blood® commentary

MYELOID NEOPLASIA

Comment on Jacquelin et al, page 2707

Accelerating myelofibrosis through loss of *Dnmt3a*

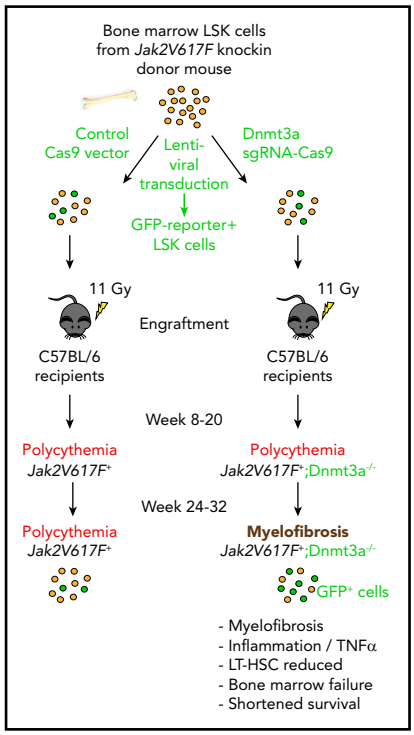
Radek C. Skoda | University Hospital Basel

In this issue of *Blood*, Jacquelin et al report that loss of DNA methyltransferase 3A (*Dnmt3a*) in hematopoietic cells expressing mutant *Jak2V617F* promotes progression from polycythemia vera to myelofibrosis leading to bone marrow failure and premature mortality.[1]

DNMT3A is an enzyme that adds methyl groups in CpG-rich regions of the chromosomes, which are involved in control of transcription. Mutations in *DNMT3A* are frequently found in normal individuals with clonal hematopoiesis of indeterminate potential,[2,3] consistent with effectively increasing the self-renewal potential of hematopoietic stem cells (HSCs).[4] Monoallelic or biallelic mutations in *DNMT3A* are found in acute myeloid leukemias and myelodysplastic syndromes and are also detectable in 7% to 10% of patients with myeloproliferative neoplasms (MPN), most frequently in combination with *JAK2V617F*.[5,6] Although the loss of DNMT3A leads to demethylation of DNA, the exact mechanism of how DNMT3A mutations contribute to the multistep genesis of cancer is still largely unknown.[4] The report by Jacquelin et al provides new insights into how the *Jak2V617F* driver mutation and the loss of *Dnmt3a* collaborate to accelerate MPN disease progression.

Jacquelin and colleagues used clustered regularly interspaced short palindromic repeats (CRISPR) with CRISPR-associated protein 9 (Cas9) technology to introduce deletions in the *Dnmt3a* gene into HSCs from a previously characterized *Jak2V617F* knockin mouse model of MPN (see figure). These targeted HSCs were then transplanted into lethally irradiated wild-type recipients. The *Jak2V617F;Dnmt3a* double-mutant HSCs exhibited a competitive advantage over the *Jak2V617F* single-mutant HSCs and over time dominated hematopoiesis in the reconstituted recipients. These double-mutant mice displayed progression from an initial polycythemia phenotype to advanced myelofibrosis accompanied by premature lethality. Hematopoiesis was oligoclonal in respect to the *Dnmt3a* mutations (ie, each recipient mouse had hematopoietic cells carrying a number of different *Dnmt3a* deletions induced by CRISPR-Cas9). Importantly, the *Dnmt3a* gene in double-mutant cells was inactivated on both chromosomes, resulting in loss of expression of the Dnmt3a protein in western blots (supplemental Figure 1B in Jacquelin et al).

