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Genetics of Myeloproliferative Neoplasms

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Abstract

In the last decade, genomic studies have identified multiple recurrent somatic mutations in myeloproliferative neoplasms. Beginning with the discovery of the JAK2 V617F mutation, multiple additional mutations have been found which constitutively activate cell-signaling pathways, including MPL, CBL, and LNK. Furthermore, several classes of epigenetic modifiers have also been identified, in MPN patients, revealing a requirement for mutations in other pathways to cooperate with JAK-STAT pathway mutations in MPN pathogenesis. Mutations in the de novo DNA methylation protein, DNMT3A, demethylation machinery, TET2 and related IDH1/2 production of onco-metabolite 2-hydroxygluterate, and polycomb complex proteins EZH2 and ASXL1 have opened new pathophysiologic clues into these diseases. The prognostic relevance of these novel disease alleles remains an important area of investigation and clinical trials are currently underway to determine if these findings represent tractable therapeutic targets, either alone, or in combination with JAK2 inhibition.

This year marks forty years since Dr. Janet Rowley published her seminal letter identifying the recurrent genetic translocation responsible for chronic myeloid leukemia (CML)[1]. This finding of the t(9;22) translocation leading to a fusion protein between Abelson leukemia virus proto-oncogene *ABL1* and breakpoint cluster region *BCR*, and the subsequent extraordinary success of the small molecule inhibitor imatinib, has been arguably one of the most important discoveries in modern oncology [2-4]. In the time since Dr. Rowley's discovery, a multitude of additional recurrent somatic mutations and fusion proteins have been discovered. CML and the other BCR-ABL1 negative myeloproliferative neoplasms (MPNs) are often characterized by mutations that lead to a growth advantage through constitutive activation of signaling pathways inherent to normal hematopoietic growth and development.

Akin to the Philadelphia chromosome in CML, discovery studies in 2005 identified the JAK2 V617F mutation[5-8] in the overwhelming majority of patients with polycythemia vera as well as 40-60% of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF)[5, 8, 9]. Such mutations have been termed "class I "mutations to distinguish such mediators of activated cellular growth signal from "class II" mutations in key regulatory elements involved in differentiation, often leading towards a myelodysplastic syndrome (MDS) phenotype. In the last decade the genetic underpinnings of MPNs have continued to expand, leading to the discovery of novel classes of genes—those involved in epigenetic regulation—thus leading to a reshaping of the two-hit model to the entire spectrum of myeloid malignancies including MPN patients. However, in contrast to BCR-ABL in CML, not all mutations in MPN patients represent the driving event towards clonal hematopoiesis, and their role in disease pathophysiology requires further investigation.

Class I Mutations: Activators of Growth

Before the advent of modern genetic and genomic tools to study malignancies, William Dameshek suggested that the various MPNs might result from an “undiscovered stimulus” [10]. The genetic basis for such stimulus was identified in 2005 by the discovery of the JAK2 V617F mutation. Multiple groups arrived at this discovery by various means (reviewed in detail by Kilpivaara and Levine[11]): the identification of uniparental disomy at common sites of LOH on chromosome 9p24[7], candidate gene approaches[5, 8], and PV cell culture studies in growth factor deficient media which failed to proliferate in the presence of JAK2 siRNA[6, 12]. Together these efforts led these different groups to identify the single most commonly mutated gene in MPN, which occurs in virtually all patients with PV and 50% of patients with ET or PMF[5, 8, 9]. Over 95% of PV patients carry the somatic JAK2 V617F mutation, whereby a guanine to thymidine transversion results in a nonsynonymous change of a valine to phenylalanine[13]. The result is constitutive activation of JAK-STAT/PI3K/AKT downstream pathways through alteration of the regulatory pseudokinase domain, resulting in phosphorylation of STAT5 and STAT3[14]. Sequencing of JAK2 V617F negative patients revealed that somatic missense, deletions, and insertions, largely in exon 12, account for nearly all of the remaining PV patients[15]. Clinical studies have suggested showed discrete associations with JAK2 mutational status and disease phenotype. PV patients with the JAK2 V617F mutation commonly present with thrombocytosis and an older age of onset than patients harboring an exon 12 mutations, but their clinical course and outcome remains similar[16, 17]. JAK2 genotyping has become a major criterion in the diagnostic schema for MPN[18], and is pathognomonic for PV in the setting of clinical polycythemia[19]. Studies in patients with ET who harbor JAK2 mutations has shown increased erythroid involvement, and may have higher risk for thrombosis[20].

This discovery, and the resultant question of how a single mutation might cause three distinct disease phenotypes led investigators to test the hypothesis that allele burden of the JAK2 mutation may contribute to this phenomenon. This hypothesis is largely driven by the observation that homozygous JAK2 V617F mutations, which occur as a result of uniparental disomy, are common in PV and PMF, but rare in ET, the quantifiable gene-dosage is thought to contribute to the disease phenotype. Although a high degree of variability has been reported in different studies, most studies suggest that the highest allele burden is found in patients with post-PV myelofibrosis (61%), whereas patients with ET have the lowest allele burden (24%). Clinical correlates of allele burden suggest a direct relationship of allele burden with splenomegaly and leukocytosis, though the relevance of JAK2 allele burden to outcome remains controversial.

