BIOGRAPHICAL SKETCH

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NAME: Paulmurugan, Ramasamy

eRA COMMONS USER NAME (credential, e.g., agency login): paulmur8

POSITION TITLE: Associate Professor of Radiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Madurai Kamaraj University, Madurai, India	B.Sc.	05/1989	Zoology, Botany, Chemistry
University of Madras, Madras, India	M.Sc.	09/1991	Biomedical Genetics
National Environmental Engineering Research Institute (NEERI), University of Madras, Madras, India	Jr. Project Fellowship	12/1993	Molecular Virology
National Environmental Engineering Research Institute (NEERI), University of Madras, Madras, India	Sr. Research Fellowship	08/1996	Molecular Virology
National Environmental Engineering Research Institute (NEERI), University of Madras, Madras, India	Ph.D.	11/1997	Biomedical Genetics (Molecular Virology

A. Personal Statement

I am an Associate Professor in the Department of Radiology at Stanford University and a member of the Canary Center for cancer early detection, the Molecular Imaging Program at Stanford (MIPS) and the Stanford BioX program. I have more than 19 years of experience in the use of *in vivo* imaging modalities (bioluminescence, fluorescence, PET, SPECT and CT) for monitoring different cellular events in mice and rats. **My research group focuses on developing new imaging assays for studying cellular signal transduction networks in cancer and other diseases.** Specifically, we apply our extensive experience in molecular biology to develop several *in vivo* imaging assays for monitoring basic cellular processes and post-translational modifications of proteins, such as protein methylation, protein phosphorylation, and protein sumoylation. We have developed split-reporter protein complementation systems for various reporter proteins (luciferases, fluorescent proteins, and thymidine kinase), and currently use them to design imaging sensors to study cellular signaling processes. **Some of the main applications of these assays include imaging the tumor microenvironment, as well as imaging cytokine signaling in cancer.** Other applications of these assays include: studying protein-protein interactions involved in estrogen receptor signaling, Nrf2-mediated antioxidant signaling in chemoresistance, p53-sumoylation mediated chemotherapy responses, NFkB mediated cytokine signaling, and signaling mechanisms associated with APP and Tau protein sumoylations in Alzheimer's disease.

We are establishing new microRNA-based reprogramming approaches in sensitizing drug-resistant cancers (breast cancer, hepatocellular carcinoma, and glioma) to commonly used chemotherapies. We mainly use microRNAs (miR-21, miR-10b, miR-122 and miR-100) that are dysregulated in cancers. To deliver intact miRNAs *in vivo*, we load miRNAs in PLGA-PEG nanoparticles and use ultrasound-microbubble (US-MB) triggered drug delivery strategies. We evaluate miRNA delivery strategies in small animal models (mice and rats) and optimize US parameters (cavitation, PRF, mechanical energy) in large animal models (pigs and dogs). We have shown tremendous progress in this area of research with a number of publications in high impact journals.

We recently identified five sense and antisense miRNAs (**miR-203, miR-218, antimiR-10b, antimiR-19b and antimiR-21**) through a rigorous analysis of miRNA expression data available in TCGA (GDC) and GEO using a biological basis-driven workflow where these microRNAs target multiple hallmarks of cancer to improve chemo- and immunotherapies in cancer. We initially identified 37 miRNAs related to several oncogenic and cytokine signaling factors. Our further screens by experimental evaluations in triple negative breast cancer (TNBC) cells of different genotypes resulted for these five important miRNA targets. In this proposed research, we will evaluate the potential of these miRNAs in preclinical animal models for their role in improving chemo- and immunotherapies in TNBC, and this strategy can be extended to other types of solid cancers.

