Priority Report

Genetic Analysis of Transforming Events That Convert Chronic Myeloproliferative Neoplasms to Leukemias

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Abstract

The oncogenic events that transform chronic myeloproliferative neoplasms (MPN) to acute myeloid leukemias (AML) are not well characterized. We investigated the role of several genes implicated in leukemic transformation by mutational analysis of 63 patients with AML secondary to a preexisting MPN (sAML). Frequent mutations were identified in TET2 (26.3%), ASXL1 (19.3%), IDH1 (9.5%), and JAK2 (36.8%) mutations in sAML, and all possible mutational combinations of these genes were also observed. Analysis of 14 patients for which paired samples from MPN and sAML were available showed that TET2 mutations were frequently acquired at leukemic transformation [6 of 14 (43%)]. In contrast, ASXL1 mutations were almost always detected in both the MPN and AML clones from individual patients. One case was also observed where TET2 and ASXL1 mutations were found before the patient acquired a JAK2 mutation or developed clinical evidence of MPN. We conclude that mutations in TET2, ASXL1, and IDH1 are common in sAML derived from a preexisting MPN. Although TET2/ASXL1 mutations may precede acquisition of JAK2 mutations by the MPN clone, mutations in TET2, but not ASXL1, are commonly acquired at the time of leukemic transformation. Our findings argue that the mutational order of events in MPN and sAML varies in different patients, and that TET2 and ASXL1 mutations have distinct roles in MPN pathogenesis and leukemic transformation. Given the presence of sAML that have no preexisting JAK2/TET2/ASXL1/IDH1 mutations, our work indicates the existence of other mutations yet to be identified that are necessary for leukemic transformation. Cancer Res; 70(2); 447–52. ©2010 AACR.

Introduction

Myeloproliferative neoplasms (MPN) are clonal disorders of hematopoiesis characterized by proliferation of mature-appearing myeloid cells and accumulation of bone marrow fibrosis (1). Genetic studies have identified recurrent somatic alterations in the majority of MPN patients, including acquired mutations in JAK2 (JAK2V617F) in 90% of polycythemia vera (PV) patients and ~50% to 60% of patients with essential thrombocytosis (ET) and primary myelofibrosis (PMF; refs. 2–5). It has long been recognized that MPN patients are at significantly increased risk for transformation to acute myelogenous leukemia (AML). Leukemic transformation occurs in 8% to 23% of MP patients in the first 10 years after diagnosis and in 4% to 8% of PV and ET patients within 18 years of diagnosis and is associated with a universally dismal prognosis (6–8). Despite the clinical importance of leukemic transformation of chronic MPNs, very little is known about the molecular mechanisms that underlie leukemic transformation in this context. Prior studies have noted that a substantial proportion of patients with JAK2V617F-mutant MPNs transform to a JAK2V617F-negative secondary AML (sAML; refs. 9, 10). This suggests the presence of other oncogenic alleles that precede JAK2V617F and/or promote leukemic transformation.

Recently, Delhommeau and colleagues identified mutations in the putative tumor suppressor TET2 in MPN patients (11). Based on the identification of TET2-mutant/JAK2wild-type and TET2-mutant/JAK2-mutant clones, but not JAK2-mutant/TET2-wild-type alleles, it is thought that TET2 mutations are acquired before JAK2V617F by the MPN clone (11). This suggests the possibility that a TET2-mutant, JAK2-wild-type clone could transform to sAML in patients with JAK2-mutant/TET2-mutant MPN. More recently, mutations in ASXL1 have been identified in MPN patients (12) and recurrent IDH1

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-09-3783

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www.aacrjournals.org

Published OnlineFirst January 12, 2010; DOI: 10.1158/0008-5472.CAN-09-3783

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mutations have been identified in AML (13). Of note, IDH1/2 mutations are commonly identified in secondary gliomas that arise from a preceding lower-grade astrocytoma (14), suggesting that IDH1/2 mutations contribute to progression to high-grade glioblastoma. Although TET2, ASXL1, and IDH1 mutations have been identified in patients with a spectrum of myeloid malignancies, the timing of acquisition of TET2/ASXL1/IDH1 mutations and the role of TET2/ASXL1/IDH1 mutations in progression from MPN to AML are not known.

Given the lack of substantive knowledge of the genes that contribute to leukemic transformations of MPN, we therefore investigated the mutational status of JAK2, TET2, ASXL1, and IDH1 in patients with leukemic transformation of MPNs.

