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Review Article

Recurring mutations in myeloproliferative neoplasms alter epigenetic regulation of gene expression

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Abstract: The prevalence of activating JAK2 mutations in myeloproliferative neoplasms (MPNs) suggests that aberrant gene expression due to deregulated signaling of the JAK2/STAT pathway plays an important role in the etiology of these diseases. While likely true, recent work has uncovered some fascinating new insights into both the function of mutationally-activated JAK2 as well as other mutated gene products in MPNs, and how these mutations may affect gene expression. In addition to being a cytoplasmic tyrosine kinase that relays signals from cytokine receptors, activated JAK2 can also function in the nucleus where it phosphorylates histones and deregulates binding of the transcriptional repressor HP1α. In addition, MPN-associated JAK2 mutants phosphorylate PRMT5 and inhibit its histone methyltransferase activity. Thus, in addition to the classical JAK/STAT pathway, JAK2 activating mutations in MPNs may deregulate gene expression by altering epigenetic mechanisms. Studies aimed at identifying the biochemical ramifications of other recurring MPN mutations also suggest deregulated epigenetic modifications may be important in MPN formation. Mutant TET2, as well as IDH1/2, impairs the hydroxylation of methylcytosine, thus affecting DNA methylation. Likewise, mutations in EZH2, a histone methyl transferase, ASXL1, which functions in chromatin modifier complexes, and the DNA methyltransferase DNMT3A, appear to inactivate the functions of these proteins toward regulating the epigenetic state of genes. Thus, it is likely that the control of gene expression by epigenetic mechanisms plays an important role in MPNs, since multiple recurring mutations in MPNs alter normal epigenetic regulatory mechanisms.

Keywords: MPN, JAK2, TET2, IDH1, IDH2, EZH2, ASXL1, DNMT3A, epigenetic, gene expression

Introduction

MPNs are a collection of hematopoietic stem/progenitor cell disorders that result in the inappropriate accumulation of myeloid cells in the blood and bone marrow [1]. The most well studied and well-known MPN is chronic myeloid leukemia (CML), whose causative agent is BCR-ABL, a fusion oncogene expressed from the chromosomal translocation-generated Philadelphia chromosome. Classic Philadelphia chromosome-negative MPNs include polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Among other clinical characteristics, polycythemia vera patients have an elevated red blood cell mass or hematocrit [1]. Essential thrombocythemia is characterized, in part, by aberrant production of platelets, while primary myelofibrosis patients exhibit elevated granulocytes as well as fibrosis of the bone marrow. These diseases are chronic disorders, that when diagnosed, generally require lifelong medical attention. Polycythemia vera and essential thrombocythemia can lead to cardiac and thrombotic complications while myelofibrosis patients can develop anemia due to bone marrow failure. Due in part to such complications, MPNs themselves can be deadly, but can also transform into acute myeloid leukemia, for which prognosis is grim [2].

Sixty years ago, William Dameshek made a prescient suggestion that these disorders were related [3]. In 2005, a point mutation in the JAK2 tyrosine kinase was discovered in each of these classic Philadelphia chromosome-negative MPNs [4-8]. This mutation changes a valine to a phenylalanine at residue 617 of JAK2. This amino acid is located in the pseudokinase domain of JAK2 and it is believed that this missense mutation reduces the ability of the pseudokinase domain to exert its negative