To understand the mechanisms of action, the authors performed a detailed gene expression, DNA methylation, and chromatin accessibility analysis. Although the global DNA methylation changes did not correlate well with alterations in gene expression, the authors found that loss of *Dnmt3a* in conjunction with *Jak2V617F* upregulated self-renewal programs mainly through regulation of chromatin topology and methylation of specific chromatin marks at enhancer sites that have been shown to regulate HSC gene expression. Thus, the loss of Dnmt3a resulted in an "enhanceropathy" driving reproducible transcriptional programs that alter stem cell properties. Surprisingly, despite upregulating self-renewal gene expression programs, the *Jak2V617F;Dnmt3a* double-mutant HSCs exhausted earlier leading to bone marrow failure and inability to engraft into secondary recipients than *Jak2V617F* single-mutant HSCs. These data suggest that double-mutant HSCs might be sensitive to drugs, such as bromodomain inhibitors, that exert activity in the context of enhancer activation.



Design and time course of the in vivo experiments. Bone marrow lineage$^-$, Sca1$^+$, c-Kit$^+$ (LSK) cells isolated from a *Jak2V617F* knockin mouse were transduced with lentivirus encoding *Cas9* and green fluorescent protein (*GFP*) reporter with nontargeting single-guide RNA (control; left) or single-guide RNA targeting *Dnmt3a* (right) and transplanted into recipient mice lethally irradiated with 11 Gy. After engraftment, both the control mice (*Jak2V617F*$^+$) and the *Dnmt3a* targeted mice (*Jak2V617F*$^+$*;Dnmt3a*$^{-/-}$) developed polycythemia, which in the *Dnmt3a* targeted mice progressed to myelofibrosis and bone marrow failure. Note that the GFP-positive *Jak2V617F*$^+$*;Dnmt3a*$^{-/-}$ cells were positively selected over time. LT-HSC, long-term hematopoietic stem cells.

The accelerated transition to myelofibrosis is reminiscent of the phenotypes observed in *Jak2V617F;Ezh2* double-mutant mice.[7-9] Indeed, Jacquelin et al found that

Jak2V617F;Dnmt3a double-mutant cells show changes in chromatin accessibility consistent with decreased repressive H3K27me3 marks that are normally deposited by the Ezh2 containing polycomb repressive complex 2 (PRC2) on histone proteins in key enhancer regions and are instead replaced by activating H3K27Ac marks histone placed by the acetyltransferases p300 and CBP. Thus, the loss of *Dnmt3a* in part mimics the loss of PRC2 activity, although at present it remains unclear how these DNA methylation and histone modification pathways interact at the molecular level. The transition to myelofibrosis in *Jak2V617F;Dnmt3a* double-mutant mice is further promoted by increased chromatin accessibility and enhancer activation in genes that orchestrate a proinflammatory program with strong upregulation of tumor necrosis factor α (TNFα) via NF-κB pathways. Increased proinflammatory cytokines as a consequence of *Dnmt3a* loss were also observed in conjunction with other activated oncogenes such as *NRas* or *FLT3-ITD*.

