Sanger Human iPS Cells, a Distinct Type of Human Pluripotent Stem cells

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Abstract:

Human pluripotent stem cells include human ES cells and iPS cells. I will present our work on producing, maintaining and differentiating a new type of human pluripotent stem cells, Sanger Human iPS Cells or SH-iPSCs. We produce SH-iPSCs by using a six-factor reprogramming cocktail including OCT4, SOX2, KLF4, MYC, RARG and NR5A2 (LRH1). SH-iPSCs grow in 2i plus LIF medium, have similar culture behavior as mouse ES cells and are receptive to genetic manipulation. They depend on JAK/STAT signaling pathway but are independent of FGF signaling to keep pluripotent. Female SH-iPSCs have two active X chromosomes. SH-iPSCs can be converted to human ES cell-like cells by culturing in FGF-containing medium. Therefore, SH-iPSCs are highly similar to mouse ES cells. We are able to efficiently differentiate SH-iPSCs to neural stem cells using a mouse ES cell differentiation protocol.

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2. Wang, W., Lin, C., Lu, D., Ning, Z., Cox, T., Melvin, D., Wang, X., Bradley, A., and Liu, P. (2008). Chromosomal Transposition of PiggyBac in Mouse Embryonic Stem Cells. Proc Natl Acad Sci USA *105*, 9290-9295.

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Education:

1992-1998 Baylor College of Medicine, Houston, Texas. USA. Ph.D.

1985-1988 Institute of Genetics, Chinese Academy of Sciences. China. M.Sc.

Research:

2010- Senior Investigator. Wellcome Trust Sanger Institute, Cambridge, United Kingdom. My lab is studying mouse genetics and stem cells. We are particularly interested in how stem cells are produced and maintained in the mouse, and how these multi-potent cells are differentiated to specific cell types. We are able to convert T lymphocytes to a new type of killer cells. In the last few years, we have developed a new reprogramming technology to rapidly and efficiently reprogramme mouse somatic cells to naïve iPS cells.

This technology also enables us to produce human iPS cells that are highly similar to naïve or ground state mouse ES cells. The new human pluripotent stem cells are therefore useful for many applications.

2003-2010 Investigator. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

1998-2003 Research Fellow. Mouse Cancer Genetics Program, National Cancer Institute at Frederick, Maryland. USA.

Primate totipotent and pluripotent cells

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Mammalian development commences with the totipotent zygote, which is capable of developing into all the specialized cells that make up a whole organism, as well as into the extraembryonic support structures necessary for fetal development. Early embryonic blastomeres, up to at least the 4-cell stage embryo, also retain totipotency. Pluripotent cells in the inner cell mass (ICM) of blastocysts are the descendants of totipotent cells and can differentiate into any cell type of a body except extraembryonic tissues.

Pluripotent cells can be isolated, adapted and propagated indefinitely in vitro in an undifferentiated state as embryonic stem cells (ESCs). ESCs retain their ability to differentiate into cells representing the three major germ layers: endoderm, mesoderm or ectoderm or any of the 200+ cell types present in the adult body. Since many human diseases result from defects in a single cell type, pluripotent human ESCs represent an unlimited source of any cell or tissue type for replacement therapy thus providing a possible cure for many devastating conditions.

Pluripotent cells resembling ESCs can also be derived experimentally by the reprogramming of somatic cells (Byrne et al., Nature, 2007). Reprogrammed somatic cells may have an even more important role in cell replacement therapies since the patient's own somatic cells can be used to make stem cells thereby eliminating immune based rejection of transplanted cells.

The ability to contribute to chimeras upon reintroduction into host embryos is the key feature of murine totipotent and pluripotent cells. We recently demonstrated that rhesus monkey ESCs and isolated ICMs failed to incorporate into host embryos and develop into chimeras (Tachibana et al., Cell, In press). However, ICMs transplanted into blastocysts formed separate viable fetuses while sharing the placental compartment of the host embryo. Monkey chimeras were produced by aggregation of totipotent cells of the 4-cell embryos.

Currently, there is little known about human and nonhuman primate embryo development and lineage specification and how closely the mouse development reflects primates. Our study presents a first glimpse at the similarities and differences between mouse and primate preimplantation embryo development and offers an important experimental model to investigate lineage commitment and interactions.

Producing primate embryonic stem cells by somatic cell nuclear transfer. Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S, Wolf DP, Mitalipov SM. Nature. 2007 Nov 22;450(7169):497-502

Totipotent but not pluripotent primate embryonic cells contribute to chimeras
Tachibana M, Sparman M, Ramsey C, Ma H, Lee HS, Penedo MC , Mitalipov S. Cell. In press

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Biography

Shoukhrat Mitalipov is an Associate Scientist in the Division of Reproductive & Developmental Sciences, Oregon National Primate Research Center, Oregon Health & Science University. He is also an Associate Professor at the Oregon Stem Cell Center and Departments of Obstetrics & Gynecology and Molecular & Medical Genetics. Dr. Mitalipov earned his Ph.D. degree in Developmental & Stem Cell Biology at the Research Center for Medical Genetics, Moscow, Russia. He came to Utah State University in 1995 to conduct a postdoctoral research training before joining the faculty at OHSU in 1998. Dr. Mitalipov is a recipient of the 2010 Discovery Award from the Medical Research Foundation of Oregon.

