論文の内容の要旨

論文題目　Somatic ASXL1 mutations in blood cells promote solid tumor progression

（ASXL1変異を伴う血液細胞が固形腫瘍の発症・進展を促進する）

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1.Introduction

During human aging, somatic mutations are accumulated in many types of cells. Recent whole-genome sequencing studies revealed that clonal expansion of blood cells with acquired somatic mutations is unexpectedly common in healthy aged individuals. This phenomenon is called clonal hematopoiesis (CHIP). CHIP carriers are at increased risk for all-cause mortality, blood cancers, and cardiovascular diseases. CHIP-associated mutations frequently occurred in genes encoding epigenetic regulators, including *DNMT3A*, *TET2*, and *ASXL1*.

Additional sex combs-like 1 (ASXL1) is a member of the mammalian ASXL family. ASXL1 regulates gene expression and signal transduction through interactions with multiple proteins, such as BAP1, EZH2, BMI1, BRD4, AKT and NONO. In addition to CHIP, *ASXL1* is frequently mutated in myeloid malignancies and associated with poor prognosis. *ASXL1* mutations are detected in the last exon, resulting in the translation of C-terminally truncated ASXL1 proteins. The pathogenic ASXL1 mutants alter epigenetic modifications, activate AKT/mTOR pathwayand disrupt paraspeckle formation. Our lab previously established conditional knock-in mice carrying a C-terminally truncated Asxl1 mutant with the floxed STOP cassette under the control of Rosa26 promoter. The Vav-Cre-Asxl1-MT$^{fl/fl}$ mice, in which the mutant Asxl1 (Asxl1-MT) was expressed specifically in hematopoietic cells, showed age-related expansion of phenotypic hematopoietic stem cells (HSCs) in native hematopoiesis, which recapitulates human ASXL1-CHIP. Clinical evidence suggests that CHIP is particularly prevalent in solid tumor patients, and its presence has an adverse impact on their overall survival. The high frequency of CHIP in solid tumor patients is correlated with primal exposure to anti-cancer therapies and smoking habits. Whether the blood cells with CHIP-associated mutations have causal effects in solid tumor progression has been unclear. A previous study using Tet2-deficient mice showed that myeloid cell-specific Tet2-deficiency inhibits melanoma progression while other studies showed that Tet2-deficiency in immune cells promotes the growth of hepatoma and lung cancer cells. Thus, it appears that Tet2-deficient immune cells create pro-tumor or anti-tumor microenvironment in a tumor-type dependent manner. The role of other CHIP-associated mutations in the development of solid tumors has not been investigated experimentally.

In this study, I assessed the role of blood cells with the *ASXL1* mutation in various mouse solid tumor models using the Asxl1-MT$^{fl/fl}$ mice crossed with Vav-Cre, LysM-Cre, and Lck-Cre mice. My data indicate that Asxl1-MT perturbs T cell development and function, which contributes to creating a pro-tumor microenvironment for solid tumors.

2. Main findings

2.1 T cells expressing Asxl1-MT promote solid tumor progression in syngeneic transplantation models

To assess the impact of the expression of ASXL1 mutation in different blood subtypes on the progression of solid tumors, I crossed *Asxl1*-MT KI mice with Vav-cre, LysM-cre or LCK-cre mice to restrict the Asxl1-MT in all blood cell lineage, myeloid cells, or T cells. Then I subcutaneously injected C57BL/6 derived solid tumor cell lines, B16F10 (*Melanoma*), LLC (Lewis *Lung Carcinoma*), and MC38(*Colon Adenocarcinoma*), into these mice respectively. I did not observe substantial changes in the growth of all these tumor cells between control and Vav-Cre-Asxl1-MT$^{fl/fl}$ and LysM-Cre-Asxl1-MT$^{fl/fl}$ and control mice. In contrast, the growth of B16F10, LLC, and MC38 cells were all accelerated in Lck-Cre-Asxl1-MT$^{fl/fl}$ mice. These data suggest that T cell-specific expression of Asxl1-MT promotes solid tumor progression, while in other types of blood cells does not.

1. Summary of the results using syngeneic transplantation models

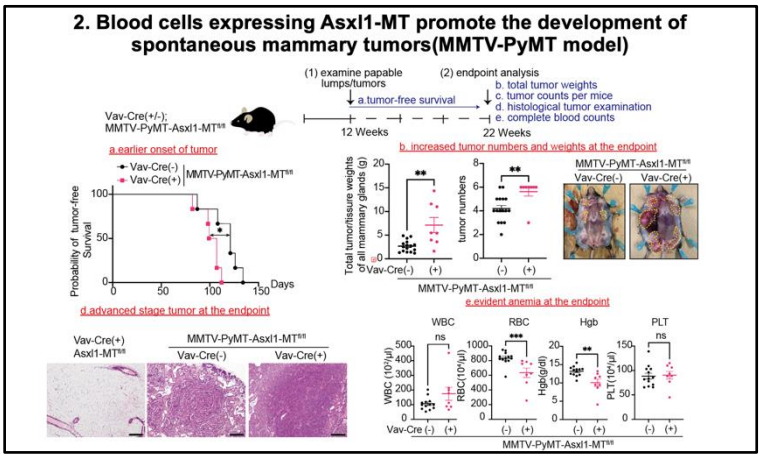
	Vav-Cre	Lysm-Cre	Lck-Cre
B16F10	NS (but tend to be promoted)	NS	Promoted(**)
LLC	NS	NS	Promoted(*)
MC38	NS	NS	Promoted(**)

T cell-specific expression of the mutant *Asxl1* promotes tumor progression in multiple solid tumor models.