Some of the key gene expression alterations leading to activated TNFα signaling and proinflammatory gene expression observed in the *Jak2V617F;Dnmt3a* double-mutant mice were also confirmed in primary cells from MPN patients with vs without mutated *DNMT3A*. In addition to advancing our understanding of the role of *Dnmt3a* loss in MPN disease progression, this study raises additional questions. In the current prognostic scores for MPN patients, loss of *DNMT3A* is not considered a "high-risk" mutation, whereas in the mouse model a strong negative effect on survival was seen. The order of acquisition of the *Jak2V617F* and *Dnmt3a* mutations may influence the severity of the phenotype. In the majority of MPN patients, the *Dnmt3a* mutation precedes the acquisition of the *Jak2V617F* mutation, whereas, as in the mouse model studied by Jacquelin et al, the *Dnmt3a* mutation is introduced on top of a preexisting *Jak2V617F* mutation. Indeed, differences in phenotypes were reported for "*JAK2*-first" vs "*DNMT3A*-first" patients with MPN.[10] Finally, this work provides a strong rationale for testing therapeutic approaches that target the activity of gene enhancers for MPN patients selected for specific mutational profiles.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Jacquelin S, Straube J, Cooper L, et al. Jak2V617F and Dnmt3a loss cooperate to induce myelofibrosis through activated enhancer-driven inflammation. *Blood*. 2018; 132(26):2707-2721.
2. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014; 371(26):2488-2498.
3. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med*. 2014; 20(12):1472-1478.
4. Brunetti L, Gundry MC, Goodell MA. DNMT3A in leukemia. *Cold Spring Harb Perspect Med*. 2017;7(2):a030320.
5. Stegelmann F, Bullinger L, Schlenk RF, et al. DNMT3A mutations in myeloproliferative neoplasms. *Leukemia*. 2011;25(7):1217-1219.
6. Abdel-Wahab O, Pardanani A, Rampal R, Lasho TL, Levine RL, Tefferi A. DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. *Leukemia*. 2011;25(7):1219-1220.
7. Shimizu T, Kubovcakova L, Nienhold R, et al. Loss of Ezh2 synergizes with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. *J Exp Med*. 2016; 213(8):1479-1496.
8. Sashida G, Wang C, Tomioka T, et al. The loss of Ezh2 drives the pathogenesis of myelofibrosis and sensitizes tumor-initiating cells to bromodomain inhibition. *J Exp Med*. 2016; 213(8):1459-1477.
9. Yang Y, Akada H, Nath D, Hutchison RE, Mohi G. Loss of Ezh2 cooperates with Jak2V617F in the development of myelofibrosis in a mouse model of myeloproliferative neoplasm. *Blood*. 2016;127(26):3410-3423.
10. Nangalia J, Nice FL, Wedge DC, et al. DNMT3A mutations occur early or late in patients with myeloproliferative neoplasms and mutation order influences phenotype. *Haematologica*. 2015;100(11):e438-e442.

DOI 10.1182/blood-2018-11-883470

LYMPHOID NEOPLASIA

Comment on Thurner et al, page 2744

Tropism of PCNSL: is it all about autoantigens?

Björn Chapuy | University Medical Center Göttingen

In this issue of *Blood*, Thurner et al identified that two-thirds of patients with primary central nervous system lymphoma (PCNSL) have B-cell receptors (BCRs) specific for 2 autoantigens, SAMD14 and neurabin-1. These antigens are predominantly expressed in the central nervous system (CNS), induced strong BCR pathway activation, and autorecognition of them is therefore an attractive explanation for the CNS tropism of the disease.[1]

PCNSL is a rare extranodal variant of a B-cell non-Hodgkin lymphoma that presents as infiltration of Epstein-Barr virus negative (EBV^-) lymphoma cells in the brain parenchyma, leptomeninges, spinal cord, or the eye without evidence of systemic disease. The most frequent histology of PCNSL is a diffuse large B-cell lymphoma (DLBCL). An etiologically different EBV^+ PCNSL occurs in immunocompromised patients. Despite its limited state, clinically PCNSL is an aggressive disease with dismal outcomes due to inferior responses to empirically derived treatment regimens. A better mechanistic understanding of the disease pathogenesis might lead to novel targeted therapeutic options for patients with unmet clinical needs.

Genetically, PCNSLs exhibit frequent biallelic inactivation of the tumor suppressor *CDKN2A*, almost uniform activation of the BCR- and Toll-like receptor signaling pathways through cooccurring *CD79B* and *MYD88* (*L265P*) mutations, and additional alterations in downstream regulators, such as inactivation of *TNFAIP3* and oncogenic mutations in *CARD11*, in addition to frequent gain of both PD-1 ligands.[2] The full coordinate genetic signature of PCNSLs has striking similarities to genetically defined ABC DLBCL subtypes (C5-DLBCL in Chapuy et al[3]; C5-DLBCL in Schmitz et al[4]).

Prior studies have established the origin of PCNSLs from germinal center experienced B cells with rearranged and