has significant consequences for the diagnostic workup of DLBCL in daily practice because DH/TH DLBCLs do not necessarily display aggressive morphological and/or immunohistochemical features, like starry sky pattern, high mitotic rate, or MYC protein overexpression.[5] This raises the question whether every DLBCL should be referred for FISH testing for *MYC*, *BCL2*, and *BCL6* rearrangements to detect DH status.

Interphase FISH on formalin-fixed paraffin-embedded tissue section is a robust technique, but is time consuming, expensive, and not widely available. However, FISH techniques in recent years have greatly improved with automatization and development of digital imaging technologies. Various strategies have been proposed to restrict FISH testing to GCB subtype, or according to Ki67 proliferative index or MYC protein expression. Some authors suggested limiting FISH to GCB and DPE DLBCLs, which would reduce FISH analysis to 15% of cases.[7] However, no consensus has been reached to date, the main reason being the lack of large cohorts of DLBCL patients with COO and FISH data to test various screening strategies.

Scott et al provide data from a large cohort of 1228 de novo DLBCLs, identified in 3 international clinical trials and a population-based registry, to evaluate the incidence of HGBL-DH/TH and the effects of screening strategies based on COO (Lymph2Cx gene expression assay and/or Hans algorithm) and/or DPE. *MYC* rearrangement (*MYC-R*) was observed in 12.2% of DLBCLs and included mostly, but not exclusively, GCB DLBCLs. *MYC* as sole genetic alteration and *MYC/BCL6* HGBL-DH included both ABC and GCB DLBCLs, whereas *MYC/BCL2* and *MYC/BCL2/BCL6* HGBL-DH/TH were exclusively GCB. In total, HGBL-DH/TH represented ~8% of tumors with DLBCL morphology (see figure).

According to the study by Scott et al, the best method for detecting all HGBL-DH/TH among tumors with DLBCL morphology is to screen all DLBCLs for *MYC* breaks. When the tumor is positive, it should be further tested for *BCL2* and *BCL6* gene alterations, which would require that the FISH technique be in pathology laboratories and that reliable *MYC* probes are used. Alternatively, restricting FISH testing to GCB DLBCLs would reduce FISH testing to half of DLBCLs and would still detect ≥99% HGBL-DH/TH with *BCL2* rearrangements. This approach is acceptable for *MYC/BCL2* HGBL-DH detection but would miss a considerable number of the uncommon *MYC/BCL6* HGBL-DH, where the prognostic value is still controversial.[5,8] In addition, this approach would miss DLBCLs with isolated *MYC* rearrangement and ABC/non-GCB phenotype. A major point of the study is to show that selecting DLBCLs on DPE status and/or COO subtyping results in missing ~35% of all HGBL-DH.

In summary, the study of Scott et al presents data on the impact of various FISH testing strategies to identify HGBL-DH/TH in tumors with DLBCL morphology. FISH testing for *MYC*, *BCL2*, and *BCL6* should be incorporated in the routine diagnostic workup of all DLBCLs in an integrated approach together with gene expression assays and next-generation sequencing. If not possible, the optimal strategy is a 2-step approach with testing for *MYC* first and to perform FISH for *BCL2* and *BCL6* if there is *MYC* rearrangement. Other screening strategies to limit the costs should be discussed in each institution depending on the local resources and with the knowledge of the limitations of each strategy as reported in this study.

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MYELOID NEOPLASIA

Comment on Peeken et al, page 2065

JAK2 and JMJD1C activate NFE2 in MPNs

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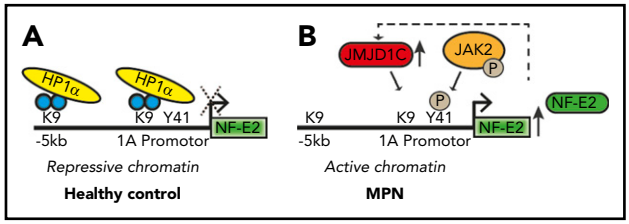
Overexpression of nuclear factor erythroid 2 (NFE2) is commonly observed in the myeloproliferative neoplasms (MPNs), especially polycythemia vera (PV) and primary myelofibrosis,[1,2] but the mechanism that drives this feature has been unclear. In this issue of *Blood*, Peeken et al reveal a 2-pronged epigenetic pathway that promotes NFE2 overexpression and disease.[3]

A hallmark of the MPNs is enhanced JAK/STAT activation, which is driven by mutations in *JAK2*, *MPL*, and *CALR*. These mutations are accompanied by dysregulated expression of many genes that modulate the disease phenotype. How enhanced JAK/STAT activation alters gene expression and how the subsequent gene dysregulation contributes to disease are important questions in the field.



