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LETTER

Animal models

ASXL1 alteration cooperates with JAK2V617F to accelerate myelofibrosis

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To the Editor:

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders. MPNs are characterized by aberrant hematopoietic proliferation of one or more hematopoietic cell lineages with increased risks of myelofibrosis (MF) progression and leukemic transformation [1]. MPNs are distinguished into three clinical entities: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [2]. The somatic mutation of JAK2V617F is considered to be the most notable landmark in the diagnosis of classic Philadelphia chromosome-negative MPNs and is present in >95% of PV patients and in ~50% of ET and PMF [3, 4]. High-throughput genomic analyses of MPN patients have identified concurrent somatic mutations of epigenetic regulators such as ASXL1, TET2, IDH, EZH2, and DNMT3A co-occurring with JAK2 mutation, which are involved in clonal evolution, disease progression, and/or poor survival in MPNs [5, 6]. ASXL1 (additional sex combs-like 1) is a polycomb group protein that putatively functions as a chromatin modifier. ASXL1 mutations were identified in all types of myeloid malignancies [7–11]. In all, 34.5% of ASXL1 mutations are found in PMF patients [11]. Despite the significant impact of ASXL1 alteration and JAK2V617F on the pathogenesis of MPNs, the importance of concomitant alterations of ASXL1 and JAK2V617F within distinct hematopoietic compartments and disease progression remains to be elucidated.

We assessed the frequency of ASXL1 mutations, the clinical features, and the cumulative MF-free survival in 95 PV patients with JAK2V617F mutations. The diagnoses were made according to the 2016 World Health Organization criteria [12]. Among the 95 PV patients, 13 patients carrying co-mutations of ASXL1 and JAK2V617F had poor MF-free survival (Fig. 1a), and the proportion of ASXL1 mutations is higher in post-PV MF (PPMF) patients (26%) than in PV patients without MF (4%) (Fig. 1b). These patients carrying co-mutations of ASXL1 and JAK2V617F had decreased level of hemoglobin (Hb), increased counts of white blood cell (WBC) and platelet (PLT), palpable splenomegaly, and clonal abnormal karyotypes when compared with PV patients with JAK2V617F mutation only (Fig. 1c–e, Supplementary Tables S1 and S2).

To further study the impact of Asxl1 alteration on disease progression in JAK2V617F-mediated MPNs and JAK2V617F-mutant hematopoietic stem and progenitor cell (HSC/HPC) function, we next crossed the $JAK2^{V617F}$ mice with $Asxl1^{+/-}$ mice and assessed hematopoietic phenotypes in vivo [13, 14]. We found that $JAK2^{V617F}$;$Asxl1^{+/-}$ mice had significantly shorter mean survival rate (~60%) than $JAK2^{V617F}$ or $Asxl1^{+/-}$ mice (Supplementary Figure S1A). To classify the hematopoietic phenotypes in $JAK2^{V617F}$;$Asxl1^{+/-}$ mice, we performed a series of