Ongoing and recently completed projects that I would like to highlight include:

5R01CA20988802 (Paulmurugan) 04/01/2017 – 03/31/2022
National Institutes of Health
Title: Therapeutic miRNA Modulation of Hepatocellular Carcinoma Using Ultrasound Guided Drug Delivery
Goal: To study therapeutic microRNAs delivered by ultrasound microbubble and PLGA-NP in orthotopic human HCC in mice and rabbit to evaluate improved drug responsive profile.
Role: PI

1R21 EB029046-01A1 (Ahmed El Kaffas (PI)/ Ramasamy Paulmurugan (Co-I))
NIH/NIBIB 07/01/2020 - 06/30/2023
Title: Development of Molecular Microbubble Probes and Ultrasound-Guidance in Immunotherapeutic Strategies
Goal: The main goal of this grant is to develop molecular microbubbles to study the immunotherapy response in cancer by ultrasound mediated contrast imaging
Role: Co-I

W81XWH-18-1-0342 (Hori) 09/01/2018 – 08/31/2022
Department of Defense Breast Cancer Research Program
A Modeling-Based Personalized Screening Strategy Combining Biomarker and Imaging Data for Breast Cancer Early Detection
Major Goals: To improve upon screening mammography and address the problems of breast cancer overdiagnosis and overtreatment by predicting when a woman's circulating biomarker measurements are abnormal, relative to her own baseline biomarker values.
Role: Co-Investigator

SCI-SPO: 133895 (Dahl/Paulmurugan) 09/01/2019 – 03/31/2022
Combining Circulating Biomarker and Imaging Data for Breast Cancer Early Detection
Major Goals: To develop novel targeted biomarker-based imaging strategy early detection of breast cancer.
Role: Multi-PI

FUS939 (Paulmurugan, Ramasamy/Dahl, Jeremy,) 10/01/2021 - 09/30/2024
Ultrasound (US)-microbubble (MB)-delivered microRNA-mediated immunomodulation for liver malignancy therapy in veterinary clinical practice
Major Goals: To longitudinally evaluate the US-MB delivered microRNA mediated enhancement of Toceranib therapy in canine model of spontaneous liver cancer.
Role: PI

5R01CA218204 (Dahl, Jeremy). 08/01/2021 – 07/31/2023
National Institutes of Health
Title: Automated Volumetric Molecular Ultrasound for Breast Cancer Imaging
Major Goals: To study B7-H3 targeted ultrasound molecular imaging of breast cancer by combining with automated volumetric analysis.
Role: Co-I

B. Positions and Honors

Positions and Employment

1996-1999	Scientist B and Head, Environmental Biotechnology Division, Rajiv Gandhi Centre for Biotechnology, Trivandrum, Kerala, India
1999-2003	Scientist C and Head, Environmental Biotechnology Division, Rajiv Gandhi Centre for Biotechnology, Trivandrum, Kerala, India
2001-2003	Post-doctoral Visiting Scientist, Crump Institute for Molecular Imaging, Department of Molecular and Medical Pharmacology, UCLA, Los Angeles, CA, USA
2003-2009	Senior Research Scientist, Department of Radiology, Molecular Imaging Program at Stanford (MIPS), Stanford University, Stanford, CA, USA
2009-2016	Assistant Professor, Department of Radiology, MIPS, Stanford University, Stanford, CA, USA

2016-2021 Associate Professor, Department of Radiology, MIPS, Stanford University, Stanford, CA, USA
2021- Professor, Department of Radiology, MIPS, Stanford University, Stanford, CA, USA

Other Experience and Professional Memberships

2001- Member, Academy of Molecular Imaging
2002- Member, Society of Molecular Imaging
2009- American Society of Gene and Cell Therapy (ASGCT)
2016- International Society for Bioluminescence and Chemiluminescence (ISBC)
2018- World Molecular Imaging Society (WMIS)

Honors

1991 Best Research Fellow, NEERI, CSIR, Nagpur, India
1999 Young Scientist Award, Government of Kerala, India
2003 Travel Award, Academy of Molecular Imaging for Best Paper Presentation, San Diego, CA, USA
2005 Travel Award, Academy of Molecular Imaging for Best Paper Presentation, Orlando, FL, USA
2005 Travel Award, Society of Molecular Imaging for Best Paper Presentation, Cologne, Germany
2006 Travel Award, Academy of Molecular Imaging for Best Paper Presentation, Orlando, FL, USA
2018 Distinguished Investigator Award, The Academy for Radiology & Biomedical Imaging Research

