BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Rong Lu

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

POSITION TITLE: Associated Professor of Stem Cell Biology and Regenerative Medicine, Biomedical Engineering, Medicine, and Gerontology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion (MM/YYYY)	FIELD OF STUDY
Lanzhou University, Lanzhou, Gansu, China PR	B.S.	06/2001	Biology
Princeton University, Princeton, NJ, USA	Ph.D.	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 blood and immune cell development and regeneration from a quantitative and systems biology perspective. My research program is driven by two overarching questions: *how do individual hematopoietic stem cells differ from one another, and how are they coordinated in sustaining a balanced blood supply?* We integrate research strategies from a broad range of disciplines including molecular biology, cell biology, systems biology, bioengineering, synthetic biology, and bioinformatics. I have ***a strong background in stem cell biology*** with specific training and *expertise in* ***systems biology and single-cell analysis***. I completed my Ph.D. studies under the guidance of Dr. Ihor R. Lemischka at Princeton University, where we carried out pioneering research in probing the gene regulatory network across the epigenome, transcriptome and proteome simultaneously in mouse embryonic stem cells (*Nature*, 2009). I then conducted my postdoctoral research with Dr. Irving L. Weissman at Stanford University, where we developed the high-throughput sequencing based clonal tracking technology that is now widely used in the field (*Nature Biotechnologies*, 2011). Currently at USC, my laboratory is one of the world's leading research groups in studying stem cells as a network in the context of development and tissue regeneration. By incorporating computational and experimental approaches, our investigations provide novel perspectives on the heterogeneity, coordination, and malfunction of hematopoietic stem and progenitor cells. Our investigations have revealed that hematopoietic stem cells (HSCs) change their differentiation programs in response to different transplantation doses (*Cell Reports,* 2016), to the addition of other hematopoietic progenitors (*Exp Mol Med.,* 2023), and to the presence of deficient HSCs (*EMBOR Reports*, 2018). We have discovered that the coordination between HSCs is achieved through distinct lineage commitment pathways that lead individual HSC clones to different levels of self-renewal and differentiation (*PNAS*, 2019). At the molecular level, we have identified four general patterns of quantitative association between the gene expression and blood production of individual HSCs *in vivo* (*Science Advances*, under revision). In addition, we have shown how individual HSC clones heterogeneously change with age and collectively contribute to the early and delayed aging phenotypes of the immune system (manuscript under review). Our recent study has revealed how HSC network responds to a Tet2 mutation (*Blood*, 2023).

My research laboratory trainees have included undergraduate students, master's graduate students, Ph.D. graduate students, post-doctoral fellows, and clinical fellows. Trainees in my lab, whether specializing in wet lab, dry lab, or a combination of both, all acquire versatile and multidisciplinary skills. I am very proud of the achievements of my trainees. The postdoctoral fellows in my lab have won two California Institute for Regenerative Medicine (CIRM) postdoctoral training grants, a Hearst Fellowship, and a research supplement award from NIH. The Ph.D. students in my lab have won one NIH T32 training award, two CIRM training awards, and three NIH F31 awards (F31CA206463, F31HL134359 and F31HL149278). All senior Ph.D. students in my lab have given 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. All four Ph.D. students who graduated from my lab have their own first-author publications (*EMBO Reports,* 2018; *Nature Protocols,* 2020; *Nature Communications,* 2021; *Blood,* 2023). Additionally, a graduate student in my lab won a Keck Genomics Partnership Grant in 2019. The undergraduate students in my lab have won two Rose Hills Science and Engineering Fellowships and five Provost's Research Fellowships. Two clinical fellows in my lab won a Broad Clinical Fellow Award (2020-2021) and a CIRM training grant (2022-2025).

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

R01AG080982 Lu, Elowitz, Lois (PI) 04/01/23 – 01/31/28
Using spatial, single-cell genomic recording to investigate age-associated clonal hematopoiesis

R35HL150826 Lu (PI) 08/01/20 – 07/31/27
Investigating the heterogeneity and coordination of hematopoietic stem cells

Leukemia & Lymphoma Society Scholar, LLS-1370-20 Lu(PI) 07/01/19 – 06/30/24
Dissecting the heterogeneity of leukemic and pre-leukemic clonal expansion to identify genes associated with leukemia relapse and genesis

NCCC Disease related Affinity Groups (DAGs) de Smith, Lu(PI) 07/01/21 – 06/30/23
The Impact of Genetic Ancestry and Childhood Leukemia Risk Alleles on Hematopoiesis in Hispanic/Latino Newborns.

