCB MLS provides all standard hematological, 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 have initiated many new generation Post Covid analyses as well as many health packages on reasonable rates round the clock and doing analysis for almost all Govt. CHC and PHC centers. The service extends to almost all Ayurveda, Siddha and Homeopathic clinics to conduct latest analysis for patients as well as research studies. Using latest cloud based software; we are able to issue duly approved reports to the persons without delay. Thus we have been approved to be an inevitable Health unit among Public.

Currently, we have 8 main Centers and 210 collection and reporting centers.Including various Govt. Offices and Clinics under western Medicine, Homeo, Ayurveada, Siddha etc. All 5 main Labs are accredited with NABH from 2019 till date. All these Labs are registered under the Clinical Establishment Act 2018.

Apart from Clinical Investigations, Short term training provides for students and staff of various centers by the dedicated and talented personnel. Having Energetic and Excellent infrastructure hands on training are also provided for B.Sc. MLT, DMLT, B.Sc and M.SC Biochemistry and Microbiology students etc with reasonable fees.

RGCB MLS is involved in investigating various types of analysis for many projects also. We are also conducting Medical Camps at request in Offices, Flat, Housing Colony etc. Recently from Covid -19 pandemic period, we have started Home collection for Clinical Investigations service for all parameters including various health checkup packages for Elders, Post Covid Analysis for post covid persons, Palliative care patients, pregnant ladies, children, and all other clinical investigations for all to provide rapid information needed to triage patients and confirm the presence of communicable disease, on CGHS rates seems to be very helpful to the public. SERVICE CHARGE is Rs. 100/- RGCB MLS is Empaneled under State Govt. CGHS and ESI hence reimbursement available. MLS gives Service with commitment to the society. While technology continue to improve the productivity of today's Laboratories, new diseases and disease epidemics requires the need for more innovative tests and testing methods, for rapid diagnosis.

Recently we have installed dedicated analyzers for doing Special investigations which are not available in Medical College TVPM and all other Centers of Govt Hospitals.

Reghuraj V, Sathiesh Kumar K B

From left: Arjun S, Pavithra Nair, Bharat Krishnan J C, Jenish Joseph, Roshna P V

NO. OF PATIENTS FROM ALL MLS CENTERS FOR THE PERIOD FROM 1ST APRIL 2022 TO 31ST MARCH 2023

Sl. No.	Departments	No. of Patients
1.	BIOCHEMISTRY	273269
2.	HAEMATOLOGY	116447
3.	IMMUNOASSAY	176105
4.	MICROBIOLOGY	47425
5.	CLINICAL PATHOLOGY	18081
6.	CYTOLOGY	564
	Sub Total	631891
	BILLED AMOUNT	11,72,13,744
	FEES RECEIVED FROM TRAINEES	13,63,500
	GRAND TOTAL	11,85,77,244

MOLECULAR FORENSICS & DNA TECHNOLOGIES (MFDT)

Front row (from left): Parvathy S Suresh, Remya R C, Preetha V Rajan, Back row (from left): Anil Kumar P, Anandhu A, Renju Krishnan R V, Ratheesh R V, Vinod Kumar S, Sureshkumar U, Johny G, Abhilash M K

All DNA related services are offered by MFDT,includes DNA fingerprinting, DNA barcoding, wild life poaching, species identification and its training. DNA fingerprinting services are exclusive to legal bodies, crime investigating and law enforcing agencies. MFDThandles samples relate to maternity/paternity disputes, crime cases, rape incidents and man missing cases. CO1-based molecular identification and DNA barcoding of fauna for species identification in wildlife forensics is a major service offered by MFDT. Other services include DNA fingerprinting of plants and animals in case-by-case manner using RAPD, AFLP or microsatellite markers and DNA barcoding.For animal identification, we useCO1 gene and for plants matK and rbcL. Facility also offers customized services like clone verification, specific amplification of target gene and sequencing.

