BIOGRAPHICAL SKETCH

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NAME: HU, Kejin

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

POSITION TITLE: Associate Professor of stem cells and regenerative medicine

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
Hong Kong Polytechnic University, Hong Kong	M.Phil.	07/1997	Fungal Biochemistry
Hong Kong University, Hong Kong	Ph.D.	05/2003	Molecular biology (shrimp)
Cornell University, Ithaca, New York	Postdoc	04/2004	Muscle development (*c elegans*)
Pittsburgh University, Pennsylvania	Postdoc	10/2006	TOR signaling (yeast)
University of Wisconsin, Madison	Postdoc	07/2007	Bone marrow Toxicity (mouse)
University of Wisconsin, Madison	Postdoc	07/2011	Pluripotent stem cells (human)

A. Personal Statement

I am an established PI in pluripotency reprogramming, Pluripotent Stem Cells (PSC) and the epigenetic readers Bromodomain and Extra Terminal (BET) proteins. Unlike other iPSC labs that mainly use the convenient mouse system, I have been focusing on human pluripotency and the more challenging human iPSC reprogramming over the last 15 years. Our episomal integration-free system has been one of the chosen methods for generating the clinical-grade human iPSCs. My method for reprogramming human blood cells into integration-free iPSCs have been used worldwide. Based on expert review, I am the first to convert primary human cancer cells (from a CML patient) into iPSCs. My human iPSC lines have been used by scientists globally (distributed by WiCell). Over the years, my lab has provided much molecular insights into human pluripotency reprogramming. My lab has discovered new reprograming factors, reprogramming promoting small molecules, approaches to overcome reprogramming barriers, and others. I proposed the new concepts of reprogramming legitimacy, reprogramome, reprogramming stress, and a revised model for mammalian totipotency. Induced cellular reprogramming has become one of the convenient and powerful tools to answer many fundamental biological questions. Over the years, my lab has provided molecular understanding of roles of BET proteins in reprogramming and established many solid research projects surrounding BET proteins. Our data indicate a novel mechanism for targeting BET proteins. Our further research may lead to novel BET inhibitors as therapeutic drugs and novel research tools.

I am a dedicated, focused, and motivated scientist with broad and solid skills, yet learn every day. In the tenure of my first R01, through the period of COVID19 pandemic, supply chain disruption and lab relocation, my small lab has published 5 original research articles, 3 book chapters, one method/tutorial article, one comprehensive review, and one tutorial book. My lab also developed one software in collaboration with Dr. Da Yan. On top of these, I have additionally edited one book for the highly regarded series of "*Methods in Molecular Biology*". During my first R01 tenure, I have also been serving the scientific community by regularly reviewing manuscripts and grant proposals, editing a book and a special issue of a journal, serving as an editorial member of a journal, hosting undergraduate students of minority in my lab, providing community service, and others. I am among a small group of PIs who are motivated to become versed in bioinformatics as a principal biomedical scientist. I am in the prime time of my scientific research career. I will continue to contribute to biomedical sciences, education, and community service.

Ongoing and recently completed projects (in the past 3 years) that I would like to highlight include:

R01, GM127411
Hu (PI)
08/01/2018-07/31/2023
"*BET regulation of mitosis and pluripotency establishment*"

U54DK126087
Yoder, Bradley (PI); Hu as a co-investigator and associate director of Core B (5%)
07/2020-02/2023
"*UAB Childhood Cystic Kidney Disease Core Center (UAB-CCKDCC)*"

R01DE028264-3
Li, Yi-Ping (PI); Hu as a co-investigator (3%)
06/01/2020 – 05/31/2021
"*gα13 signaling attenuates periodontal inflammation and alveolar bone loss in the mouse model of age-associated periodontitis*"