regulation on the tyrosine kinase domain [9]. Thus, the kinase activity of JAK2-V617F is elevated compared to wild type JAK2 and JAK2-V617F exhibits transforming activity in hematopoietic cell lines [5-8]. JAK2-V617F is present in about 95% of polycythemia vera patients and about 50% of essential thrombocythemia and primary myelofibrosis patients [10]. Interestingly, in the small percentage of polycythemia vera patients that lack the JAK2-V617F mutation, *JAK2* mutations in exon 12 (e.g K539L) have been detected [11]. These exon 12 mutations of *JAK2* also lead to constitutive kinase activity and transforming activity. Expression of MPN-associated JAK2 mutants in mouse models leads to recapitulation of human MPN-like disorders, thus demonstrating that these mutations likely play a bona fide role in the etiology of these diseases [11-16]. It is believed that essentially all polycythemia vera patients have either the V617F mutation of JAK2 or have a JAK2 exon 12 mutation. In essential thrombocythemia, the thrombopoietin receptor (Mpl) is mutated in about 5% of patients [1, 17, 18]. Like mutationally activated JAK2, mutated Mpl leads to elevated signaling through the JAK2/STAT pathway and also induces an MPN-like phenotype in mice [17]. The conclusion from these and many others studies is that aberrant activation of JAK2 contributes to the development of MPNs.

Because of the abundance of data demonstrating the relationship between aberrant JAK2 activation and MPNs, JAK2 inhibitors have been developed and are being evaluated in clinical trials for MPNs [19-21]. The pioneering clinical success of the kinase inhibitor, imatinib, in the treatment of CML has supported optimism that JAK2 inhibitors can ultimately provide a viable treatment option for MPNs [22]. The current status of JAK2 inhibitors in clinical trials is reviewed elsewhere, but suffice it to say that these inhibitors ameliorate symptoms in patients without inducing clinical remission [19, 20]. Thus, a further understanding of the molecular aspects of MPNs may provide new insights that can be utilized to identify additional therapeutic approaches that can be used alone or in combination with JAK2 inhibitors to treat MPNs.

In addition to mutations in *JAK2*, numerous other mutations recur in MPNs. These include mutations in *LNK*, *TET2*, *IDH1*, *IDH2*, *CBL*, *EZH2*, *ASXL1*, and *DNMT3A*, among others [23-35]. These mutations likely contribute important roles to the development of MPNs. A common theme that ties a number of these MPN mutations together is that they deregulate epigenetic modifications, and thus have great potential to alter gene expression. This review summarizes a number of recent important findings that provide mechanistic insight into how such recurring mutations can lead to epigenetic deregulation and subsequent alteration of gene expression.

JAK2 localizes to the nucleus and inhibits the tumor suppressive function of HP1α

Signaling by the JAK2 tyrosine kinase is classically understood as a plasma membrane-initiated event where JAK2 is activated by membrane-bound receptors. Activated JAK2 phosphorylates various substrates, including the receptor it is bound to as well as STAT proteins. Phosphorylation of STAT proteins induces their translocation into the nucleus where they function as transcription factors, regulating the expression of genes through STAT-binding DNA sequences [36]. Thus, JAK2 signaling is initiated at the plasma membrane in response to ligand-mediated activation of receptors and transduces a signal via STAT proteins into the nucleus. Indeed, STAT5 has been shown to be required for JAK2-V617F-mediated transformation [37]. However, another mechanism by which JAK2 can regulate gene expression has been uncovered. Intriguingly, Dawson et al. identified JAK2 in the nucleus of various hematopoietic cells [38]. These cells included myeloid cell lines, such as various JAK2-V617F+ patient-derived cell lines (e.g. HEL), as well as CD34+ cells from peripheral blood of a patient with a JAK2-V617F+ MPN. With no knowledge of a function for JAK2 in the nucleus, they determined that JAK2 could phosphorylate histone H3 at tyrosine-41 (H3Y41) [38]. Cytokine signals that activate JAK2 induced H3Y41 phosphorylation in multiple cell lines while small molecule JAK2 inhibitors were found to inhibit tyrosine phosphorylation of H3Y41. Importantly, phosphorylation of H3Y41 inhibited the binding of the transcriptional repressor heterochromatin protein-1α (HP1α) [38]. Dawson et al. investigated HP1α as a potential player in JAK2 signaling in the nucleus because previous work by Shi et al. identified the loss of HP1 binding to heterochromatin in response to activated JAK (Hopscotch, hop) in Drosophila [39]. When Daw-