Merkin Scholar Lu(PI) 10/01/18 – 12/31/21
Investigating hematopoietic stem cells at the single cell and clone levels

Eli and Edythe Broad Innovation Awards Lu, Elowitz (PI) 02/01/20 – 12/31/21
Identifying the spatial organization of hematopoietic stem and progenitor cells in the bone marrow

R01HL138225 Lu (PI) 08/28/17 – 05/31/20
Tracing the Developmental Origin of Hematopoietic Stem Cell Heterogeneity

R01HL135292 Lu (PI) 08/01/17 – 07/31/20
Investigating the Heterogeneous Aging of Individual Hematopoietic Stem Cell Clones

NIH-R01-HL138225 and NIH-R01-HL135292 were relinquished due to the award of NIH-R35-HL150826.

K99/R00HL113104 Lu (PI) 06/01/12 – 02/28/18
Lineage Bias and Clonal Expansion of Hematopoietic Stem Cell Differentiation

Citations:

1. 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.
2. 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.
3. Contreras-Trujillo H, Eerdeng J, Akre S, Jiang D, Contreras J, Gala B, Vergel-Rodriguez MC, Lee Y, Jorapur A, Andreasian A, Harton L, Bramlett CS, Nogalska A, Xiao G, Lee JW, Chan LN, Müschen M, Merchant AA, **Lu R**. Deciphering intratumoral heterogeneity using integrated clonal tracking and single-cell transcriptome analyses. *Nature Communications*. 2021 Nov 11;12(1):6522. doi: 10.1038/s41467-021-26771-1. PMID: 34764253; PMCID: PMC8586369
4. Bramlett C, Eerdeng J, Jiang D, Lee Y, Garcia I, Vergel-Rodriguez M, Condie P, Nogalska A, **Lu R**. RNA splicing factor Rbm25 underlies heterogeneous preleukemic clonal expansion in mice. Blood. 2023 Jun 15;141(24):2961-2972. doi: 10.1182/blood.2023019620. PMID: 36947858.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

04/2013 – 12/2013 Instructor, Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA

01/2014 – 12/2021	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	Adjunct Professor, Department of Biomedical Engineering, University of Southern California, Los Angeles, CA
01/2018 – Present	Adjunct Professor, Department of Medicine, University of Southern California, Los Angeles, CA
10/2018 – 12/2021	Richard N. Merkin Assistant Professor in Regenerative Medicine, Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA
03/2019 – Present	Adjunct Professor, Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA
12/2021 – Present	Associate Professor (tenured), 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
2014 – Present	Reviewer, *Cell Stem Cell, Cell Reports, PNAS, Nature Methods, Nature Biotechnology, Nature Communications, Stem Cell Research, Stem Cell Report, Blood, Experimental Hematology, Stem Cell Research, Aging, Plos Biology, Genome Biology, Nucleic Acids Research, Biology of Blood and Marrow Transplantation, Translational Research, Cell.*
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
2018	Grant Reviewer, University of California, Irvine, Institute for Clinical and Translational Science (ICTS)
2019	Grant Reviewer, USC Core Instrumentation grant
2019	Grant Reviewer, USC internal grant, MHI program
2020 – 2026	Standing member, BBHV Study Section, NIH
2021	Grant Reviewer, Saban Research Institute's Intramural Awards Study Section, Children's Hospital Los Angeles, USC
2022	Grant Reviewer, Norris Cancer Center Core Grant (CCSG) Developmental Funds Review Committee, USC
2022	Grant Reviewer, CIRM training grant, Ph.D. program in Development, Stem Cells and Regenerative Medicine, USC

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, 2020	Eli and Edythe Broad Innovation Awards
2018	Richard N. Merkin Assistant Professorship
2019	Leukemia & Lymphoma Society Scholar
2020	NIH/NHLBI Emerging Investigator Award (R35)

C. Contributions to Science

1. As an independent investigator at USC, my research addresses 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 cell (HSC) clones*** *in vivo* using mouse models. We found that the differentiation programs of individual HSCs change in response to the transplantation dose, to the addition of other hematopoietic progenitors, and to 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 of these studies.

   a. 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.

   b. 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.

   c. Wang Z, Jiang D, Vergel-Rodriguez M, Nogalska A, **Lu R**. Lineage tracking to reveal the fate of hematopoietic stem cells influenced by $Flk2^-$ multipotent progenitors after transplantation. Exp Mol Med. 2023 Jan;55(1):205-214. doi: 10.1038/s12276-022-00922-w. Epub 2023 Jan 13. PMID: 36639717; PMCID: PMC9898540.

