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
spleenomegaly. 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 32Dc13 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-binding 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-binding 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 HOXA9s 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.