The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia


Activating mutations in tyrosine kinases have been identified in hematopoietic and nonhematopoietic malignancies. Recently, we and others identified a single recurrent somatic activating mutation (JAK2V617F) in the Janus kinase 2 (JAK2) tyrosine kinase in the myeloproliferative disorders (MPDs) polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. We used direct sequence analysis to determine if the JAK2V617F mutation was present in acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), T-cell ALL, and chronic lymphocytic leukemia (CLL). Analysis of 222 patients with AML identified JAK2V617F mutations in 4 patients with AML, 3 of whom had a preceding MPD. JAK2V617F mutations were identified in 9 (7.8%) of 116 CML/malignancy samples, and in 2 (4.2%) of 48 MDS samples. We did not identify the JAK2V617F disease allele in B-lineage ALL (n = 83), T-cell ALL (n = 93), or CLL (n = 45). These data indicate that the JAK2V617F allele is present in acute and chronic myeloid malignancies but not in lymphoid malignancies. (Blood. 2005; 106:3377-3379)

Introduction

Constitutive activation of tyrosine kinases by chromosomal translocation, interstitial deletion, internal tandem duplication, and amino acid substitution have been observed in hematopoietic malignancies including acute myeloid leukemia (AML) and myeloproliferative disorders (MPDs). These mutant kinases are attractive therapeutic targets, as demonstrated by the efficacy of imatinib in BCR-ABL–positive chronic myelogenous leukemia (CML), as well as in MPD associated with activating alleles involving PDGFRα or PDGFRβ. In addition, activating mutations in the FLT3 receptor tyrosine kinase are the most common genetic event in acute myeloid leukemia (AML), and specific inhibitors of the FMS-like tyrosine kinase 3 (FLT3) have entered late-stage clinical trials. Although mutations in tyrosine kinases and in other genes have been identified in a subset of MPD and AML, in many cases the genetic events that contribute to the molecular pathogenesis of these diseases remain unknown.

Recently, we and others identified a recurrent somatic activating mutation in the JAK2 tyrosine kinase in polycythemia vera (PV), essential thrombocythemia (ET), and myeloid metaplasia with myelofibrosis (MMM). This mutation results in a valine to phenylalanine substitution at codon 617 within the Jak homology domain 2 (JH2) pseudokinase domain of Janus kinase 2 (JAK2).
Expression of the JAK2V617F kinase in vitro demonstrates constitutive activation and factor-independent growth,10,11 and expression of JAK2V617F in a murine bone marrow transplant model results in erythrocytosis in recipient mice.11 These data suggest that JAK2V617F is a constitutively active tyrosine kinase and that activation of the JAK2 tyrosine kinase by the V617F mutation is an important pathogenetic event in PV, ET, and MMM.

The identification of a single disease allele in 3 related myeloid diseases suggests that the JAK2V617F mutation may be important in the pathogenesis of additional hematopoietic malignancies. In addition, the TEL-JAK2 and PCM1-JAK2 fusions have been identified in patients with MPD, AML, and acute lymphoblastic leukemia (ALL),14-16 and activation of the JAK–signal transducer and activator of transcription (STAT) pathway is observed in hematopoietic and nonhematopoietic malignancies.17 This led us to search for JAK2V617F mutations in chronic myelomonocytic leukemia (CMLML), atypical (BCR-ABL negative) CML (aCML), AML, myelodysplastic syndrome (MDS), ALL, and chronic lymphocytic leukemia (CLL). In this report, we identified JAK2V617F mutations in a subset of CML/aCML, AML, and MDS but not in B-lineage ALL, T-cell ALL, or CLL. These results demonstrate that the JAK2V617F mutation contributes to the pathogenesis of a spectrum of myeloid diseases including MPD and AML but not to ALL.

Study design

Patient samples and isolation of genomic DNA

All patients provided informed consent. DNA isolated from blood and bone marrow samples from 222 patients with AML, from 48 patients with MDS, from 83 patients with B-lineage ALL, from 93 patients with T-cell ALL, and from 45 patients with CLL were included in this study. DNA from 78 patients with CMLML or aCML and RNA from 38 patients with CMLML/aCML were included in this study. DNA was isolated from blood and bone marrow samples using the QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, CA), and cDNA was synthesized from RNA using random hexamer priming.

JAK2V617F sequence analysis

Amplification and sequencing of JAK2 exon 14 was performed as previously described10 using primers exon 14F (5′GTAAGACGGCCGCTTGGCTGAAGTAAAGTGCAT, forward) and exon 14R (5′CAGGAAAACAGCTGATCCTTGCAATAG, reverse) and using a specific forward sequencing primer (5′AGTTTTGTTGTTGTTAAAAAA, reverse) and a reverse primer. Amplification and sequence analysis of cDNA samples was performed using polymerase chain reaction (PCR) primers RT-F1 (5′CCCTCAGTGGGACAAAGAAGAAC3′) and RT-R1 (5′CCCAATCGCAGTGGGACAAAGAAGAC3′) and performing bidirectional sequence traces was performed using Mutation Surveyor version 2.28 (SoftGenetics, State College, PA). Candidate mutations were reamplified and sequenced from the original DNA sample. Sequence analysis of the entire open reading frame of JAK2 was performed using M13-tailed primers as previously described.10