Materials and Methods

Patient samples. Peripheral blood and/or bone marrow were collected from 63 MPN/AML patients from the M.D. Anderson Cancer Center (49 patients with DNA only at leukemic transformation and 14 patients for whom DNA was available at MPN diagnosis and at leukemic transformation) and from additional 47 de novo AML patients and 47 patients with AML transformed from myelodysplastic syndrome (MDS) collected at Memorial Sloan-Kettering Cancer Center. Informed consent was obtained from all patients. Genomic DNA was extracted from viably frozen bone marrow mononuclear cells, peripheral blood granulocytes, and/or paraffin-embedded tumor tissue. Patient details are in Supplementary Materials and Methods and Supplementary Table S3. Mutational analysis was done in 36.8% of patients with AML transformed from MPNs.

Sequence analysis of TET2, ASXL1, JAK2, and IDH1. DNA sequencing methods, primer sequences, and genomic coordinates of all amplicons sequenced for TET2, ASXL1, JAK2, and IDH1 are in Supplementary Materials and Methods and Supplementary Table S3. Mutational analysis was done as described previously (15). Missense mutations that were not in dbSNP7 nor could be shown to be somatic in paired tumor/normal or MPN/AML samples were censored. For paired samples where a putative mutation was seen in only one member of the pair, resequencing was done on nonamplified DNA from both samples. Frameshift mutations were validated by cloning and sequencing individual isolated colonies (TOPO TA cloning, Invitrogen).

Results

TET2, ASXL1, JAK2, and IDH1 mutations are common in patients with sAML. We identified JAK2V617F mutations in 36.8%, TET2 mutations in 26.3%, ASXL1 mutations in 19.3%, and IDH1 mutations in 9.5% of sAML patients. Mutations in ASXL1 were heterogeneous deletions or nonsense mutations presumed to truncate the plant homeodomain finger domain (12). In contrast, mutations in TET2 were found throughout the coding region as frameshift (n = 2), nonsense (n = 4), or somatic missense (n = 15) mutations in evolutionarily conserved regions.

JAK2-wild-type and JAK2V617F-mutant sAML were both observed in patients with a history of antecedent PV, ET, and PMF, and we did not observe any correlation between specific MPN diagnosis and mutations in JAK2, TET2, ASXL1, or IDH1. All eight possible genotype combinations of JAK2, TET2, and ASXL1 were observed in sAML patients (Table 1), showing that these alleles are not mutually exclusive. We most frequently identified patients who were wild-type for JAK2, TET2, and ASXL1 (40.4%) or who were JAK2V617F positive with wild-type TET2 and ASXL1 (19.3%). However, we identified patients who were JAK2/ASXL1 mutant, JAK2/TET2 mutant, or TET2/ASXL1 mutant, and we found three sAML patients (5%) with mutations in all three genes (JAK2, TET2, and ASXL1). IDH1 mutations were present in 9.5% of patients and were most commonly observed in patients wild-type for JAK2, TET2, and ASXL1 (four patients), with the exception of one patient who acquired IDH1R132C and JAK2V617F mutations.

The mutational frequency of TET2, ASXL1, and IDH1 mutations did not differ between AML transformed from MPN and an independent cohort of 47 de novo AML patients and 47 patients with AML transformed from MDS (P > 0.05, Fisher’s exact test).

Table 1. Analysis of JAK2, TET2, ASXL1, and IDH1 mutational status in 63 sAML samples reveals all eight possible combinations of genetic states for JAK2, TET2, and ASXL1

<table>
<thead>
<tr>
<th>No. samples</th>
<th>JAK2</th>
<th>TET2</th>
<th>ASXL1</th>
<th>IDH1</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
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<td>10</td>
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<td>6</td>
<td>WT</td>
<td>Mutant</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>5</td>
<td>Mutant</td>
<td>Mutant</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>4</td>
<td>WT</td>
<td>WT</td>
<td>Mutant</td>
<td>WT</td>
</tr>
<tr>
<td>4</td>
<td>WT</td>
<td>WT</td>
<td>Mutant</td>
<td>WT</td>
</tr>
<tr>
<td>3</td>
<td>Mutant</td>
<td>Mutant</td>
<td>WT</td>
<td>WT</td>
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<tr>
<td>3</td>
<td>Mutant</td>
<td>Mutant</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>2</td>
<td>WT</td>
<td>Mutant</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>1</td>
<td>Mutant</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
</tbody>
</table>

NOTE: Samples with missense mutations, which could not be verified as somatic mutations, were excluded from analysis.

Abbreviation: WT, wild-type.


