"The 88th of Stem Cell Biology and Regenerative Medicine Forum"

Date：Feb 22nd (Mon) 2016
Time : 13:00 ～ 14:30
Place：Auditorium in 1st Building

（Internal Speaker）

13:00-13:30 Lin Huan-Ting(Division of Stem Cell Processing The Institute of Medical Science The University of Tokyo)
An assessment of the effects of ectopic gp91phox expression in XCGD iPSC-derived neutrophils

（External Speaker）

13:30-14:30 Yoshihiro Takihara (Department of Stem Cell Biology, Research Institute of Radiation Biology and Medicine, Hiroshima University)
Geminin, a molecular switch turning on and off quiescence or blood cell production in hematopoietic stem cells

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Hosted by Center for Stem Cell Biology and Regenerative Medicine

---Information---

* Please register attendance at the reception desk.
* Next forum (the89th) will be held on Mar 10th 18:00～19:30.
* Please contact tatsu-m@ims.u-tokyo.ac.jp, for Forum speaker recommendations

An assessment of the effects of ectopic gp91phox expression in XCGD iPSC-derived neutrophils

Lin Huan-Ting (Division of Stem Cell Processing The Institute of Medical ScienceThe University of Tokyo)

Disease modeling using patient autologous iPSCs as an exploratory platform has promising applications in elucidating pathophysiological mechanisms and methodological development. X-linked chronic granulomatous disease (XCGD) is unique amongst monogenetic primary immunodeficiency diseases since the appearance of functional neutrophils in the peripheral blood following hematopoietic stem cell gene therapy is only transient. One possibility is the occurrence of detrimental effects secondary to ectopic gp91phox expression in neutrophils, which has not been demonstrated under formal experimental settings. Since hematopoiesis occurs largely in the bone marrow, modeling XCGD using iPSCs allows the process of differentiation to be studied intensely *in vitro*. In this study, alpharetroviral vectors carrying a ubiquitous promoter were used to drive the "ectopic" expression of codon optimized gp91phox cDNA. In the mature fraction of neutrophils differentiated from transduced XCGD-iPSCs, cellular recovery in terms of gp91phox expression and reactive oxygen species production was abruptly lost before cells had fully differentiated. Most critically, ectopic gp91phox expression could be identified clearly in the developing fraction of the transduced groups, which appeared to correspond with reduced cell viability. It is possible that this impedes further differentiation of developing neutrophils. Therefore affording cellular protection from the detrimental effects of ectopic gp91phox expression may improve XCGD clinical outcomes.

Geminin, a molecular switch turning on and off quiescence or blood cell production in hematopoietic stem cells

Yoshihiro Takihara (Department of Stem Cell Biology, Research Institute of Radiation Biology and Medicine, Hiroshima University)

Hematopoietic stem cell (HSC) transplantation therapy has provided an epochal clinical outcome. Currently three kinds of different cellular sources, bone marrow cells, peripheral blood stem cells and umbilical cord blood, are available for the transplantation. A technology for expanding HSCs *ex vivo* could not only advance HSC transplantation therapy but may pave a way to further application of HSCs for advanced immunotherapy and gene therapy. There developed has, however, been no practically available technology for the expansion. Since hematopoietic cytokines and niche molecules have been shown not to be enough for the expansion, we have focused on the cell intrinsic factors. By utilizing biochemical as well as genetic approaches, we previously demonstrated that Polycomb-group complex 1 and Hoxb4/Hoxa9 regulate HSCs through the ubiquitin proteasome system-mediated direct regulation of Geminin protein and proposed a hypothesis that Geminin acts as a key regulator for determining cell fate of HSCs, *i.e.,* cellular quiescence, self-renewal and differentiation. Geminin negatively regulates DNA replication and chromatin remodeling through the direct interaction with Cdt1 and Brahma/Brg1, respectively. We then generated a recombinant Geminin protein fused with a membrane translocating motif (MTM) of FGF4, which was designated as cell-penetrating (CP-) Geminin. We have demonstrated that amount as well as timing of Geminin expression in NIH 3T3 cells was altered by direct transduction of a CP-Geminin protein. Biologically, CP-Geminin transduction controlled the cell cycle in NIH 3T3 cells, *i.e.,* CP-Geminin efficiently suppressed G_0/G_1 to S-phase transition to keep cellular quiescence. We currently aim at regulating activity as well as expression of Geminin to perform detailed verification of our hypothesis described above and further to generate a new strategy for manipulating the HSC activity.