son et al. identified genes whose expression were decreased by JAK2 inhibition, they determined that not all of these genes contained JAK2-responsive STAT5-binding sites and thus presumably are not regulated by the classical JAK2/STAT5 pathway [38]. They demonstrated that the histones near the transcriptional start site of one of these genes, *lmo2*, had decreased H3Y41 phosphorylation and increased HP1α binding in response to JAK2 inhibition. Thus, Dawson et al. have provided evidence of a mechanism by which genes may be regulated by non-classical JAK2 signaling [38]. That is, activated JAK2 phosphorylates H3Y41, resulting in the inhibition of the binding of the transcriptional repressor HP1α, thus enhancing gene expression (**Figure 1**). While a potential role for *lmo2* in leukemogenesis has been previously identified, the identity and potential roles of other genes regulated by JAK2 in this manner remain to be determined [40]. Since HP1α inhibits gene expression and mitotic recombination, Dawson et al. suggest JAK2 inhibition of these HP1α tumor suppressive functions may contribute to the increased gene expression and genomic instability associated with mutationally activated JAK2-associated hematopoietic malignancies [38, 41, 42]. Further support for the importance of nuclear JAK2 in JAK2-driven disease comes from a previous study in Drosophila. Shi et al. demonstrated that mutational activation of the Drosophila JAK kinase hop resulted in a global disruption of gene silencing [39]. In fact, they concluded that the levels of HP1 protein could dictate the outcome of hop-driven hematopoietic disease in Drosophila. That is, low HP1 led to an enhancement of hop-driven disease while elevated HP1 suppressed disease. It will be important to determine if JAK2-mediated regulation of HP1α activity is necessary for mammalian induced JAK2-driven malignancies and if inactivating mutations or decrease in HP1α protein could enhance disease formation.

Work by Rinaldi et al. confirmed nuclear localization of JAK2 in CD34+ cells of JAK2-V617F MPN samples but not JAK2-WT MPNs [43]. Interestingly, their work suggests that as cells become more differentiated, significantly less JAK2 is present in the nucleus, with localization being primarily cytoplasmic. That is, more mature cells such as erythrocytes, granulocytes, and megakaryocytes of JAK2-V617F+ MPN patients exhibited less nuclear JAK2 than cytoplasmic JAK2. Rinaldi et al. also made the observation that treatment of cells with a JAK2-selective kinase inhibitor reverted localization of JAK2 from the nucleus to the cytoplasm [43]. These results suggest that perhaps activated JAK2 translocates to the nucleus while inactive JAK2 remains cytoplasmic. It is possible nuclear JAK2 plays a role in the development of blood cells from the immature state, while more mature cells do not require nuclear JAK2. Rinaldi et al. have suggested that JAK2 nuclear translocation inhibitors could be developed as therapies for JAK2-driven hematopoietic malignancies [43]. Given the potential effects of nuclear JAK2 inducing gene expression by inhibiting the tumor suppressive function of HP1α, this indeed may be possible. JAK2 kinase inhibitors may lead to the loss of JAK2 in the nucleus [43]. Such inhibitors may reduce the phosphorylation of important substrates through direct inhibition of kinase activity as well as by preventing JAK2 access to important nuclear substrates such as histone H3.

Since both wildtype JAK2 and JAK2-V617F require a functional receptor-interacting FERM domain for activation, JAK2 activation presumably occurs at the plasma membrane followed by translocation into the nucleus [44]. It has been well documented that certain tyrosine kinase receptors can translocate to the nucleus after activation [45]. Perhaps JAK2-bound receptors translocate to the nucleus after activation, carrying activated JAK2 with it. Alternatively, it is possible that JAK2 present in the nucleus becomes activated by an unknown mechanism. Clearly additional work remains to be done to elucidate these aspects of the function of nuclear JAK2.

