Capturing and characterising naïve pluripotency in early mammalian embryos

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Embryonic stem (ES) cells derived from blastocysts of mice and humans differ in their morphology, culture requirements and molecular profile. However, pluripotent cell lines produced from post-implantation mouse epiblasts resemble human ES cells (Tesar et al., 2007). Therefore, it is postulated that during the derivation process explanted human embryos progress to a post-implantation-like state that is primed for differentiation, whereas explanted mouse blastocysts can be captured in a naïve, pre-implantation-like state (Nichols and Smith, 2009). The efficiency of capturing this state can be significantly improved by inhibiting the Erk and GSK3 pathways during culture of mouse embryos (Ying et al., 2008), but so far no naïve human ES cells have been derived directly from blastocysts using this regime, suggesting that the control of developmental progression differs between these species. We have previously shown that multiple ES cell clones can be derived from peri-implantation mouse epiblasts by plating single cells into medium supplemented with Erk and GSK3 inhibitors (Nichols et al., 2009). We are currently applying this system to establish the timing of acquisition and loss of ES cell forming ability from single cells from the blastocyst to early post-implantation stages in order to define the mechanism by which naïve pluripotency is established. In parallel, we are using single cell molecular profiling to create a blueprint for naïve pluripotency in the mouse which we will use to probe for an equivalent state in primate embryos. We hope to use the results to devise more informed protocols for the derivation of naïve ES cells from other mammals, including humans.

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JENNIFER NICHOLS, CURRICULUM VITAE

Career history

2006-present: Group Leader, Wellcome Trust Centre for Stem Cell Research, University of Cambridge.

1995-2006: Post-Doctoral Research Fellow with Professor Austin Smith, University of Edinburgh.

1990-1995: Research Assistant/Graduate Student with Dr. Austin Smith, University of Edinburgh.

1981-1990: Research Assistant to Professor Richard Gardner, Oxford University.

Qualification

PhD (1995, University of Edinburgh)
'A Study of the Expression and Function of Differentiation Inhibiting Activity and its Receptor in the Early Mouse Embryo'

Research Interests

Mechanisms that establish and maintain pluripotency in the early embryo and during the formation of embryonic stem cells in mammals

Additional Responsibilities

Academic coordinator of transgenics facility for the Cambridge Stem Cell Intitiative
Member of ethics review committee, University of Cambridge
Committee member of British Society for Developmental Biology
Co-organiser of 'ES cells as a model for mammalian development' workshops in Latin America since 2006
Organiser of ES cell derivation course and workshop, July 2010
Teaching on various international courses
Undergraduate and graduate student teaching and supervision in Cambridge and abroad

Awards

NC3Rs '3Rs' prize 2009 for research to reduce, refine or replace the use of animals in biomedical research, £10,000
Elected Fellow of the Society of Biology, 2010

Pluripotency-associated transcription factor network

Hitoshi Niwa
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The generation of induced pluripotent stem (iPS) cells by introduction of 4 transcription factors into somatic cells revealed that the transcription factors have primary role to determine pluripotency. Moreover, since the introduced transgenes are silenced in the resulting iPS cells, it is suggested that the exogenous transcription factors act as switches to turn on the endogenous transcription factor genes, which form the autonomous network to maintain their expressions in the exogenous signal-dependent manner. We have analyzed the character of the pluripotency-associated transcription factor network in mouse embryonic stem (ES) cells. Mouse ES cells continue self-renewal in the presence of the cytokine leukemia inhibitory factor (LIF). We previously confirmed the function of the canonical pathway composed by Jak kinases and Stat3, but recently found that the LIF signal integrates into the transcription factor network via three intracellular signal transduction pathways including PI3K-Akt and MAPK pathways. Why is the network so complex? One possible answer is that the complex structure of the network contributes to make binary decisions of pluripotent stem cells to self-renew or differentiate with canceling the noise of signal integration. Now we are trying to dissect the network structure more precisely by analyzing the functions of each components as well as monitoring the dynamics in the time course of differentiation after withdrawal of LIF or perturbation of particular component. I will introduce our recent progress in this field.

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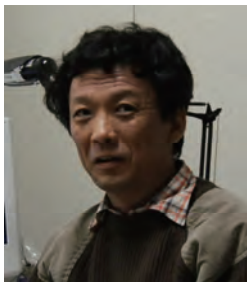

1983-1989	Nara Medical University (MD)
1989-1993	Kumamoto University Graduate School of Medicine (PhD)
1993-1994	Research Associate, Department of Developmental Genetics, Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine
1994-1996	Postdoctoral Fellow, Centre for Genome Research, University of Edinburgh
1996-2001	Research Assiociate, Department of Nutrition and Physiological Chemistry, Osaka University Graduate School of Medicine
2001-	Laboratory Head, Laboratory for Pluripotent Cell Studies, RIKEN Center for Developmental Biology
2002-	A guest professor, Laboratory for Developmental and Regenerative Medicine, Kobe University Graduate School of Medicine
2009-	Project Leader, Laboratory for Pluripotent Stem Cell Studies, RIKEN CDB