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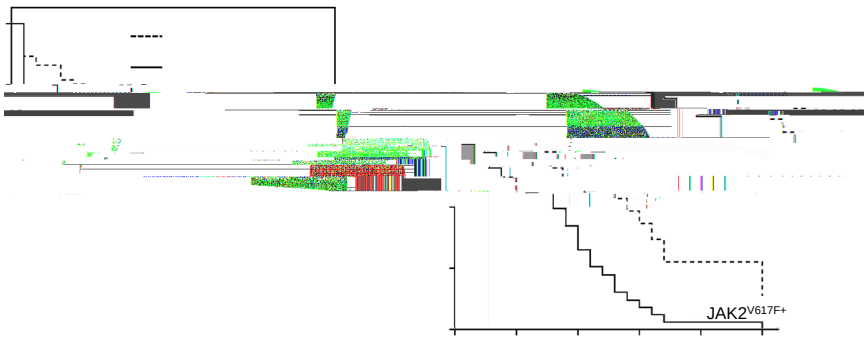
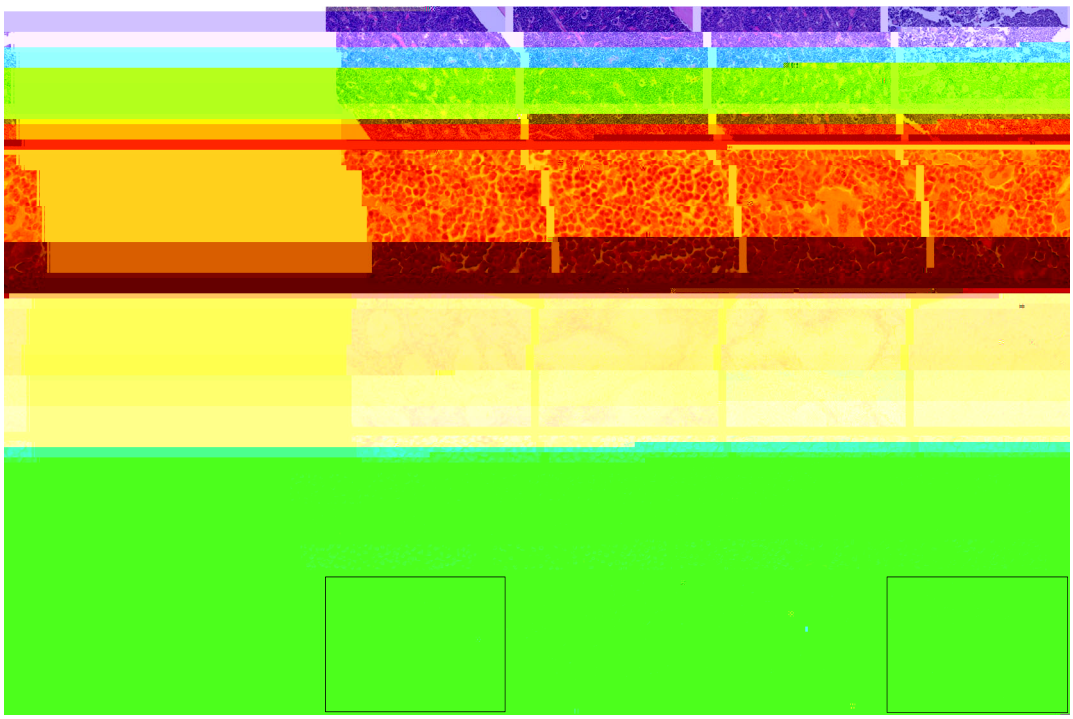


analyses including necropsy, histology, and flow cytometry on peripheral blood (PB), bone marrow (BM), and spleen. Fibrosis was assessed at each specific time points. We found that both JAK2^{V617F};Asxl1$^{+/-}$ and JAK2^{V617F} mice developed progressive MPN, including PV, ET, and MF (Supplementary Figure S1B). Notably, 5 (26%) of the 19 JAK2^{V617F};Asxl1$^{+/-}$ mice developed MF at 2–3 months of age, which was much earlier than JAK2^{V617F} mice (1 (6%) of the 18 mouse develop MF at 6 months of age) (Fig. 2a). Additionally, 3 (12%) of the 26 JAK2^{V617F};Asxl1$^{+/-}$ mice, but not the littermate controls, progressed/transformed to secondary acute myeloid leukemia (Fig. 2a, Supplementary Figure S1B–C).

The average numbers of WBC, neutrophil, and PLT were significantly higher in the PB of JAK2^{V617F};Asxl1$^{+/-}$ mice compared with the wild-type (WT) group at the age of 2–3 months. In contrast, there was no significant difference in the WBC and neutrophil counts in the JAK2^{V617F} mice at the age of 2–3 months, and from 4–6 months of age, the JAK2^{V617F} mice started to exhibit higher counts of WBC and neutrophil (Fig. 2b–d). The Hb levels in JAK2^{V617F};Asxl1$^{+/-}$ mice with MF were the lowest among all the groups of mice at the age of 18 months (Supplementary Figure S2A). The JAK2^{V617F};Asxl1$^{+/-}$ mice exhibited splenomegaly (Supplementary Figure S2B). The histologic analysis of the femur sections from JAK2^{V617F};Asxl1$^{+/-}$ mice displayed megakaryocytic hyperplasia (Fig. 2e). Spleen sections from JAK2^{V617F};Asxl1$^{+/-}$ mice showed a disrupted splenic architecture and prominent megakaryocytes and myeloid precursors (Fig. 2e). Reticulin staining of femur sections revealed extensive fibrosis in the BM of JAK2^{V617F};Asxl1$^{+/-}$ mice at the age of 3 months (Fig. 2e). Consistently, flow cytometric analysis demonstrated that JAK2^{V617F};Asxl1$^{+/-}$ mice had a significant expansion of erythroid precursors (Ter119^{+}CD71^{+}) in the BM and spleen compared with the JAK2^{V617F} and WT groups (Supplementary Figure S2C). The PB smear of JAK2^{V617F};Asxl1$^{+/-}$ mice contained more bluish red blood cells compared with WT PB smear, suggesting an impaired red blood cell differentiation (Supplementary Figure S2D). Interestingly, the frequency of CD41^{+}CD61^{+} megakaryocytic precursors was significantly increased in the BM of JAK2^{V617F};Asxl1$^{+/-}$ mice by flow cytometric analysis compared with WT and JAK2^{V617F} mice (Supplementary Figure S2E). These data demonstrate that concurrent haploinsufficiency of Asxl1 and JAK2V617F enhances megakaryopoiesis and increases erythroid precursors in the BM and spleen, which may accelerate MF development in vivo.