Citation:

a. **Kejin Hu**. (2014) All roads lead to induced pluripotent stem cells: the technologies of iPSC generation. *Stem Cells and Development*, 23(12) 1285-1300.
b. **Kejin Hu**. (2019) On mammalian totipotency: What is the molecular underpinning for the totipotency of zygote? *Stem Cells and Development*. 28(14):897-906. (Highlighted by the editor-in-chief).
c. **Kejin Hu.** as the editor (2021). "*Nuclear Reprogramming*", a volume of the series of "*Methods in Molecular Biology*". Vol. 2239. DOI: 10.1007/978-1-0716-1084-8.
d. **Kejin Hu.** (2022) RNA-seq analyses using HPC, a tutorial for bench scientists. Amazon, BookBaby, ISBN, 9781667864471; ASIN, B0BHZVWFNL

B. Positions, scientific appointments, and Honors

Positions and Scientific Appoints

03/01/2023-	Associate Professor, Stem Cells & Regenerative Medicine, Department of Microbiology, Immunology and Genetics, University of North Texas Health Science Center, Fort Worth
10/2020-02/2023	Associate Professor, Stem Cells & Regenerative Medicine, University of Alabama at Birmingham (UAB), Birmingham, AL, USA
2020-2023	Associate director of core B, UAB Childhood Cystic Kidney Disease Core
2020-2022	Guest editor for "*Cellular Reprogramming and Tissue Repair*", a special issue for "Methods and Protocols".
2020	Member of the grant reviewer panel, Canada Research Chairs Program (Canada)
2020	Member of the grant reviewer panel, Medical Research Council (UK)
2019-2020	Editor for "Nuclear Reprogramming", a volume of "*Methods in Molecular Biology*".
2018	Grant reviewer for Alabama Children's Hospital Foundation
2017-2020	Member of remote grant reviewer panel, European Research Council
2017	Grant reviewer for Alabama Children's Hospital Foundation
2016-present	Member of the editorial board, "*Stem Cells and Development"*
2016	Member of one of the grant review panels, New York Stem Cell Science
2012-present	Member of International Society for Stem Cell Research
2011-2020	Assistant Professor, stem cell and molecular biology, University of Alabama at Birmingham
2015	Member of the grant review panel, Medical Research Council (UK)
2007- 2011	Postdoc, Stem Cell Biology, University of Wisconsin-Madison, Madison, WI, USA
2006- 2007	Postdoc, Bone Marrow Toxicology, University of Wisconsin-Madison, WI, USA
2004-2006	Postdoc, TOR Signaling in yeast, Pittsburgh University, Pittsburgh, PA, USA

2003-2004 Postdoc, Muscle Development in *C elegans*, Cornell University, Ithaca, NY, USA
1998/3-9 Teaching assistant at Zoology Department, Hong Kong University, Hong Kong.

Honors

1) Top 10 cited list for articles published in *Methods and Protocols* during 2020-2021, MP news, 09/30/2022. My tutorial remains among the top 10 cited papers published in MP.
2) Invited webinar speaker, by 'The Scientist". "Stem Cells: Opportunities, Hurdles, and Promises". November 14th, 2018.
3) Invited and paid symposium speaker by *10 years of iPSC technology Beijing*, October 13, 2016.
4) UAB provost faculty development award, 2016.
5) Alabama Institute of Medicine (AIM) Junior Investigator Award, 2014.
6) Student travel award to attend conference abroad, 2001, by Hong Kong University
7) Scholarship for Ph.D. program (1999-2003), Hong Kong University (not by the advisor's grants).
8) Student international conference travel award, 1997, by The Hong Kong Polytechnic University
9) Scholarship for MPhil. program (1995-1997), Hong Kong Polytechnic University (not by advisor's grants).

C. Contribution to Science (*, corresponding author)

1. Defining the roles of BET proteins in human cellular reprogramming (and beyond)

Our story of BET proteins follows a typical and classic scientific journey, in which seeking answers leads to an unexpected discovery; and this "answer" raises more questions, motivates further research, and hence leads to more discoveries. In seeking for new reprogramming factors, my lab screened a human kinase cDNA library and unexpectedly discovered that the uncharted short isoform of human BRD3 possesses reprogramming activity (I named it as BRD3R, GenBank #, KR633047). My lab also revealed that BRD3R enhances mitosis of the reprogramming cells. Interestingly, we found that mild chemical BET inhibitions promote reprogramming and dampen the somatic transcription. We further demonstrated that BRD2, BRD3 and BRD4 all harbor reprogramming activities, but their ET tails and bromodomains inhibit/mask their reprogramming activities. During this endeavor, our data allow me to propose formally the new concept of reprogramming stress. Critically, we showed that those dominant negative (DN) BET fragments mitigate stress responses over-activated by the Yamanaka reprogramming factors. My lab further provided evidence that targeting BRD2 can enhance reprogramming and reduce the oxidative stress (unpublished data). Our exploration of BET roles in reprogramming, transcription, stress response, inflammation, and others will lead to many more discoveries in the years to come.

a) Z Shao, R Zhang, A Khodadadi-Jamayran, B Chen, M Crowley, M Festok, D Crossman, T Townes, **Kejin Hu ***. (2016) The Acetyllysine Reader BRD3R Promotes Human Nuclear Reprogramming and Regulates Mitosis. *Nature Communications*, 7:10869
b) Z Shao, C Yao, A Khodadadi-Jamayran, W. Xu, T Townes, MR Crowley, **Kejin Hu** *. (2016) Reprogramming by de-bookmarking somatic transcriptional program via targeting the BET bromodomains. *Cell Reports*, 16(12): 3138-3145.
c) M Hossain, R Cevallos, R Zhang, **Kejin Hu** * (2023) Attenuating iPSC reprogramming stresses with dominant negative BET peptides. *iScience*. 20(1): 105889.

2. Establishment of an integration-free reprogramming system for normal and diseased human primary cells.

In 2006, a groundbreaking technology, Induction of Pluripotent Stem Cells (iPSC) by transgene overexpression, was developed. However, the original iPSC reprogramming used integration viral vectors to deliver multiple genes for reprogramming because iPSC reprogramming takes 10-30 days to complete and transient transfection cannot provide such a lasting expression of transgenes. Integration of transgenes poses several problems including insertion mutagenesis, residual expression of the transgenes after completion of reprogramming, and re-activation of the transgenes during the differentiation process of iPSCs and/or in iPSC-derived cells. Residual expression of the pluripotent genes (reprogramming factors) is detrimental as well because the expression levels of the pluripotent genes in PSCs need to be maintained in a narrow window. Re-activation of reprogramming factors is also dangerous because the reprogramming factors are all

oncogenic to some extent with MYC as the most notorious one. To address the integration issue, we used episomal plasmids to deliver reprogramming factors (*Science* article below. Please note that Yamanaka's group published a similar episomal reprogramming method in *Nature Methods*, doi: 10.1038/nmeth.1591, in 2011, two years later than our publication). Episomal plasmids can replicate in human cells but exist as extrachromosomal entities, so they provide a lasting expression, but do not integrate into the transfected genomes. In addition, upon completion of reprogramming, episomal plasmids will gradually be lost from iPSCs due to their imperfect partitioning. *Our system is used worldwide, and has been chosen as one of the preferred tools to generate clinical-grade iPSC lines*. With the then newly developed episomal system, I further developed an efficient protocol (100x more efficient) to generate human iPSCs from human blood cells (bone marrow or cord blood) frozen in the freezer for more than 10 years. The iPSC lines I have generated are widely used by fellow scientists as shown by royalty I have been receiving every year (Four of my human iPS cell lines have been distributed by WiCell), and by high citation numbers of my publications (see citation counts in my Google Scholar profile). I am the first one to convert primary human cancer cells (from a CML patient) into iPSCs.