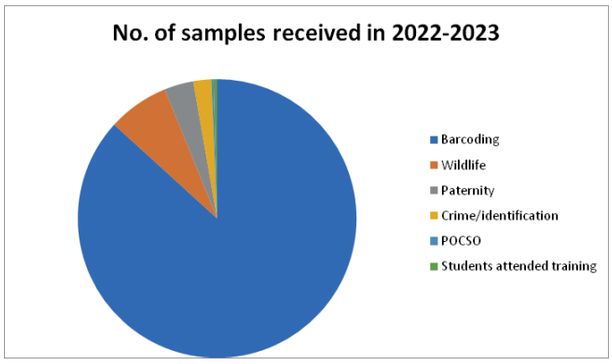
The facility also offers extensive hands on training on DNA fingerprinting, DNA sequencing, its analysis using bioinformatics tool and DNA barcoding.This programme is helpful to lot of students, teachers and researchers from various universities and research institutes. Details about various DNA fingerprinting/barcoding services and training programs are available in our website.

In the current year we analyzed more than 42cases related to human identification, maternity/paternity and relationship disputes forwarded by various Courts from different districts of Kerala, Child welfare Committee and Kerala Women's Commission. In addition we have helped police department byanalyzing12 forensic cases to identify the body recovered from the crime site.

We have also received more than 114cases related to wildlife poaching forwarded from various forest range offices through court. Wildlife 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, cooked or degraded 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. Through our research activities, we are also trying to enrich the database by adding new DNA sequence information.

Sl. No.	Description of Case	No. of Cases	No. of Samples
1.	Paternity	38	115
2.	Crime/Identification	14	71
3.	POCSO	2	8
4.	Wildlife	114	240
5.	DNA Barcoding	837	2936
6.	Students attended training	14 (Applications)	14



Total revenue generated during the period: INR 1,69,86,085/-

Major Clients for Paternity/ Crime/ POCSO/Wildlife

1.	Family Court's of Kerala
2.	High Court of Kerala
3.	Kerala Women's Commission
4.	Child Welfare Committee
5.	Judicial First Class Magistrate, Androth Island, Lakshadweep (POCSO)
6.	District & Sessions Court, Kavaratti, Lakshadweep (POCSO)
7.	Chief Judicial Magistrate, Amini Island, Lakshadweep (POCSO)
8.	All Judicial Court's of Kerala for Crime Cases
9.	All Judicial Court's of Kerala for Wildlife Cases
10.	Special Court for Forest Offences, Nagercoil, Tamil Nadu
11.	Additional Chief Judicial & Judicial Magistrate of First Class, Chintamani, Karnataka.

Currently we are conducting 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 the period. We have received more than 837cases for DNA Barcoding/Fingerprinting/Sequencing analysis from various research institutions, colleges and universities from all over India.

Major Clients for DNA barcoding

1.	Botanical Survey of India, West Bengal &Shillong
2.	Zoological Survey of India, West Bengal & Calicut
3.	Cochin University of Science & Technology, Kochi
4.	Kerala University of Fisheries & Ocean Studies, Kochi
5.	Bharathidasan University, Trichy
6.	Bharathiyar University, Coimbatore
7.	National Centre for Coastal Research, Chennai
8.	Mormugao Zonal Base of Fishery Survey of India, Goa
9.	College of Agriculture, Vellayani, Thiruvananthapuram
10.	College of Agriculture, Vellanikkara, Thrissur
11.	Integrated Farming System Research Station, Thiruvananthapuram
12.	Rice Research Station, Alappuzha
13.	ICAR Sugarcane Breeding Institute, Kannur
14.	Kerala Veterinary & Animal Science University, Thrissur
15.	College of Dairy Science & Technology, Wayanad
16.	Assam Agricultural University, Jorhat
17.	Punjab University, Punjab
18.	Kerala Forest Research Institute, Thrissur
19.	CSIR-NIIST, Pappanamcode, Thiruvananthapuram
20.	College of Science, Albaha University, Saudi Arabia
21.	JamiatAlkhaneel Tree Trading Palms, UAE
22.	Vidyasagar University, West Bengal
23.	Rajasthan Agriculture University, Udaipur
24.	College of Dairy & Food Technology, MaharanaPratap University of Agriculture and Technology, Rajasthan
25.	University of Jammu & Kashmir
26.	University of Kerala.
27.	Pepper Research Station, Kannur
28.	Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Thiruvananthapuram
29.	Institute of Animal Health & Veterinary Biologicals, Thiruvananthapuram
30.	Annamalai University, Tamil Nadu

