BIOGRAPHICAL SKETCH

NAME: Sriram Vaidyanathan

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

POSITION TITLE: Postdoctoral Scholar

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
Purdue University	B.S	12/2010	Biomedical Engineering
University of Michigan	M.S.E	04/2013	Biomedical Engineering
University of Michigan	PhD	04/2016	Biomedical Engineering
Stanford University	Postdoc		Genome Editing

A. Personal Statement

I am a postdoctoral scholar working with Dr. Matthew Porteus. My primary research interest over the past decade has been gene therapy. As a doctoral student, I studied the intracellular transport of non-viral gene delivery vectors to optimize delivery. I joined the Porteus lab to further my interest in gene therapy by applying CRISPR/Cas9 based genome editing for monogenic diseases. The Porteus lab has used chemically modified guide RNAs (MS-sgRNA) with Cas9 protein in combination with rAAV6 donor templates to achieve targeted HR mediated insertion in 30-70% hematopoietic stem cells.

Over the past three years, I have focused on optimizing the gene editing of airway basal stem cells to correct mutations that cause cystic fibrosis. The protocols I have helped develop enable us to correct the F508del mutation (a 3 bp deletion) with very high efficiency (>40% allelic correction) and partially restore CFTR function. Our goal in this current project is to leverage this expertise in gene editing to develop a genome editing system that safely and efficiently inserts the CFTR cDNA in the CFTR locus in human airway basal stems cells. Successful editing of airway stem cells would then be followed up by studies to transplant edited stem cells in sinuses of CF patients to treat CF sinusitis. The platform will also then be used to tackle the more challenging problem of transplanting edited cells in the lungs. My training in the Porteus lab will complement my doctoral training in non-viral gene delivery by enabling me to develop expertise in topics such as stem cell biology, genome editing of stem cells, electrophysiology, single cell genomics and advanced microscopy.

In the long-term, my goal is to use my training in bioengineering, drug delivery and genome editing to develop treatments for congenital and degenerative disorders. Over the past two years, I have developed an interest in cystic fibrosis (CF) and I hope to pursue an independent academic research career centered around CF.

B. Positions and Honors

07/2016 – Postdoctoral Scholar, Department of Pediatrics, Stanford University

Other Experience and Professional Memberships

2010-Present Tau Beta Pi Engineering Honor Society
2014-Present Member, American Chemical Society
2018 Eureka Certificate Course in Translational Medicine

Honors

2010 Distinction, Purdue University
2011 Rollin M. Gerstacker Fellowship
2012 Vaughan Symposium Poster Travel Award
2013 Vaughan Symposium Oral Presentation Travel Award
2014 Rackham Graduate Student Research Grant
2017 School of Medicine Dean's Fellowship
2017 Stanford Spectrum Pilot Grant
2019 Cystic Fibrosis Foundation Postdoctoral Fellowship
2019 Cystic Fibrosis Research Institute Postdoctoral Fellowship (Declined)
2020 American Society of Cell and Gene Therapy, Meritorious Abstract Travel Award

C. Contribution to Science

1. **Optimizing gene editing in airway and other primary stem cells:** As a postdoctoral scholar in the Porteus lab, I have led our efforts to optimize gene editing in airway basal stem cells. In addition, I have contributed to other efforts to optimize editing in hematopoietic stem cells and identifying optimal Cas9 delivery strategies that showed improved editing and reduced immune responses. In collaboration with Trilink Biotechnologies, I tested the influence of various chemical modifications, HPLC purification and sequence modifications on the activity of Cas9 mRNA in CD34+ hematopoietic stem and progenitor cells. The results showed that uridine depletion of the Cas9 mRNA sequence resulted in improved editing efficiency and reduced innate immune responses. In a complementary study, we investigated the transcriptional responses in CD34+ cells treated with Cas9 mRNA and Cas9 ribonuclear protein (RNP). We discovered that the cells showed the least immune apoptotic responses to Cas9 RNP.

    a. **Vaidyanathan, S.*,** Salahudeen, A.A.*, Sellers, Z.S.*, Bravo D.T.*, Choi S.C., Batish A., Le W., Baik S., De La O S., Kaushik M.P., Galper N., Lee C.M., Teran C.A., Yoo J.H., Bao G., Chang E.H., Patel Z.M., Hwang P.H., Wine J.J., Milla C.E., Desai T.J,* Nayak J.V,* Kuo C.J,* Porteus M.H.* Highly Efficient Repair of the ΔF508 Mutation in Airway Stem Cells of Cystic Fibrosis Patients with Functional Rescue of the Differentiated Epithelia. *Cell stem cell., **2020**,26,* pp 161-171 **Equal contributions*
    b. **Vaidyanathan, S.***, Azizian, K.T.*, Haque, A.A.*, Porteus, M.H., McCaffrey, A.P et al. Uridine Depletion and Chemical Modification Increase Cas9 mRNA Activity and Reduce Immunogenicity Without HPLC Purification. *Mol. Ther. Nucleic Acids.*, **2018**, 12, pp 530-542. PMCID: PMC6076213.* Equal contributions
    c. Cromer, M.K., **Vaidyanathan, S.**, Ryan, D.E., Bruhn, L. and Porteus, M.H et al. Global Transcriptional Response to CRISPR/Cas9-AAV6-Based Genome Editing in CD34+ Hematopoietic Stem and Progenitor Cells. *Mol. Ther,* **2018** PMCID: PMC6171165
    d. Camarena, J., Charlesworth, C.T., Cromer, M.K., **Vaidyanathan, S.**, Bak, R.O., Porteus, M, et al. Priming Human Hematopoietic Stem and Progenitor Cells for Cas9/Sgrna and rAAV6-Mediated Homologous Recombination. *Mol. Ther. Nucleic Acids.*, **2018**, 12, pp 89-104 PMCID: PMC6023838