Following the identification of JAK2 mutations, candidate gene approaches were applied to additional JAK-STAT signaling pathways to account for the 50% of ET and PMF patients wild type for JAK2. Mutations in several other receptor tyrosine kinase proteins were discovered in the thrombopoietin receptor, MPL[21], and Casitas B-lineage lymphoma protooncogene, CBL[22]. MPL mutations were identified in 3% of patients with ET and 10% of patients with PMF and correlate with older age, female gender, lower hemoglobin, and more pronounced thrombocytosis[21, 23]. CBL mutations, initially discovered in juvenile myelomonocytic leukemia, have been found in 3% of patients with PMF[24].

Class III Mutations: Epigenetic Modifiers

In the context of large-scale efforts to sequence large portions of the genome, multiple recurrent somatic mutations have been discovered in genes responsible for epigenetic regulation. Such mutations had been long been known to be involved in AML (reviewed in

detail by Shih et al.[25]), particularly *MLL* translocations, which harbor a poor prognosis[26, 27]. However, *MLL* abnormalities are not present in MPNs, thus it was initially believed that mutations in epigenetic modifiers were a transformative event seen in MPN patients who progress to AML, and not in patients with chronic phase MPN. More recently several such mutations have been identified in MPNs, having a marked presence, as well, in MDS/MPN overlap syndromes.

The epigenetic regulation of DNA methylation of CpG islands is a complex, highly regulated process that involves both de novo methylation events as well as maintenance of post-replicative methylation from the parental strand template. De novo methylation events are carried out by the DNA methyltransferease, DNMT3A. Mutations in DNMT3A are common in AML and have been linked with anthracycline resistance and poor prognosis[28, 29]. Although far more common in AML, DNMT3A mutations have been reported in 7-15% of MPN patients[30, 31]. Though several studies seem to suggest a prognostic significance in AML, there is no data regarding the relevance of DNMT3A mutations to phenotype, time to transformation, or survival in MPN.

DNA de-methylation similarly has a well-regulated and organized pathway involving conversion of 5-methylcytosine to 5-hydroxymethylcytosine as an intermediate step. 5-hmC has been shown to be associated with increased gene expression in an embryonic stem cell model and to induce demethylation, as maintenance methylation via DNMT1 is unable to recognize 5-hmC in the post replicative step. Based on mapping minimally deleted regions of loss of heterozygosity on chromosome 4q24 by SNP-based array technology, recurrent mutations in TET2, the protein responsible for 5mc to 5hmc conversion, were identified in MPN and MDS patients[32]. TET2 is mutated in multiple solid tumor malignancies and a broad spectrum of myeloid diseases including in 10-20% of MPN[33]. No prognostic significance has been associated with TET2 mutations in MPN. A requisite cofactor for TET2-mediated conversion of 5mC to 5hmC is α-ketogluterate, the product of an essential oxidative step of isocitrate in the Krebs cycle. Originally discovered in Glioblastoma [34], mutations in two isoforms of the enzyme isocitrate dehydrogenase (IDH) have been identified in patients with myeloid malignancies. These mutations result in expression of enzymes with altered enzymatic activity and produce an onco-metabolite, 2-hydroxygluterate (2-HG), which poisons the catalytic activity of TET2[35, 36]. IDH mutations have been reported in 2-5% of MPN[37], and PMF patients harboring IDH mutations are associated with earlier transformation to AML and poor overall survival[38]. Mutations in TET2 and IDH 1/2 have been found to be mutually exclusive[29] and share unique patterns of DNA methylation as well as gene expression, suggesting their shared mechanism in disease biology[39]. Emerging studies have identified several other proteins whose activity is affected by 2-HG. Notably the jumonji-domain-containing (JMJC) family, which are histone demethylase proteins, are also inhibited by 2-HG[40].

Mutations in histone modifying genes have been described in MPNs, particularly in the polycomb group proteins (PcG), EZH2, and the polycomb repressive ubiquitinase component, ASXL1[41]. EZH2 represents the enzymatic component of the PRC2 complex, which acts as the methyltranferase at H3K27. Loss of function EZH2 mutations identified in MPN patients have been suggested to decrease the transcriptionally repressive H3K27 trimethylation chromatin mark[42, 43]. EZH2 mutations are more frequent in PMF than the other MPNs (5-7%), but rare EZH2 mutations have been reported in both PV and ET. One recent report suggested that EZH2 mutant PMF had higher IPSS risk and worse overall survival[44]. ASXL1 mutations are more common than EZH2 mutations in all three MPNs, and occur in 5-25% of PV, 5-10% of ET, and 13-23% of PMF patients[45]. The exact mechanisms of ASXL1 mutant MPN are less well known, though recent studies have suggested a critical role in mediating PRC2 function, likely due to its role in recruitment of

the PRC2 complex[46, 47]. A marked increase in HOXA gene transcription has been associated with ASXL1 loss of function. Such transcriptional patterns have suggested a poor prognosis in AML[48], though no distinct clinical prognostic association between HoxA gene expression and outcome has been reported in MPN.