2.2 Blood cells expressing Asxl1-MT promote the development of spontaneous mammary tumors

In the syngeneic tumor models, tumors are generated by subcutaneous implantation of established tumor cell lines. Therefore, the models used above do not reflect tumor progression in relevant organ-specific environments. To assess the role of Asxl1-MT expressing blood cells on tumor development in the correct microenvironment, I next used the spontaneous mouse breast cancer model induced by the polyoma middle T antigen (PyMT) driven by the murine mammary tumor virus promoter (MMTV). I crossed Vav-Cre-Asxl1-MT$^{fl/fl}$ mice with MMTV-PyMT mice to generate Vav-Cre-MMTV-PyMT-Asxl1-MT$^{fl/fl}$ mice, in which PyMT and Asxl1-MT were expressed in the mammary gland and blood cells, respectively. I observed earlier onset of tumors, increased tumor numbers and weights at the endpoint in Asxl1-MT mice compared with control mice. These data suggest that the expression of Asxl1-MT in blood cells creates a pro-tumor microenvironment in the spontaneous breast cancer model. Interestingly, I also found that Asxl1-MT mice developed modest anemia at the endpoint which is likely relevant with the advanced-stage of mammary tumors that developed in Asxl1-MT mice. Next, I examined the infiltrated immune cells inside

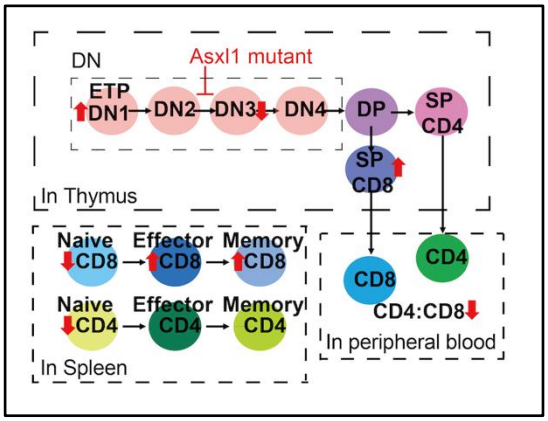
2. Blood cells expressing Asxl1-MT promote the development of spontaneous mammary tumors(MMTV-PyMT model)



the tumors using flow cytometry. I observed a significant reduction of infiltrated $CD3^+$ and $CD4^+$ T cells in mammary tumors of Asxl1-MT mice. Although the number of $CD8^+$ T cells was not significantly reduced in tumors of Asxl1-MT mice, expression of an exhaustion marker PD-1 was upregulated in them. These data indicate that Asxl1-MT-expressing T cells have an exhausted phenotype and impaired ability to infiltrate the tumor site.

2.3 Asxl1-MT perturbs T cell development and induces inflammation, mitochondrial dysregulation in T cells

The results described above indicate that Asxl1-MT promotes solid tumor progression through T cell dysregulation. To address the effect of Asxl1-MT in T cell development, I first analyzed the number and subset distribution of thymocytes and the peripheral organs in control and Vav-Cre-Asxl1-$MT^{fl/fl}$ mice. Expression of Asxl1-MT did not change the absolute numbers of thymocytes and the $CD4^-CD8^-$ double-negative (DN) fraction. However, the proportion of DN1 cells, especially $lineage^-c\text{-}Kit^+$ early T-cell precursor (ETP) population, was significantly increased in Asxl1-MT-expressing thymi. In contrast, $CD44^-CD25^+$ DN3 cells were decreased in Asxl1-MT-expressing thymi, indicating a developmental defect from DN1 to later stages. Thus, the expression of Asxl1-MT alters the intrathymic differentiation of immature T cells. In the peripheral organs. Expression of Asxl1-MT significantly decreased CD4/CD8 ratio in peripheral blood of Vav-Cre-Asxl1-$MT^{fl/fl}$ mice due to the slight increase and decrease of $CD8^+$ and $CD4^+$ cells, respectively. Intriguingly, I also found a dramatic reduction of naïve $CD4^+$ and $CD8^+$ T cells, an increase of effector $CD4^+$ and $CD8^+$ T cells, and an increase of memory $CD8^+$ T cells in the spleen of Vav-Cre-Asxl1-$MT^{fl/fl}$ mice. The reduction of peripheral naïve T cells with a relative increase of memory/effector T cells are typical immunosenescent features that are observed in aged T cells. Taken together, these data suggest that mutant Asxl1 perturbs T cell development in the thymus and induces naïve-memory imbalance in peripheral organs.



Furthermore, RNA-sequence data revealed that Asxl1-MT upregulated genes related to IL-2-STAT5 signaling and inflammatory responses in both $CD4^+$ and $CD8^+$ T cells. Activation of the IL-2-STAT5 pathway and upregulation of the pro-inflammatory genes indicate the chronic-activation and the exhaustion phenotype of Asxl1-MT-expressing T cells. Our lab previously showed that Asxl1-MT activates mitochondrial dysregulation as well as overproduction of ROS in HSCs. Consistent with the phenotypes of Asxl1-MT expressing HSCs, I observed increased mitochondrial membrane potential in $CD4^+$ and $CD8^+$ Asxl1-MT expressing T cells. Asxl1-MT also increased the intracellular ROS level in $CD4^+$ T cells and tended to increase it in $CD8^+$ T cells. Collectively, these data suggest that Asxl1-MT provokes inflammation and mitochondrial dysregulation in T cells, thereby accelerating T cell aging.

3. Summary

In this study, I showed that CHIP-associated Asxl1-MT induces T cell dysregulation and promotes tumor progression in multiple solid tumor models. my findings raise the possibility that blood cells with ASXL1 mutations exacerbate solid tumor progression in ASXL1-CHIP carriers.