"ASXL1"-erating inflammation and bone marrow fibrosis in myeloproliferative neoplasms

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Received: August 17, 2022.
Accepted: August 19, 2022.
Early view: August 25, 2022.

https://doi.org/10.3324/haematol.2022.281634

In this issue of *Haematologica*, Shi *et al.* report on a critical role of *ASXL1* mutations in driving bone marrow fibrosis via a EGR1-TNFA axis in both murine models and patients with primary myelofibrosis.[1]

Additional sex combs like 1 (*ASXL1*) mutations are among the most common molecular biological abnormalities in patients with primary myelofibrosis, but the effect of these mutations on prognosis remains controversial. Recent studies demonstrated that *ASXL1* mutations alone are not detrimental but confer a worse prognosis when associated with a mutation in TP53 or high-risk genes.[2] In line with these findings, it was demonstrated that *ASXL1* mutations are early driver events in primary myelofibrosis but might be acquired later in the disease course of secondary myelofibrosis.[3] This raises the question of the effect of *ASXL1* mutations on hematopoietic stem and progenitor cells. In their study, Shi *et al.* sought to shed light on the mechanism of aberrant lineage differentiation and transcription deregulation related to *ASXL1* mutations in myeloproliferative neoplasms (MPN), using patients' biopsies and the hematopoietic-specific Vav-Cre-driven murine model named *Asxl1*$^{-/-}$ *Jak2*^{VF}.

In their article, Shi and colleagues[1] once again confirm that *ASXL1* mutations, regardless of the "MPN driver" mutation, are associated with a more severe disease phenotype (e.g., larger spleens, higher fibrosis grades, lower hemoglobin) and higher monocyte frequency but do not specifically differentiate between primary myelofibrosis and secondary myelofibrosis or additional mutations. The hematopoietic-specific *Jak2*^{VF} murine model with deletion of *Asxl1* represents a model for early acquisition of *ASXL1* mutations comparable to *ASXL1* being an early event in primary myelofibrosis.[3] In line with their own and earlier clinical data, Shi *et al.* demonstrate that loss of *Asxl1* triggers earlier onset of fibrosis and a generally more severe phenotype and also induces a differentiation bias towards the monocyte/macrophage lineage. Monocytosis in patients with primary myelofibrosis was previously associated with inferior survival[4] and could be explained by a more severe inflammatory state. As *ASXL1* mutations were associated with monocytosis in patients and the murine model, the authors explored the hypothesis of monocyte-derived fibrocytes contributing to more severe fibrosis. Fibrocytes are still only very broadly defined as spindle-shaped cells expressing markers of both hematopoietic cells (CD34, CD43, CD45, CD68, LSP-1, and major histocompatibility complex class II) and stromal cells (collagen I, collagen III, and fibronectin) and have been associated with primary myelofibrosis.[5] Shi *et al.* show an association of an increased frequency of fibrocytes in patients carrying an *ASXL1* mutation when compared to controls but functional evidence of active extracellular matrix production of these cells contributing to fibrosis still remains to be demonstrated. Surprisingly, the authors did not find a significant difference in Gli1^{+} and LepR^{+} staining in their relatively small cohort of patients (n=4 *ASXL1*^{mut} *vs.* n=8 *ASXL1*^{WT}) which were previously reported to expand as fibrosis-driving cells in response to a MPN clone.[6,7] This might be due to the fact that both are known to be expressed at low levels and are difficult to detect by immunofluorescence without signal amplification. Another critical point is the preparation of tissue, specifically fixation and decalcification, which have significant impact on bone marrow staining. Recent work by van Egeren and colleagues[8] just described a population of CD34^{-} bone marrow monocytes using single-cell RNA sequencing and found that the *JAK2* mutation increased expression of intermediate monocyte genes and the fibrocyte-associated surface protein SLAMF7 in these cells. It would now be interesting to explore if there is also an association with *ASXL1* co-mutations.

Shi *et al.* sought to dissect transcriptional differences upon co-mutation/loss of *Asxl1* in their murine model. Using bulk RNA sequencing of the heterogeneous population of cKit^{+} hematopoietic stem and progenitor cells, the authors show that inflammation-related pathways such as Nfκb, TNFα and IL-17, are upregulated in *Asxl1*$^{-/-}$ *Jak2*^{VF} bone marrow ckit^{+} cells and confirmed higher serum levels of TNFβ in ASXL1 mutant patients and

Asxl1$^{-/-}$ *Jak2*^{VF} mice. Given the strong association they observed between the double mutants/co-mutations, it would have been of particular interest to determine the effect of the co-mutation on CD14^{+} monocytes, for example, and not only progenitor cells. Interestingly, Shi and colleagues observed and validated the upregulation of Egr1 in LSK, GMP and monocytes of *Asxl1*$^{-/-}$ *Jak2*^{VF} mice. This is an interesting link to fibrosis as Egr1 expression was described in solid organ fibrosis to be induced by fibrogenic (pro-inflammatory) stimuli and to regulate the expression of extracellular matrix components, matrix remodeling enzymes and fibrogenic cytokines such as TGF-β, leading to myofibroblast differentiation. Shi *et al.* further leveraged RNA sequencing, assay for transposase-accessible chromatin (ATAC) sequencing and chromatin immunoprecipitation sequencing to investigate the transcriptional and epigenetic alterations in *Asxl1*$^{-/-}$ *Jak2*^{VF} double mutants and highlight increased chromatin accessibility associated with increased levels of histone marks on enhancers, also specifically on the *Egr1* locus. This is a strong point towards a role of EGR1 in more advanced fibrosis.

Recent pivotal studies have transformed our understanding of mutation acquisition in MPN[9,10] and the timing of acquisition of an *ASXL1* mutation in MPN patients seems to be crucial for the phenotype. This raises the question of what role the timing of *ASXL1* mutations in MPN has on disease and fibrosis initiation and progression, and if similar pathways and genes are activated. The "ASXL1-erating" effect on fibrosis kinetics in MPN was clearly demonstrated and it will be interesting to see in the future the functional effect of an EGR1/TNFα axis which could potentially act as a point of therapeutic intervention.

Disclosures

No conflicts of interest to disclose.

Contributions

HG and RKS wrote and edited the manuscript.

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