Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Signaling
幹細胞シグナル制御分野

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Our major interest is to elucidate the mechanisms of pluripotency, self-renewal and the control of cell division and differentiation of hematopoietic stem and progenitor cells. We have developed the retrovirus-mediated efficient gene transfer and several functional expression cloning systems, and utilized these system to our experiments. We are now conducting several projects related to stem cells to characterize stem cells, clarify underling mechanisms of maintenance of pluripotency, and differentiation.

1. Developing Analysis Tools for Cell Cycle and Cell Division of Hematopoietic Stem Cells: MgcRacGap-hmKusabiraOrange2 (MRG-hmKuO2) fusion protein for midbody marker.

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Previously, we reported that MgcRacGap is a marker for midbody and that MgcRacGap-mVenus fusion protein visualized asymmetric inheritance and release of midbody during cytokinesis (Nishimura et al., 2013). We retrovirally introduced MRG-hmKuO2 into hematopoietic stem cells (HSCs), in order to examine whether midbody asymmetric inheritance and release is involved with asymmetric division of HSCs. HSCs showed high frequency of midbody release during cytokinesis in culture. Interestingly, one daughter cell releasing midbody differentiated earlier than the other daughter cell inheriting midbody. We generated Cre-inducible MRG-hmKuO2 mouse line. Briefly, the MRG-hmKuO2 fusion gene is inserted into Rosa26 locus following a loxP-NEO-STOP-loxP cassette, in order to visualize asymmetric inheritance and release of midbody in vivo without retroviral infection. Crossing MRG-hmKuO2 mice with Vav-Cre mice, MRG-hmKuO2 nicely marked midbody asymmetric inheritance and release in HSCs in culture. We are planning to do paired-daughter assay using HSCs from MRG-hmKuO2 mice to examine whether inheritance and release of midbody link to asymmetric division of HSCs. Given that some problems were found in this new mouse line, we are planning to establish surrogate experimental models for this.

Next we performed long-term, live single-cell imaging and tracking during HSC division with future cell-fate quantification and MRG inheritance of daughter cells. We expressed MRG-hmKuO2 in mouse HSCs, and quantified their inheritance during HSC division in vitro. We observed the linkage between midbody release and stem/progenitor cell potential. That was more obvious after 1st division and the tendency was decreased by increasing dvision times. We also counted lineage marker positive cells 4 days after culture. The detection antibodies (CD16/32 for myeloid cells and CD71 for erythroid progenitor cells) were put in culture at the beginning of the culture. Lineage restriction was not linked to the inheritance of midbody. These results indicated that midbody release would be one of features of keeping stemness in HSC asymmetric division and dose not affect lineage commitment.

2. Developing Analysis Tools for Cell Cycle and Cell Division of Hematopoietic Stem Cells and Leukemic Stem Cells: A novel G_0 marker (G_0M), mVenus-p27K- and its transgenic mouse

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One of the common features of the stem cells is that they are in quiescent (G_0) phase of cell cycle. Several reports indicate that tissue specific stem cells like hematopoietic stem cells (HSCs) and cancer stem cells are in G_0 phase.

We have developed a novel G_0 marker (G_0M), mVenus-p27K- (Oki et al, 2013). The G_0M clearly marked the cells in G_0 and very early G_1 in NIH3T3 cells. To examine G_0 status in HSCs, we generated a G_0M mouse line where hamtopoietic cells express mVenus-p27K- fusion gene. Interestingly, three different fractions (G_0M-high (70%), G_0M-low (20%), G_0M-negative (10%)) were identified in the HSC fraction (CD150+CD48-cKit+Sca-1+Lineage-). G_0M-high/low fractions but not G_0M-negative fraction showed an ability to reconstitute multi-lineage blood cells. BrdU-label retaining assay, a method for detection of dormant cells in various tissues, showed that G_0M-high population contained dormant functional HSCs and G_0M-low population contained active functional HSCs. Single-cell RNA sequence (scRNA-seq) analysis showed that G_0M-high cells expressed well-known HSC-related genes including *Hlf*, *Ifitm1*, *Mpl* and *Ly6a*. On the other hand, highly expressed genes in G_0M-low cells included genes associated with cell cycle or differentiation, such as *Gata1*, *Itga2b* and *Cdk6*. Small-cell Mass Spec analysis showed that Cdk6 protein was detected in G_0M-low fraction, but not in G_0M-high fraction. Taken together, these data exhibited that G_0M could discriminate dormant and active functional HSCs in the conventional HSC fraction. Moreover, high-throughput small molecule screening revealed that high concentrations of cytoplasmic calcium ($[Ca^{2+}]_c$) were linked to dormancy of HSCs. Of note, upregulation $[Ca^{2+}]_c$ by thapsigargin, a sarco/endoplasmic reticulum calcium-ATPase (SERCA) inhibitor, which increases $[Ca^{2+}]_c$ by leaking calcium from ER, could enhanced bone marrow multi-lineage reconstitution ability of LT-HSCs. These findings indicate that G_0M separates dormant and active adult HSCs, which are regulated by Cdk4/6 and $[Ca^{2+}]_c$.

To get more insight about the regulaton of stemness, we performed RNA-Seq analysis between dormant and active adult HSCs and found that their gene expression differecies were small. This result encouraged us to assess their enhancer expression differencies, as gene expressions are regulated by enhancers in general. We identified about 400 enhancers highly expressed in doramnt HSCs. Using public ChipSeq data of histons and HSC-specific transcription factors, we identified about 100 dormant HSC-specific enhancers. Now we are planning to further identify functional enhancers by manupurating those enhancers in HSCs.

Publications

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