BIOGRAPHICAL SKETCH
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NAME: Sivakamasundari V

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

POSITION TITLE: Basic Life Science Research Scientist

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
National University of Singapore	B.S (Hons.)	06/2007	Life Sciences (Biomedical science)
National University of Singapore	Ph.D.	01/2013	Biological Sciences
Genome Institute of Singapore	Postdoctoral	02/2015	Stem cells and Regenerative Biology
The Jackson Laboratory	Associate Research Scientist	2015-2018	Single Cell Biology Laboratory
Stanford University	Basic Life Science Research Scientist	2018-Present	Institute for Stem Cell Biology & Regenerative Medicine

A. Personal Statement

My research interests are focused on understanding the molecular basis of early development and stem cells, as it is often aberrations in stem cells or signaling mechanisms between tissues that lead to diseased states, including tumor development and cancer progression. Knowledge of stem cells and development is also critical to develop appropriate cell-based therapies for various diseases or injuries. In my doctoral work, I was involved in a project elucidating the gene regulatory network of osteo-chondrogenic pathways in the axial skeleton. I investigated the roles of numerous transcription factors (TFs) in a cell-type specific manner, using mouse models, fluorescence assisted cell sorting (FACS) and high-throughput genomic approaches (microarray and chromatin immunoprecipitation-sequencing (ChIP-seq)). By combining a traditional technique with the then state-of-the art genomic methods, we revealed previously under-appreciated roles of *Pax1* and *Pax9* TFs in the regulation of key chondrogenic genes and their gene dosage effects at a molecular level in embryonic intervertebral disc (IVD). We also showed the pleiotropic molecular roles of *Bapx1* in various tissues as well as its co-regulatory role with *Sox9* (master regulator of chondrogenesis) in controlling cell fate and morphogenesis of vertebral column. I transitioned into stem cell biology and single-cell transcriptomics for my postdoctoral training, which primarily involved studying the efficiency and dynamics of *in vitro* derivation of trophectoderm (TE) cells from embryonic stem cells using single cell RNA-sequencing (RNA-seq). Concurrently, we performed a time-course study elucidating the transcriptional dynamics of BMP signaling–mediated immediate response genes using single-cell profiling and ChIP-seq. My expertise in single cell transcriptomics led to my involvement in another collaborative project where we successfully delineated the commitment of dendritic cell (DC) progenitor subsets (cDC1 and cDC2) in the bone marrow itself and the transcriptional signature defining the transition of macrophage DC progenitors (MDPs) into common DC progenitors (CDPs) and then pre-DCs. As an Associate Research Scientist at the Single Cell Biology Laboratory (SCBL, The Jackson Laboratory), my work was focused on circulating tumor cell and cancer biology, as well as establishing protocols for the SCBL. I established and optimized various protocols, such as the droplet-based single cell RNA-seq (Drop-seq,

Macosko et al 2015) method by Macosko et al, plate-based single cell RNA-seq, as well as tissue dissociation and single cell enrichment protocols for a variety of tissue types. I have also established CTC-derived PDX models at JAX – the first of this model to be generated at JAX. I utilized the CTC-derived PDX models and single cell transcriptomics (Chromium Platform, 10xGenomics) and proteomics (CyTOF and imaging mass cytometry) to decipher the heterogeneity in tumor initiating cells within the CTCs present in patient blood samples and the *in vivo* selection of subclones. In addition to these, I also used Imaging Mass Cytometry (IMC) to profile human kidney tissues to decipher the transcriptome and proteome at single cell resolution. In my current work at Stanford University, I am focused on understanding how Hedgehog signaling between epithelial and mesenchymal tissues instruct maintenance of stem/progenitor cells in adult tissue homeostasis as well as immune modulation to maintain tissue integrity upon injury (gastrointestinal and salivary glands) by utilizing various lineage tracing models, single cell transcriptomics, CUT-and-RUN chromatin immunoprecipitation techniques. Further, I aim to develop and optimize the culture conditions to maintain salivary gland progenitors *in vitro*, which will be useful for transplantation therapy to regenerate salivary glands post radiation therapy or injury. My background in developmental, stem and cancer cell biology, and proficiency in a wide variety of techniques, equip me well to execute any research project that require expertise in both traditional animal models and state-of-the art next-generation sequencing and imaging techniques.

