論文の内容の要旨

論文題目　The molecular mechanism of leukemogenesis promoted by mutant ASXL1

（変異型 ASXL1 が白血病を促進する分子メカニズム）

氏名　浅田修平

Additional sex combs like 1 (ASXL1) is a member of the ASXL family and is involved in epigenetic regulation. ASXL1 mutations occur frequently in myeloid neoplasms including myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML), and acute myeloid leukaemia (AML) and are associated with poor prognosis. How ASXL1 mutations induce myeloid transformation is not fully understood. Previous studies have reported that ASXL1 knockdown and genetic deletion of *Asxl1* in haematopoietic cells promotes myeloid transformation, indicating that mutations in ASXL1 produce loss of function. However, a growing body of evidence suggests that *ASXL1* mutations in fact result in gain of function. Experiments using mouse bone marrow transplantation models have revealed that forced expression of a C-terminally truncated ASXL1 mutant in haematopoietic progenitor cells induces MDS-like diseases, and accelerates AML development in concert with Nras or SETBP1 mutations. In patients with *ASXL1* mutations, the mutations are typically heterozygous and occur near the 5′ end of exon 12, thus producing C-terminally truncated ASXL1 proteins are indeed expressed in MDS cells. Thus, whether

ASXL1 mutations promote myeloid transformation via a gain- or loss-of-function remains an unresolved question.

In the present study, I found a mutually reinforcing effect between a C-terminally truncating ASXL1 mutant (ASXL1-MT) and the histone H2A deubiquitinase Brca-1 associated protein 1 (BAP1). I revealed that BAP1 strongly stabilized ASXL1-MT, but not wildtype ASXL1 and induced its monoubiquitination at lysine 351. The monoubiquitinated ASXL1-MT in turn enhanced the catalytic function and nuclear retention of BAP1. Thus, ASXL1-MT forms a hyperactive complex with BAP1 and removes efficiently global ubiquitination from histone H2A at lysine 119 (H2AK119ub) in cells.

I next assessed the role of ASXL1-MT and BAP1 in hematopoietic differentiation using *in vitro* cell culture assays with cell lines (32Dcl3 and TF1), mouse primary bone marrow (BM) cells, and human primary CD34+ cord blood (CB) cells. These assays revealed that ASXL1-MT alone moderately, and coexpression of ASXL1-MT and BAP1 dramatically impaired differentiation of hematopoietic stem/progenitor cells towards granulocytes, macrophages, mast cells, and matured erythroid cells. Interestingly, ASXL1-MT/BAP complex did not inhibit, but rather promoted monocytic differentiation, which may account for the frequent detection of *ASXL1* mutations in patients with chronic myelomonocytic leukemia (CMML).

I then assessed the role of ASXL1-MT and BAP1 in myeloid leukemogenesis. Coexpression of ASXL1-MT and BAP1 enhanced colony forming capacity of mouse BM cells, but was not sufficient to immortalize them. Next, I examined the potential cooperation between ASXL1-MT and RUNX1-ETO in promoting myeloid leukemogenesis because *ASXL1* mutations have been frequently found in RUNX1-ETO leukemia patients. Forced expression of ASXL1-MT significantly accelerated the development of leukemia driven by RUNX1-ETO9a (a shorter isoform of RUNX1-ETO with a strong leukemogenic activity) in a mouse BM transplantation model, and also promoted the growth of RUNX1-ETO-expressing human CB cells *in vitro*. These growth-promoting effects of ASXL1-MT were further enhanced by BAP1 coexpression in RUNX1-ETO leukemia cells. Thus, ASXL1-MT/BAP1 complex promotes myeloid leukemogenesis in cooperation with RUNX1-ETO.

Mechanistically, I identified posterior *HOXA* cluster genes and *IRF8* as direct targets of ASXL1-MT/BAP1 complex. RNA-seq analysis and ChIP-qPCR assays revealed that ASXL1-MT/BAP1 complex bound to the promoter regions of *Hoxa5, Hoxa7, Hoxa9, and Irf8* genes, and induced upregulation of these genes through removal of H2AK119ub. The upregulation of posterior *HOXA* genes and *IRF8* likely contribute to the ASXL1-MT/BAP1-induced leukemogenesis and monopoiesis, respectively.

Finally, I performed endogenous Bap1 depletion using CRISPR/Cas9 system in murine

leukemia cells transformed by combined expression of ASXL1-MT and a SETBP1 mutant (SETBP1-D868N). Strikingly, Bap1 depletion in the cells dramatically reduced leukemogenicity. In addition, Bap1 depletion decreased posterior *Hoxa* gene expression in the cells. I then examined whether Bap1 is also required for leukemogenicity of MLL-fusion leukemia, a well-known leukemia subtype characterized by Hox gene dysregulation. Again, Bap1 depletion profoundly reduced colonogenicity and posterior *Hoxa* gene expression in mouse BM cells transformed by MLL-AF9. I also confirmed the critical roles of endogenous BAP1 to support the growth of human myeloid leukemia cell lines with ASXL1 mutations (Kasumi-1, MEG-01 and TS9;22) or MLL-fusions (MOLM-13 and THP1).

In summary, my study demonstrated the critical role of BAP1 in ASXL1-MT-induced aberrant myeloid differentiation, myeloid leukemogenesis via upregulation of *HOXA* genes and *IRF8*. Targeting enzymatic activity of BAP1, which has long been considered a tumor suppressor, can be a promising therapeutic strategy for myeloid neoplasms with ASXL1 mutations, and potentially for a broad range of myeloid neoplasms with HOX dysregulation.