International Patents Filed

2007 R. Paulmurugan, S.S. Gambhir. Self-complementing firefly luciferase enzyme fragments for studying cellular events in living animals (Publication #: 20070161067)
2007 S.S. Gambhir, R. Paulmurugan. Protein Phosphorylation Imaging Systems, Methods of Making Phosphorylation Imaging Systems, and Methods of Use Thereof. US Patent #: 7834148 (Publication #: 20070275428)
2008 S.S. Gambhir, R. Paulmurugan. Ligand-regulable transactivation systems, methods of use thereof, methods of detecting estrogen receptor ligands, and methods of differentiating estrogen receptor ligand agonists and antagonists. US Patent #: 7709253 (Publication #: 20080034445)
2009 R. Paulmurugan, S.S. Gambhir. Estrogen receptor intramolecular folding systems, Estrogen receptor intramolecular folding sensors, Methods of use thereof, methods of detecting ER ligands, and methods of detecting ER agonists and antagonists. No Patent # (Original Publication #: 20090044286; also published as 8178654 B2)
2009 T. F. Massoud, R. Paulmurugan, S.S. Gambhir: Split Herpes Simplex Virus Type 1 Thymidine Kinase for Noninvasive PET Reporter Complementation Imaging of Protein-Protein Interactions No Patent # (Publication #: 20090075313)
2010 S.S. Gambhir, R. Paulmurugan. Ligand-regulable transactivation systems, methods of use thereof, methods of detecting estrogen receptor ligands, and methods of differentiating estrogen receptor ligand agonists and antagonists. US Patent #: 8076159 (Publication #: 20100169993)
2011 S. Bhaumik, S.S. Gambhir, R. Paulmurugan, S. Yaghoubi, B-C Ahn, N. Parashurama. Composition and Method for Imaging Stem Cells. (Publication #: 20110059439)
2021 R. Paulmurugan, Tarik F Massoud, and Uday Kumar Sukumar. SARS-CoV-2 Vaccine (041243-563P01US)
2021 R. Paulmurugan, Tarik F Massoud, and Uday Kumar Sukumar. Cell-Derived Nanovesicles for In Vivo Transport and Delivery of Therapeutic Materials (041243-581P01US)

C. Contribution to Science

1. **Reporter gene assays for imaging protein-protein interactions.** My early publications address my training in molecular virology and molecular biology. Later I used my experience in molecular biology to develop reporter gene assays/sensors for studying protein-protein interactions in living animals by molecular imaging. The sensors are mainly based on reporter protein complementation. From these studies I developed optimal split-reporter complementation systems for various optical reporters, such as firefly luciferase, renilla luciferase, gaussia luciferase, enhanced green fluorescent protein, and monomeric red fluorescent protein, and for PET reporter, the Thymidine Kinase (HSV1-sr39TK).

   a. **Paulmurugan R**, Umezawa Y, Gambhir SS. Noninvasive imaging of protein-protein interactions in living subjects by using reporter protein complementation and reconstitution strategies. Proc Natl Acad Sci USA. 2002 Nov 26;99 (24):15608-13. PMCID PMC137764.

b. **Paulmurugan R**, Gambhir SS. Monitoring protein-protein interactions using split synthetic renilla luciferase protein-fragment-assisted complementation. Anal Chem. 2003 Apr 1;75:1584-9. PMCID: PMC4154785.
c. **Paulmurugan R**, Massoud TF, Huang J, Gambhir SS. Molecular imaging of drug-modulated protein-protein interactions in living subjects. Cancer Res. 2004 Mar 15;64(6):2113-19.PMCID: PMC4154786.
d. Nishihara R, **Paulmurugan R**, Nakajima T, Ishikawa Y, Yamamoto E, Hiruta Y, Iwasawa N, Nishiyama S, Citterio D, Sato M, Kim SB and Suzuki K. A bioluminescence assay platform with coelenterazine analogues for deep-tissue imaging in vivo at near infrared wavelength. *Theranostics*, 2019 Apr 13;9(9):2646-2661. PMCID: PMC6525985.