RESEARCH ENGINEERING AND TECHNICAL SERVICES

Front row (from left): Sreelekshmi S, Rajasekharan K, Ancy Prince T S, Sreekanth S L, Jobin T J
Back row (from left): Sajin G S, Aneesh R, Jacob J

Front row (from left): Lekshmi C, Sajan I X, Arunima B
Back row (from left): Arun R, Renadeep C S Nair, Manoj Kumar K, Unniraj S, Sajith Kumar S

Research Engineering Service Department has been playing an important role since inception of the Institute, and its contributions in the growth of the Institute are not neglectable. Having operations in three campuses, it ensures uninterrupted supports, with no compromise on quality and standards at any level of its functioning, which directly contributes to the outcome of the various Research activities. The ultimate goal of the department is to support all divisions, striving to achieve the Mission & Vision of the Institute, impartially and upholding the highest standards of integrity.

The prime responsibility of the Department encompasses installation, care &maintenance as well as service of all sophisticated and general research Equipments, includingthat of Central Instrumentation Facilities at Main Campus and C2 Campus at Akkulam.

This Division also maintains a well equipped engineering workshop with facilities for repair of sophisticated instrumentation systems. It helps to curtail,the down time of the sophisticated instruments and heavy repair costs. InhouseEngineer's expertise to fix highly complicated hardwareissues helps the institute to save heavily on AMC and CAMC's to be signed with respective suppliers of the equipments. On top of the above, the Division also undertakes customisation by designing, fabrication& modification of components of research automations to meet end users requirements and convenience.

It also extends its support in procurement, by analysing the need of the user department, understanding the currently available technology, features and future upgradation/ customisation possibilities, and prepare necessary technical specifications within the budget, to initiate purchase processes through out the year.

Division has well equippedinhouse Calibration facilities, mainly for pipettes, electronic balances, centrifuges, autoclaves, freezers, incubators, PCRs etc., .This facility includes standards and measuring instruments with proper calibration certificates from Govt. recognised National Calibration and Acreditation Agencies. Department has been calibrating and certifying instrumentsof various laboratoriesaspiring for NABL accreditation.

Research Engineering Services has also been offering training programs for Degree and Post graduate students on operation, application, calibration and maintanance of various instrumentation systems used in Biotechnology and Life Science Research.Research Engineering Services had trained 63 Students during the financial year 2022-23.

Front row (from left): Radhika U, Arya P Nair, Sreelekshmi A S, Aswathy G Raj, Soumya S P
Back row (from left): Amal V, Sidharth Achuthan , Ancy Prince T S, Rahul C S Nair

Apart from the above, the Division also maintains Computers andSecurity surveillance systems, Biometrictime attendance recorders, Conferencing facilities, Communication systems, Liquid Nitrogen Plant, Auditoriums, Convention Centre, 11KV Electrical Substations&Central AC plants which includes Power Transformers, Distribution Transformers, DG sets, Protection & Control Equipments, Medium & High Voltage Switchgears, Chillers, UPS & Batteries, Passenger Lifts and elevators. Department also takes a role in Automation with PLC/DCS/Scada Control Systems.

Research Engineering services plays an instrumental role in recognizing and adapting cutting edge technologies to facilitate instrumentation systems to reduce man machine interface.

Front row (from left): Bibin Dev, Ratheesh Kumar A, Ajay S Kumar, Rajasekharan K, Ancy Prince T S, Arun R, Sujith SV
Back row (from left): Akhilesh P M, Mohammed Sanofar S, Jagatheswara Kumar,Sajith Mohan M L, Jaslee N, Ajun Babu, Aseem A, Shaji S

INFORMATION TECHNOLOGY AND DATA MANAGEMENT GROUP

From left: Durga Prasad C, Anand Mohan, Lekshmi R, Rajasekharan K, Remya Rajan, Gowrisankar S P

The IT infrastructure of RGCB's main campus includes 11 Servers, more than 450 Desktops, and Laptops, Network Printers, etc., and houses of one of the best computing networks with constant up-gradation in a bid to provide the 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 staff and research scholars' problems. It is managed and actively supported by experienced engineers in the IT Department. 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, WINDOWS platforms and includes software development for research groups.