To determine the effect of Asxl1 alteration on JAK2^{V617F} HSC/HPCs, we performed flow cytometric analyses and found that the frequencies of short-term (ST)- HSC and megakaryocyte/erythroid progenitor (MEP) were significantly increased in the BM of JAK2^{V617F};Asxl1$^{+/-}$ mice compared with those in WT mice, while the frequency of multipotent hematopoietic progenitors (MPP) was significantly decreased in the BM of JAK2^{V617F};Asxl1$^{+/-}$ mice compared with that in WT mice (Supplementary Figure S3A–B). Colony-forming unit (CFU) assays



revealed the total colony number and replating capacity in the BM cells of $JAK2^{V617F}$;$Asxl1^{+/-}$ mice was significantly higher compared with those in the WT group of mice (Supplementary Figure S3C–D). Furthermore, we observed the frequencies of burst-forming unit-erythroid (BFU-E), CFU-erythroid (CFU-E), and CFU-megakaryocyte (CFU-MK) colonies were significantly increased in response to a series of doses of erythropoietin (EPO) in the spleen and BM cells from $JAK2^{V617F}$;$Asxl1^{+/-}$ mice (Fig. 2f, Supplementary Figure S3E). Notably, EPO-independent BFU-E and CFU-E formation, a hallmark feature of PV [15], were also higher in the spleen and BM cells from $JAK2^{V617F}$;$Asxl1^{+/-}$ mice compared with other groups of mice (Fig. 2g, Supplementary Figure S3E). Thus Asxl1 alteration cooperates with JAK2V617F mutation leading to biased lineage skewing, favoring erythroid and megakaryocytic differentiation.

We have reported that leukemic transformation in MDS/MPN can occur in the aged $Asxl1^{+/-}$ mice (>16 month old) [14]. In the current study, we found that three $JAK2^{V617F}$;$Asxl1^{+/-}$ mice developed myeloid leukemia at the age of 6–8 months, which is much earlier than that found in $Asxl1^{+/-}$ mice. Two of the $JAK2^{V617F}$;$Asxl1^{+/-}$ mice also had intestinal myeloid sarcoma, which were verified by histology and flow cytometric analysis (Supplementary Figure S4A–C). The moribund leukemic $JAK2^{V617F}$;$Asxl1^{+/-}$ mice had blast cells in PB, >20% blast cells in BM, and splenomegaly (Supplementary Figure S4D–G). The histologic analyses of femur sections revealed an increase of megakaryocytes and a decrease in erythroid islands of these $JAK2^{V617F}$;$Asxl1^{+/-}$ mice but not in any other groups of mice. The spleen sections of $JAK2^{V617F}$;$Asxl1^{+/-}$ mice showed a disrupted architecture with an increased proportion of myeloid cells. Reticulin staining showed extensive BM fibrosis in $JAK2^{V617F}$;$Asxl1^{+/-}$ mice (Supplementary Figure S4H). These data indicate that Asxl1 alteration cooperates with JAK2V617F mutation to accelerate myeloid leukemic transformation.

In summary, PV patients with co-mutations of ASXL1 and JAK2V617F had a poor MF-free survival. Likewise, Asxl1 loss accelerates MF in JAK2V617F-driven MPN in mice. $JAK2^{V617F}$;$Asxl1^{+/-}$ mice induces megakaryocytic hyperplasia and can transform to myeloid leukemia. Future studies using the Asxl1 and $JAK2^{V617F}$ co-mutated mice to further investigate the cooperative effect between ASXL1 mutant and JAK2V617F in the progression of myeloid malignancies are warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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