Results and discussion

Table 1 details the results of genotypic analysis for the JAK2V617F allele in 607 patients with hematopoietic malignancies. We identified heterozygous JAK2V617F mutations in 4 patients with AML, 3 of whom had clinicopathologic evidence of a pre-existing MPD. One patient presented at age 60 years with a chronic MPD most consistent with ET and secondary myelofibrosis and subsequently transformed to AML, which was associated with deletion 5q, monosomy 7, and trisomy 8. A second patient presented at age 81 years with CMLML with normal cytogenetics, and subsequently transformed to AML. A third patient presented at age 57 years with PV with normal cytogenetics; the patient was treated with P12 2 years after being diagnosed with PV and transformed to AML 3 years later, which was associated with t(1;7)(cen;cen) at transformation. The remaining patient with AML and a JAK2V617F mutation presented at age 63 years with AML with morphologic evidence of granulocytic and megakaryocytic dysplasia but did not have evidence of a pre-existing chronic MPD. Given the possibility that there may be additional JAK2 mutations in AML, we resequenced all coding exons of JAK2 in 93 AML samples and did not identify additional JAK2 mutations. Although JAK2V617F mutations are rare in de novo AML (~0.5%), activating KIT mutations are also rare (1.6%) in our AML series (data not shown). In addition, the observation that 3 patients with AML and JAK2V617F mutations initially presented with an MPD suggests that the JAK2V617F allele is most common in patients who transform to AML from a pre-existing MPD.

Heterozygous JAK2V617F mutations were identified in 2 (4.2%) of 48 MDS cases. One patient presented at age 66 years with MDS with bone marrow dysplasia and 3% bone marrow blasts, and at the time his sample was obtained he had transformed to refractory anemia with excess of blasts in transformation (RAEB-t) with 25% bone marrow blasts; the second patient presented at age 74 years with refractory anemia and 7% bone marrow blasts. Sequence analysis of DNA samples from 78 patients with CMLML/aCML samples identified heterozygous JAK2V617F mutations in 7 samples and a homozygous mutation in 1 sample. All CMLML/aCML patients with JAK2V617F mutations had a normal diploid karyotype, and clinical characteristics are listed in Table 2. Sequence analysis of exons 12 to 19 (encoding the JH2 pseudokinase domain) and exon 22 (encoding the activation loop) in 32 CMLML/aCML samples did not identify additional JAK2 mutations. Analysis of cDNA from an additional 38 patients with CMLML/aCML identified a heterozygous mutation in one additional patient with aCML; in total, 9 (7.8%) of 116 patients with CMLML/aCML had JAK2V617F mutations. Two patients with CMLML and heterozygous JAK2V617F mutations also had KRAS or NRAS mutations. This observation suggests that activation of the JAK-STAT pathway and the RAS–mitogen-activated protein kinase (MAPK) pathway can cooperate in CMLML, and suggests that the possibility that additional mutations in JAK2V617F mutant hematopoietic progenitors may direct the eventual disease phenotype in specific patients.

The identification of a single disease allele in 3 different MPDs prompted us to search for JAK2V617F mutations in CMLML/aCML, AML, and MDS. The presence of JAK2V617F mutations in CMLML/aCML, MDS, and AML demonstrates that the same
genetic event can play a role in the pathogenesis of a wide spectrum of myeloid malignancies. We believe these observations warrant a comprehensive search for activated tyrosine kinases in MPD and AML, as there are likely additional unidentified genetic events with biologic and therapeutic significance. In contrast, we did not identify JAK2V617F mutations in B-lineage ALL (83 patients), T-cell ALL (93 patients), or CLL (45 patients). Additional in vitro and in vivo studies are needed to determine the cause of the specificity of JAK2V617F for myeloid diseases, as second mutations, host modifiers, differential cytokine receptor expression, and other factors may influence the ultimate phenotype of hematopoietic progenitors that acquire the JAK2V617F mutation. These data also suggest that different genetic events may lead to JAK-STAT pathway activation in different malignancies. Amplification of the JAK2 locus has been described in Hodgkin disease and medistinal B-cell lymphoma,\textsuperscript{18,19} and biallelic inactivating mutations in sup-
pressor of cytokine signaling-1 (SOCS-1), a negative regulator of JAK2, have been identified in mediastinal B-cell lymphoma.\textsuperscript{20} Genomic analysis of JAK2 and of other JAK-STAT pathway members may lead to the identification of mutations of the JAK-STAT pathway in lymphoid diseases and other malignancies.

Note added in proof. While this article was in preparation, two reports described infrequent JAK2V617F mutations in CMML/aCML and MDS\textsuperscript{21} and 20% of patients with unclassified MPD.\textsuperscript{22}

### Acknowledgments

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### References


### Table 2. CMML/aCML: JAK2V617F positive cohort

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<th>Progression to AML</th>
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The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia