Brief report

Clonal analysis of \textit{TET2} and \textit{JAK2} mutations suggests that \textit{TET2} can be a late event in the progression of myeloproliferative neoplasms

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Somatic mutations in \textit{TET2} occur in patients with myeloproliferative neoplasms and other hematologic malignancies. It has been suggested that \textit{TET2} is a tumor suppressor gene and mutations in \textit{TET2} precede the acquisition of \textit{JAK2-V617F}. To examine the order of events, we performed colony assays and genotyped \textit{TET2} and \textit{JAK2} in individual colonies. In 4 of 8 myeloproliferative neoplasm patients, we found that some colonies with mutated \textit{TET2} carried wild-type \textit{JAK2}, whereas others were \textit{JAK2-V617F} positive, indicating that \textit{TET2} occurred before \textit{JAK2-V617F}. One of these patients carried a germline \textit{TET2} mutation. However, in 2 other patients, we obtained data compatible with the opposite order of events, with \textit{JAK2} exon 12 mutation preceding \textit{TET2} mutation in one case. Finally, in 2 of 8 patients, the \textit{TET2} and \textit{JAK2-V617F} mutations defined 2 separate clones. The lack of a strict temporal order of occurrence makes it unlikely that mutations in \textit{TET2} represent a predisposing event for acquiring mutations in \textit{JAK2}.

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Table 1. Characteristics of MPN patients with mutations in TET2

<table>
<thead>
<tr>
<th>UPN</th>
<th>Diagnosis</th>
<th>JAK2-V617F, percentage T</th>
<th>TE2 mutation</th>
<th>Disease duration, mo</th>
<th>Age at diagnosis, y</th>
<th>Complications</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>p010</td>
<td>Male</td>
<td>30%</td>
<td>V617F</td>
<td>75</td>
<td>55</td>
<td>None</td>
<td>Aspirin, hydroxyurea</td>
</tr>
<tr>
<td>p036</td>
<td>Male</td>
<td>38%</td>
<td>V617F</td>
<td>66</td>
<td>75</td>
<td>NA</td>
<td>None</td>
</tr>
<tr>
<td>p191</td>
<td>Male</td>
<td>100%</td>
<td>V617F</td>
<td>59</td>
<td>66</td>
<td>TA, intracranial bleeding, pulmonary</td>
<td>Aspirin, hydroxyurea, oral anticoagulants</td>
</tr>
<tr>
<td>p209</td>
<td>Male</td>
<td>75%</td>
<td>V617F</td>
<td>38</td>
<td>64</td>
<td>Post-PV myelofibrosis, arterial occlusion</td>
<td>Aspirin, hydroxyurea</td>
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<tr>
<td>p226</td>
<td>Female</td>
<td>82%</td>
<td>V617F</td>
<td>30</td>
<td>59</td>
<td>None</td>
<td>Aspirin, hydroxyurea</td>
</tr>
<tr>
<td>p227</td>
<td>Female</td>
<td>82%</td>
<td>V617F</td>
<td>40</td>
<td>66</td>
<td>None</td>
<td>Aspirin, hydroxyurea</td>
</tr>
<tr>
<td>p234</td>
<td>Male</td>
<td>20%</td>
<td>V617F</td>
<td>19</td>
<td>75</td>
<td>None</td>
<td>Clopidogrel, hydroxyurea</td>
</tr>
</tbody>
</table>

UPN indicates unique patient number; PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; and NA, not applicable.

Figure 1. Analysis of single colonies for mutations in TET2 and JAK2. Mononuclear cells from peripheral blood were grown in methylcellulose in the presence or absence of erythropoietin. Single burst-forming units erythroid (BFU-E), endogenous erythroid colonies (EEC), and colony-forming units granulocytes (%T) are shown above the corresponding boxes. Light blue arrows indicate the suggested order of mutation events. (A) Patterns compatible with biclonal disease; yellow boxes, germline TET2; red box, germline JAK2. The inactivated IDS allele is marked with an arrow. (B) Patterns compatible with JAK2-V617F mutations occurring before JAK2-V617F. The sequencing chromatograms for patient p226 show the presence of TET2 mutation in DNA from hair follicles, T cells, and granulocytes, demonstrating the germline origin of the mutation. Allele-specific PCR assay for the X-chromosomal gene IDS is shown for p226. The genomic DNA from patient p226 was heterozygous for a C/T single nucleotide polymorphism (not shown). The relative expression of the 2 IDS alleles was determined by comparing the C and T peak intensities obtained by the allele-specific reverse-transcribed PCR assay in T cells and granulocytes. The skewing of expression toward the C-allele is shown for 10 individual colonies (gray area). The inactivated IDS allele is marked with an arrow. (C) Patterns compatible with JAK2-V617F mutations occurring before TET2 was mutated. Patient p021 carries 2 JAK2 mutations, JAK2-V617F and JAK2-N542-E543del, but the TET2 mutation can be only found together with the deletion in exon 12 of JAK2. In patient p191, the sequencing chromatogram for the SNP rs34402524 located in TET2 is shown for one BFU-E colony marked in red. The presence of a heterozygous SNP excludes the possibility that this colony is the product of a mitotic recombination event. (D) Location of mutations in the TET2 protein in patients from whom data on single colonies are available. Mutations from this study are shown above the protein strand, and mutations analyzed in previous publications,22,23 are shown below. The gray boxes represent regions conserved between the different TET family members; blue boxes, TET2 mutations that occur before JAK2 mutations; yellow boxes, TET2 mutations that occurred after JAK2; white boxes, TET2 and JAK2 mutations compatible with biclonal disease; and red box, germline TET2 mutation.