IT department design, develop, update, host and support 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, ecomplaint portal, yearly portals for updating annual reports, SAC and integrating payment gateway for various web applications.eOffice complete suite implemented.

Internet facilities are provided throughout the campus through 1 Gbps, and 100 Mbps leased lines from NKN and BSNL. Internet facility for Akkulam campus also has the 100Mbps leased line.RGCB has invested in a high-speed Fibre Optic Backbone with high-end security for networking across the campus. Wireless connectivity is provided at strategic locations to provide Internet access to the faculty.

The Information Technology Division of Bio-innovation Centre at KINFRA,Kazhakuttom, uses cutting-edge technology to provide high-quality services and capabilities to different research groups. It includes servers with active directory domain infrastructure, secured network with state of the art firewall system,100Mbps leased line, and 100Mbps broadband line with failover backup connection

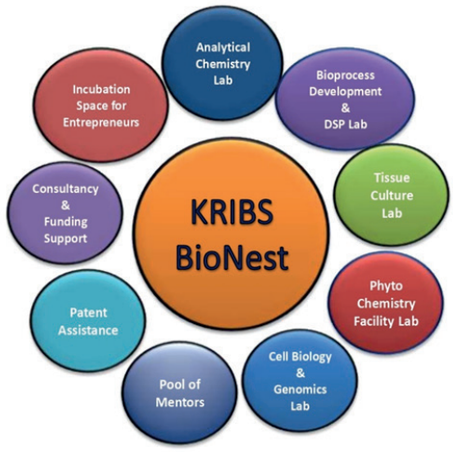
KRIBS – BIONEST

Front row (from left): Roshna S Nair, Smitha.S, Charutha
Back row (from left): Manoj.A, Antony K P

Dr N P Anish

Dr T R Santhosh Kumar

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. This is a unique facility designed to provide infrastructure and scientific support to enable researchers and investors entrepreneurs looking to transform biology, medical based technologies and innovations into real and mature big business. With a total floor area of over 44,000 square feet, it offers over 17,000 square feet of bio incubation space and a common laboratory measuring 1000 square feet to the Start-ups housed within the facility.



The broad business verticals/laboratories include Analytical Chemistry, Molecular Biology, Phytochemistry and Bioprocess Engineering.BioNest has been able to collaborate with a number of industries to set up Corporate Research Operations. During last year, 20 startups were incubated physically and 8 were in virtual mode. All the Start-ups launched different products in various streams; biotechnology, healthcare, nutraceuticals, cosmetics, agriculture, etc., and many of their products are under different stages of development.

Many entrepreneurs explored various marketing strategies and succeeded in getting their products known in both national and international markets. Beyond the support provided to the startups incubated inhouse, KRIBS-BioNest also facilitated numerous external entrepreneurs/researchers for various R & D activities. The highly equipped Biotech incubation facility in KRIBS BIONEST attracts students/faculties/researchers/entrepreneurs from all over the country. During the year more than 250 visitors explored the facility and interacted with the incubated startups.

List of New incubatees in KRIBS-BioNest 2022-2023

Sl. No	Name
1.	M/s Pharmacon care Pvt. Ltd
2.	M/s. Solaire Initiative
3.	M/s. Clean Conscious
4.	M/s. Bionre Laboratories
5.	M/s. Agro-Bio Tech Research Centre

Regarding the IP creation by the incubatees, M/s. Heka Medicals India Pvt Ltd has developed "Heka Flow- High flow Nasal oxygen therapy device" and owned Indian patent for the invention "IOT enabled novel high flow nasal oxygen delivery system" vide no. 431088 dated 04/05/2023. The present invention relates to the field of high flow nasal oxygen (HFNO) delivery system for persons suffering from respiratory and breathing disorders, the system comprises of an electric powered high-flow oxygen/air supply unit, a humidifier, a heated wide bore tube, a touch function display unit for user interface that houses a flow button, a temperature button, a FiO2 button, a SpO2 button, a flow meter, button and rox index button wherein the humidified air is warmed and fed to the patient through a heated tube and a nasal cannula and the IoT enabled HFNO system monitors in real time the functioning of the system using customized web based portals that simultaneously display the status of plurality of patients under HFNO therapy.