Published OnlineFirst January 12, 2010; DOI: 10.1158/0008-5472.CAN-09-3783
Mutational analysis of paired MPN and sAML samples identifies acquisition of TET2 mutations at leukemic transformation of MPN. To assess the chronology of mutational events in leukemic transformation of MPNs, we examined the mutational status of JAK2, TET2, ASXL1, and IDH1 in paired samples for which DNA was available from both the MPN and the AML phase of disease. The identity of the paired samples was verified by Sequenom single-nucleotide polymorphism genotyping, showing that the likelihood of a match occurring by chance was <1 × 10^{-13}. We identified four patients with JAK2V617F-positive MPN and a JAK2V617F-negative sAML clone (Fig. 1A; Table 2). In one case, we identified an ASXL1 mutation in both the JAK2V617F-positive MPN and the JAK2V617F-negative sAML clone, consistent with transformation of a pre-JAK2V617F, ASXL1-mutant hematopoietic progenitor.

The only gene in this sample set where mutations were found at a statistically significant difference between MPN

Table 2. Mutational analysis of JAK2, TET2, ASXL1, and IDH1 in 14 paired samples of chronic MPNs transformed to AML

<table>
<thead>
<tr>
<th>Sample</th>
<th>MPD</th>
<th>Genotype during MPN</th>
<th>Genotype during AML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>JAK2 V617F</td>
<td>TET2 WT</td>
</tr>
<tr>
<td>1</td>
<td>MF</td>
<td>V617F</td>
<td>WT</td>
</tr>
<tr>
<td>2</td>
<td>MF</td>
<td>V617F</td>
<td>WT</td>
</tr>
<tr>
<td>3</td>
<td>MF</td>
<td>V617F</td>
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<td>WT</td>
<td>WT</td>
</tr>
<tr>
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<td>PV</td>
<td>V617F</td>
<td>WT</td>
</tr>
<tr>
<td>9</td>
<td>PV</td>
<td>V617F</td>
<td>WT</td>
</tr>
<tr>
<td>10</td>
<td>PV</td>
<td>V617F</td>
<td>WT</td>
</tr>
<tr>
<td>11</td>
<td>PV</td>
<td>V617F</td>
<td>WT</td>
</tr>
<tr>
<td>12</td>
<td>ET</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>13</td>
<td>PV</td>
<td>V617F</td>
<td>WT</td>
</tr>
<tr>
<td>14</td>
<td>MF</td>
<td>V617F</td>
<td>WT</td>
</tr>
</tbody>
</table>

Frequency of mutation: 78.6% 0% 21.4% 0% 50% 42.9% 28.6% 0%

NOTE: The presence of mutations, which were discordant between paired samples, is shaded in gray.
and sAML was TET2, where mutations were far more likely at leukemic transformation (P = 0.016, Fisher’s exact test). We did not identify any cases with TET2 mutations in both a JAK2V617F-positive MPN and the paired JAK2V617F-negative sAML; however, we identified two patients with TET2 mutations in the JAK2V617F-negative sAML, which were not present in the paired JAK2V617F-positive MPN (Fig. 1A). We also identified three patients with TET2 mutations in the JAK2V617F-positive sAML clone, which were not present in the JAK2V617F-positive MPN (Fig. 1B). These data show that TET2 mutations can be acquired at the time of leukemic transformation of a MPN clone and that this can occur in a JAK2V617F-positive or JAK2V617F-negative hematopoietic progenitor (Fig. 1B and C). This is in contrast to previous data suggesting that TET2 can be acquired before JAK2V617F in MPN pathogenesis (11).

With the exception of a single patient who acquired both TET2 and ASXL1 mutations at transformation, all patients with an ASXL1 mutation in the chronic MPN state had retention of the ASXL1 mutation at leukemic transformation (Fig. 1B). Interestingly, we identified one patient with JAK2 and ASXL1 mutation in their MPN clone who then acquired a TET2 mutation at leukemic transformation (Fig. 1B). In this case, the patient had a JAK2V617F mutation and a 23-bp deletion in ASXL1 at both MPN diagnosis and leukemic transformation, whereas a missense mutation in TET2 was seen only at transformation. The 23-bp deletion in ASXL1 was validated by subcloning of genomic DNA (Fig. 1D). Although we identified sAML patients with mutations in JAK2, TET2, and ASXL1, we did not identify MPN patients with mutations in all three genes. In addition, 2 of 14 paired MPN/AML samples were wild-type for JAK2, TET2, and ASXL1 in both MPN and leukemic states. We did not identify IDH1 mutations in any paired MPN/AML samples.