2. **Imaging cellular signaling networks in living animals.** The molecular imaging assays and sensors I developed using various bioluminescence reporters were later applied to studying the biological complexities associated with estrogen receptor signaling, activation of Hsp90 chaperon complex, activation c-myc, and phosphorylation of Akt. Estrogen receptors respond and activate downstream target genes' expressions in response to estrogen. While estradiol is an endogenous estrogen that regulates estrogen receptor pathways, various estrogen analogues, estrogen mimetics, and environmental estrogens also significantly activate estrogen receptor signaling and induce carcinogenesis. In addition, the recent discovery of the second estrogen receptor, the estrogen receptor beta (ER-β), adds additional complexity to ER biology. I used reported complementation to study ligand-induced ER-folding and ER-α/ER-β cross-talk in cells and living animals. Similarly, Hsp90 α/β -p23 interaction and Akt phosphorylation systems were used for drug screening.
   a. **Paulmurugan R**, Gambhir SS. An intramolecular folding sensor for imaging estrogen receptor-ligand interactions. Proc Natl Acad Sci USA. 2006 Oct 24;103 (43):15883-8. PMCID: PMC1635097.
   b. Chan CT, **Paulmurugan R**, Gheysens OS, Kim J, Chiosis G, Gambhir, SS. Molecular imaging of the efficacy of heat shock protein 90 inhibitors in living subjects. Cancer Res. 2008 Jan 1;68(1):216-26. PMCID: PMC4146344.
   c. **Paulmurugan R**, Tamrazi A, Katzenellenbogen JA, Katzenellenbogen BS, Gambhir SS. A human estrogen receptor (ER) alpha mutation with differential responsiveness to nonsteroidal ligands: novel approaches for studying mechanism of ER action. Mol Endocrinol. 2008 Jul;22(7):1552-64. PMCID: PMC2453600.
   d. Chan CT, **Paulmurugan R**, Reeves RE, Solow-Cordero D, Gambhir SS. Molecular imaging of phosphorylation events for drug development. Mol Imaging Biol. 2009 May-Jun;11(3):144-58. PMCID: PMC4154800.

3. **Imaging Nrf2-Keap1 antioxidant signaling in drug resistance and cancer therapy.** Transcription factor Nrf2 plays a major role in maintaining intracellular ROS level to maintain cellular homeostasis. Activation of Nrf2 by antioxidants is a potent way to improve β-cell function in diabetes. However, Nrf2 demonstrates the opposite effect in cancer therapy and drug resistance. Hence, we are working on finding the mechanism to activate/inhibit the Nrf2 pathway to improve cancer therapy.
   a. **Paulmurugan R**, Oronsky B, Brouse CF, Reid T, Knox S, Scicinski J. Real time dynamic imaging and current targeted therapies in the war on cancer: a new paradigm. Theranostics. 2013 May 25;3(6):437-47. PMCID: PMC3677414.
   b. Ramkumar KM, Sekar TV, Bhakkiyalakshmi E, Foygel K, Rajaguru P, Berger F, **Paulmurugan R**. The impact of oxidative stress on islet transplantation and monitoring the graft survival by non-invasive imaging. Curr Med Chem. 2013; 20(9):1127-46.