M/s. Greenovative Foods Pvt Ltd has filed an Indian patent for the invention "Electro-thermo-mechanical equipment for texturizing plant proteins into fibrous structures for use as meat substitutes" vide application No: 202341013597 dated 28/02/2023. Another incubatee, M/s. SCOPEFUL BIORESEARCH PVT LTD, filed two Indian patents on their inventions such as Coconut sapbased composition with high nutritional and therapeutic value (No.: 202341038080 Dated: 02/06/2023) and "Methods for extracting sweetening components from stevia" (No. 202341040641 dated 14/06/2023).

Four of the incubatees such as M/s. ZyGene Biotechnologies (P) Ltd., M/s. Bio Mount Nutrients, M/s. Homey Kitchen Diners LLP and M/s. Greenovative Foods Pvt Ltd protected their product name etc under Trademark Act.

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

Sl. No	Product Name	Name of Incubate	Uses
1.	Melanin	M/s. Avisa Biotech Pvt.Ltd.	UV Blocking
2.	Turmericoil	M/s. Spiceor BioNutrilities Pvt.Ltd.	Cancer preservativecapsule production
3.	Curcumin	M/s. Spiceor Bio Nutrilities Pvt.Ltd.	Cancer preservative capsuleproduction
4.	Maracujaoil	M/s. Spiceor BioNutrilities Pvt.Ltd.	Cosmetics Products Manufacturing
5.	Oleoresins	M/s. Spiceor Bio Nutrilities Pvt.Ltd.	Used in food grade ingredient companies and cosmetic industries
6.	ZEROL	M/s. Bodina Naturals Pvt.Ltd	Disinfection
7.	ZEROL GARGLE	M/s. Bodina Naturals Pvt.Ltd	Antiviral Antibacterial Gargle
8.	Blacumin	M/s. Kerala Nutraceuticals Pvt.Ltd.	Health Managemt
9.	Vegflax	M/s. Kerala Nutraceuticals Pvt.Ltd.	For Omega3 Deficiency
10.	Spirulina ExtraCoated	M/s. Phytocom Pharmaceuticals PrivateLimited	Aquatic Fish Seed
11.	Activgra Plus	M/s. Phytocom Pharmaceuticals Private Limited	Stress Management
12.	Jaiva Rich	M/s. BIO Mount Nutrients LLP	Organic Manure forplants
13.	Nucleosieve DNA	M/s. Primordia Life Sciences Pvt.Ltd.	DNAExtraction
14.	Nucleosieve RNA	M/s. Primordia Life Sciences Pvt.Ltd.	RNAExtraction
15.	Antifat	M/s. Kerala Remedies	Obesity Managemen
16.	Grapelina	M/s. Kerala Remedies	Hypertension Management
17.	SafeTouch	M/s. Scire Sciences PvtLtd	Disinfection
18.	SCIENRICH	M/s. Scire Science Pvt.Ltd.	Skincare purpose
19.	ATTAIN	M/s. Scire Science Pvt.Ltd.	Food Item
20.	Thermo Seller	M/s. Scire Tech	Rearch Purpose
21.	GT Caps	M/s. Kerala Remedies	Anti-Aging
22.	GC Plants	M/s. Green Clones	Agri/Horti/InvitroPlants
23.	Infantwarmer	M/s. Biophoton Technologies PvtLtd	Infantwarmer
24.	Biosan	M/s. Biophoton Technologies PvtLtd	Mobile Phone Disinfection
25.	Aque Sense	M/s. Klonos LifeSciences Pvt.Ltd.	Coliform Testingkits
26.	Multivitamin Gummies	M/s. Ebrilive Healthcare PvtLtd	Vitamin Supplement
27.	Polyclonal Antibodies	M/s. Prayaga Scientific Laboratories Pvt.Ltd.	Polyclonal Abs
28.	Monoclonal Antibodies	M/s. Prayaga Scientific Laboratories Pvt.Ltd.	Monoclonal Abs
29.	Neem Pappaya Hand Sanitizers	M/s. Bipha Drug Labs PvtLtd	Disinfection
30.	Zymag (Nucleic acid extraction kit – Magnetic bead based technology	M/s. ZyGene Biotechnologies (P) Ltd	Nucleic acid extraction kit

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. This is a unique facility designed to provide infrastructure and scientific support to enable researchers and investors entrepreneurs looking to transform biology, medical based technologies and innovations into real and mature big business. With a total floor area of over 44,000 square feet, it offers over 17,000 square feet of bio incubation space and a common laboratory measuring 1000 square feet to the Start-ups housed within the facility.

