The chronic myeloproliferative disorders and mutation of JAK2: Dameshek’s 54 year old speculation comes of age

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In 1951, William Dameshek speculated on the common origin of the chronic myeloproliferative disorders—polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (IMF), and chronic myelogenous leukemia (CML). Subsequent work suggested that all arose from the hematopoietic stem cell. About 20 years ago the oncogene responsible for CML, bcr-abl, was identified, and more recently the mutant genes that cause hypereosinophilic syndrome and systemic mast cell disorder have been discovered. However, until very recently, the origin of PV, ET, and IMF have defied molecular explanation. In 2005, four separate groups working on tyrosine kinase signal transduction reported a gain-of-function, valine-to-phenyalanine, mutation at position 617 in the JH2 domain of the Janus kinase (JAK) 2 cytoplasmic tyrosine kinase. This mutation requires the presence of the erythropoietin, thrombopoietin, or granulocyte-colony stimulating factor receptor/s for function, the mutation leads to functional hyperactivity and appears responsible for hematopoietic growth factor hypersensitivity, the most characteristic finding in these disorders. Virtually all patients with PV and substantial proportions of those with ET and IMF have now been shown to harbor this mutation. The mutant kinase appears to be a useful diagnostic test for myeloproliferative disorders and may have prognostic value. Future research will undoubtedly focus on the development of specific inhibitors as therapeutic agents as well as answering a number of questions that remain regarding the role of signal intensity, genotypic and phenotypic expression and the possible involvement of additional as yet unidentified mutations in these disorders.

Key words: JAK2 mutation; myeloproliferative disorders; essential thrombocytosis; polycythemia vera; idiopathic myelofibrosis.
INTRODUCTION

In 1951, in the journal *Blood*, William Dameshek speculated on the origins of the chronic myeloproliferative disorders (MPD). The World Health Organization now defines seven distinct chronic MPDs, of which the molecular origins of three, hypereosinophilic syndrome, systemic mast cell disorder, and chronic myelogenous leukemia, have been described. However, three major disorders, polycythemia vera (PV), idiopathic myelofibrosis (IMF), and essential thrombocythemia (ET), until very recently have defied a molecular explanation. All three are chronic marrow diseases characterized by unregulated growth of one or more blood cell lineages that may extend to extramedullary sites. They arise in a single multilineage hematopoietic stem or progenitor cell, and the symptoms and signs of these disorders are a manifestation of excess blood cells or their functional dysregulation.

UNDERSTANDING THE MOLECULAR LESION OF MYELOPROLIFERATIVE DISORDERS

There has been incremental understanding of some of the more rare but familial MPDs. A famous Finnish pedigree of individuals with familial erythrocytosis was described by de la Chapelle and colleagues and commented upon by Longmore in 1993. We now know that this disorder is due to a truncation of the erythropoietin (EPO) receptor, leading to reduced signal extinction. Prchal and colleagues have also contributed to the understanding of familial polycythemia in describing a mutation in the von Hippel-Lindau (VHL) protein that leads to isolated erythropoiesis in the Chuvash population of Central Europe. In this condition, a dysfunctional VHL reduces the capacity to degrade the primary transcription factor for EPO, hypoxia-inducible factor (HIF)$\alpha$, which drives expression of EPO. One other instance of a familial MPD, familial thrombocytosis, has now been explained by one of several mutations in the thrombopoietin (TPO) gene. These mutations all act in the same way, to increase the translational efficiency of TPO messenger RNA into protein—by altered splicing or a deletion or a stop codon—leading to a chronic MPD. However, despite these interesting insights into how normal physiological processes can be disturbed and cause disease, it is also clear that most of the chronic MPDs are diseases of cytokine/growth factor signaling.

SIGNALING ABNORMALITIES

A number of signaling abnormalities lead to MPDs in mice. These include enhanced signaling from the EPO receptor, much like the family pedigree described above, loss of an inhibitory phosphatase, up-regulated expression of TPO or the TPO receptor, transgenic marrow expression of TPO, and loss of another inhibitory phosphatase, among others.