Although well described for its canonical role for its signal transduction, JAK2 has more recently also been shown to have direct epigenetic functions. JAK2 phosphorylates the arginine methyltransferase, PMRT5. In its phosphorylated form, interaction with MEP50 is blocked, resulting in decreased arginine methylation of histones H2A/H4, leading towards a skewed erythropoiesis[49]. Additional studies have shown that JAK2 can localize to the nucleus through a yet, unknown mechanism[50], which results in phosphorylation of H3-Y41 and reduced the binding of the transcriptional silencer HP1α[51].

Mutations Enriched in Blast Phase MPN

Despite better characterization of the genetic background of MPN, the contribution of specific somatic genetic events to transformation to AML has not been well understood. Transformative events either leading towards a blast phase MPN or leukemogenesis do not seem to be explained by the individual genetic alterations which characterize the disease. However, the frequency of specific somatic mutations, including IDH1/2, TET2, and EZH2, are more frequent in blast-phase MPN[30, 31, 38, 44, 45, 52, 53]. In addition, two recently identified somatic mutations are most common in patients with post-MPN AML. LNK, a negative regulator of JAK2[54], is mutated in 13% patients with blast phase MPN, but is rarely targeted by somatic mutations in chronic phase MPN[55, 56]. In addition, mutations in the zinc finger IKZF1, known to be prevalent in blast phase CML and Ph+ ALL, have been identified in 19% of blast phase MPN whereas they are almost never observed in chronic phase MPN[57].

Genetic Basis for Therapy

Shortly after the discovery of the JAK2 V617F mutation, multiple small molecule inhibitors were developed for therapeutic use, several of which are currently under clinical investigation. To date, only one JAK kinase inhibitor has been approved by the FDA, ruxolitinib, a JAK1 and JAK2 inhibitor, approved in August 2011 for use in intermediate and high-risk PMF and post PV/ET myelofibrosis. The approval of this agent was based upon two phase III clinical trials (COMFORT I and II) which showed ruxolitinib was effective in reducing spleen size and reduction in symptoms[58, 59]. Ruxolitinib induces a reduction in leukocytosis and thrombocytosis, and anemia/thrombocytopenia was a dose limiting adverse effect for 63% of patients in the phase I/II trial which is attenuated with more flexible dosing regimens. Long-term follow-up has shown a small, but statistically significant survival advantage for patients treated with ruxolinitib compared to those treated with placebo[58] (COMFORT I), or best supportive care[59] (COMFORT II). The mechanisms for these effects have yet to be fully elucidated.

Selective JAK2 inhibitors[60, 61] (SAR302503 and BMS911543), combination JAK2/JAK3 inhibitor[62] (CEP701), and combination JAK2/TYK2 inhibitor[63] (pacritinib) have shown clinical efficacy in phase I/II trials. Similarly, combination therapies of JAK2 inhibition with inhibitors of Aurora kinase[64], PI3K/AKT/mTOR[65], and HSP90[66-68] have all shown to act synergistically in preclinical studies to decrease JAK2 V617F clonal proliferation. Although no specific targets of epigenetic mutations seen in MPN have been developed, several attempts to use epigenetically active drugs, such as hypomethylating agents and histone deacetylase inhibitors have been used in MPN[69]. Preclinical studies show efficacy both alone and in combination with JAK2 inhibitors[70].

One important question with regard to JAK inhibitors and other targeted therapies is whether somatic mutations in the JAK-STAT pathway, or in specific epigenetic regulators, influence the response to targeted therapies. This has not been well elucidated to date, and there is a pressing need for detailed genomic profiling of large MPN patient cohorts and clinical trial cohorts to identify novel factors which predict outcome, risk of leukemic transformation, and therapeutic response in the different MPNs. As similar efforts have begun to show significant benefit in MDS and AML, it is important for similar efforts in the MPN field to delineate whether genomic profiling can be used to improve outcomes for MPN patients.

Conclusions

The molecular pathogenesis MPN have become more clearly annotated with regard to the role of recurrent somatic and germline mutations which both drive unrestricted outgrowth and alter transcriptional programming though epigenetic modification. While the clinical utility of this information remains in its infancy, novel therapeutic agents aimed at the aberrant underlying processes have merit and promise and are being tested in mechanism based trials including rational combination therapies. This has led to optimism that outcomes for MPN patients will substantially improve as we develop therapies based on insights into the genetic basis of these myeloid neoplasms.

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