Research Overview

Our overall research goal is to use molecular and cellular approaches to answer scientifically and clinically pertinent questions regarding gamete, embryo and stem cell biology. The main focus of several ongoing projects is to understand the mechanisms of genetic and epigenetic reprogramming of aged somatic cells to the totipotent and pluripotent states following somatic cell nuclear transfer (SCNT). Specifically, we are interested in the role of mitochondria and mitochondrial (mt)DNA in reprogramming and re-setting the developmental program in experimental pluripotent stem cells derived from aged somatic cells. Another objective is to develop efficient protocols for deriving human pluripotent stem cells via SCNT for patients carrying mtDNA mutations.

Several other projects in the lab are focused on the assessment of the safety and efficacy of stem cell based therapies by transplantation studies in a clinically relevant nonhuman primate model. The overall goal of these studies is to take advantage of recent developments in our lab that allowed for the first time derivation of immuno-matched pluripotent cells by SCNT or iPS approaches, suitable for autologous transplantation into existing monkeys.

Our lab is also investigating novel germ line gene therapy approaches for the treatment of inherited human diseases. Particularly, mutations in mtDNA contribute to a diverse range of still incurable human diseases and disorders including neurodegenerative diseases, myopathies, diabetes, blindness, cancer and infertility. Our team recently demonstrated that the mitochondrial genome could be efficiently replaced in mature nonhuman primate oocytes by chromosome transfer from one egg to an enucleated, mitochondrial-replete egg. The reconstructed oocytes with the mitochondrial replacement were capable of supporting normal fertilization, embryo development and produced healthy offspring. This discovery suggest that the nuclear genetic material from a patient's egg containing mtDNA mutations could be removed, and transplanted into an enucleated egg containing normal mtDNA donated by a healthy female. A child born following fertilization with the husband's sperm would be free of risk from maternal mtDNA mutations as well as the authentic biological child of the patients.

Genetically modified non-human primate models in life science

Erika Sasaki

Central Institute for Experimental Animals

Transgenic mice have contributed immensely to biomedical science. However, the genetic and physiological differences between primates and mice including their physiologic functions hamper the extrapolation of results from mouse disease models to direct clinical applications in humans. Thus, the development of non-human primate models that mimic various human physiologic function including neuronal system would accelerate the advance of biomedical research. In particular, genetically modified primates would be a powerful human disease model for basic neuroscience, preclinical study for newly developed therapies or drugs.

The common marmoset (*Callithrix jacchus*), a new world primate has a number of advantages as an experimental animal, including small body size (300–450 g), high fertility, and early sexual maturity. The common marmoset has attracted considerable attention as a potentially useful biomedical research animal in fields such as neuroscience, stem cell research and regenerative medicine.

Recently, we have established techniques to produce transgenic marmosets using self-inactivating lenti-viral vector containing the EGFP transgene into marmoset embryos[1)]. With this success of the first transgenic primate, research into human disease, physiology, and the development of drug therapies and their validation is expected to increase. In addition, we also have established two kinds of pluripotent stem cells; embryonic stem (ES) cells and developed iPS cells from the marmoset[2, 3)]. Both of these pluripotent stem cell lines offered much hope to the numbers of patients who could benefit from tissue transplants. Furthermore these pluripotent stem cells also show interesting characteristics to study reproductive biology because pluripotent stem cells can produce genetically modified animals in several ways.

These marmoset transgenic technologies, the pluripotent stem cells of the common marmoset brain will provide an excellent model to study in the field of neuroscience.

1. Sasaki, E., et al.: Nature, 459: 523-7, 2009
2. Sasaki, E., et al.: Stem Cells, 23: 1304-13, 2005
3. Tomioka, I., et al.: Genes Cells, 15: 959-69, 2010

Curriculum Vitae

Erika Sasaki, Ph.D.

Academic Appointments

2010-	Department Head, Department of Applied Developmental Biology, Central Institute for Experimental Animals
2007-	Associate Professor, Keio Advanced Research Centers (KARC), Keio University, Tokyo, Japan
2007-2010	Laboratory head, Laboratory of Applied Developmental Biology, Department of Marmoset research
2004-2007	Assistant professor, Department of Anatomy, School of Medicine, Keio University, Tokyo, Japan
2003-2007	Project leader, Research Group of non-human primate reproduction and development, Laboratory of non-human primate research, Division of Biomedical Science, Central Institute for Experimental Animals
2002-2003	Research Associate, Medical Institute of Bio Regulation, Kyusyu University
2001-2002	Research Associate, Institute of Medical Science, University of Tokyo
1996-2000	Postdoctoral fellow, Department of Animal Science, University of Guelph, Canada
1995-1996	Research fellow, Japan Science and Technology Agency (JST)
1994-1995	Young Scientist Research fellow, Japan Society for the Promotion Science

Education

1995	Ph.D.	Department of agricultural and forestry science University of Tsukuba, Tsukuba, Japan
1992	M.S.	Department of agricultural and forestry science University of Tsukuba, Tsukuba, Japan
1989	B.S.	Department of agricultural and forestry science University of Tsukuba, Tsukuba, Japan