Thirty years ago, Prchal described the phenomenon of endogenous erythroid colony formation when bone marrow or blood progenitor cells grow into colonies in the absence of exogenous EPO. Subsequently, not just erythroid hypersensitivity, but all of the myeloid lineages were found to display enhanced proliferation in response to various growth factors. From this came the notion that the chronic MPDs were all manifestations of a growth factor hypersensitive state.
In normal physiology, a panoply of signaling pathways are triggered in response to the binding of EPO, TPO, and granulocyte-colony stimulating factor (GCSF) to their cognate receptors (Figure 1). The kinase, Janus Kinase 2 or JAK2, then triggers the signaling molecules phosphoinositol-3 kinase, Ras, and MAP kinases, and signal transducers and activators of transcription, the STAT proteins. Over the last 10 years, a number of investigators have demonstrated that at least four of these molecules, or ones that lie immediately downstream of them, are constitutively activated in patients with PV or ET. Constitutive activation ultimately leads to cellular proliferation and dysregulation of programmed cell death. These findings provide a molecular basis for the notion that growth factor signaling is altered in patients with MPDs.

**IDENTIFICATION OF THE JAK 2 MUTATION**

The logical next step was to identify the origins of chronic activation of signaling in MPDs. In 2005 four different groups independently identified a single, valine-to-phenylalanine somatic mutation at position 617 (V617F) in the JAK2 kinase. This mutation is widespread in patients with chronic acquired MPDs. JAK2V617F is present in a fraction of the myeloid cells of virtually every patient with PV, and about half of patients with ET or IMF bear this mutation (Table 1). The incidence of JAK2V617F in MPDs has been confirmed in a number of subsequent studies, which have also verified its absence in normals and rarity in other hematological disorders.

**Figure 1.** Activation of signaling molecules in myeloproliferative disorders. In normal physiology, EPO, TPO, and granulocyte-colony stimulating factor (GCSF) binding to their respective receptors generate signals that are transduced through intracellular signaling pathways. The responses generated by the growth factors simultaneously stimulate multiple activators such as Janus Kinase 2, which then triggers phosphoinositol-3 kinase, Ras, and MAP kinases leading to growth, and the STAT proteins, leading to gene transcription, as shown in the cartoon above. Four of these molecules, outlined in red, are constitutively activated in patients with PV or ET. IRS-P, phosphorylated insulin receptor substrate; MAPK, mitogen activated protein kinase; PI, phosphoinositol-3; STAT, signal transducer and activator of transcription; γ-P, phosphorylated tyrosine.
ACTIVITY OF THE JAK 2 KINASE

Normal JAK2 kinase is tethered to the cytokine receptors for EPO, TPO or G-CSF through the amino terminal FERM (family of 4.1-Ezrin-Radixin-Moesin) domain. The kinase portion resides at the carboxyl terminus of the protein, termed the JH1 domain, which is adjacent to a catalytically inactive pseudokinase domain (JH2). JH2 is important for the inhibition of basal Jak activity, but the mechanism of this regulation had remained elusive. V617 resides within the pseudokinase, JH2, domain, a residue that is highly conserved between species. In 2003, Saharinen and colleagues assessed the kinase activity of expressing either the JH1 domain alone, or the JH1 and the JH2 domains together; phosphotyrosine blotting gauged activity of the JH1 kinase. When expressed alone, JH1 displayed a highly robust phosphorylation signal, but the combination protein, the JH1 and the JH2, was scarcely active. This indicated that the JH2 domain is a regulatory subunit for the JH1, and thus a loss of function mutation in this regulatory subunit likely accounts for the etiology of MPDs.