The significance of JAK2-mediated epigenetic regulation of gene expression in human hematopoietic cells remains unknown, although the novelty and potential importance of this discovery is intriguing. This finding now suggests that there may be a significantly larger number of genes that can be regulated by JAK2 signaling (and thus aberrantly regulated in JAK2-driven diseases including MPNs) than previously thought based on the presence of canonical STAT5 binding sites. Recent work in embryonic stem cells has confirmed that JAK2-V617F controls gene expression via H3Y41 phosphorylation and that other JAK family members likely function similarly [46]. Important questions re-

main including: what other genes may be regulated by JAK2 phosphorylation of histone H3; how these genes play a role in malignancy; the importance of inactivation of HP1α tumor suppressive activity to JAK2-driven disease; the mechanism by which JAK2 translocates to the nucleus; if other tyrosine kinases function in a similar manner to JAKs to regulate gene expression; and if MPNs that do not have JAK2 mutations utilize a mechanism of JAK2-mediated epigenetic regulation of gene expression. At the rate JAK2 discoveries are being made it would not be surprising for many of these questions to be answered in the near future.

MPN-associated JAK2 mutants regulate PRMT5 activity by phosphorylation

The basic mechanistic understandings of the JAK/STAT pathway have become well established since the initial discovery of the Janus kinases two decades ago. The discovery of the constitutively activated JAK2-V617F mutant and its obvious importance in myeloid malignancies has allowed researchers to re-visit older findings and study them in the context of mutationally activated JAK2 and its associated diseases. In 1999, a type II arginine methyltransferase, PRMT5, was identified as a JAK2-binding protein utilizing a yeast two-hybrid screen to identify JAK2-interacting partners [47]. Since then, however, the significance of this interaction has remained unknown. A recent report by Liu et al. has now established a potentially significant mechanistic relationship between JAK2 and PRMT5 [48]. In this report, the interaction of JAK2 with PRMT5 was addressed in the context of wildtype JAK2 as well as two MPN-associated JAK2 mutants, V617F and K539L. While wildtype JAK2 was confirmed to interact with PRMT5, both JAK2-V617F and JAK2-K539L exhibited enhanced interaction with PRMT5. This interaction was detected not only in the cytoplasm, but also in the nucleus, further suggesting a role for JAK2 in the nucleus. Activated JAK2 proteins can phosphorylate PRMT5 *in vitro* and PRMT5 appears to be a target of JAK2 kinase activity in cells. Importantly, Liu et al. demonstrated that phosphorylation of PRMT5 by activated JAK2 inhibited the methyltransferase activity of PRMT5 toward histone substrates (H2A/H4) (**Figure 1**) [48]. JAK2 kinase inhibitor studies suggested that in cells expressing activated JAK2 mutants, symmetric dimethylation of histone H2A/H4 R3 was under the control of JAK2 kinase activity. The authors also demonstrated that phosphorylation of PRMT5 by activated JAK2 disrupts the ability of PRMT5 to interact with MEP50, a protein required for PRMT5 activity in the methylosome complex [49]. Thus, inhibition of PRMT5 methyltransferase activity by JAK2-mediated phosphorylation likely occurs, at least in part, through disruption of the PRMT5/MEP50 complex. Studies in primary cells demonstrated that knockdown of endogenous PRMT5 promoted hematopoietic progenitor growth and erythroid differentiation while overexpression of PRMT5 inhibited hematopoietic progenitor growth and erythroid differentiation [48]. Importantly, in JAK2-mutated MPN patient cells PRMT5 showed increased tyrosine phosphorylation compared to control cells. Moreover, overexpression of a tyrosine phosphorylation mutant of PRMT5 (and to a lesser extent wildtype PRMT5) in CD34+ cells from JAK2-V617F+ patients blocked erythroid colony formation of these cells. This suggests inhibition of PRMT5 by phosphorylation by mutationally activated JAK2 may play a role in mutant JAK2-driven erythropoiesis. Finally, Liu et al. demonstrated that inhibition of JAK2-V617F and knockdown of PRMT5 in JAK2-V617+ cells resulted in a set of ninety genes that were reciprocally regulated [48]. That is, these genes represent putative genes that may be regulated by PRMT5 methyltransferase activity that is controlled by JAK2 phosphorylation in JAK2-V617+ cells, thus adding further evidence JAK2-mediated control of gene expression may be, in part, by regulating PRMT5 activity.

