GCOE Program, The University of Tokyo

Center of Education and Research for the Advanced Genome-Based Medicine

For personalized medicine and the control of worldwide infectious diseases

No advance registration required. Free Admission

IMSUT & RCAST Global COE Program

Mini-Symposium

Reprogramming, Genome editing and Epigenetics

June 18th , 2012 14:00 - 17:00

Venue : 8th floor Tommy Hall, Research Hospital, Institute of Medical Science, The University of Tokyo (IMSUT)

14:00 - 15:00

Atsushi Iwama

Professor of Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University

Role of the polycomb group proteins in the maintenance of hematopoietic stem cells and restriction of tumor development

15:00 - 16:00

Linzhao Cheng

Professor of Medicine, Oncology and Gyn/Ob; Edythe Harris Lucas and Clara Lucas Lynn Chair in Hematology, Associate Director for Basic Research, Division of Hematology in Department of Medicine; Stem Cell Program in the Institute for Cell Engineering, Johns Hopkins University School of Medicine, USA

Human Cell Engineering: Cellular Reprogramming and Genome Editing

16:00 - 17:00

Gerald de Haan

Professor of Molecular Stem Cell Biology, European Research Institute for the Biology of Aging, University Medical Center Groningen, Groningen, the Netherlands

Polycomb Cbx orthologs mediate the balance between Hematopoietic Stem Cell self-renewal and differentiation

Host : Hiromitsu Nakauchi, Professor and Director, Center for Stem Cell Biology and Regenerative Medicine, IMSUT

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Shirokane Campus: Shirokanedai Station (Subway Namboku Line & Mita Line), Exit 2; about 3 minutes walk

“Role of the polycomb group proteins in the maintenance of hematopoietic stem cells and restriction of tumor development”

Atsushi Iwama, Professor of Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University

Presentation Abstract:

Polycomb-group (PcG) proteins form the polycomb repressive complexes (PRC) 1 and 2, functioning as transcriptional repressors through histone modifications. They have been implicated in the maintenance of hematopoietic as well as leukemic stem cells, by repressing the transcription of tumor suppressor genes, namely Ink4a and Arf, and thus have been characterized as oncogenes. Of great interest, however, inactivating mutations of EZH2 have been identified in patients with myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN), showing that PcG genes also have a tumor suppressor function. However, the mechanism by which PcG genes exert their tumor suppressor function remains to be clarified.

We have recently shown that loss of the PRC1 gene Bmi1 in mice causes pathological hematopoiesis that mimics human primary myelofibrosis (PMF), a disease categorized as a MPN. The loss of Bmi1 is self-limiting due to derepression of the Ink4a/Arf locus. However, deletion of both Ink4a and Arf in Bmi1-deficient mice substantially restores the defective self-renewal capacity of HSCs. To investigate the role of Bmi1 in hematopoiesis, we characterized Bmi1-/-Ink4a-Arf-/- hematopoietic cells transplanted into wild-type recipient mice. Loss of Bmi1 augmented reconstituting capacity of BM cells, established marked extramedullary hematopoiesis, and eventually induced lethal myelofibrosis. Myelofibrosis is caused by excessive release/leakage of growth factors by an abundance of necrotic megakaryocytes. Indeed, Bmi1-/-Ink4a-Arf-/- hematopoietic cells induced abnormal megakaryocytopoiesis. We further identified that the oncogene Hmga2 is a direct target of Bmi1 that contributes to the PMF-like disease. Loss-of-function mutations of EZH2 have been reported in MPN patients including PMF patients. Notably, recipient mice reconstituted with Ezh2-deficient BM cells developed mild but significant MPN-like disease and some of them developed myelofibrosis. These data suggest that impaired function of PcG proteins could accelerate the development of myelofibrosis via derepression of oncogenes, such as HMGA2.

Collectively, our findings indicate that PcG proteins antagonize the development of MPN in the absence of their major tumor-suppressor targets. In such situations, the tumor cells with impaired PcG function could out-compete cells with normal PcG function since the effects of de-repressed oncogenes appear to supersede the effects of de-repressed tumor suppressor genes.