Molecular modeling of the JAK2 kinase suggests that the JH2 domain mutation lies adjacent to the site that stabilizes the activation loop of the kinase in the inactive conformation. A mutation at this point conceivably destabilizes this conformation, allowing the loop to flip into the active conformation, removing its regulation and leading to its constitutive activity.

JAK2 MUTATION AND DISEASE

The question now remains as to whether there is relationship of these findings to disease. William Vainchenker’s laboratory tested factor-dependent cells that contain the EPO receptor transduced either with the wild-type or the mutant JAK2. When transduced with the wild-type gene, they observed no constitutive JAK2 activity, but with the mutant JAK2 construct, “spontaneous” activity was observed. In keeping with these observations, downstream signaling molecules such as STAT5, normally inactive in the absence of EPO, were very active in the absence of EPO. More recently, the Lodish and Gilliland laboratories have identified the specific receptors that can support this activity; they demonstrated that if a cell with only the interleukin-3 receptor is transduced with a mutant kinase, there is no activation. But if the EPO, TPO, or G-CSF receptor is in the cell, brisk activation of the kinase is seen. This observation highlights that cell context is also a vital contributor to disease.

The ultimate proof of the pathophysiological relevance of JAK2V617F was first supplied by Vainchenker. Wild-type kinase transfected into hematopoietic stem cells and then transplanted into lethally irradiated murine recipients results in normal

<table>
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<tr>
<th>Abnormalities of JAK2 in chronic MPD.</th>
<th>Proportion (95% CI)</th>
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<tr>
<td>Polycythemia vera</td>
<td>97% (93–100)</td>
</tr>
<tr>
<td>Essential thrombocytopenia</td>
<td>57% (43–70)</td>
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<tr>
<td>Idiopathic myelofibrosis</td>
<td>50% (26–74)</td>
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<td>Controls</td>
<td>0%</td>
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erythropoiesis. In contrast, transfection of mutant JAK2 kinase in the same cells produces constitutive tyrosine phosphorylation that promotes cytokine hypersensitivity and induces substantial erythrocytosis (hematocrit ~ 60% within a month). This model established that expression of JAK2V617F in the hematopoietic stem cell is fully capable of inducing polycythemia.

CLINICAL SIGNIFICANCE

At present, while this exciting finding helps explain, or at least contributes to the explanation of how uncontrolled myeloproliferation occurs in the MPDs. Moreover, the presence of this mutant kinase appears to provide some prognostic information for the subsequent clinical course of an individual patient with JAK2V617F+ MPD. In a modest-sized series of patients, those with the mutant kinase present in their hematopoietic cells had a higher incidence of complications than the 50% of patients with ET or myelofibrosis who did not have the kinase. These findings are confirmed in some studies, but not in others. A larger cohort of patients or more careful analysis will be required to understand the prognosis of having this mutant kinase, and the implications of having one or two copies of the mutant allele as sometimes occurs.

JAK 2 MUTATION AND ET

The murine models may be developed to show characteristics of human MPD disease. One of these murine lines developed a phenotype, transplantable from mouse to mouse, with ET and high platelet counts. Analysis of the mice showed that it was expressing the lowest level of the mutant JAK2 kinase of all the other strains of mice examined. These findings contribute to the data suggesting that perhaps the level of the JAK2 kinase expressed in the stem cells dictates phenotype as opposed to another mutation. Vainchenker’s interpretation is that the level of expression of JAK2 is responsible for the ET phenotype. The cause of an IMF phenotype is less clear, as this disease can be viewed also as an over production of megakaryocytes. The subsequent overproduction of cytokines in the marrow seems likely to be responsible for the fibrosis.