2. **Optimizing non-viral gene delivery:** My research as a doctoral student investigated the intracellular transport of non-viral gene delivery materials to the nucleus. Successful *in vivo* delivery of DNA/RNA

therapies is limited by the lack of safe and effective delivery agents. Although non-viral vectors pose fewer safety concerns than viral vectors, non-viral strategies are much less effective in delivering the therapeutics into the target cells. Our long-term goal was to understand the intracellular transport of non-viral gene delivery complexes and use this knowledge to develop more effective non-viral delivery agents. Our hypothesis was that the polymer-plasma membrane interactions were critical for enabling the transport of the delivered cargo to the cytosol and nucleus. To test this hypothesis, I studied the interactions of polymers and polymer-DNA complexes with plasma membrane using an automated patch clamp device. I complemented this method by tracking the subsequent transport of intact DNA molecules within the cell. My work showed that DNA release by effective vectors correlates with the portioning of free vectors in the plasma membrane. Hence, new vectors must be optimized for their interaction with intracellular lipid bilayer, as opposed to the current focus on endosomal buffering activity of vectors, to optimize the transport of intact DNA into the cytosol and the nucleus.

a. **Vaidyanathan S,** Orr B.G and Banaszak Holl M.M. Role of Cell Membrane–Vector Interactions in Successful Gene Delivery. *Acc. Chem. Res.*, **2016**, *49* (8), pp 1486–1493.
b. **Vaidyanathan S,** Chen J , Orr B.G and Banaszak Holl M.M. Cationic Vector Intercalation into the Lipid Membrane Enables Intact Polyplex DNA Escape from Endosomes for Gene Delivery. *Mol. Pharmaceutics*, **2016**, *13* (6), pp 1967–1978.
c. **Vaidyanathan S,** Merzel R.L, Banaszak Holl M.M. et al. Quantitative Measurement of Cationic Polymer Vector and Polymer/pDNA Polyplex Intercalation into the Cell Plasma Membrane. *ACS Nano.* **2015**, 9 (6), pp 6097–6109. PMCID: PMC4771022.
d. Rattan R, **Vaidyanathan S**, Wu G. S.-H., et al. Polyplex-Induced Cytosolic Nuclease Activation Leads to Differential Transgene Expression. *Mol Pharm*. 2013, 10, 3013-3022. PMCID: PMC3776314.

3. **Characterize cellular uptake of dendrimer-fluorophore conjugates:** In addition to the contributions described above, with a team of collaborators I also studied the intracellular behavior of dendrimers (a class of drug delivery polymers) with precise ratios of dyes. The *in vivo* behavior of polymer drug delivery agents is routinely studied using fluorophore/polymer conjugates consisting of a mixture of moieties with different polymer/dye ratios. The interpretation of these results requires the assumption that the presence of the dyes does not influence the behavior of the polymeric carries. Our work showed that the cellular uptake and intracellular behavior of dendrimer/dye conjugates was different at different dendrimer/dye ratios. Moreover, the trends change based on dendrimer size and cell lines. Hence, our work suggests that a thorough characterization of these materials is necessary while using dyes to study the transport of polymer drug delivery systems.
    a. **Vaidyanathan S,** Kaushik M.P, Dougherty C.A, Rattan R, Goonawardena S.N, Banaszak Holl M.M, Monano J, DiMaggio S. Increase in Dye: Dendrimer Ratio Decreases Cellular Uptake of Neutral Dendrimers in RAW Cells. *ACS Biomater. Sci. Eng.*, **2016**, *2* (9), pp 1540–1545. PMCID: PMC5342898.
    b. Dougherty, C.A*, **Vaidyanathan S*** and Banaszak Holl M.M. Fluorophore: Dendrimer Ratio Impacts Cellular Uptake and Fluorescence Lifetime. *Bioconjugate Chemistry.***2015**, 26 (2), pp 304–315. PMCID: PMC4636191. (* co-first author)

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/sriram.vaidyanathan.1/bibliography/public/

D. Additional Information: Research Support

Cystic Fibrosis Foundation Postdoctoral Research Fellowship Vaidyanathan, S (PI)
05/01/2019- 04/30/2021
Genome edited airway stem cells as a durable cell-based therapy for CF
Goals: The development of a genome editing platform to correct CF causing mutations in airway basal stem cells.