DNA Methylation and Hydroxymethylation in Development

Toru Nakano, M.D.
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Epigenetic regulation, which plays pivotal roles in development and cell differentiation, consists of DNA and histone modifications. In contrast to various histone modifications, methylation of 57 position of cytosine (5MC) had been known as only one kind of DNA modification for long time. Drastic change of DNA methylation, namely global DNA demethylation, takes place for capturing totipotency soon after fertilization. During this process, demethylation of maternal genome is delayed by the phenomenon of “epigenetic asymmetry” and some genes such as IAP retrotransposons and imprinted genes are escaped from the so called “active demethylation” process. We revealed that methylation of maternal genome and these genes was protected by PGC7/Stella, a maternal factor essential for early embryogenesis, from active demethylation. For the protection, PGC7/Stella recognizes chromatin containing di-methylated lysine 9 of histone H3 (H3K9me2).
Recently, another DNA modification, hydroxymethylation of 5’ position of cytonsine, has emerged as a major issue of active DNA demethylation. Several reports strongly suggest that Tet enzymes catalyze hydroxylation of methylated cytosine and the resultant 5-hydroxymethyl cytosine (5HmC) is an intermediate of active demethylation. Among three Tet’s (Tet1 ~ Tet3), early embryos mainly express Tet3 and the enzyme is essential for the active demethylation. Nuclear localization of Tet3 is also regulated by PGC7/Stella via binding of PGC7/Stella to H3K9me2 and is required for the appropriate DNA demethylation in early embryogenesis. In addition to the zygotes, the data of function of Tet in fetal glia cell differentiation will be presented and discussed (in collaboration with Prof. Tetsuya Taga of Tokyo Medical and Dental University)

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5-hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming *Nature Commun,* 2:241, 2011

Curriculum Vitae

Toru Nakano, M.D.
Professor
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Toru Nakano is a Professor of the Department of Pathology, Osaka University Medical School, Osaka Japan. He received his M.D. from Osaka University Medical School in 1981. After working as a physician for three years, he worked from 1984 to 1988 at the Medical School where he was engaged in the transplantation experiments of mast cells and hematopoietic stem cells. From 1989, he joined to European Molecular Biology Laboratory (EMBL) as a visiting scientist and was involved in the viral leukemogenesis of chicken. As a staff scientist, he next went on to work, first as an assistant professor (1990) and then as a lecturer (1991) at the Faculty of Medicine, Kyoto University, on a project studying the molecular mechanisms of hematopoesis using his unique *in vitro* differentiation induction method from mouse ES cells. He took a professor position at the Research Institute for Microbial Diseases, Osaka University in 1995 and started his study of germ cell development. In 2004, he was appointed as a professor at the Graduate School of Frontier Biosciences and Medical School, Osaka University. His major interest is “How various kinds of cells are produced from single totipotent cells, zygotes?” Based on the interest, he has been studying epigenetic modification, especially DNA methylation, in early embryogenesis and in spermatogenesis. To be more precisely, his recent and major scientific theme s are the regulation of DNA methylation in early embryogenesis and *de novo* DNA methylation of male germ cells by germ cell specific small RNA, pi-RNA (piwi interacting RNA).

Trend of pluripotent stem cell-derived gamete research

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Pluripotent stem cell (PSC)-derived gamete research has potential both for understanding basic mechanisms of differentiation and maintenance of gamete-producing (germline) cells and overcoming clinical problems related to infertility. For example, PSC-derived germ cells purified from various culture stages enable us to explore molecular mechanisms such as developmental network and epigenetic regulation of lineage-specific genes, which are essential for understanding germline-specificity. Culture system of functional maturation of PSC-derived gametes (eggs and sperm) *in vitro* will provide valuable techniques for overcoming various difficulties in researches for reproduction of human as well as experimental animals.

Another advantage of this line of research is possibility to realize the alternation of generations without growth and sexual maturation of individual bodies. Thus technical revolution is required for the reproductive engineering to generate genetically modified animals using non-human primate species, which must serve as much more valuable models for human diseases or biomedical researches than those of mouse. Similarly, further studies using monkey iPS-derived cells will play a crucial role for preclinical assessment of their safety and efficacy prior to application of iPS cell-based therapies.

Based on those scientific and social meanings, PSC-derived gamete research becomes the object of public attention, and the extensive approaches in some years ahead have accelerated the progress. Nevertheless, there are several differences found in the reproductive system between mouse and primate animals, which will be a current problem awaiting solution to be solved.

Curriculum Vitae

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Senior Researcher 1987 - 2009
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Project Professor 2009 - 2011
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Project Professor 2011-present
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