TET2 and ASXL1 mutations can predate acquisition of JAK2V617F mutations and MPN diagnosis. We also analyzed serial samples from a patient who initially presented with follicular lymphoma and then presented with PMF 6 years later. Analysis of DNA from bone marrow mononuclear cells during complete remission from follicular lymphoma revealed a heterozygous nonsense mutation in TET2 as well as a 23-bp deletion in ASXL1 (Fig. 2). At this time, there was no evidence of the JAK2V617F mutation. Six years later, the patient developed PMF by clinical parameters and bone marrow examination. DNA from bone marrow mononuclear cells and peripheral blood granulocytes from this period of disease revealed development of the JAK2V617F mutation as well as an additional inactivating mutation in TET2, and continued presence of the preceding inactivating mutation in TET2 and ASXL1.
Discussion

Although many investigators have done mutational analysis of large MPN and AML cohorts, previous studies have not addressed the mutational order of events that occur in leukemic transformation from MPN. In this study, we provide insight into the mutational events that contribute to the transformation from MPN to AML. We identified frequent mutations in JAK2, TET2, ASXL1, and IDH1 in sAML derived from MPN; with the exception of JAK2, the mutational frequency of these genes was similar in de novo and sAML derived from MPN or MDS. The presence of concurrent mutations in TET2, ASXL1, and JAK2 in sAML suggests that these mutations have nonoverlapping contributions to myeloid transformation.

By studying paired samples from patients with MPN who later developed AML, we were able to make several important observations. First, we found evidence of acquisition of TET2 mutations at leukemic transformation not present in the predominant MPN clone. In some cases, TET2 mutations were present in the JAK2V617F-positive AML and not in the paired JAK2V617F-positive MPN, and we also identified patients with JAK2-wild-type, TET2-wild-type MPN who transformed to JAK2-wild-type, TET2-mutant AML. Our data are most consistent with the likelihood that TET2 mutations are acquired after JAK2V617F or other myeloproliferative disorder (MPD) disease alleles at the time of leukemic transformation of a JAK2V617F-positive or JAK2V617F-negative MPN clone. In contrast, ASXL1 mutations were present at all phases of disease, and we identified one patient with an ASXL1 mutation in their MPN clone that subsequently acquired a TET2 mutation at leukemic transformation. This suggests that ASXL1 mutations may precede the acquisition of JAK2 or even TET2 mutations in myeloid transformation and has a distinct role in myeloid transformation.

The mutational analysis of the patients in our cohort was done using Sanger-based sequencing, which is able to detect 15% to 20% mutant alleles (16). Our study cannot exclude the possibility that parallel sequencing technologies (17) can be used to identify rare mutant clones not present in the bulk of the clinical isolate. Nonetheless, our data show that the mutational analysis of the dominant MPN and AML clone can be used to provide insight into the pathogenesis of transformation from MPN to AML.

We also identified a patient in whom TET2 and ASXL1 mutations were present before the development of clinically evident MPN and before acquisition of the JAK2V617F mutation. This finding is consistent with the hypothesis that alterations in TET2 and ASXL1 can, in some cases, occur at an early stage of myeloid neoplasia and that acquisition of additional mutations leads to development of clinically manifest disease phenotypes. Functional studies are needed to delineate the role of TET2 and ASXL1 in normal and malignant hematopoiesis and specifically in transformation from MPN to AML.

It is important to note that many patients were wild-type for JAK2, TET2, ASXL1, and IDH1, consistent with the presence of unidentified lesions that contribute to leukemic transformation. One previous report identified mutations in p53 and K/N-Ras, but not in RB1, in chronic myelogenous leukemia (CML) blast crisis samples not present in matched chronic-phase CML samples (6). Subsequent studies using genetic/epigenetic platforms to study paired MPN/AML samples will identify novel somatic alterations that contribute to leukemic transformation.

Disclosure of Potential Conflicts of Interest

There are no relevant conflicts of interest to disclose.

Grant Support

NIH and Starr Foundation (R.L. Levine). O. Abdel-Wahab is supported by the American Society of Hematology Research Training Award for Fellows and by the Clinical Scholars Program at Memorial Sloan-Kettering Cancer Center. R.L. Levine is an Early Career Award recipient of the Howard Hughes Medical Institute and is the Geoffrey Beene Junior Chair at Memorial Sloan-Kettering Cancer Center.

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Received 10/13/09; revised 11/23/09; accepted 11/23/09; published OnlineFirst 1/12/10.

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Cancer Res 2010;70:447-452. Published OnlineFirst January 12, 2010.

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