The ability of mutant JAK2 to inhibit PRMT5 activity resulting in myeloproliferation and erythroid cell growth suggests this may be an important mechanism utilized by mutant JAK2 proteins to induce MPN formation. While JAK2 regulates gene transcription through activation of the STAT pathway, regulation of gene expression by JAK2-mediated inhibition of PRMT5 activity may play a vital role in JAK2-mediated MPN formation (**Figure 1**). However, it remains to be determined if PRMT5 inhibition is required for MPN formation. Also, it is not known if activated JAK2 phosphorylates PRMT5 in the nucleus and/or in the cytoplasm. In addition, there may be other non-histone substrates of PRMT5 that may contribute to the regulation of mutant JAK2-driven myeloproliferation/erythropoiesis. Finally, although Liu et al. suggest wildtype JAK2 does not phosphorylate PRMT5, it is possible

that PRMT5 phosphorylation by JAK2 may occur in JAK2 mutation-negative MPN samples, perhaps through constitutive JAK2 activation induced by upstream signals (e.g mutant Mpl) or through other tyrosine kinases [48]. Together with JAK2-mediated inhibition of HP1α activity, as described by Dawson et al., regulation of the arginine methyltransferase activity of PRMT5 by mutationally activated JAK2 further demonstrates that JAK2 may be regulating gene expression through epigenetic modifications (**Figure 1**), in addition to the classical JAK/STAT pathway, and this regulation may play a role in MPN formation by mutant JAK2 proteins [38, 48].

TET2 and IDH1/2 mutations in MPNs compromise TET2 enzymatic activity

TET2 mutations are found in approximately 10-15% of Philadelphia chromosome-negative MPNs, in addition to other myeloid malignancies [26, 27]. These mutations, often involving both *TET2* alleles, include point mutations, deletion mutations, and frameshift mutations, suggesting they are likely inactivating mutations and that TET2 functions as a tumor suppressor. However, details regarding the significance of these mutations had been lacking until recently. Previous work aimed at understanding the functions of TET1 and TET2 determined that these related enzymes catalyzed the hydroxylation of 5-methylcytosine (5mC) to generate 5-methylhydroxycytosine (5hmC) [50, 51]. Ko et al. recently confirmed this activity of TET2 by expressing TET2 in cells and observing an increase in the levels of 5hmC and a decrease in 5mC [52]. However, when TET2 proteins containing mutations that are found in myeloid malignancies were expressed in cells, the increase in 5hmC and decrease in 5mC were not observed. This suggests such mutations inactivate the catalytic activity of TET2. Ko et al. then analyzed genomic DNA from patients with various myeloid malignancies and determined that *TET2* mutations associated with lower levels of 5hmC compared to controls [52]. Knockdown of TET2 in hematopoietic progenitor cells led to a skewing of differentiation toward monocyte/macrophage cells, suggesting TET2 impairment may affect myelopoiesis. This conclusion is similar to that of Delhommeau et al. who observed greater human cell reconstitution, consisting primarily of myeloid cells, when CD34+ MPN cells containing a *TET2* mutation were engrafted in NOD-SCID mice, compared to the same cells from MPN patients with wildtype *TET2* [26]. Finally, Ko et al. analyzed the status of DNA methylation with respect to *TET2* mutations in myeloid malignancies [52]. The surprising finding was that *TET2* mutations, which associate with low 5hmC, also associate with low levels of methylated DNA. Why low 5hmC results in lower DNA methylation remains unknown, especially since it has been postulated that 5hmC may be a precursor/intermediate step of demethylation [53]. Thus, lower 5hmC should result in a higher level of methylation. One suggestion by the authors is that 5hmC may direct the methylation of DNA and the loss of TET2 activity would result in a loss of this positive regulation of methylation [52]. While these remains unknown, it is clear that *TET2* mutations in MPNs likely impede 5hmC formation and this may lead to aberrant DNA methylation, which could have important ramifications on gene expression (**Figure 1**).