Two epigenetic pathways converge to control NFE2 expression in the MPNs. (A) In healthy hematopoietic cells, NFE2 expression is maintained at a low level by HP1α binding to unphosphorylated H3Y41 and by the presence of dimethylated H3K9. (B) In MPN cells, NFE2 expression is upregulated by both a reduction in HP1α binding, which is associated with increased phosphorylation of H3Y41 by JAK2^{V617F} and by demethylation of H3K9. The figure has been adapted from Figure 6 in the article by Peeken et al that begins on page 2065.

A notable example of a gene that is dysregulated in the MPNs is NFE2, whose overexpression was found to cause a myeloproliferative disease that resembles the MPNs in animal models.[4,5] To study how increased levels of NFE2 contribute to the MPNs, Peeken et al analyzed published data regarding NFE2 chromatin immunoprecipitation sequencing and identified 60 epigenetic regulators as presumptive targets. Among these genes, they focused on the histone demethylase JMJD1C, which is reported to convert H3K9 from a mono- or dimethylated state to an unmethylated state, allowing for increased gene transcription.[6] Consistent with it being a direct NFE2 target, JMJD1C expression was elevated in NFE2 transgenic mice, and its messenger RNA levels correlated with those of NFE2 in PV patients. Peeken et al further demonstrated that the increased levels of JMJD1C were associated with decreased H3K9 methylation along the NFE2 locus; this finding suggests that there is a positive feedback loop that augments NFE2 expression (see figure).

Prior studies demonstrated that, beyond enhancing STAT signaling, nuclear JAK2 phosphorylates Y41 on histone H3[7]; this event impairs binding of the heterochromatin protein-1α (HP1α) to chromatin, which reduces heterochromatin formation and causes increased gene expression. Peeken et al found that HP1α binding along the NFE2 locus was reduced in PV patients. Reduction in HP1α binding and decreased methylation of H3K9 seem to cooperate to increase NFE2 activity. Further evidence for this dual epigenetic action to enhance NFE2 expression was obtained from studies with the epigenetic modulator decitabine. Treatment of MPN cell lines with decitabine reversed aberrant histone methylation at the NFE2 locus, increased HP1α binding, and shut down NFE2 expression. The levels of JMJD1C were similarly reduced. This effect was likely due in part to the ability of decitabine to suppress JAK2 activity.

JMJD1C has been shown to be essential for RUNX1-RUNX1T1 and MLL-AF9 leukemia,[8,9] but what is its role in the MPNs? Peeken et al demonstrate that knockdown of JMJD1C preferentially suppresses cytokine-independent growth of JAK2^{V617F}-expressing Baf/3 cells, suggesting that inhibiting this demethylase may provide therapeutic benefit.

The study by Peeken et al answers one important question about NFE2 regulation but raises several others. First, although JMJD1C knockdown suppressed the growth of Baf/3 cells, to what extent does the observed increase in JMJD1C contribute to disease progression in human MPNs? Second, is this suppression of cell growth upon JMJD1C knockdown a consequence of decreased NFE2 expression? Third, whereas Peeken at al demonstrated that decitabine reduced NFE2 expression, 5-azacitidine showed limited clinical activity in a phase 2 trial as a single agent.[10] However, the degree of NFE2 knockdown was not examined in patients treated with 5-azacitidine. The results of Peeken et al raise a question: To what extent does increased NFE2 contribute to MPN pathogenesis, especially given the observation that it is sufficient to cause an MPN-like disease and progression of acute myeloid leukemia in animal models?[4,5] Targeting NFE2 will prove challenging, but genetic studies in mice will shed light on this issue. Finally, 60 epigenetic regulators were identified as potential targets of NFE2; therefore, it is likely that there are other targets beyond JMJD1C that contribute to NFE2 dysregulation and the pathogenesis of the MPNs. Although JMJD1C is a putative demethylase for H3K9, inhibitors of the protein have not been developed; thus, an approach to targeting this gene in patients is not feasible at this time. Future studies to identify the other relevant NFE2 targets will increase our understanding of the disease and may reveal more tractable therapeutic targets.

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