Patient p191 displayed colonies positive for JAK2-V617F without the TET2 mutation, and all colonies with mutated TET2 were also positive for JAK2-V617F. Unfortunately, 9 single nucleotide polymorphisms (SNPs) in the vicinity of JAK2-V617F were noninformative to provide evidence for a possible biclonal acquisition of JAK2-V617F. The transition from heterozygous to homozygous JAK2-V617F appears to have independently occurred twice. Indeed, the analysis of individual colonies homozygous for JAK2-V617F revealed the presence of 2 subclones with different sizes of the 9p uniparental disomy (UPD) region (supplemental Figure 1A). By analyzing an informative SNP in exon 11 of TET2 (Figure 1B), we can exclude the possibility that the JAK2-V617F positive and TET2-L1210P-negative colonies arose through mitotic recombination, in which the TET2-L1210P was lost. In patient p021, 2 independent clones were present: a smaller clone positive for JAK2-V617F and a larger clone positive for a JAK2 exon 12 mutation (JAK2-N542-E543del).22 Interestingly, the TET2 mutation in this patient occurs only in combination with the JAK2 exon 12 mutation, and the TET2 mutation in this patient was acquired after the JAK2 exon 12 mutation. A third pattern consisted of biclonal disease, as illustrated in patient p010 with colonies positive either for JAK2-V617F or TET2, but absence of double-positive colonies (Figure 1C). A similar biclonal pattern was previously described in one patient with del1q20q and JAK2-V617F, consistent with the presence of a predisposition to independently acquire 2 otherwise rare somatic events.21 Patient p234 also shows a pattern compatible with biclonal disease.
However, to progress to the double-positive stage, either JAK2 or TET2 must have mutated twice independently. Again, SNP analysis excluded the possibility that the JAK2-V617F+ and TET2-G1275E− colonies arose through mitotic recombination (Figure 1C). Because it is unlikely that TET2 independently mutated twice at the exact same position (TET2-G1275E), we conclude that the majority of colonies first acquired TET2-G1275E followed by JAK2-V617F. A second independent event produced the subclone that is positive for JAK2-V617F only. The SNPs in close proximity (rs10974944 and rs12343867) were noninformative and were not part of the recently described GGCC or 46/1 JAK2 haplotype (data not shown).24,25

In 4 of 8 patients, we observed a small number of colonies that were homozygous for the TET2 mutations. Gene copy number analysis revealed that p209 and p191 retained 2 copies of the TET2 gene, whereas in p225 loss of one copy of the TET2 gene was found, indicating that the normal TET2 allele was lost through a deletion (supplemental Figure 1B). In patient p234, homozygosity was achieved through UPD in some colonies and deletion in other colonies. Colonies homozygous for the TET2 mutations from all 4 patients displayed loss of heterozygosity of SNPs or microsatellite markers in the TET2 locus, as expected for a deletion or UPD at chromosome 4q (supplemental Figure 1B).

The heterozygous TET2-D1858fs mutation in patient p226 was also present in DNA from hair roots, indicating that the mutation was germline. This 4-bp deletion, located in the C-terminal conserved domain of TET2, results in a frame shift and premature stop and is probably functionally relevant. The same mutation was also found in buccal DNA from an asymptomatic sister of p226 (data not shown). This appears to be the first report of a germline mutation in TET2. Accordingly, all colonies in this patient were positive for the TET2 mutation. The X-chromosome inactivation pattern in individual colonies from p226 with wild-type JAK2, as determined by scoring a C/T polymorphism in the 3′-untranslated region of the IDS mRNA,18,20 revealed a strong skewing (10 of 10 expressed the C allele of IDS), indicating that these progenitors were of clonal origin (Figure 1A). The finding of clonality suggests that this patient has a second significant disease clone, which does not carry a mutation in JAK2.

We showed that a JAK2 exon 12 mutation preceded the TET2 mutation in p021. In addition, the data in p191 are compatible with JAK2-V617F preceding TET2. A similar conclusion was reached in one patient with familial MPN positive for TET2 and JAK2-V617F mutations.26 In 4 of 8 patients, some colonies carried a homozygous TET2 mutation that was the result of the loss of the wild-type allele through deletion or UPD. The percentage of such homozygous colonies in all 4 patients was very low (<5%), opening the possibility that the loss of the wild-type TET2 may not provide an additional competitive advantage. The location of the TET2 mutation, which has been analyzed using single colony assays, is summarized in Figure 1D. Most of the TET2 frame shift and nonsense mutations occurred in patients in whom TET2 preceded the acquisition of JAK2. The lack of a strict temporal order of occurrence resembles the findings obtained for del20q and makes it unlikely that mutations in TET2 represent a predisposing event for acquiring JAK2.

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Authorship

Contribution: F.X.S. performed research, analyzed data, and wrote the paper; R.L., S.L., and H.H.-S. performed research; T.L. and A.T. provided clinical data; and R.C.S. designed research, analyzed data, and wrote the paper.

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