Work published concurrently by Figueroa et al. demonstrated that *IDH1/2* mutations and *TET2* mutations were mutually exclusive in AML patients [54]. Normally, (isocitrate dehydrogenase) enzymes catalyze the production of alpha-ketoglutarate (αKG). However, IDH1/2 mutant proteins found in cancer results in the production of 2-hydroxyglutarate (2HG) instead of αKG [55, 56]. Since TET2 utilizes αKG as a cofactor for its enzymatic activity, Figueroa at al. postulated that the aberrant production of 2HG in cells expressing IDH1/2 mutants might interfere with TET2 activity [54]. This is exactly what they observed, that is, expression of a mutant IDH1 protein, which leads to production of 2HG, but not wildtype IDH1, which produces αKG, blocked the formation of 5hmC by TET2. Interestingly, *IDH1/2* mutations and *TET2* mutations associated with a hypermethylated phenotype in AML patients, as would be expected if 5hmC was a precursor to demethylation [54]. Importantly, these methylation profiles of *IDH1/2* mutant and *TET2* mutant samples were overlapping, suggesting these proteins function in the same pathway. Figueroa et al. also knocked down TET2 expression in mouse bone marrow cells and demonstrated a decrease in myeloid differentiation [54]. While these findings apparently contrast with the results of Ko et al., it is possible that loss of TET2 reduces differentiation, but the phenotype of those cells that do differentiate is skewed toward the myeloid lineage [52, 54]. Together, the data by Figueroa et

al. suggest that both *TET2* mutations and *IDH1/2* mutations induce a similar biochemical result, that is, inhibition of TET2 enzymatic activity (**Figure 1**), thus giving biochemical support to the genetic data that demonstrate these mutations are mutually exclusive in AML [54]. It is interesting that the AML study of Figueroa et al. identified hypermethylation in mutant *IDH1/2* and mutant *TET2* cells and the study of Ko et al., which focused on myelodysplastic syndrome (MDS) and MPN patients, identified hypomethylation in cells with mutated *TET2* [52, 54]. These disparate observations may be due to the different diseases studied. Nonetheless it is apparent that *TET2* mutations as well as *IDH1/2* mutations deregulate biochemical reactions that methylate DNA. Thus, mutant forms of these proteins likely contribute to altered DNA methylation and subsequent gene expression in myeloid diseases including MPNs.

Other MPN mutations that may alter gene expression via epigenetic modifications

Mutations in *JAK2*, *TET2*, and *IDH1/2* are not the only mutations in MPNs that appear to regulate epigenetic modifications. Mutations in other genes such as *EZH2*, *ASXL1*, and *DNMT3A* have also been identified in MPNs and the products of these genes function as epigenetic modifiers [31-33, 35]. EZH2 (enhancer of zeste homolog 2) functions as part of the polycomb repressor complex 2 that serves as a histone methyltransferase [57]. EZH2 is the catalytic component of this complex and its methylation of histone H3 lysine 27 induces gene silencing. Point mutations identified in *EZH2* in MPNs (the study also included MDS samples) affect the EZH2 enzymatic domain and inactivate the enzyme [31]. Frameshift mutations were also prevalent, resulting in carboxy-terminal truncation of the protein due to early translation termination. This suggests inactivation of EZH2 activity may result in aberrant histone methylation and thus deregulation of gene silencing (**Figure 1**). Interestingly, *EZH2* has also been described as an oncogene [58]. However, the inactivating mutations described in myeloid disorders, which include bi-allelic mutations, suggest that EZH2 functions as a tumor suppressor in myeloid cells [31]. The ability of EZH2 to function as a tumor suppressor or an oncogene is likely cell type and mutation specific. ASXL1 (additional sex combs-like 1), is a polycomb group protein that presumably functions in chromatin modifier complexes [59]. Multiple mutations in *ASXL1* were identified in MDS, including frameshift mutations that would result in the disruption of the plant homeodomain (PHD) located at the carboxy-terminus of ASXL1 [60]. PHDs function as modified chromatin readers that bind to modified (e.g. methylated) histone proteins [61]. Thus, it is presumed that disruption of the PHD of ASXL1 would have deleterious effects on its function in the context of chromatin modifier complexes. Such mutations would be expected to alter normal mechanisms of epigenetic modifications to chromatin and thus influence the regulation of gene expression (**Figure 1**). *ASXL1* mutations that likely inactivate the protein have since been identified in MPN patients, suggesting inactivation of ASXL1 may play a role in these diseases [32, 33]. Finally, inactivating mutations in DNMT3A, DNA methyltransferase 3A, have also been identified in MPNs, further supporting the common theme that recurring mutations in MPNs target genes whose protein products regulate epigenetic control of gene expression (**Figure 1**) [34, 35].