4. **Imaging histone methylation in living animals.** Post-translational modifications in histone proteins play major roles in chromatin organization and transcriptional regulation of gene expression in cells. However, while some histone modifications, such as acetylation, phosphorylation, sumoylation, ubiquitination, and citrullination have been well-characterized and documented in the literature, histone methylations that occur in the N-terminal lysine residues of histone 3 and histone 4, which control chromatin structure and gene expression in cells, are poorly understood. We are developing molecular imaging biosensors to study histone methylations in living animals.
   a. Sekar TV, Foygel K, Gelovani JG, **Paulmurugan R**. Genetically encoded molecular biosensors to image

histone methylation in living animals. Anal Chem. 2015 Jan 20;87(2):892-9. PMCID: PMC4303335.

b. Sekar TV, Foygel K, Devulapally R, **Paulmurugan R**. Degron protease blockade sensor to image epigenetic histone protein methylation in cells and living animals. ACS Chem Biol. 2015 Jan 16;10(1): 165-74. PMCID: PMC4301175.

5. **MicroRNAs (miRNAs) and gene-directed enzyme prodrugs therapies for cancer treatment.** MiRNA modulation is a new molecular approach for treating various cancers. MiRNAs are a group of gene expression regulators that play a profound role in the pathogenesis and progression of cancers when aberrantly expressed. In cancers, tumor suppressive miRNAs are significantly under-expressed, while oncogenic miRNAs are highly overexpressed. Both also regulate chemoresistance genes. Therapeutic restoration of both miRNAs has the potential to not only slow cancer growth and metastasis, but also render these tumors sensitive to chemo- and radiotherapies. A major challenge to clinical translation of the miRNA therapy is a safe and efficient approach for *in vivo* delivery. My lab is currently working with ultrasound-microbubbles (US-MB) in combination with polymeric nanoparticles to load and deliver microRNAs *in vivo*. Similarly, gene-directed enzyme prodrug therapy is another efficient strategy for cancer therapy where tumor-targeted delivery of an exogenous gene that codes for an enzyme capable of converting a non-toxic prodrug into an activated cytotoxic agent is used to kill cancer cells. We recently showed that HSV1-sr39TK and NTR enzymes expressed as a fusion protein (TK-NTR) can efficiently kill tumor cells in a subcutaneous and metastatic xenograft model of a human TNBC tumor (MDA MB231) in mice. In these studies, we achieved a complete removal of tumors by injecting just two doses of prodrug combinations (GCV and CB1954).

   a. Devulapally R, Sekar NM, Sekar TV, Foygel K, Massoud TF, Willmann JK, **Paulmurugan R***. Polymer nanoparticles mediated co-delivery of antimiR-10b and antimiR-21 for achieving triple negative breast cancer therapy. ACS Nano. 2015 Mar 24;9(3):2290-302. PMCID: PMC4374409.

   b. Bose RJC, Uday Kumar S, Zeng Y, Afjei R, Lau K, Bermudez A, Habte F, Pitteri SJ, Sinclair R, Willmann JK, Massoud TF, Gambhir SS and **Paulmurugan R**. Tumor Cell-Derived Extracellular Vesicle Coated Nanocarriers: An Efficient Theranostic Platform for Tumor-Specific Delivery of Therapeutic MicroRNAs and Imaging Agents. *ACS Nano*. 2018 Oct 22. PMID:30346694.

   c. Wischhusen JC, Chowdhury SM, Lee T, Wang H, Bachawal S, Devulapally R, Afjei R, Sukumar UK, **Paulmurugan R**. Ultrasound-mediated delivery of miRNA-122 and anti-miRNA-21 therapeutically immunomodulates murine hepatocellular carcinoma in vivo. *J. Control Release*, 2020, 10;321:272-284. PMCID: PMC7170770.

   d. Sukumar UK, Wang H, Telikcho A, Thierry B, Samir C, Massoud TF, Dhal J, **Paulmurugan R**. Ultrasound Triggered Co-delivery of Therapeutic microRNAs and TK-p53-NTR Gene Therapy Vector by PLGA-PEG-PEI Nanoparticles in Mice for Improved Cancer Therapy. *Advanced Therapeutics*. Early View, First published: 05 March 2021. **[Cover Image]**

   * Corresponding author

Complete List of Published Work:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Paulmurugan%20R%5BAuthor%5D&cauthor=true&cauthor_uid=20639890