a. **V Sivakamasundari***, Mohan Bolisetty*, Santhosh Sivajothi*, Shannon Bessonett, Diane Ruan, Paul Robson. Comprehensive Cell Type Specific Transcriptomics of the Human Kidney. 2017. doi: https://doi.org/10.1101/238063 (In review). *- equal contribution.
b. Nichane M, Javed A, **Sivakamasundari V**, Ganesan M, Ang LT, Kraus P, Lufkin T, Loh KM, Lim B. (2017) Isolation and 3D expansion of multipotent Sox9+ mouse lung progenitors. Nature methods, 14(12):1205-1212.
c. Nathan Lawlor, Joshy George, Mohan Bolisetty, Romy Kursawe, Lili Sun, **Sivakamasundari V**, Ina Kycia, Paul Robson, Michael L. Stitzel. Single cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes. Genome Res. 2017 Feb;27(2): 208-222.
d. **Sivakamasundari V**, Kraus P, Sun W, Hu X, Lim SL, Prabhakar S, Lufkin T. (2016) A developmental transcriptomic analysis of Pax1 and Pax9 in embryonic intervertebral disc development. Biol Open. 2016; PMID: 28011632
e. Analysis of single cell response to BMP stimuli reveals transcriptional dynamics of first responders. **Sivakamasundari V**, Mohan Bolisetty, Paul Robson. CSHL Single Cell Analysis 2015 meeting (Poster).
f. Andreas Schlitzer, **Sivakamasundari V***, Jinmiao Chen*, Hermi Rizal Bin Sumatoh, Jaring Schreuder , Josephine Lum, Benoit Malleret, Sanqian Zhang, Anis Larbi, Francesca Zolezzi, Laurent Renia, Michael Poidinger, Shalin Naik, Evan Newell, Paul Robson and Florent Ginhoux. (2015) Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. Nat Immunol. Jul;16(7):718-28.
g. Chatterjee Sumantra, **Sivakamasundari V**, Yap Sook Peng, Kraus Petra, Kumar Vibhor, Xing Xing, Lim Siew Lan, Sng Joel, Prabhakar Shyam, Lufkin Thomas. (2014) *In vivo* genome-wide analysis of multiple tissues identifies gene regulatory networks, novel functions and downstream regulatory genes for Bapx1 and its co-regulation with Sox9 in the mammalian vertebral column. BMC Genomics. 15(1):1072.
h. **Sivakamasundari V***, Chan HY*, Yap SP, Xing X, Kraus P, Lufkin T (2011). New Bapx1 (Cre-EGFP) mouse lines for lineage tracing and conditional knockout studies. Genesis. 2012 Apr; 50(4):375-83. (* equal contribution)

B. Positions and Honors

Positions and Employment

2007-13	Research Officer, Stem Cell and Developmental Biology, Genome Institute of Singapore, PI: Thomas Lufkin
2013-15	Postdoctoral Fellow, Genome Institute of Singapore, Supervisor: Paul Robson
2015-2018	Associate Research Scientist, Single Cell Biology Laboratory, The Jackson Laboratory. PI: Paul Robson
2018-present	Basic Life Science Research Scientist, Institute for Stem Cell Biology & Regenerative Medicine, PI(s): Philip A Beachy and Kyle M Loh

Other Experience and Professional Memberships

2013-14 Member, Stem Cell Society (Singapore)
2014-15 Member, International Society for Stem Cell Research (ISSCR)
2016 Member, American Association for Cancer Research (AACR)

Honors

2010-13 Scientific Staff Development Award (SSDA) (A*STAR) (PhD scholarship)

C. Contribution to Science

1. **Stem cell and developmental biology -** A regulatory network of TFs orchestrate the differentiation of mesenchymal stem cells towards a chondrogenic or osteogenic lineage in axial skeletal development. To elucidate this network, we adopted a traditional mouse embryonic stem cell (mESC) based gene targeting and high-throughput gene expression and TF binding profiling approach. My initial part of the work involved optimizing strategies for and generating ideal mouse models for knock-in and knock-out of TFs using EGFP insertion. We published our results on the comparison of F2A vs. IRES based methods for concatenating a TF and a reporter in vivo. We also successfully generated mouse models of various TFs involved in the osteo-chondrogenic pathways, namely Sox9, Bapx1, Pax1 and Pax9. These publications and mouse models are a valuable resource for easy isolation of TF-specific cell types from various tissues that would allow comprehension of their tissue-specific roles. We demonstrated the utility of our approach and models in my thesis work on the in vivo gene dosage effect of Pax1 and Pax9 on intervertebral disc at a molecular level. We revealed the integrated roles of Pax1, Pax9 with the Sox5/6/9 (Sox trio) and TGFB/BMP pathways in early IVD development. We identified the existence of novel negative feedback loop between the Pax1/Pax9 and Sox trio that highlights the spatio-temporal regulation of early IVD development. We also showed the pleiotropic molecular roles of Bapx1 in chondrogenic (hindlimg, forelimb, vertebral column) and non-chondrogenic tissues (spleen, gut). We further identified the in vivo co-regulatory roles of Bapx1 and Sox9 in cell fate decisions and preventing premature chondrogenic differentiation of sclerotomal cells in the vertebral column.

