Pathogenetic Role of JAK2 V617F Mutation in Chronic Myeloproliferative Disorders

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The molecular pathogenesis of chronic myeloproliferative disorders (MPDs) is poorly understood. The hematopoietic progenitor cells of patients with polycythemia vera (PV) or essential thrombocythemia (ET) are characterized by hypersensitivity to hematopoietic growth factors and formation of endogenous erythroid colonies. Recently, 4 groups reported almost simultaneously Janus kinase 2 (JAK2) V617F mutation in more than 80% of PV patients, 30% of patients with ET and in about 50% of patients with idiopathic myelofibrosis. The identification of the JAK2 mutation represents a major advance in the understanding of the molecular pathogenesis of MPDs that will likely permit a new classification and the development of novel therapeutic strategies for these diseases. [*J Chin Med Assoc* 2007;70(3):89–93]

Key Words: essential thrombocythemia, JAK2 mutation, myeloproliferative disorders, polycythemia vera

Introduction

Chronic myeloproliferative disorders (MPDs), first proposed by Dameshek in 1951, are clonal hematopoietic stem cell disorders characterized by proliferation of 1 or more myeloid cell lineages in the bone marrow and increased numbers of mature and immature cells in peripheral blood.[1] According to the World Health Organization classification, MPDs include polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF) and chronic myeloid leukemia (CML), plus rarer subtypes such as chronic neutrophilic leukemia, hypereosinophilic syndrome and chronic eosinophilic leukemia. These diseases overlap with myelodysplastic/myeloproliferative diseases such as atypical CML and chronic myelomonocytic leukemia, in which proliferation is accompanied by dysplastic features or ineffective hematopoiesis in other lineages.[2] The clinical pictures of these disorders share many features: genesis in a single, multipotent hematopoietic stem cell that assumes dominance over nontransformed progenitors; hypercellularity of the marrow, with apparently unstimulated overproduction of 1 or more of the formed elements of blood; and increased risk of thrombosis and bleeding, spontaneous transformation to acute leukemia and marrow fibrosis.[3]

Molecular Pathogenesis of Chronic MPDs

During the past 5 decades, many achievements have been made in understanding the pathogenesis of PV, ET and IMF. Both PV and ET are characterized by increased sensitivity of committed hematopoietic cells to their respective primary humoral growth factors: erythroid precursors to erythropoietin (Epo) in PV and megakaryocytes to thrombopoietin (Tpo) in ET. *In vitro*, Epo-independent (endogenous) erythroid colony formation and also Tpo-independent megakaryocyte colony formation are found in both PV and ET.[4] However, no mutations of Epo and Tpo or their respective receptors, Epo-R and Tpo-R, have yet been identified in PV and ET. Moreover, subsequent studies revealed that marrow and blood cells from patients with PV were hypersensitive not only to Epo or Tpo but also to several other hematopoietic growth factors, including interleukin 3 (IL-3), stem cell factor (SCF),

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granulocyte-macrophage colony-stimulating factor (GM-CSF) and insulin-like growth factor-1 (IGF-1).[5–8] These findings suggest that events downstream from receptor engagement might be responsible for endogenous erythroid colony formation in PV or ET.

The Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway plays a central role in initiating signal transduction from hematopoietic growth factor receptors. JAK2, like the other members of the JAK family, has an enzymatically active kinase domain (JAK homology 1 [JH1]) and a catalytically inactive pseudokinase domain (JH2). The JH2 domain has an autoinhibitory function that normally suppresses the kinase activity of JAK2.[9] A previous study has demonstrated that inhibitors of JAK2 can repress the Epo-independent differentiation of erythroid progenitors in PV, and constitutive activation of STAT3 has also been reported in patients with PV.[10] JAK2 therefore represented a logical target for identifying the molecular abnormalities of PV and ET.[11] In 2005, JAK2 V617F mutation was identified in patients with chronic MPDs by using different approaches.[12–15] James et al found that a kinase inhibitor of JAK2 or knockdown of JAK2 (wildtype) expression using small interfering RNA technology could inhibit the formation of Epo-independent erythroid colonies that are a hallmark of PV.[12] This led to the sequencing of JAK2 and the detection of a mutation in the JH2 pseudokinase domain of the JAK2 gene. However, Kralovics et al had previously identified loss of heterozygosity of a region on chromosome 9p in PV and identified a 6.2-Mbp region common to all of the 51 patients who were screened. As this region contained JAK2, with its known role in erythropoiesis, this was screened further for mutations.[13] Three other groups targeted JAK2 as part of a general sequencing screen of tyrosine kinases and phosphatases in MPDs.[14–16] As shown in Table 1, all of the recently published studies showed that the majority of patients with PV (65–97%) have the JAK2 V617F mutation. In contrast, only 23–57% of patients with ET have the JAK2 mutation.[11,12,14–16] Our group reported that the frequency of JAK2 V617F mutation could be detected in 81%, 61% and 33% of Taiwanese patients with PV, ET and IMF, respectively.[17] The mutation is somatic and has not been detected in any normal individuals or patients with secondary erythrocytosis. The mutation was also rarely detected in patients with myelodysplastic syndrome, in patients with acute myelogenous leukemia with or without antecedent PV or IMF, or in patients with lymphoid leukemia.[18–20] The differences in reported rates are likely due to at least 3 reasons: (1) the stringency of the criteria used to diagnose PV; (2) the sensitivity of the method used to detect mutations; and (3) the source of DNA. Direct sequencing techniques are used in the detection of JAK2 V617F mutation in most studies. However, they are likely to have a lower sensitivity than techniques that employ polymerase chain reaction (PCR) amplification of the mutant allele, such as allele-specific PCR or amplification refractory mutation system PCR.[21] The majority of studies have used peripheral blood neutrophils, as they are thought to be derived from the same clonal progenitor that is transformed in PV. The JAK2 V617F mutation has been detected in progenitors and myeloid cells including cells with hematopoietic stem cells, common myeloid progenitor and megakaryocyte–erythroid progenitor phenotypes as well as colony-forming cells and more mature progenies, such as neutrophils and platelets.[15,22,23] So far, mutant JAK2 V617F has not been reported in T or B lymphocytes.[12,24] The JAK2 V617F mutation is a gain-of-function mutation in that it releases the autoinhibitory action of JH2 and thereby results in expression of a constitutively activated JAK2 tyrosine kinase. JAK2 V617F may thus bind to a receptor (e.g. Epo-R or Tpo-R) and recruit STATs in the absence or in the presence of only trace quantities of hematopoietic growth factor (e.g. Epo or Tpo). An *in vitro* study has demonstrated that the expression of the mutated

Table 1. Frequency of JAK2 V617F mutation in chronic myeloproliferative disorders

Study	Polycythemia, *n* (%)	Essential thrombocythemia, *n* (%)	Idiopathic myelofibrosis, *n* (%)
James et al[12]	40/45 (89)	9/21 (43)	3/7 (43)
Kralovics et al[13]	83/128 (65)	21/93 (23)	13/23 (57)
Levine et al[14]	121/164 (74)	37/115 (32)	16/46 (35)
Baxter et al[15]	71/73 (97)	29/51 (57)	8/16 (50)
Zhao et al[16]	20/24 (83)		
Hsu et al[17]	25/31 (81)	23/38 (61)	2/6 (33)
Jones et al[18]	58/72 (81)	24/59 (41)	15/35 (43)
Total	418/537 (77)	143/377 (37)	57/133 (42)

JAK2 (but not wildtype JAK2) induced Epo hypersensitivity and Epo-independent survival of cultured cell lines.[12] Clinical study also confirmed that the presence of the JAK2 V617F mutation is correlated with other biological phenomena such as polycythemia rubra vera-1 (PRV-1) expression and endogenous erythroid colony formation.[25,26] A small interfering RNA (siRNA), used to knockdown JAK2 expression, can further block endogenous erythroid colony formation in the cells from PV patients with the JAK2 V617F mutation.[12] The *in vivo* effect of the mutation was demonstrated by the development of erythrocytosis in mice that received transplants with bone marrow containing the JAK2 V617F mutation, but not wildtype JAK2.[12] Recently, Kralovics et al[26] further demonstrated that the alterations in the expression of the biological and epigenetic markers in patients with PV and ET, including deregulated expression of Bcl-x, an inhibitor of apoptosis, overexpression of the PRV-1 and transcription factor NF-E2 genes and impaired expression of Tpo-R[26–30] are due to the activation of the JAK/STAT pathway through the JAK2 V617F mutation. This evidence strongly indicates that the JAK2 V617F mutation has a direct causative role in the pathogenesis of MPDs.

