"The 89th of Stem Cell Biology and Regenerative Medicine Forum"

Date：Mar 23rd (Wed) 2016
Time : 13:00 ～ 14:30
Place：8th floor Hospital Building

（Internal Speaker）

13:00-13:30 Kimihito Cojin Kawabata (Institute of Medical Science, Division of Cellular Therapy, The University of Tokyo, Tokyo, Japan)
ATP-binding Cassette Transporter G2 (Abcg2) Plays Oncogenic Roles in Myelodysplastic Syndrome in Mouse BMT Model and Characterizes Advanced MDS in Clinical Specimens

（External Speaker）

13:30-14:30 Susie Nilsson (Office of the Chief Executive Science Leader, Manufacturing, CSIRO)
Therapeutic targeting and rapid mobilization of endosteal HSC using a small molecule integrin antagonist

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Hosted by Center for Stem Cell Biology and Regenerative Medicine

---Information---

* Please register attendance at the reception desk.
* Next forum (the90th) will be held on Apr 14th 18:00～19:30.
* Please contact tatsu-m@ims.u-tokyo.ac.jp, for Forum speaker recommendations

ATP-binding Cassette Transporter G2 (Abcg2) Plays Oncogenic Roles in Myelodysplastic Syndrome in Mouse BMT Model and Characterizes Advanced MDS in Clinical Specimens

Kimihito Cojin Kawabata (Institute of Medical Science, Division of Cellular Therapy, The University of Tokyo, Tokyo, Japan)

A histone H3 Lysine 27 (H3K27)-methyltransferase, enhancer of zeste homolog 2(EZH2) is known as a tumor-associated gene. Physiological role of EZH2 is an enzymatic component of polycomb repressive complex 2 (PRC2) to inhibit expression of target genes. While EZH2 plays oncogenic roles by repressing the expression of tumor suppressors in solid tumors and some lymphomas, it plays rather tumor-suppressive roles in myeloid malignancies. We have generated a short-form EZH2 that lacks the catalytic SET domain (EZH2-dSET). Using this EZH2 mutant we could produce serially transplantable MDS-like diseases. Microarray analysis using the MDS-like bone marrow cells enabled us to identify novel targets of EZH2 in MDS tumorigenesis, including ATP-binding cassette (ABC) transporters. Either in our clinical specimens or TCGA public database, ABCG2 high expressions were observed in MDS samples but not in de novo AML and CML samples. The results from clinical data also indicate a link between U2AF1 mutations and ABCG2 expression via disrupted EZH2. Intriguingly, with Abcg2 expression alone, primary bone marrow cells could produce an MDS-like cytopenic disease in our BMT model. Those Abcg2-MDS mice had severe cytopenia due to inefficient hematopoiesis. Using histological, *in vivo* live imaging, and *in vitro* analyses of bone marrow, altered microenvironments in Abcg2-MDS mice were suggested. In conclusion, a short form of EZH2 upregulates Abcg2 expression resulting in MDS-like disease in model mice. ABCG2 is a gene specific to advanced MDS in clinical samples, and its overexpression leads to cytopenic disease showing inefficient hematopoiesis.

Therapeutic targeting and rapid mobilization of endosteal HSC using a small molecule integrin antagonist

Susie Nilsson (Office of the Chief Executive Science Leader, Manufacturing, CSIRO)

The collection of haematopoietic stem cells (HSC) for bone marrow transplantation relies on their mobilisation into the peripheral blood (PB). Traditionally, this has been routinely achieved using granulocyte-colony stimulating factor (G-CSF). However, G-CSF-based mobilisation requires multiple doses over a number of days, is known to alter the function of the HSC niche as well as bone formation, can cause bone pain and spleen enlargement as well as other rare but life threatening complications. Recently the use of small molecules for HSC mobilisation has been explored, such as the CXCR4 antagonist AMD3100 (Plerixafor/Mozobil). However, clinical mobilisation with AMD3100 is only effective in combination with G-CSF and the search for rapid, selective and G-CSF independent mobilisation regimes remains a topic of interest. Recently, we have shown that inhibiting $\alpha_9\beta_1/\alpha_4\beta_1$ integrins with a small molecule antagonist (*N*-(**B**enzene-sulfonyl)-L-prolyl-L-**O**-(1-**P**yrrolidinylcarbonyl)tyrosine; BOP) rapidly mobilises HSC with long-term multi-lineage engraftment potential. Additive augmentation of the engraftment of PB HSC was observed when BOP was co-administered with AMD3100. This combination effectively out-competed PB HSC mobilised with 4 days of G-CSF treatment in murine competitive transplant models. Subsequently, we demonstrated enhanced mobilisation using the small molecule combination could be recapitulated in humanized NODSCIDIL2R$\gamma^{-/-}$ mice, where a significant increase in PB $CD34^+$ stem and progenitor cells were observed after treatment with BOP and AMD3100. To assess the binding activity of this class of integrin antagonists on stem cell populations *ex vivo* and *in vivo*, we synthesized a related fluorescent analogue (R-BC154). Using R-BC154, we showed that this class of antagonists preferentially bind mouse and human HSC *via* intrinsically activated $\alpha_9\beta_1/\alpha_4\beta_1$ integrins within the endosteal niche; the region most closely associated with the bone/BM interface. More recently, we have applied this concept to the chemosensitisation of acute lymphoblastic leukaemia, whereby quiescent BM leukaemic cells were effectively dislodged from their protective endosteal BM microenvironment rendering them susceptible to chemotherapy. The results support the use of dual $\alpha_9\beta_1/\alpha_4\beta_1$ integrin inhibitors as effective, rapid and transient mobilisation agents with promising clinical applications in stem cell therapies.