JAK 2 MUTATION AND POLYCLONAL OR MONOCLONAL ABNORMALITIES

The discovery of JAK2V617F has provided new insights into clonality and evolution in the MPDs. Using quantitative PCR several investigators have shown that approximately one third of patients with PV have greater than one mutant allele per cell. Since the mutation is not inherited, but rather arises by spontaneous mutation in a somatic hematopoietic stem cell, the finding of two mutant alleles per cell at first was puzzling. It is now known that the development of a second mutant allele in a single cell arises by uniparental disomy, i.e., a crossover event occurs in a cell that has a single mutation, with two mutant alleles segregating to one daughter cell, with the other cell reverting to two wild type JAK2 alleles. It is presumed that a cell that bears two mutant alleles now has a proliferative advantage over all the surrounding cells that bear only one mutant allele, so those cells progressively occupy a greater proportion of the marrow stem cells, ultimately becoming the only stem cell giving rise to progeny. At this point,
a secondary clonal disorder is established. However, it should be noted that patients with ET or IMF very rarely, if ever, develop two mutant alleles. The explanation for this is uncertain at present.

**JAK 2 MUTATION AND PHENOTYPIC EXPRESSIONS IN MYELOPROLIFERATIVE DISORDERS**

A number of factors are probably involved in the evolution of the chronic MPD into a myelofibrosis stage and then transformation to leukemia. Among these factors are cytokines released from the chronically stimulated megakaryocytes. Prior to elucidation of the mutant JAK2 disorder, the best mouse model of myelofibrosis was chronic transgenic expression of TPO. Within a few months of expression of TPO in the bone marrow of the transgenic mouse, a very aggressive myelofibrosis and osteosclerosis develop. Furthermore, when transgenic mice that develop myelofibrosis were crossed with transforming growth factor-beta (TGF-β) null mice, myelofibrosis failed to develop, or was minimal, despite megakaryocyte hypertrophy and thrombocytosis equivalent or higher than the simple TPO transgenic mice. Thus, it is presumed that JAK2V617F-driven, megakaryocyte-derived TGF-beta is responsible for myelofibrosis in the human MPDs. Patients with PV often also suffer from thrombotic complications including the Budd-Chiari syndrome; in fact, in the absence of leukemic transformation triggered by the use of genotoxic agents to control the myeloproliferation, thrombotic manifestations are the most common cause of death in patients with MPDs. As with myelofibrosis, the thrombotic diatheses in PV likely results from mutant JAK2 driven functional cellular activation; however, whether the platelet, leukocyte or some other cell is primarily responsible for the hypercoagulable state seen in patients with MPDs is uncertain at present. Finally, although leukemic transformation in the MPDs has become a rare event once it was recognized that genotoxic agents should be avoided in such patients, there is an enhanced risk of acute myeloid leukemia in such patients. Clonal evolution is thought responsible for this event.

**CONCLUSIONS**

Even though much has been revealed by the finding of the mutant kinase domain and its constitutive activity in erythropoiesis, many questions still remain. Is this single mutation responsible for three MPDs, based on the level of signaling, or the level of expression? The recent finding that many patients with JAK2V617F have a higher proportion of cells that are clonal, than carry the mutant kinase, implies that a precursor event (mutation) occurred giving rise to clonality, and once that clone became established in the marrow one of these cells then acquired the JAK2V617F mutation, giving it a survival advantage. The nature of the first event leading to clonality could vary amongst patients with PV, ET and IMF, accounting for the presence of JAK2V617F in three different diseases. Additionally, the contribution of the mutant kinase towards the development of thrombotic complications in PV, myelofibrosis, or leukemic transformation needs to be further studied. So far we know that the mutant JAK2 kinase is present in most patients with PV and at least half of patients with ET and IMF, but not in atypical MPDs, myelodysplastic syndromes, or most patients with acute leukemia. The altered JAK2 is responsible for endogenous erythroid colony formation. The mutation is also responsible for the myeloproliferation and is also likely responsible for the clonality characteristics of these disorders. The mutant kinase has already shown to be a very useful diagnostic test for
MPDs, and it is likely to provide prognostic value. This finding will certainly rekindle efforts to develop a specific JAK2 inhibitor, ideally one that differentially active only against the mutant kinase, lest it cause global myelosuppression.

REFERENCES