2. Recent studies from our group and others show that individual HSCs self-renew and differentiate substantially differently from one another in both mice and humans. To understand ***the cellular heterogeneity***, my lab further improved the quantification and sensitivity of the genetic barcode based clonal tracking technology that I had developed as a post-doc. We identified the cellular origins of clonal dominance and lineage bias, and uncovered the lineage commitment pathways that lead HSC clones to different levels of self-renewal and blood production under various transplantation conditions. We also showed that HSC clones heterogeneous expand upon Tet2 knockout and that their expansion levels are associated with the expression of RNA splicing factors such as Rbm25. Using a patient-derived xenograft model of B-cell acute lymphoblastic leukemia, we found that leukemia cells with distinct chemotherapy resistances exhibited unique gene expression signatures prior to chemotherapy treatments. These findings provide new technologies and conceptual frameworks for understanding cellular heterogeneity in tissue regeneration and leukemia. I am the primary investigator of these studies.

   a. **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

   b. Bramlett C, Jiang D, Nogalska A, Eerdeng J, Contreras J, **Lu R**. Clonal tracking using embedded viral barcoding and high-throughput sequencing. *Nature Protocols.* 2020 Apr;15(4):1436-1458. Epub 2020 Mar 4. doi: s41596-019-0290-z. PMID: 32132718.

   c. Contreras-Trujillo H, Eerdeng J, Akre S, Jiang D, Contreras J, Gala B, Vergel-Rodriguez MC, Lee Y, Jorapur A, Andreasian A, Harton L, Bramlett CS, Nogalska A, Xiao G, Lee JW, Chan LN, Müschen M, Merchant AA, **Lu R**. Deciphering intratumoral heterogeneity using integrated clonal tracking and single-cell transcriptome analyses. *Nature Communications*. 2021 Nov 11;12(1):6522. doi: 10.1038/s41467-021-26771-1. PMID: 34764253; PMCID: PMC8586369.

   d. Bramlett C, Eerdeng J, Jiang D, Lee Y, Garcia I, Vergel-Rodriguez M, Condie P, Nogalska A, **Lu R**. RNA splicing factor Rbm25 underlies heterogeneous preleukemic clonal expansion in mice. Blood. 2023 Jun 15;141(24):2961-2972. doi: 10.1182/blood.2023019620. PMID: 36947858.

3. My lab at USC has been actively collaborating with other research groups to ***study HSCs using various experimental and animal models***. 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. With Dr. Cynthia Dunbar's lab at NIH, we used the *rhesus macaque* autologous transplantation model to identify new characteristics of HSC differentiation at the clonal level. With Dr. Keyue Shen's lab at USC, we used a supported lipid bilayer (SLB) to study the roles of membrane-bound factors in regulating HSCs. These studies revealed new insights into HSC regulatory mechanisms from various perspectives. I am a collaborator in these studies and provide supports on mouse models, HSC biology, and the clonal tracking technology.
    a. 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
    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. Hao J, Zhou H, Nemes K, Yen D, Zhao W, Bramlett C, Wang B, **Lu R**, Shen K. Membrane-bound SCF and VCAM-1 synergistically regulate the morphology of hematopoietic stem cell. J Cell Biol. 2021 Oct 4;220(10):e202010118. doi: 10.1083/jcb.202010118. PMID: 34402812.
4. 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*. <u>During my postdoctoral training at Stanford</u>, I developed an ***innovative cellular tracking technology*** with single-cell sensitivity and high-throughput capacity using genetic barcoding and next generation sequencing. 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 is now widely used by many research labs. In addition, I collaborated with two engineering groups to develop ***micro-fluidic devices*** to study molecular regulation of single cells. 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 cellular and molecular regulation at a refined resolution.
    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. **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.
    c. 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.
    d. 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.
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*** <u>during my PhD study at Princeton</u>. 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. Our discovery of discrepancies between the transcriptome and the proteome inspired a battery of studies on post-transcriptional regulation in stem cells. 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/