TECHNOLOGY INTERVENTIONS FOR TRIBAL HERITAGE RESILIENCE OF KERALA

Scientific validation and conservation of tribal heritage is multifaceted and crucial for several reasons including conservation of biodiversity and cultural diversity, sustainable resource management, empowerment and recognition of communities etc. The Science& Heritage Research Initiative (SHRI) Program by Department of Science and Technology, Government of India is providing grant-in-aid support to RGCB for the studies on Tribal Heritage of Kerala.

This project serves as platforms for intergenerational knowledge transfer and creating livelihood opportunities by bridging the traditional knowledge with modern technological interventions. The Project team has made substantial progress in the three targeted districts such as Thiruvananthapuram, Idukki, and Wayanad in terms of documenting traditional knowledge, identifying technology gaps and revamping the tribal traditional practices. Tribal communities are still practicing traditional health care systems using medicinal plants for the prevention and control of not only humans but also for various livestock diseases. The ethnoveterinary practices were documented through direct interviews with the tribal healers. One of the herbal formulations for wound healing was selected for scientific validation in invitro and in vivo systems and the results were presented as “Scientific Validation of an Ethnoveterinary Formulation Used for Wound Healing” in the International Bioresource Conclave and Ethnopharmacology Congress – 2023. There are about 38 species of medicinal plants belonging to 30 families used in ethno-veterinary practices that were documented and identified. The outcome of the study was presented in the 35th Kerala Science Congress (2023) and received the Best Poster Presentation Award. The studies on “Ethnobotanical Exploration of Medicinal Plants and Promotion of Tribal Health Care Practices” was also presented in the International Bioresource Conclave and Ethnopharmacology Congress- 2023.

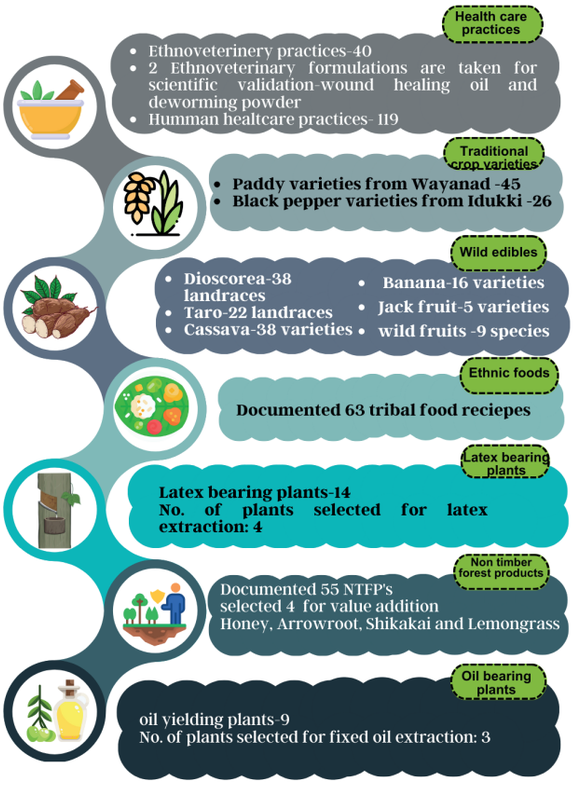
As part of ethnic food documentation, 85 tribal informants were interviewed from 9 tribal communities and documented the details of wild edibles, food recipes and food culture. The folk taxonomy and morphological analysis data on wild tubers especially, Dioscorea varieties used by the tribal communities were presented in the 35th Kerala Science Congress (2023). Saplings of wild edibles were also collected during documentation and established three demonstration plots and nurseries in the three targeted districts with the participation of tribal communities. These plots serve as conservation/knowledge/promotion centres for wild edibles, and also provide additional income to the community through the sale of saplings. These activities in the domain of biodiversity conservation were displayed at the Kerala Biodiversity Congress of 2023, which was honoured with the 2nd Best Stall Award under the 'Government Institutions' category. Furthermore, a tribal SHG, ‘NellaraPattika Varga Karshakasangam’ supported under the project received second best stall award under the ‘Farmers’ category for their commendable efforts incollection and exhibition of wild tubers at the congress.

