BIOGRAPHICAL SKETCH

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NAME: Nakauchi, Hiromitsu

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POSITION TITLE: Professor of Genetics

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Yokohama City University School of Medicine.	M.D.	03/1978	Medicine
Harvard Medical School	Visiting student	09/76-08/77	Medicine
Department of Medicine, Yokohama City University Hospital	Internship	03/1979	Internal Medicine
Department of Immunology, Faculty of Medicine, University of Tokyo	Ph.D.	03/1983	Immunology
Department of Genetics, Stanford University School of Medicine	Postdoctoral Fellow	12/1985	Molecular Immunology

A. Personal Statement

I am a physician scientist with a broad background in stem cell biology, hematology, and immunology, with specific training and expertise in *in vivo* approaches and molecular biology, within a long scientific career of innovative and high-impact research.

After my postdoctoral training at the Laboratory of the late Prof. Len Herzenberg at Stanford University, I returned to Japan to establish my own laboratory. As a faculty in Japan, I have supervised over 70 postgraduate students at Juntendo University School of Medicine, Tsukuba University, and the University of Tokyo. I focused on creating a safe, collaborative, and stimulating environments for all of my students to let them experience the enjoyment of research. Many of the graduates have gone on to work as PIs at universities and research institutions, while others have gone on to become experts in scientific ethics, patents, and physician scientists.

Since joining the Stanford faculty in 2014, I have supervised and am still supervising in total of eleven postdocs and five graduate students. Of the seven postdocs who have already left my lab, five of them have become faculties (two of them with K99 awards), one of them has become a senior scientist in industry, and another one has returned to clinical practice as a surgeon. One of my main responsibilities as the PI of my laboratories, both at the University of Tokyo and Stanford University, is to meet with my lab members and stand side-by-side to discuss their current progress of projects and long-term future plans. I have also utilized the virtual platform, in the midst of the pandemic, to create a rigorous learning environment for my lab members in both Japan and the U.S., in which they collaborate together to strengthen their abilities to review and give critiques to the existing literature or to have a single lab member give a presentation of its current project. This thought-provoking environment allows to strengthen the connections of lab members internationally.

During my postdoctoral training at the Laboratory of the late Prof. Len Herzenberg at Stanford University, I successfully isolated the CD8 gene. After returning to Japan, I shifted my main field of interest toward hematopoietic stem cells (HSCs). I developed methods to phenotype mouse HSCs and demonstrated that a single HSC can reconstitute the entire hematopoietic system. The ability to prospectively isolate and clonally analyze mouse HSCs was a forerunner of single cell biology and contributed to biology at large. Recently, after many years of effort, my laboratory succeeded in expanding functional HSCs ex vivo. This represents the first

culture platform to achieve bona fide ex vivo HSC self-renewal, with broad application to HSC biology and exciting translational implication. Outside of HSC biology, my laboratory has pioneered the use of pluripotent stem cells (PSCs) as a source of functional cell types and organs for transfusion and transplantation. My laboratory was responsible for establishing key methods for *in vitro* generation of human platelets and T cells for clinical transfusion and immunotherapy, respectively. Additionally, I established methods to generate complex solid organs from PSCs by using *in vivo* developmental organ niches. These diverse research achievements at the interface of stem cell biology, hematology, and immunology highlight my credentials of innovative and transformative research.

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

R01 DK116944
Nakauchi (PI)
08/15/2018 – 05/31/2022
Valine as a Metabolic Modulator of Hematopoiesis

R01 HL147124
Nakauchi (PI)
09/06/2018 – 08/31/2022
Modulating HSC-niche interactions to understand aging and improve transplantation

1R01DK121851-01A1
Nakauchi (PI)
06/01/2020 – 05/31/2023
Understanding the developmental xenobarrier

1R21OD030009-01
Nakauchi (PI)
07/15/2020 – 04/30/2022
Selectable non-mosaic embryo editing

Citations (last 5 years):

a. Nishimura T, Suchy FP, Bhadury J, Igarashi KJ, Charlesworth CT, **Nakauchi H**. (2021). Generation of Functional Organs Using a Cell-Competitive Niche in Intra- and Inter-species Rodent Chimeras. Cell Stem Cell. 28(1):141-149. PMCID: PMC8025673.
b. Wilkinson AC, Ishida R, Kikuchi M, Sudo K, Morita M, Crisostomo RV, Yamamoto R, Loh KM, Nakamura Y, Watanabe M, **Nakauchi H***, Yamazaki S* (2019). Long-term ex vivo hematopoietic stem cell expansion affords nonconditioned transplantation. Nature. 571(7763):117-121. PMCID: PMC7006049.
c. Yamamoto R, Wilkinson AC, Ooehara J, Lan X, Lai CY, Nakauchi Y, Prichard J, **Nakauchi H** (2018). Large-scale clonal analysis resolves aging of the mouse hematopoietic stem cell compartment. Cell Stem Cell 22(4):600-607. PMCID: PMC5896201.
d. Yamaguchi T, Sato H., Kato-Itoh M, Goto T, Hara H, Sambo M, Kobayashi T, Mizuno N, Yanagida A, Umino A, Ohta Y, Hamanaka S, Masaki H, Rashid D, Hirabayashi M, **Nakauchi H** (2017). Interspecies organogenesis generates autologous functional islets. Nature. 542(7640):191-196.

B. Positions, Scientific Appointments, and Honors

Positions, Scientific Appointment

2022-present	Distinguished Professor, Stem Cell Therapy Laboratory, Advanced Research Institute, Tokyo Medical and Dental University
2017 -2022	Project Professor, Division of Stem Cell Therapy, Distinguished Professor Unit, IMSUT
2017-2018	Scientific Advisory Board, World Premier International Research Center Initiative (ASHBi, Kyoto University)
2015 -present	Advisory board, RIKEN Biosystems Dynamics Research, Japan
2014 -present	Professor, Department of Genetics, Institute for Stem Cell Biology and Regenerative Medicine, Stanford University

2010 -2013 Guest Professor, University of Ulm, Germany
2008 -2017 Founding Director, Center for Stem Cell Biology and Regenerative Medicine, IMSUT
2008 -2013 Leader, University of Tokyo, iPS Research Core Facility Program of the Project for Realization of Regenerative Medicine, The Ministry of Education, Culture, Sports, Science and Technology, Japan
2005 -2013 Advisory board, RIKEN Research Center for Allergy and Immunology 2007 -2010
2004 -2008 Board of Directors, International Society of Stem Cell Research, Member, Board of Directors
2004 -2007 American Society of Hematology, Member, International Members Committee
2004 -2007 Advisory board, CONSERT (Concerted Safety & Efficiency Evaluation of Retroviral Transgenesis in Gene Therapy of Inherited Disease) by the European Union
2002 -2008 Professor, Laboratory of Stem Cell Therapy, Center for Exp. Medicine, Institute of Medical Science, University of Tokyo (IMSUT)
1999 -2002 Founding Director, Laboratory Animal Resource Center, University of Tsukuba
1994 -2002 Professor, Department of Immunology, Institute of Basic Medical Sciences, University of Tsukuba
1987 -1995 Associate team leader, Team leader, Laboratory of Cell Growth and Differentiation, The Institute of Physical and Chemical Research (RIKEN)
1986 -1987 Assistant Professor, Department of Immunology Juntendo University School of Medicine

Honors

2015 Ernest McCulloch Memorial Lecture, International Society for Stem Cell Research
2015 Japanese Society of Hematology Award
2014 Research Leadership Award, California Institute of Regenerative Medicine
2011 Royan International Research Award, Royan Institute
2009 Donald Metcalf Lecture Award, International Society for Experimental Hematology
2004 Erwin von Baelz Prize
1989 Yokohama Medical Award

C. Contributions to Science

1. Isolation, Characterization, and Expansion of Mouse Hematopoietic Stem Cells: I introduced the use of fluorescent activated cell sorting (FACS) as a clone-sorting device into the field of hematology and stem cell biology, to physically separate single cells for precise clonal level analyses. In my first published study in this discipline, I used clonal culture of FACS clone-sorted cells to demonstrate the presence of CD34+ colony forming cells in human peripheral blood. This provided the scientific basis for peripheral blood hematopoietic stem cell (HSC) transplantation. I then combined molecular and cellular techniques to isolate a truncated form of the erythropoietin receptor that plays a role in the regulation of erythrocyte number. Next, I determined the phenotype of mouse HSCs and demonstrated that a single FACS-purified HSC can reconstitute the entire hematopoietic system (a). This became a landmark paper demonstrating the robustness of stem cells and the importance of clonal analysis, and a pioneering work in single cell biology. The establishment of a method to prospectively isolate HSCs by FACS enabled quantitative and precise analysis of individual HSCs, which are now widely used by the stem cell community. By combining this with a paired daughter cell assay, my group showed that lineage commitment can take place asymmetrically at the level of HSCs and that HSCs are heterogeneous in their repopulating activity. Furthermore, we pioneered the study of aging by revealing an age-dependent increase in myeloid-biased hematopoietic stem cells at clonal level in aged BM. My group has also performed molecular analysis on highly purified HSCs. In collaboration with Brian Sorrentino's group, we identified *bcrp1* as responsible for the HSC side-population (SP) phenotype and characterized SP and non-SP cells in the mouse bone marrow. We also clarified the role of the polycomb group protein Bmi1 in HSC self-renewal and discovered that an adaptor molecule, Lnk, is a negative regulator of HSC self-renewal. Most recently, we have developed a chemically-defined albumin-free ex vivo HSC expansion culture system that enables expansion of functional mouse HSCs – up to 900-fold over 28 days. This is the first time that long-term HSC expansion has been achieved and offers a powerful new platform to study and perturb HSCs ex vivo, including the ability to perform HSCT using nonconditioned recipients through high dose transplantation.

a. Osawa M, Hanada K, Hamada H, **Nakauchi H** (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science. 273(5272):242-245. PMID: 8662508
b. Yamazaki S, Ema H, Karlsson G, Yamaguchi T, Miyoshi H, Shioda S, Taketo MM, Karlsson S, Iwama A,

Nakauchi H (2011). Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell. 147(5):1146-1158. PMID: 22118468

c. Yamamoto R, Wilkinson AC, Ooehara J, Lan X, Lai CY, Nakauchi Y, Prichard J, **Nakauchi H** (2018). Large-scale clonal analysis resolves aging of the mouse hematopoietic stem cell compartment. Cell Stem Cell 22(4):600-607. PMCID: PMC5896201

d. Wilkinson AC, Ishida R, Kikuchi M, Sudo K, Morita M, Crisostomo RV, Yamamoto R, Loh KM, Nakamura Y, Watanabe M, **Nakauchi H***, Yamazaki S* (2019). Long-term ex vivo hematopoietic stem cell expansion affords nonconditioned transplantation. Nature. 571(7763):117-121. PMCID: PMC7006049

2. Identification and Characterization of HSC Niche Components: Self-renewal and differentiation of stem cells are tightly regulated by the stem cell microenvironment (i.e., the "stem cell niche"). Using highly purified HSCs, my group determined that most HSCs in the bone marrow niche are usually quiescent and only undergo cell division about once every 3 weeks. We then demonstrated that HSCs are protected from internal or external stress through a mechanism similar to that of *C. elegans* and hibernating squirrels, whereby TGF-beta (among other factors) induces the HSC hibernation in the bone marrow niche. We further identified non-myelinating Schwann cells, the glial cells in bone marrow, as responsible for regulation of HSC hibernation. This was an unexpected finding; a novel interface between the hematopoietic and neural systems, with potential therapeutic intervention. Recently, we provided evidence that amino acids are also constituents of the HSC niche. We found that the essential amino acid valine is indispensable for the proliferation and maintenance of mouse and human HSCs. Dietary restriction of valine can be even used to empty the mouse bone marrow of HSCs and affords (both allogenic and autogenic) donor-HSC engraftment without chemotherapy or irradiative myeloablation, suggesting that use of dietary valine restriction as a conditioning regimen may reduce iatrogenic complications in HSC transplantation. This discovery of the valine dependency of HSCs opens up a new paradigm for amino acid metabolism in the regulation of adult stem cell function and prospects for novel therapeutic intervention.

a. Yamazaki S, Iwama A, Takayanagi S, Morita Y, Eto K, Ema H, **Nakauchi H** (2006). Cytokine signals modulated via lipid rafts mimic niche signals and induce hibernation in hematopoietic stem cells. The EMBO Journal. 25(15):3515-3523. PMCID: PMC1538571

b. Yamazaki S, Iwama A, Takayanagi S, Eto K, Ema H, **Nakauchi H** (2009). TGF-beta as a candidate bone marrow niche signal to induce hematopoietic stem cell hibernation. Blood. 113(6):1250-1256. PMID: 18945958

c. Yamazaki S, Ema H, Karlsson G, Yamaguchi T, Miyoshi H, Shioda S, Taketo MM, Karlsson S, Iwama A, **Nakauchi H** (2011). Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell. 147(5):1146-1158. PMID: 22118468

d. Taya Y, Ota Y, Wilkinson AC, Kanazawa A, Endo TA, Watarai H, Kasai M, **Nakauchi H***, Yamazaki S*. (2016). Depleting dietary valine permits non-myeloablative mouse hematopoietic stem cell transplantation. Science. 354(6316):1152-1155. PMID: 27934766

3. Generation of functional hematopoietic cells from PSCs**:** Blood transfusion is a standard therapeutic intervention but depends on a limited supply of donated blood and carries risks such as immune reactions and infections. To address these limitations, I investigated pluripotent stem cells as a potential source of blood cells. Our initial target was platelets because they are difficult to preserve and often in demand. We established an *in vitro* culture system whereby human embryonic stem cells (hESCs) can be differentiated into hematopoietic progenitors within "sac"-like structures, termed "embryonic stem sacs" (ES-sacs). The cells inside ES-sacs were capable of differentiating into mature megakaryocytes and functional platelets. My group further determined that a metalloproteinase inhibitor effectively prevents non-specific platelet activation during culture. We also found that human induced pluripotent stem cells (hiPSCs) could be used for platelet production in the same culture system. These results indicated that hESCs and hiPSCs are potential sources for unlimited platelet production and may replace donated sources. Most recently, by modifying this *in vitro* culture system, we established a novel approach to rejuvenate antigen-specific T cells for adoptive immunotherapy. Knowing that T cell receptor specificity is genomically encoded, we established iPSCs from a patient's antigen specific CD8+ T cell clone that was specific for an HIV peptide. The T cell-derived iPSCs were then redifferentiated into CD8+ T cells. These cells possessed antigen-specific killing activity and exhibited TCR gene rearrangement patterns identical to those of the patient's original T cell clone. These findings suggest that iPSC technology allows rejuvenation of antigen-specific immune cells and may provide a novel approach to adoptive immunotherapy.

a. Ando M, Nishimura T, Yamazaki S, Yamaguchi T, Kawana-Tachikawa A, Hayama T, Nakauchi Y, Ando J, Ota Y, Takahashi S, Nishimura K, Ohtaka M, Nakanishi M, Miles JJ, Burrows SR, Brenner MK, **Nakauchi H**. (2015) A Safeguard System for Induced Pluripotent Stem Cell-Derived Rejuvenated T Cell Therapy. *Stem Cell Reports*. 5(4):597-608. PMCID: PMC4624898

b. Nakamura S, Takayama N, Hirata S, Seo H, Endo H, Ochi K, Fujita K, Koike T, Harimoto K, Dohda T, Watanabe A, Okita K, Takahashi N, Sawaguchi A, Yamanaka S, **Nakauchi H**, Nishimura S, Eto K (2014). Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell*. 14(4):535-548. PMID: 24529595

c. Nishimura T, Kaneko S, Kawana-Tachikawa A, Tajima Y, Goto H, Zhu D, Nakayama-Hosoya K, Iriguchi S, Uemura Y, Shimizu T, Takayama N, Yamada D, Nishimura K, Ohtaka M, Watanabe N, Takahashi S, Iwamoto A, Koseki H, Nakanishi M, Eto K, **Nakauchi H** (2013). Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation. *Cell Stem Cell*. 12(1):114-126. PMID: 23290140

d. Takayama N, Nishikii H, Usui J, Tsukui H, Sawaguchi A, Hiroyama T, Eto K. and **Nakauchi H.** (2008). Generation of functional platelets from human embryonic stem cells in vitro via ES-sacs, VEGF-promoted structures that concentrate hematopoietic progenitors. *Blood*. 111:5298-306. "PMID": 18388179.

4. Generation of Functional Organs from ES/iPSCs: In the course of the above research, I realized the importance of the niche not only for HSC engraftment but also for the development of various stem/progenitor cells into organs. Organogenesis is so complex that recapitulating it *in vitro* to generate a functional organ has been impossible. Thus, current stem cell therapy mainly targets diseases that can be treated by cell therapy. To address the shortage of organ donors, my group established a proof-of-principle model to generate a donor-cell–derived solid organ *in vivo*, using the blastocyst complementation technique using a pancreatogenesis-disabled *Pdx1-/-* mouse embryo as a host. We first demonstrated the generation of a functional and mouse-sized rat pancreas in a mouse. We next demonstrated the generation of a rat-sized mouse pancreas in a rat. Transplantation of mouse islets isolated from this rat-sized mouse pancreas cured drug-induced diabetic mice without a need of ongoing immunosuppression, a proof-of-principle data demonstrating usefulness of an organ generated from iPS cells via interspecies organogenesis. More recently, we have developed a novel in vivo organogenesis approach using Igf1r-/- animal as a host for multi-organ generation. This generation of organs using ES/iPSCs and interspecific blastocyst complementation *in vivo* provides a novel strategy for understanding organogenesis and a novel approach to organ supply. We have since extended this to the pig to generate functional pancreas.

a. Kobayashi T, Yamaguchi T, Hamanaka S, Kato-Itoh M, Yamazaki Y, Ibata M, Sato H, Lee YS, Usui J, Knisely AS, Hirabayashi M**, Nakauchi H**. (2010). Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. *Cell*. 142(5):787-799.

b. Matsunari H, Nagashima H, Watanabe M, Umeyama K, Nakano K, Nagaya M, Kobayashi T, Yamaguchi T, Sumazaki R, Herzenberg LA, **Nakauchi H** (2013). Blastocyst complementation generates exogenic pancreas in vivo in apancreatic cloned pigs. *Proceedings of the National Academy of Sciences of the United States of America*. 110(12):4557-4562.

c. Yamaguchi T, Sato H., Kato-Itoh M, Goto T, Hara H, Sambo M, Kobayashi T, Mizuno N, Yanagida A, Umino A, Ohta Y, Hamanaka S, Masaki H, Rashid D, Hirabayashi M, **Nakauchi H** (2017). Interspecies organogenesis generates autologous functional islets. Nature. 542(7640):191-196.

d. Nishimura T, Suchy FP, Bhadury J, Igarashi KJ, Charlesworth CT, **Nakauchi H**. (2021). Generation of Functional Organs Using a Cell-Competitive Niche in Intra- and Inter-species Rodent Chimeras. *Cell Stem Cell*. 28(1):141-149.

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