a) J Yu, **Kejin Hu**, K Smuga-otto, S Tian, R Stewart, I Slukvin, J Thomson* (2009) Human Induced Pluripotent Stem Cells free of vector and transgene sequences. *Science*, 324:797-80.
b) **Kejin Hu**, J Yu, K Suknuntha, S Tian, K Montgomery, K Choi, R Stewart, J Thomson, I Slukvin* (2011) Efficient reprogramming of mononuclear cells from archived normal and neoplastic human bone marrow and cord blood with non-integrating episomal constructs. *Blood*, 117:e109-e119.
c) **Kejin Hu**, I Slukvin (2013) Generation of transgene-free lines from human normal and neoplastic blood cells using episomal vectors. *Methods in Molecular Biology*, 997:163-176.
d) **Kejin Hu**, I, Slukvin (2013) Induction of pluripotent stem cells induction from umbilical cord blood. *Review in Cell Biology and Molecular Medicine*, doi, 10.1002/3527600906.mcb.201200006, Wiley-VCH

3. Providing insights into the black box of cellular reprogramming

The conversion of somatic cells into the pluripotent state is a very complicated cellular process, which involves extensive changes in almost all aspects of cell biology. Pluripotency reprogramming remains as a black box to scientists and serves as a convenient model for study of general cell biology, epigenetics, transcription, molecular biology, biochemistry, and others. My lab has been using human iPSC reprogramming as a model to answer many fundamental questions in biology and biomedical sciences. Over the years, I established the concepts of reprogramome and reprogramming legitimacy. These concepts allow us to reveal the significance of WNT reprogramming in pluripotency establishment, and to provide some molecular explanations to both the potency and limitations of the Yamanaka factors. Guided by these concepts, my lab has also discovered that ribosome biogenesis is the first major cellular process that is properly reprogrammed (earlier than MET). With these concepts, we also found that many transcriptional factors that are responsive to the Yamanaka factors are successfully reprogrammed at the early stage of reprogramming. We have also revealed that KLF4 quickly reprograms the pluripotent surface marker gene *PODXL*, which encodes the carrier protein of TRA-1-60 and TRA-1-81 (Kang et al. 2016, full citation not included due to limit in items for each contribution section).

a) **Kejin Hu***, L Ianov, D Crossman. (2020) Profiling and quantification of pluripotency reprogramming reveal that WNT pathways and cell morphology have to be reprogramed extensively. *Heliyon*. 6(5):e04035.
b) **Kejin Hu***. (2020) A PIANO (Proper, Insufficient, Aberrant, and NO reprogramming) to the Yamanaka factors in the initial stages of human iPSC reprogramming. *IJMS*, *21*(9), 3229
c) **Kejin Hu***. (2020) Quick, coordinated and authentic reprogramming of ribosome biogenesis during iPSC reprogramming. *Cells*, *9*(11), 2484
d) R Cevallos, Y Edwards, J Parant, B Yoder, **Kejin Hu***. (2020) Human transcription factors responsive to initial reprogramming predominantly undergo legitimate reprogramming during fibroblast conversion to iPSCs. *Scientific Reports*. 10:19710.

4. Establishment of a model for flow of mRNA back into the genome and intron elimination in evolution

Jaenisch et al. recently reported that COVID19 RNA can integrate into the patient genomes via reverse transcription. As early as 2006, I have already developed a model for flow of mRNA back into their genomes via reverse transcription. In 2014, I have already warned that the reprogramming mRNAs could have a risk of insertion into the reprogrammed genome albeit remote. During evolution, many introns are found to be lost precisely without any scar. How introns are eliminated from the intron-containing genes is not well understood. I

was puzzled by this phenomenon when I cloned a functional gene, as a PhD student, for which only one of its introns is missing with other introns intact. To explain this phenomenon, I established a model as that: mRNA is reverse transcribed into cDNA, and cDNA undergoes homologous recombination with the genomic copy of the same gene. This homologous recombination is responsible for the loss of the introns in many species. My model further provides insights into how the overwhelming pseudogenes in the human genome (and other genomes) are generated by retrotransposition.