“Human Cell Engineering: Cellular Reprogramming and Genome Editing”

Linzhao Cheng, Ph.D., Professor of Medicine, Oncology and Gyn/Ob; Edythe Harris Lucas and Clara Lucas Lynn Chair in Hematology, Associate Director for Basic Research, Division of Hematology in Department of Medicine; Stem Cell Program in the Institute for Cell Engineering, Johns Hopkins University School of Medicine, USA

Presentation Abstract:

Human induced pluripotent stem cells (iPSCs) that are functionally similar to embryonic stem cells (ESCs) hold great potential for cell and gene therapies, disease modeling and drug development in addition to human biology. The earliest success was achieved by using adherent fibroblastic cells and retroviral vectors that transduce fibroblasts very efficiently. It is also highly desirable to reprogram postnatal blood cells, including those from cord blood (CB) and adult peripheral blood (PB) that are easily accessible and less exposed to environmental mutagens. In 2009, we and others first achieved the reprogramming of human postnatal blood cells using the 4 reprogramming factors delivered by retroviral vectors. We also found that reprogramming efficiencies of CB and PB CD34+ cells are higher than age-matched fibroblastic cells including marrow-derived MSCs. This may result from an epigenetic profile of hematopoietic CD34+ cells that appears closer to iPSCs/ESCs than that of fibroblasts/MSCs to iPSCs/ESCs. We and others then attempted to reprogram postnatal human blood cells as well as adherent cells by OriP/EBNA1 episomal vectors. We reported recently generation of integration-free human iPSC cells by episomal vectors from adult blood cells, which appears to be more efficient than age-matched fibroblastic cells such as MSCs. Mononuclear cells from several milliliters of peripheral blood cells were cultured for a few days before one-time transfection by plasmid DNA. Sufficient numbers of iPSC candidates emerged after 2 weeks and many proved to be bona fide iPSCs after expansion and characterization. The derived iPSCs can efficiently differentiate to various cell types including neuronal, glial and neural crest cells. The whole genome sequencing of 3 iPSC lines (reprogrammed by 1-3 plasmids) revealed that they lacks vector integration or V(D)J genomic rearrangements. Low incidence of genetic variation (mostly heterozygous point mutations) in the nuclear and mitochondrial genomes is found in these integration-free iPSC lines reprogrammed by plasmid expression. The details of whole-genome sequencing analysis will be discussed.

The self-renewable iPSCs also allow us to achieve precise gene targeting to create or correct a mutation by homologous recombination (HR). The HR-mediated gene targeting is currently very low (typically 10e-6) for non-transformed human cells, so that this approach can only apply effectively to stem cells that proliferate extensively in culture and allow clonal expansion. Aided by zinc finger nuclease that makes DNA double strand break at a pre-selected site and thus enhances HR by >100-fold, we achieved specific gene targeting at several endogenous loci in human iPSCs derived from adult SCD and X-CGD patients. Various strategies for efficient gene targeting at a mutation site or a genetically safe harbor will be discussed.

With the advent of both breakthroughs, i.e., cellular reprogramming to make iPSCs from easily accessible somatic cells and genome editing to correct a mutation or create a designer DNA change, we are at the dawn of human cell engineering to develop more relevant disease models for research and novel treatments by cell and gene therapy.

“Polycomb Cbx orthologs mediate the balance between Hematopoietic Stem Cell self-renewal and differentiation”

Gerald de Haan, Ph.D., Professor of Molecular Stem Cell Biology, European Research Institute for the Biology of Aging, University Medical Center Groningen, Groningen, the Netherlands

Presentation Abstract:

A precise balance between self-renewal and differentiation of adult stem cells is essential for tissue homeostasis. Here we show that in the hematopoietic system this process is governed by Polycomb Chromobox (Cbx) proteins. Cbx7 shows a hematopoietic stem cell (HSC) specific expression pattern, and its overexpression induces self-renewal and leukemia upon transplantation. This effect is dependent on integration into Polycomb Repressive Complex-1 (PRC1) and requires H3K27me3 binding. In contrast, overexpression of Cbx2, Cbx4 or Cbx8 result in differentiation and HSC exhaustion. ChIP-seq analysis shows that Cbx7 and Cbx8 share ~95% of their targets; we identified approximately 200 differential targets. Whereas genes explicitly targeted by Cbx8 are highly expressed in HSCs and become repressed in progenitors, Cbx7 targets show the opposite expression pattern. Thus, Cbx7 can preserve HSC selfrenewal by repressing progenitor-specific genes. Taking together, the presence of distinct Cbx proteins confers target selectivity to PRC1 and provides a molecular balance between self-renewal and differentiation of HSCs.