Targeting epigenetic modifications to treat MPNs

The recent descriptions that epigenetic modifications may be altered in MPNs due to the direct result of recurring MPN mutations further strengthen the argument that epigenetic altering therapeutic strategies may be valid options to treat these diseases. A recent report demonstrated that a treatment regimen involving chromatin-modifying agents (including histone deacetylase and DNA methyltransferase inhibition) could inhibit MPN patient JAK2-V617F+ hematopoietic progenitor cells from repopulating mice in a transplant system [62]. This treatment led to increased apoptosis of JAK2-V617F+ CD34+ cells compared to normal CD34+ cells, suggesting JAK2-V617F+ MPN cells may be more sensitive to treatment with agents that deregulate epigenetic modifications. Thus, it is quite possible that the potential reversal of MPN mutation-induced changes in gene expression may be a therapeutic strategy that can be exploited to treat MPN patients. Both basic and clinical trials utilizing agents that target epigenetic modifiers (e.g. histone deacetylase inhibitors) are ongoing, in order to ascertain if such approaches may be beneficial to MPN patients [63-67]. However, such ap-

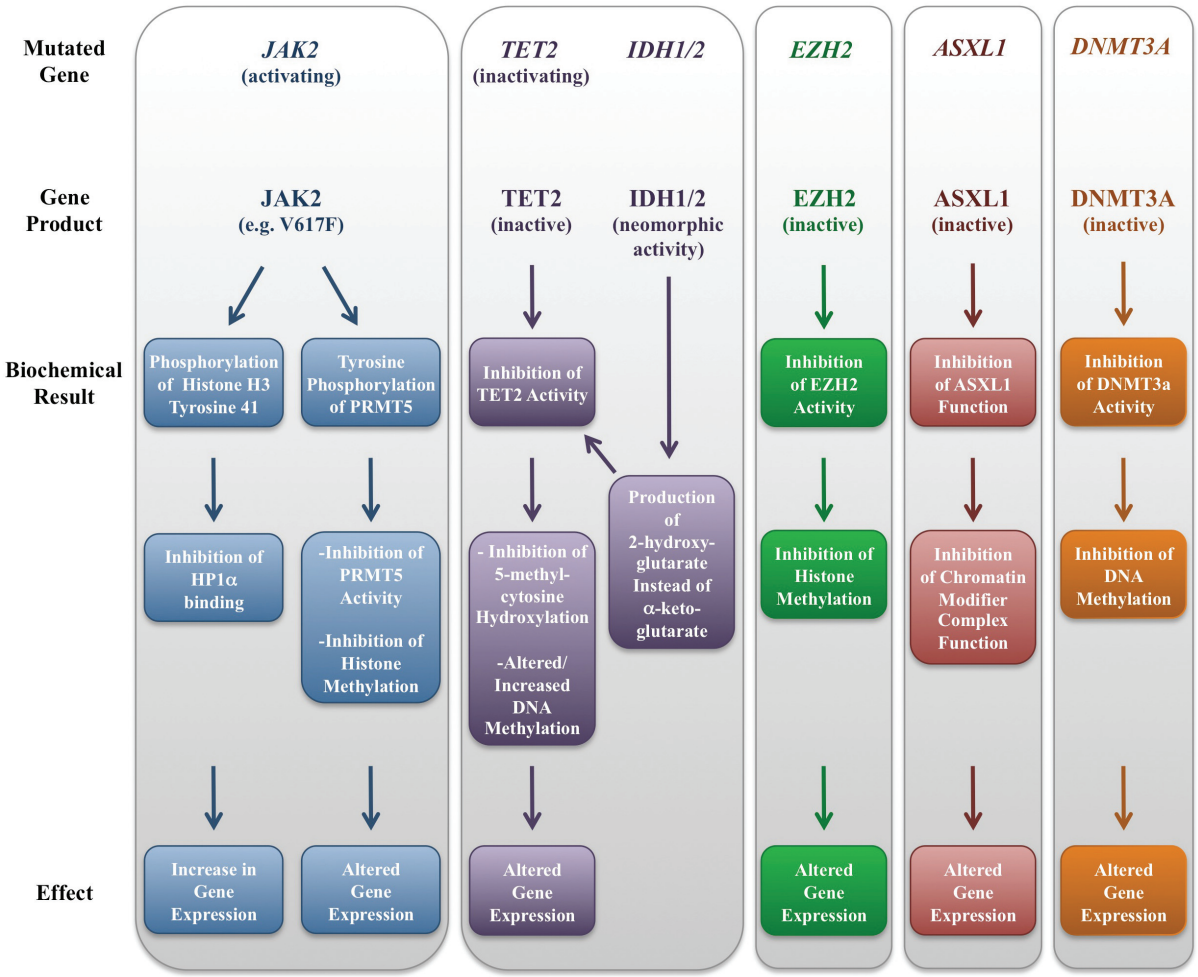

Figure 1. Recurring Mutations in MPNs Alter Epigenetic Mechanisms of Gene Expression. Recurring mutations in MPNs include mutations in *JAK2*, *TET2*, *IDH1/2*, *EZH2*, *ASXL1*, and *DNMT3A*. These mutations alter specific biochemical functions of their gene products, resulting in deregulation of gene expression through altered epigenetic mechanisms. In some cases, it is not clear if mutations and the subsequent