a. **Kejin Hu***, PC Leung. (2006) Complete, precise and innocuous loss of multiple introns in the currently intronless, active cathepsin L-like genes, and inference from this event. *Molecular Phylogenetics and Evolution,* 38, 685-696.
b. **Kejin Hu***. (2006) Intron exclusion and the mystery of intron loss. *FEBS Letters*, 580(27), 6361-6365.
c. **Kejin Hu***. (2008) Homologous recombination and innocuous intron elimination. *In* Genetic Recombination Research Progress. Pp. 323-333. Nova Science Publishers, ISBN: 978-1-6045-482-2.
d. **Kejin Hu*.** (2014) Vectorology and factor delivery system in induced pluripotent stem cell reprogramming. *Stem Cells and Development*, 23(12): 1301-1315.

5. Empower and enable fellow scientists

I am a dedicated and focused scientist, leading a small lab**.** While funding does not allow for investigation of high-risk projects, I made some of my hypotheses public and other well-funded labs may resonate and want to explore it. My model about mammalian totipotency is such a hypothesis I made public (citation 1 below). Based on extensive literature review and my extensive and diverse scientific knowledge, I proposed an unconventional model about mammalian totipotency in which three types of totipotency were formulated: genetic, transcriptional/epigenetic, and biochemical totipotency. I further proposed that the mouse transcriptional/epigenetic totipotency exits in the 4- to 8-cell stages of embryogenesis. Under such a model, I hypothesize that the mammalian totipotent cells can be captured in tissue culture as we have achieved for the pluripotent stem cells (ESCs and iPSCs). My model may inspire some well-funded labs to investigate into this topic and may lead to new technology in the area of totipotent stem cells.

I am a motivated scientist and avidly learn every day. Over the years, I have become very proficient in R programming, Linux and bash scripting, high-performance computing, RNA-seq analyses pipeline, big data management and visualization, and other bioinformatics skills. Running a small lab without the luxury of dedicated bioinformatics service, I understand the pain of many other labs with similar difficulty. Therefore, I edited and published my lab tutorials of bioinformatics. These tutorials are written in layman language with the bench scientists in my mind. These tutorials have been motivating, enabling, and empowering bench scientists. I am one of those scientists who publicly promote boxplots over the simple bar plots because using boxplots is more scientifically rigorous. Boxplots provide at least 5-point statistic summary of data while bar graph provide one only. My tutorials have been well received by fellow scientists.

I have also converted my reprogramming procedures and methods into published protocols, and scientists around the world benefit from such sharing of skills and experiences. Furthermore, I edited a method book, and a special issue of "*Methods and Protocols*". These works have been enabling the scientific community.

a. **Kejin Hu***. (2019) On mammalian totipotency: What is the molecular underpinning for the totipotency of zygote? *Stem Cells and Development*. 28(14), 897-906. (Highlighted by the editor-in-chief in the form of a news release by the journal.).
b. **Kejin Hu***. (2020) Become competent in one day in preparation of boxplots and violin plots for novices without prior R experience. *Methods and Protocols*. 3(4):64. (Among the list of the top 10 cited articles published in MP).
c. **Kejin Hu***. (2021) Become competent in one day in preparation of RNA-seq heat maps for novices without prior R experience. *Nuclear Reprogramming – Methods in Molecular Biology***,** Chapter 17, vol. 2239:269-303. (Scientists and students around the world email me for a copy of this chapter).
d. **Kejin Hu**, I Slukvin (2013) Generation of transgene-free lines from human normal and neoplastic blood cells using episomal vectors. *Methods in Molecular Biology*, 997:163-176. (Among one of the most purchased chapters of this volume based on the website of this book).

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/1PGmVc6FkJCkk/bibliography/public/