BIOGRAPHICAL SKETCH

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NAME: Rong Lu

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POSITION TITLE: Richard N. Merkin Assistant Professor of Stem Cell Biology and Regenerative Medicine, Biomedical Engineering, and Medicine

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion (MM/YYYY)	FIELD OF STUDY
Lanzhou University, Lanzhou, Gansu, China PR	BS	06/2001	Biology
Princeton University, Princeton, NJ, USA	PhD	06/2007	Molecular biology Embryonic stem cells
Stanford University, Stanford, CA, USA	Postdoctoral	03/2013	Cell biology Hematopoietic stem cells

A. Personal Statement

My lab studies stem cell coordination, regulation and malfunction from a single cell perspective. Currently, our research is focused on understanding the mechanisms underlying the differences between individual hematopoietic stem cells (HSCs) and how heterogeneous HSCs are coordinated during blood production. We use mouse and human HSCs as our model systems and integrate research strategies from a broad range of disciplines including molecular biology, cell biology, systems biology, bioengineering, and bioinformatics. We have established a novel experimental platform centered on a ***quantitative in vivo clonal tracking technology*** that I developed. This technology integrates three cutting-edge techniques: DNA "barcoding", viral labeling, and high-throughput sequencing. It requires a combination of expertise in molecular biology to construct and process the DNA barcodes, in cell biology to label and transplant the cells, and in systems biology and bioinformatics to analyze the data. I developed a strong background in molecular biology from my Ph.D. training under the guidance of Dr. Ihor R. Lemischka at Princeton University and expertise in cell biology during my postdoctoral training under the mentorship of Dr. Irving L. Weissman at Stanford University. I am proficient with several programming languages, including C/C++, R, Matlab, and Python. I can independently design and carry out the advanced statistical analyses required for modern quantitative biology. With this unique combination of skills, I designed a barcode tracking technology to quantify the proliferation, differentiation, engraftment and migration of single cell clones in a high-throughput manner. The original description of this technology was published in *Nature Biotechnology*, highlighted by *Cell Stem Cell* and *Nature Methods*, and rated "Exceptional" by the *Faculty of 1000*. This technology has been adopted by many research labs. When single cell RNA-seq and ATAC-seq techniques emerged, I quickly adapted our clonal tracking to the new research opportunities and integrated single cell molecular analyses into our experimental platform. I have been actively involved in every aspect of the wet lab and dry lab research in my group.

I have ***a strong background in stem cell biology*** with specific training and *expertise in* ***systems biology and single-cell analysis***. I have been an active researcher in the stem cell field for the past fifteen years, working on embryonic stem cell and hematopoietic stem cell models. I received my initial training in systems biology during my Ph.D. thesis project where I studied the gene regulatory network of mouse embryonic stem cells. I designed and conducted experiments to collect and verify data for the epigenome, transcriptome and proteome in mouse embryonic stem cells. I also designed and programmed a novel algorithm to determine coordinated interactions between different regulatory levels. This work was published in *Nature* and highlighted by *Nature Biotechnology*. Our discovery of discrepancies between the transcriptome and the proteome inspired a battery of studies on post-transcriptional regulation in stem cells.

During my postdoctoral training, I continued to investigate stem cells using the mouse hematopoietic stem cell transplantation system. At Stanford, I developed the ***cross-disciplinary skills*** necessary to meet the challenges of single cell studies. Through collaborations with Dr. Stephen R. Quake's lab at Stanford's Department of Bioengineering and with Dr. Luke Lee's lab at UC Berkeley, I become proficient with next generation high-throughput sequencing technology and micro-fluidic devices. The former was essential to developing the single cell tracking technology mentioned previously. The latter has helped me integrate cutting-edge droplet-based single cell RNA sequencing technology with our barcode tracking system (manuscript under preparation).

Independence: Since I started as a tenure-track assistant professor at USC in 2014, I have been running an independent research lab. I have successfully obtained two R01 grants from NIH/NHLBI. I am the sole key

personnel in both grants. Neither is co-sponsored by a co-PI or a co-I. My lab has already published three papers (in *Cell Reports, EMBO Reports,* and *PNAS*) where I am the corresponding author.