A pivotal aspect of the project is genetic profiling of wild tubers. Genomic DNA from 41 samples, representing a diverse array of 8 Dioscorea species was isolated and underwent genotyping using molecular markers to analyse the intricate genetic makeup of these landraces. This was presented at the 35th Kerala Science Congress 2023, and honoured with the ‘Best Poster Presentation Award’. To add to the scroll, the work was also published as "F-ISSR Marker-Based Genotyping of Wild Tuber Population of Dioscorea Exclusive to Tribal Utility in Kerala, India" in the International Journal of Current Microbiology and Applied Sciences (Volume 12, Number 8; 2023).

More than 70 ethnic food recipes from various tribal communities of the target Districts were documented and analysed the botanical sources and its already reported therapeutic/nutritional properties. Nutritional profiles of selected wild tubers, Dioscorea and Musa varieties were also performed. A series of awareness classes on the importance of ethnic food were conducted and established three ethnic food processing units with tribal Self-Help Groups. In order to promote and popularize the ethnic food culture and to get its health and nutritional benefits to the modern society, value-added products using underutilized and locally available resources were also initiated. Two value added products such as Jackfruit Choco-cookie and Health Mix from four varieties of millets and various pulses were developed.

Fixed oils from wild plants is an under-explored area and the indigenous communities have depended on various wild sources to meet their oil requirement as edible, medicinal and lighting purposes. The project team documented nine oil-yielding plants and three of them were explored scientifically. Fixed oils from the seed kernels of Garcinia gummi gutta, Sarcostigmaklenii, Hydnocarpuspentandra were extracted under lab conditions. The physio-chemical analysis of the extracted oils were performed and the fatty acid compositions were identified with Gas chromatography – Mass spectrometry technique. Further, the biological properties, such as anti-bacterial, anti-inflammatory, and antioxidant studies of the extracted fixed oil were also carried out.

As of now, about 250 tribal families are directly benefiting from the project activities, by harnessing their traditional knowledge and skills to enhance their livelihoods. With judicious technology interventions, it is witnessed that the heritage is not just a past memory, it can make wonders in all aspects of modern society as well.



Stat on Documentation of Traditional Knowledge

Inauguration of wild edibles Demonstration plot and Nursery at Kottoor, Thiruvananthapuram by Prof. Chandrabhas Narayana.

Ethnic Food Awareness Program conducted at Pattayakudi, Idukki

Inauguration of NTFP value addition unit at Makkimala, Wayanad.

Project team at Idukki (Left to Right) Jishnu Janardhanan, Hari M K, Anu Theresa Antony, Rajesh K T

Project team at Wayanad (Right to Left) Mr. Abin Abraham, Ms. Roshni S, Mr. Syam Sankaren and Mr. Sebastine A. C.

Project team at Thiruvananthapuram :
Back row (from left): Mahesh K B, Adharsh Sen Madhu, Professor Chandrabhas Narayana, Dr Anish N P, Dr Manoj P
Front row (from left): Deepa Mathews, Dr Archana S, Mariya Mary Gigi, Deepthi Mohan, Aparna M, Harikrishnan P

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 the cafeteria. 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"

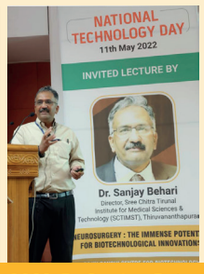
Main Campus:
From row (from left): Akhil M K, Aravindh U,
Rejani T, Sandhya R, Presanna T R, Meera R P, Abhinand C S,
Najeem M, Suresh Kumar R
Back row (from left): Tyagaraj, Gopakumar M S, Sajith C,
Retheesh S, Byju S, Manoj Kumar R

Akkulam Campus:
From Left: Ananthakumar A, Deepak, Sivaprasad
Nanthakumar A, Rahul.S, Sunilkumar K, Anoop. KC, Shankar
Manoj .S

RGCB EVENTS 2022 - 23



APRIL 2022 | Inauguration of Lemongrass Oil Extraction Unit:
Professor Chandrabhas Narayana, inaugurated the unit on 7th April 2022 at Valad, Wayanad.