However, several questions remain unanswered: (1) how might a single mutation give rise to at least 3 different diseases (PV, ET and IMF) and also to some other atypical MPDs; and (2) why don't all patients demonstrate this mutation? In a recent study, Campbell et al demonstrated that V617F mutation-positive ET patients had multiple features resembling PV, with significantly increased hemoglobin, neutrophil counts, bone marrow erythropoiesis and granulopoiesis, more venous thromboses and a higher rate of PV transformation than those without the mutation.[31] V617F mutation-positive ET patients also had lower serum Epo and ferritin concentrations than did mutation-negative patients.[31] Nonetheless, V617F mutation-negative ET patients did show many clinical and laboratory features that were characteristic of MPDs, including cytogenetic abnormalities, hypercellular bone marrow with abnormal megakaryocyte morphology, PRV overexpression, growth of Epo-independent erythroid colonies, and risk of myelofibrotic or leukemic transformation.[31] This evidence implies that JAK2 V617F-positive ET and PV form a biological continuum, with the degree of erythrocytosis determined by physiologic or genetic modifiers.[31] This model suggests that, in patients at the thrombocythemia end of the continuum, the effects of the V617F mutation on erythropoiesis are constrained by physiologic mechanisms, including Epo suppression and depleted iron stores, or by genetic modifiers, either acquired or constitutional. Acquisition of homozygosity for the V617F mutation may favor development of a polycythemic phenotype since homozygosity for mutant JAK2 occurs in approximately 30% of patients with PV, but is rare in ET.[12–15] Gender may influence presentation of V617F-positive disease, since PV is more common in men, whereas V617F-positive thrombocythemia is more common in women.[32,33] The animal model also suggests that genetic modifiers affect the hematopoietic phenotype of the JAK2 mutation. A recent study has demonstrated that expression of JAK2 V617F in mouse bone marrow results in polycythemia in different strains, but that associated leukocytosis is strain-dependent.[34] In those PV or ET patients without JAK2 V617F mutation, disease alleles other than JAK2 V617F might be involved, however, Levine et al failed to identify any mutations by exon sequence analysis of the activating loops and autoinhibitory domains of other tyrosine kinases in granulocyte DNA samples from PV patients.[14] Other mutations may occur in pathways that interact with JAK/STAT signaling or in other effector proteins, including adapter molecules that facilitate JAK/STAT pathway activation.[35] Mutations in any one of several known negative regulators of the JAK/STAT pathway might be likewise operative in the other PV or ET patients without JAK2 V617F mutation.

JAK2 V617F Mutation in the Diagnosis and Treatment of PV and ET

This breakthrough discovery has had a great impact in the diagnosis of chronic MPDs. In the newly proposed diagnostic criteria for PV, presence of the JAK2 V617F mutation has been integrated as a major criterion (Table 2).[36] It has been suggested that JAK2 V617F mutation analysis can be used to help screen individuals with polycythemia and that this may reduce the need for further investigations, such as red cell mass and bone marrow biopsy.[24,37] However, the presence of a JAK2 V617F mutation alone does not distinguish PV from IMF or ET.[38] In ET, where differentiating a primary proliferative condition from a reactive one is notoriously difficult, the use of JAK2 mutation analysis may assist in identifying patients with a stem cell disorder. However, it has to be remembered that patients without a JAK2 mutation can still have a primary MPD. Recently, the Medical Research Council Primary Thrombocythemia-1 Trial studied the effect of JAK2 V617F mutation on treatment outcome in patients with ET and PV, demonstrating that JAK2 V617F mutation-positive patients were much more sensitive

Table 2. Proposed diagnostic criteria for polycythemia vera (PV)[36]

A1.	Raised red cell mass (> 25% above predicted, or hematocrit ≥ 60 in males or > 0.56 in females)*
A2.	Absence of causes of secondary erythrocytosis (normal arterial oxygen saturation and no elevation of serum erythropoietin)†
A3.	Palpable splenomegaly
A4.	Presence of JAK2 V617F mutation or other cytogenetic abnormality (excluding B-cell receptor-ABL gene [BCR-ABL]) in hematopoietic cells
B1.	Thrombocytosis (platelets $> 400 \times 10^9$/L)
B2.	Neutrophilia (neutrophils $> 10 \times 10^9$/L; $> 12.5 \times 10^9$/L in smokers)
B3.	Radiologic splenomegaly
B4.	Endogenous erythroid colonies or low serum erythropoietin

Criteria for a diagnosis of PV: A1 + A2 + either another A or 2 of B

**These hematocrit values are invariably associated with a raised red cell mass in an adult population; †note that it is possible in rare cases for PV to coexist with a cause of secondary erythrocytosis.*

to hydroxyurea, but not to anagrelide, than those without the JAK2 V617F mutation.[31] Furthermore, the rate of arterial thrombosis appeared to be lower in JAK2 V617F-positive patients receiving hydroxyurea compared to those receiving anagrelide, an effect that was not evident in JAK2 V617F-negative patients.[31] These findings further support the concept of classifying patients as JAK2 V617F-positive or -negative during diagnosis and designing individualized treatment strategies for PV or ET patients.

The identification of JAK2 V617F mutations in MPDs has stimulated a great deal of effort in screening and developing specific inhibitors for clinical use. It is certain that the next few years will bring further developments in this fast-evolving field. However, as the management of many PV patients with conventional therapies such as phlebectomy, aspirin and hydroxyurea has a reasonable outcome with little hematologic toxicity and relatively modest costs, extensive evaluation of the potential hematologic toxicity and a cost–benefit analysis will be needed before inhibitors of the JAK signaling pathway can be introduced into clinical practice, especially for MPD patients with the JAK2 V617F mutation.

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