Mentoring: During the past 5 years at USC, I have mentored 9 undergraduate students, 8 master's students, 4 PhD students, and 3 postdoctoral fellows in my lab. These include 3 minority students and 2 students who receive federal work-study. Currently, there are 1 undergraduate student, 2 master's students, 3 PhD students, and 2 postdoctoral fellows in my lab. The 2 domestic postdoctoral fellows in my lab have both won the California Institute for Regenerative Medicine (CIRM) postdoctoral training grant, and one has won a Hearst Fellowship as well. The 2 domestic PhD students in my lab have won 1 NIH T32 training award and 2 NIH F31 awards (F31CA206463 and F31HL134359). The work of all 3 PhD students has been selected for oral presentations at prestigious national and international meetings, including the International Society for Stem Cell Research (ISSCR) meeting in 2017, the American Society of Hematology (ASH) meetings in 2016 and 2017, and the International Society for Experimental Hematology (ISEH) meeting in 2016. During these meetings, they won a first prize for the top poster award at the ISEH meeting and an Abstract Achievement Award at the ASH meeting. The undergraduate students in my lab have won a Rose Hills Science and Engineering Fellowship and three Provost's Research Fellowships.

Teaching: I teach a 2-unit one-semester course every academic year for master's students. I also give lectures to PhD students. I have served on the thesis committees for 4 PhD students, qualifying exam committees for 4 PhD students, and dissertation committee for 1 PhD student. I was a judge for the annual Graduate Research Symposium at USC in 2014.

Service: I am the faculty director of the FACS Core and oversee its operation. At the department level, I served on the faculty annual merit review committee in 2015 and 2018, and on the junior faculty recruitment committee in 2017 and 2018. I have been serving on the faculty panels for the USC postdoctoral career development seminars since it started in 2017. I served on the qualifying exam study section for the PhD Programs in Biomedical and Biological Sciences in 2015 and 2018. For the community, I frequently contribute "What I'm Reading" articles for the USC Stem Cell website to introduce the latest stem cell research. I have also hosted 3 local high school students for summer projects in my lab. I was invited to comment on new technologies by 2 journals: *The Scientist* and *Bio Techniques*. Selected as an example of a successful young investigator, I was interviewed by *Cell Stem Cell* to share my experiences in setting up my lab. (The Path to My Lab's First Paper: Rong Lu, Alvaro Rada-Iglesias, and Jennifer Phillips-Cremins, *Cell Stem Cell*. 2016 Dec 1; 19(6)683-685. doi: 10.1016/j.stem.2016.11.016).

Collaboration: I have been actively collaborating with researchers of complementary expertise, particularly on translational research projects. For example, in collaboration with Dr. Keyue Shen from USC's Biomedical Engineering Department, we are creating artificial HSC niches that can controllably maintain and expand HSCs *in vitro* (Eli and Edythe Broad Innovation Awards, 2016; Rose Hills Foundation Research Fellowship, 2017; NIH-R21-EB024748, 2017-2020). In collaboration with Dr. Qi-Long Ying from my department, we developed a new strategy to expand human granulocyte macrophage progenitors *in vitro* (Whittier Foundation Translational Research Grant, 2017; Eli and Edythe Broad Innovation Awards, 2018). In collaboration with Dr. Akil Merchant from USC's Division of Hematology, we identified clonal evolution patterns of human acute lymphoblastic leukemia cells during relapse following various clinical treatments (oral presentation at ASH, 2017; F31CA206463, 2016-2019). In collaboration with Dr. Cynthia Dunbar at NIH, we used the *rhesus macaque* autologous transplantation model to identify new characteristics of HSC differentiation at the clonal level (*Cell Stem Cell*, 2014; *Blood*, 2017; *Journal of Experimental Medicine*, 2018; *Blood*, 2018; *Science Immunology*, 2018). In collaboration with Dr. David Bryder at Lund University in Sweden, we used induced pluripotent stem (iPS) cells to show that functional changes to HSCs during aging can be reversed (*Nature Communications,* 2017). I frequently participate in seminars and research with hematologists from USC Keck Hospital, Los Angeles County + USC Medical Center, Children's Hospital Los Angeles, USC Norris Comprehensive Cancer Center, City of Hope, and Cedars-Sinai Medical Center. These interactions have improved the design of our experiments and will provide opportunities to quickly translate our findings into clinical applications.

Institutional Support: USC and the Department of Stem Cell Biology and Regenerative Medicine have provided me with strong support and a rich scientific environment. In addition to the outstanding research facilities and support personnel, I have a strong team of mentors. My departmental colleagues all work on stem cells of various tissues and organs, and have provided me with many helpful comments and suggestions regarding the proposed research during our bi-weekly faculty lunch discussions.

I took maternity leaves after giving birth to my daughter in September 2014 and my son in March 2017.

B. Positions and Honors

Positions and Employment

04/2013 – 12/2013	Instructor, Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA
01/2014 – Present	Assistant Professor, Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA

07/2015 – Present	Faculty Director, FACS Core, Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA
07/2016 – Present	Assistant Professor (by courtesy), Department of Biomedical Engineering, University of Southern California, Los Angeles, CA
01/2018 – Present	Assistant Professor (by courtesy), Department of Medicine, University of Southern California, Los Angeles, CA
10/2018 – Present	Richard N. Merkin Assistant Professor in Regenerative Medicine, Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA

Other Experience and Professional Memberships

2005 – Present	Member, International Society for Stem Cell Research (ISSCR)
2011 – Present	Member, American Society of Hematology (ASH)
2014 – Present	Member, USC Norris Comprehensive Cancer Center, USC
2014 – Present	Reviewer, *Cell Stem Cell, Cell Reports, PNAS, Journal of Experimental Hematology, Nature Methods, Stem Cell Research, Stem Cell Report, Blood, Stem Cell Research, Aging*
2015	External reviewer, Human Frontier Science Program (HFSP) Career Development Awards, France
2018	Member, Local Organizing Committee for International Society for Experimental Hematology (ISEH) meeting
2018	Temporary reviewer, MCH Study Section, NIH

Honors

1998 – 2001	First-class Scholarship, Lanzhou University
2000	Baogang Educational Fund for Exceptional Student, National Education Department of China PR
2000, 2001	Sanhao (Outstanding) Student, Lanzhou University
2001	Outstanding Graduate, Lanzhou University
2003	Johnston Fund, Princeton University
2011	Best Poster Award, Gordon Conference on Stem Cells & Cancer
2011, 2012	Abstract Achievement Award, American Society of Hematology (ASH) Meeting
2012	Stanford University Best Postdoctoral Research Award
2012	NIH Pathway to Independence Award (K99/R00)
2016, 2018	Eli and Edythe Broad Innovation Awards
2018	Richard N. Merkin Assistant Professorship

C. Contributions to Science

1. During my five years as an independent investigator, my research has addressed an important yet poorly understood question of how stem cells are coordinated to ensure proper tissue size and function. We study ***the coordination between individual hematopoietic stem cells*** (HSCs) *in vivo* using mouse models. We found that most HSCs do not supply every blood cell type over the long term post transplantation. We also found that the differentiation programs of individual HSCs change in response to the transplantation dose, and in the presence of mutant HSCs that lack the capacity to produce every blood lineage. These studies indicated that HSC clones can sense the presence of other HSC clones and adapt their differentiation programs. I am the primary investigator in these studies.
   a. **Lu R**. Sleeping beauty wakes up the clonal succession model for homeostatic hematopoiesis. *Cell Stem Cell*. 2014 Dec 4;15(6):677–8. doi: 10.1016/j.stem.2014.11.015. PMID: 25479744.
   b. Brewer C, Chu E, Chin M, **Lu R**. Transplantation dose alters the differentiation program of hematopoietic stem cells. *Cell Reports*. 2016 May 24;15(8):1848-57. doi: 10.1016/j.celrep.2016.04.061. PMID: 27184851.
   c. Nguyen L, Wang Z, Chowdhury AY, Chu E, Eerdeng J, Jiang D, **Lu R**. Functional compensation between hematopoietic stem cell clones *in vivo*. *EMBO Reports*. 2018 May 30; pii: e45702. doi: 10.15252/embr.201745702. PMID: 29848511.
2. Cellular heterogeneity plays a prominent role in stem cells, but research in this area is limited by technical hurdles in assaying single cells *in vivo*. To overcome these limitations, I developed an ***innovative cellular tracking technology*** with single-cell sensitivity and high-throughput capacity using genetic barcoding and next generation sequencing. I made the first direct measurement of *in vivo* HSC differentiation at the clonal level through multiple stages of lineage commitment. I discovered the cellular origins of lineage bias and

clonal dominance during HSC differentiation. I also found that the standard radiation conditioning regimen used in virtually all HSC studies and clinical applications induces radical changes to HSC differentiation at the clonal level. These studies revealed unexpected clonal-level HSC behaviors that are undetectable by conventional population-level studies. I am the lead author of these studies.

a. **Lu R**, Neff NF, Quake SR, Weissman IL. Tracking single hematopoietic stem cells *in vivo* using high-throughput sequencing in conjunction with viral genetic barcoding. *Nature Biotechnology*. 2011 Oct 2;29(10):928–33. doi: 10.1038/nbt.1977. PMID: 21964413.
b. Wahlestedt M, Erlandsson E, Kristiansen T, **Lu R**, Brakebusch C, Weissman IL, Yuan J, Martin-Gonzalez J, Bryder D. Clonal reversal of aging-associated stem cell lineage bias via a pluripotent intermediate. *Nature Communications*. 2017 Feb 22;8 doi: 10.1038/ncomms14533. PMID: 28224997
c. **Lu R***, Czechowicz A, Seita J, Jiang D, Weissman IL*. Clonal-level lineage commitment pathways of hematopoietic stem cells in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2019 Jan 22;116(4):1447-1456. doi: 10.1073/pnas.1801480116. PMID: 30622181. *co-corresponding authors

3. In collaboration with Dr. Cynthia Dunbar's lab at NIH, we used the ***rhesus macaque* autologous transplantation model** to provide more translational insights into our findings in mice. We identified the temporal and spatial distributions of HSPC clones. In particular, we found a distinct lineage origin for natural killer cells and for T cells immediately after HSPC transplantation. I am a collaborator and provided key technical support for the clonal tracking in these studies.
   a. Wu C*, Li B*, **Lu R***, Koelle SJ, Yang Y, Jares A, Krouse AE, Metzger M, Liang F, Loré K, Wu CO, Donahue RE, Chen IS, Weissman I, Dunbar CE. Clonal tracking of rhesus macaque hematopoiesis highlights a distinct lineage origin for natural killer cells. *Cell Stem Cell*. 2014 Apr 3;14(4):486-99. doi: 10.1016/j.stem.2014.01.020. PMID: 24702997. * Equal contribution.
   b. Koelle SJ, Espinoza DA, Wu C, Xu J, **Lu R**, Li B, Donahue RE, Dunbar CE. Quantitative stability of hematopoietic stem and progenitor cell clonal output in rhesus macaques receiving transplants. *Blood*. 2017 Mar 16;129(11):1448-1457. doi: 10.1182/blood-2016-07-728691. PMID: 28087539.
   c. Wu C, Espinoza DA, Koelle SJ, Potter EL, **Lu R**, Li B, Yang D, Fan X, Donahue RE, Roederer M, Dunbar CE. Geographic clonal tracking in macaques provides insights into HSPC migration and differentiation. *Journal of Experimental Medicine*. 2018 Jan 2;215(1):217-232. doi: 10.1084/jem.20171341. PMID: 29141868.
   d. Yu KR, Espinoza DA, Wu C, Truitt L, Shin TH, Chen S, Fan X, Yabe I, Panch S, Hong SG, Koelle S**, Lu R**, Bonifacino A, Krouse A, Metzger M, Donahue RE, Dunbar CE. The impact of aging on primate hematopoiesis as interrogated by clonal tracking. *Blood*. 2018 Jan 2; pii: blood-2017-08-802033. doi: 10.1182/blood-2017-08-802033. PMID: 29295845.
   e. Wu C, Espinoza DA, Koelle SJ, Yang D, Truitt L, Schlums H, Lafont BA, Davidson-Moncada JK, **Lu R**, Kaur A, Hammer Q, Li B, Panch S, Allan DA, Donahue RE, Childs RW, Romagnani C, Bryceson YT, Dunbar CE. Clonal expansion and compartmentalized maintenance of rhesus macaque NK cell subsets. Science immunology. 2018 Nov 2;3(29): doi: 10.1126/sciimmunol.aat9781. PMID: 30389798.

4. To meet the challenges of ***single-cell gene expression analysis***, miniaturization is an effective way to increase analysis sensitivity and throughput. I collaborated with two engineering groups to develop micro-fluidic devices. With Dr. Stephen R. Quake's lab at Stanford, we developed a microfluidic device that can analyze the epigenetics of small cell populations. With Dr. Luke Lee's lab at UC Berkeley, we developed a microfluidic device that can measure single cell RNA expression in a high-throughput manner. These new tools provide the high-throughput and high-sensitivity necessary to study gene regulatory mechanisms at a refined resolution. I am a collaborator and provided key support on mouse models and biological insights in these studies.
   a. Wu AR, Hiatt JB, **Lu R**, Attema JL, Lobo NA, Weissman IL, Clarke MF, Quake SR. Automated microfluidic chromatin immunoprecipitation from 2,000 cells. *Lab on a Chip*. 2009 May 21;9(10):1365–70. doi: 10.1039/b819648f. PMID: 19417902.
   b. Dimov IK, **Lu R**, Lee EP, Seita J, Sahoo D, Park SM, Weissman IL, Lee LP. Discriminating cellular heterogeneity using microwell-based RNA cytometry. *Nature Communications*. 2014 Mar 25;5:3451. doi: 10.1038/ncomms4451. PMID: 24667995.