    a. Nichane M, Javed A, **Sivakamasundari V**, Ganesan M, Ang LT, Kraus P, Lufkin T, Loh KM, Lim B. (2017) Isolation and 3D expansion of multipotent Sox9+ mouse lung progenitors. Nature methods, 14(12):1205-1212.
    b. **Sivakamasundari V**, Kraus P, Sun W, Hu X, Lim SL, Prabhakar S, Lufkin T. (2016) A developmental transcriptomic analysis of Pax1 and Pax9 in embryonic intervertebral disc development. Biol Open. 2016; PMID: 28011632.
    c. Chatterjee Sumantra, **Sivakamasundari V**, Yap Sook Peng, Kraus Petra, Kumar Vibhor, Xing Xing, Lim Siew Lan, Sng Joel, Prabhakar Shyam, Lufkin Thomas. (2014) *In vivo* genome-wide analysis of multiple tissues identifies gene regulatory networks, novel functions and downstream regulatory genes for Bapx1 and its co-regulation with Sox9 in the mammalian vertebral column. BMC Genomics. 15(1):1072.
    d. **Sivakamasundari V**, Petra Kraus, Song Jie and Thomas Lufkin (2013) Pax1EGFP: New wildtype and mutant EGFP mouse lines for molecular and fate mapping studies. Genesis. 51(6):420-9
    e. Chan HY*, **V Sivakamasundari***, Xing X, Kraus P, Yap SP, Ng P, Lim SL, Lufkin T (2011) Comparison of IRES and F2A-based locus-specific multi-cistronic expression in stable mouse lines. PLoS One. 6(12):e28885. (* equal contribution)
    f. Yap SP, Xing X, Kraus P, **Sivakamasundari V**, Chan HY, Lufkin T. (2011) Generation of mice with a novel conditional null allele of the Sox9 gene. Biotechnol Lett. 33(8):1551-8.

2. **Pre-implantation development and signaling pathways.** The efficiency and ability to derive trophectoderm (TE) cells from human embryonic stem cells (hESCs), *in vitro*, has often been debated. Using single cell transcriptomics we sought to assess the efficiency of established TE differentiation protocol that involves inhibition of FGF signaling and stimulation of BMP pathway. Concurrently, we also captured the dynamic transcriptional states during BMP-mediated differentiation and coupled it with ChIP-seq of BMP effector (SMAD1/5/8p) to identify the immediate early, delayed and secondary responders of BMP stimulus. In conjunction with RNA-flow cytometry for validation, we found that all the cells responded to BMP stimuli within 1-4h based on the expression trend of immediate early genes. While there was heterogeneity in response in the initial 4h of stimulation, all the cells upregulated known BMP direct targets

and TE markers by 48h (commitment stage), indicating uniformity in differentiation. With our two-pronged approach, we demonstrated the ability to identify the early and late responding cells at single cell level.

   a. Analysis of single cell response to BMP stimuli reveals transcriptional dynamics of first responders. **Sivakamasundari V**, Mohan Bolisetty, Paul Robson. CSHL Single Cell Analysis 2015 meeting (Poster).

3. **Single cell transcriptomics** – Single cell profiling has an unparalleled ability to unravel the transcriptional kinetics of a cell during differentiation, diseased states or identify cell types in a heterogenous tissue - aspects which are often masked in bulk transcriptome analyses. Fluidigm's C1™ microfluidic platform was one of the pioneering technologies in the field of single cell RNA-seq. We employed this technology in a number of studies to successfully: identify single cell response to BMP stimuli in hESCs; delineate early priming of sub-populations of dendritic cell progenitors in the bone marrow in contrast to peripheral tissues; and decipher the transcriptomic programs of different human islet cell types, including the molecular aberrations in diseased state (type 2 diabetes). We continue to take full advantage of the leading-edge single cell sequencing platforms such as the Chromium™ Single Cell Instrument (10X Genomics) to address emerging questions in various developmental systems (kidney, lung, gastrointestinal system) and diseases (bladder cancer, small cell lung cancer, renal cell carcinoma, pancreatic cancer, chronic kidney diseases).