MAY 2022 | National Technology Day Celebrations on May 11, 2022. Dr Sanjay Behari, Director, SCTIMST, Thiruvananthapuram was the chief guest


JUNE 2022 | International Yoga Day was celebrated on 21st June 2023. The theme of this year's worldwide Yoga Day is "Yoga for Humanity".


JULY 2022 | RGCB conducted an awareness program on GATI (Gender Advancement for Transforming Institutions) initiative on 15th July 2023. Dr. Navjot Khosa IAS, The Hon. District Collector of Thiruvananthapuram and Dr.Divya S.Iyer IAS, The Hon. District Collector of Pathanamthitta were the invited dignitaries.

RGCB EVENTS 2022 - 23

JULY 2022 | RGCB organized a one-day symposium on Biotechnology for sustainable development-2022 and felicitation to senior scientists on 26th July 2022.

AUGUST 2022 | Freedom 5K run as a part of Azadi Ka Amrit Mahotsav to commemorate the 75th anniversary of Indian Independence

AUGUST 2022 | Celebration of 76th Independence Day. Professor Chandrabhas Narayana, Director hoisted the National Flag

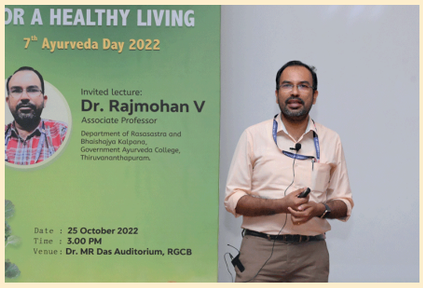
RGCB EVENTS 2022 - 23


SEPTEMBER 2022 | Celebration of the harvest festival of Kerala "Onam" in a traditional manner. Her Highness Pooyam Thirunal Gouri Parvathi Bayi was the chief guest.



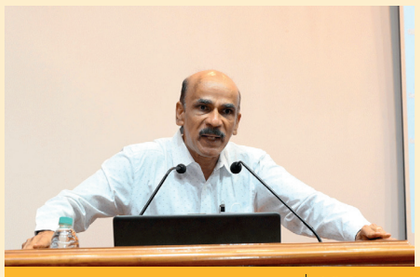
SEPTEMBER 2022 | RGCB bid farewell to the second batch of M.Sc Biotechnology (2020-2022).





OCTOBER 2022 | 75th Ayurveda Day Celebration. Dr. Rajmohan V was the chief guest

RGCB EVENTS 2022 - 23



NOVEMBER 2022 | Vigilance Awareness Week Invited talk by Mr. A. Hemachandran IPS, Ex DGP, Kerala




NOVEMBER 2022 | RGCB Foundation Day Lecture was delivered by the chief guest Dr. Tapas K Kundu (JNCASR, Bangalore)



NOVEMBER 2022 | November 30, 2022, Dr. Rajesh S Gokhale, Secretary, Department of Biotechnology visited RGCB

RGCB EVENTS 2022 - 23

NOVEMBER 2022 | Ramalingaswami Re-Entry Fellowship & MK Bhan- Young Researcher Fellowship Joint Conclave

JANUARY 2023 | To commemorate the 75 years of Independence, a Science Museum at the Government Higher Secondary School, Meppadi, Wayanad, the Aspirational district of Kerala was established and handed over to school authorities.

JANUARY 2023 | Professor Chandrabhas Narayana unfurled the National Flag on the occasion of 74th Republic Day of our nation

RGCB EVENTS 2022 - 23

FEBRUARY 2023 | Science Day Celebration: Dr. A Ajayaghosh, Former Director, CSIR-NIIST, was the chief guest.

MARCH 2023 | International Women's Day 2023. Mrs. Santha Jose, founder of ASRAYA was the chief guest.

ANNUAL REPORT COMPILATION COMMITTEE 2022-2023

Dr. K.B. Harikumar, Scientist E-II
Mr. R. Kumar, Deputy Controller of Finance
Ms. R. Lekshmi, Manager (Technical Services)
Ms. Ramya Rajan, Engineer (IT)
Mr. S. Vivek Hari, Project Management Assistant

RGCB

DISCOVERIES FOR A
BETTER TOMORROW

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