5. At a time when genome-wide studies were on the rise and epigenome analyses had just emerged, I studied mouse embryonic stem cells at the genomic scale using ***molecular and systems biology approaches***. I developed innovative strategies to determine the coordination between the epigenome, the transcriptome, and the proteome during differentiation. I found that more than 40% of the changes at the

protein level are not reflected by changes at the mRNA level during embryonic stem cell differentiation. I also found that chromatin reconfiguration is preceded by transcription factor mediated regulatory events during cell fate alteration. These findings revealed how the gene regulatory network of stem cells is coordinated at different molecular regulatory levels. I am the lead author of these studies.

a. Ivanova N, Dobrin R, **Lu R**, Kotenko I, Levorse J, Decoste C, Schafer X, Lun Y, Lemischka IR. Dissecting self-renewal in stem cells with RNA interference. *Nature*. 2006 Aug 3;442(7102):533–8. doi:10.1038/nature04915. PMID: 16767105.
b. Guan Y, Myers CL, **Lu R**, Lemischka IR, Bult CJ, Troyanskaya OG. A genomewide functional network for the laboratory mouse. *PLOS Computational Biology.* 2008 Sep 26;4(9):e1000165. doi: 10.1371/journal.pcbi.1000165. PMID: 18818725.
c. **Lu R**, Markowetz F, Unwin RD, Leek JT, Airoldi EM, Boyer LA, Troyanskaya OG, Whetton AD, Lemischka IR. Systems-level dynamic analyses of fate change in murine embryonic stem cells. *Nature*. 2009 Nov 19;462(7271):358–62. doi:10.1038/nature08575. PMID: 19924215.

Complete List of Published Work: https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47387216/

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

Ongoing Research Support

R01HL135292 Lu (PI) 08/01/17 – 07/31/22
Investigating the Heterogeneous Aging of Individual Hematopoietic Stem Cell Clones
The goal is to investigate how individual HSCs differentially age.
Role: PI

R01HL138225 Lu (PI) 08/28/17 – 05/31/21
Tracing the Developmental Origin of Hematopoietic Stem Cell Heterogeneity
The goal is to investigate how diverse differentiation programs of HSCs arise during development.
Role: PI

R21 EB024748 Shen (PI) 08/01/17 – 04/30/20
Artificial Stromal Cell for Hematopoietic Stem Cell Expansion
The goal is to create artificial substrates that can controllably maintain and expand HSCs *in vitro.*
Role: Co-Investigator

Eli and Edythe Broad Innovation Awards Ying, Lu (PI) 01/01/18 – 12/31/18
Engineering Human Granulocyte Macrophage Progenitors for Cancer Immunotherapy
The goal is to engineer human progenitor cells to treat cancer.
Role: PI

Completed Research Support

NIH-K99/R00-HL113104 Lu (PI) 06/01/12 – 02/28/18
Lineage Bias and Clonal Expansion of Hematopoietic Stem Cell Differentiation
The goal is to characterize HSC differentiation at the clonal level.
Role: PI

Whittier Foundation Translational Research Grant Ying, Lu (PI) 03/01/17 – 02/28/18
Expansion and Characterization of Human Granulocyte Macrophage Progenitors
The goal is to expand human granulocyte macrophage progenitors *in vitro.*
Role: PI

Eli and Edythe Broad Innovation Awards Shen, Lu (PI) 01/01/16 – 12/31/16
Microscale Engineering of Hematopoietic Stem Cell Niches in a Dish
The goal is to engineer HSC niche to maintain and expand hematopoietic stem cells *in vitro.*
Role: PI

CIRM-TG2-01159 10/01/09 – 05/31/12
Heterogeneity of Hematopoietic Stem Cells
The goal is to examine the heterogeneity of HSCs.
Role: Award Recipient

NIH-T32AI07290 09/01/07 – 08/31/09
Early Development of Hematopoietic Stem Cells
The goal is to determine how epigenetic mechanisms regulate HSCs during early development.
Role: Award Recipient