alteration in epigenetic control of genes lead to an increase or decrease in gene expression. DNA and histone methylation generally, but not always, have a repressive effect on gene expression, and thus mutations that increase or decrease DNA or histone methylation would presumably have a corresponding effect on gene expression.

proaches will likely have effects at the epigenetic level as well as at the protein level, as histone deacetylases have both epigenetic and non-epigenetic (non-histone) targets [67].

Summary

The discovery of the JAK2-V617F mutation in MPNs led to the intuitive presumption that gene expression controlled by deregulated JAK2/STAT signaling plays a role in these diseases. However, recent work suggests mutated JAK2 also likely deregulates gene expression through epigenetic mechanisms. These mechanisms include tyrosine phosphorylation of histones resulting in inhibition of binding of the transcriptional repressor HP1α, as well as tyrosine phosphorylation of PRMT5, which inhibits the histone methyltransferase activity of PRMT5 (**Figure 1**). The alteration of epigenetic controls in MPNs is further highlighted by recurring mutations in numerous other genes, including *TET2*, *IDH1/2*, *EZH2*, *ASXL1*, and *DNMT3A* (**Figure 1**). These studies lend additional support for an important role of mutationally induced aberrant epigenetic regulation of gene expression in MPNs. In addition to targeting JAK2 activity with JAK2 inhibi-

tors, therapeutic approaches that reverse effects of aberrant epigenetic alterations in MPN cells may prove to be effective treatment options for MPN patients.

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