   a. **V Sivakamasundari***, Mohan Bolisetty*, Santhosh Sivajothi*, Shannon Bessonett, Diane Ruan. Paul Robson. Comprehensive Cell Type Specific Transcriptomics of the Human Kidney. 2017. doi: https://doi.org/10.1101/238063 (In review). *- equal contribution.
   b. **V Sivakamasundari**, Mohan Bolisetty, Santhosh Sivajothi, Paul Robson. Resolving cellular complexity in renal cell carcinoma with droplet-based single cell transcriptomics. Research Annual Meeting 2017; 2017 Apr 1-5; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2017;77(13 Suppl):Abstract nr 3976. doi:10.1158/1538-7445. AM2017-3976.
   c. Nathan Lawlor, Joshy George, Mohan Bolisetty, Romy Kursawe, Lili Sun, **Sivakamasundari V**, Ina Kycia, Paul Robson, Michael L. Stitzel. Single cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes. Genome Res. 2017 Feb;27(2): 208-222.
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   e. Andreas Schlitzer, **Sivakamasundari V***, Jinmiao Chen*, Hermi Rizal Bin Sumatoh, Jaring Schreuder , Josephine Lum, Benoit Malleret, Sanqian Zhang, Anis Larbi, Francesca Zolezzi, Laurent Renia, Michael Poidinger, Shalin Naik, Evan Newell, Paul Robson and Florent Ginhoux. (2015) Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. Nat Immunol. Jul;16(7):718-28.

4. **Cancer biology** –Small cell lung cancer (SCLC) is an aggressive and highly metastatic form of lung cancer. I generated patient derived circulating tumor cell xenograft (PDCX) models from circulating tumor cells (liquid biopsies) and in collaboration with researchers at MD Anderson, utilized them to identify and decipher the heterogeneity of tumor initiating cells present during the course of treatment (naïve vs. chemoresistant recurrent stage) at a single cell level. Generating CTC-derived xenografts is an extremely challenging pursuit and several labs attempt to perform this within 1h of patient blood draw to ensure success of PDX derivation. At JAX, I optimized the protocols and tested various CTC enrichment strategies to derive PDCX models from patient samples shipped over long distance and have successfully established the first of JAX's PDCX models. I also employed various latest single cell technologies including single cell mass cytometry (CyTOF) and imaging mass cytometry (IMC) to understand the signaling pathways that are critical in instilling the tumor initiating properties of the cancer cells and those that drive chemoresistance in SCLC. These models have great utility in deriving patient-specific treatment modalities. Besides SCLC, we also used single cell approach to investigate the cell types that compose the tumor and its environment in renal cell carcinoma (RCC), in order to decipher the underlying mechanism of tumorigenesis and the key players in metastatic progression. With our single cell data we also aim to develop methods to deconvolute bulk transcriptomic data on RCC.

   a. **V Sivakamasundari***, Mohan Bolisetty*, Santhosh Sivajothi*, Shannon Bessonett, Diane Ruan, Paul Robson. Comprehensive Cell Type Specific Transcriptomics of the Human Kidney. 2017. doi: https://doi.org/10.1101/238063 (Submitted, in review). *- equal contribution.

b. C. Allison Stewart*, Carl M. Gay[1*], Yuanxin Xi*, **Sivakamasundari V**., Junya Fujimoto, Pan Tong, Lixia Diao, Mohan Bolisetty, Patrice Lawson, Francesca Iommelli, Veerakumar Balasubramaniyan, John Stewart, Hai Tran, Bingliang Fang, Jianjun Zhang, John de Groot, Stephen G. Swisher, Jack A. Roth, John V. Heymach, Ignacio Wistuba, Paul Robson, Jing Wang, Lauren Averett Byers. Intratumoral heterogeneity predicts resistance in CTC-derived models of small cell lung cancer. (Submitted manuscript, Nature, May 2018).
c. **V Sivakamasundari**, Mohan Bolisetty, Santhosh Sivajothi, Paul Robson. Resolving cellular complexity in renal cell carcinoma with droplet-based single cell transcriptomics. Research Annual Meeting 2017; 2017 Apr 1-5; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2017;77(13 Suppl):Abstract nr 3976. doi:10.1158/1538-7445. AM2017-3976.

Complete List of Published Work in MyBibliography:

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D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

NA