論文の内容の要旨

論文題目　C-terminal-truncating *ASXL1* mutations induce MDS via inhibition of PRC2

（*ASXL1* 遺伝子の C 末端欠失型変異は PRC2 を阻害し MDS を惹起する）

氏名　井上大地

Recurrent mutations of *ASXL1* (*Additional sex combs-like1*) are found in various hematological malignancies including myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia, and acute myeloid leukemia (AML) with myelodysplasia-related changes. Additionally, *ASXL1* mutations are linked with adverse survival in a variety of myeloid malignancies. A previous study demonstrated that loss of ASXL1 in mice promotes myeloid transformation by impairing polycomb repressive complex 2 (PRC2)-mediated gene repression at a number of critical loci and leads to myeloid transformation. However, most *ASXL1* mutations are heterozygous and located in the 5’ region of the last exon, indicating a dominant-negative or gain-of-function feature of a truncated ASXL1 protein. Therefore, we investigated if the C-terminal truncated form of ASXL1 (ASXL1-MT) contributes to the development of myeloid malignancies. To this end, we examined the effects of ASXL1-MT using *in vitro* and *in vivo* experiments.

In *in vitro* experiment, expression of ASXL1-MT inhibited G-CSF-induced myeloid differentiation of 32Dcl3 cells. In a mouse bone marrow transplantation (BMT) model, ASXL1-MT induced multilineage dysplasia, differentiation block, slowly progressive pancytopenia, BM hyperplasia and

splenomegaly. The transduced mice died of severe anemia after a long latency (median survival, 400.5 days), and some of the mice progressed to overt leukemia. Thus, the current model displays all of the features of human MDS. In addition, ASXL1-MT collaborated with N-RAS-G12V, which confers a proliferative advantage, in inducing progression of N-RAS-G12V-induced myeloproliferative neoplasm (MPN) to AML, suggesting that ASXL1-MT contributes to leukemic transformation by inhibiting differentiation of MPN cells.

To clarify the molecular mechanism for differentiation block and MDS development induced by ASXL1-MT, we performed expression profiles of 32Dcl3 cells transduced with ASXL1-MT and BM cells of the MDS mice. Of note, gene set enrichment analysis (GSEA) of BM cells of the MDS mice indicated that ASXL1-MT induced an expression profile which inversely correlated with known PRC target genes. In fact, ASXL1-MT remarkably derepressed expression of posterior *Hoxa* genes, including *Hoxa5*, *Hoxa9* and *Hoxa10*, which are epigenetically silenced by PRC2 in mature cells. In consistent with this, H3K27me3 was globally reduced in ASXL1-MT transduced cells. We also found ASXL1-MT as well as wild type ASXL1 (ASXL1-WT) can bind to EZH2 and, importantly, co-expression of ASXL1-MT with ASXL1-WT efficiently inhibited the binding between ASXL1-WT and EZH2, suggesting a dominant-negative role of ASXL1-MT against the PRC2 function. Using a chromatin immunoprecipitation (ChIP) assay, we confirmed that H3K27me3 and Ezh2-bindig profoundly decreased around the promoter regions of *Hoxa5*, *Hoxa9*, and *Hoxa10* in the MDS mice, correlating with the

upregulation of their mRNA expression. On the other hand, we found that ASXL1-MT reduced the expression of *Clec5a*, a type 2 transmembrane receptor and that this reduction was associated with differentiation block of the 32Dcl3 cells. Moreover, utilizing an shRNA or a mutant form of *Clec5a*, we identified that *Clec5a* plays essential roles in myeloid differentiation of 32Dcl3 cells.

Lastly, we searched for microRNAs deregulated by ASXL1-MT since a large subset of microRNAs are found to be transcriptionally regulated by PRC2. Among upregulated microRNAs related to myeloid malignancies, we found that miR-125a targeted 3'UTR of *Clec5a* gene, repressed *Clec5a* expression and inhibited granulocytic differentiation in vitro. Intriguingly, H3K27me3 and Ezh2-bindig greatly decreased around the miR-125a gene in the BM cells of the MDS mice, similar to the results of ChIP assays around *Hoxa* genes.

The present results indicate that ASXL1-MT which results in a truncated protein product may (1) inhibit PRC2-function by impairing the interaction of EZH2 with the ASXL1-WT and (2) promote myeloid transformation through impaired PRC2-mediated repression of posterior *HOXA*s and miR-125a, and subsequent suppression of *CLEC5A*. *HOXA9* and *CLEC5A* expression were shown to be high and low, respectively, in MDS patients with ASXL1-MT. Our data provide evidence for a novel axis in MDS pathogenesis and implicate both mutant forms of ASXL1 and miR-125a as therapeutic targets in MDS.