Correspondence

To the editor:

Failure of the Ipsogen MutaScreen kit to detect the JAK2 617V>F mutation in samples with additional rare exon 14 mutations: implications for clinical testing and report of a novel 618C>F mutation in addition to 617V>F

The JAK2 617V>F point mutation is an important diagnostic tool for Philadelphia chromosome–negative myeloproliferative disorders. JAK2 mutation analysis has been endorsed by the World Health Organization for diagnosing polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis.1

On March 30, 2006, Ipsogen announced a license agreement for the worldwide and exclusive rights on a mutation in the JAK2 gene.2 Options for clinical laboratories performing JAK2 617V>F mutation testing are to use commercial kits from Ipsogen or to pay Ipsogen an upfront fee plus royalty payments for each test performed if their kits are not used. We compared the JAK2 Ipsogen MutaScreen kit & Reference Scale kit (MSPP-03), which is a TaqMan allelic discrimination assay that contains fluorescent probes specific for wild-type (617V) and mutant (617F) alleles, with results previously obtained using a laboratory-developed melt-curve assay,3 and found agreement in 58 of 60 cases.

The 2 discordant samples (Figure 1) had atypical melt-curves, suggestive of novel mutation(s). Sequence analysis from patient no. 1 with PV showed homozygosity for 2 mutations in codon 617 (1849G>T, 1851C>T) and 1 in codon 618 (1852T>C) resulting in 617V>F and 618C>R mutations.4

Patient no. 2 with ET and on Hydrea was heterozygous for 2 mutations: 617V>F (1849G>T) and 618C>F (1853G>T). Patient no. 1 failed to give a fluorescent signal using the Ipsogen kit; patient no. 2 incorrectly genotyped as “wild-type.” To differentiate nonamplification (patient no. 1) from failure of the mutant probe to bind to the mutant allele (patients no. 1 and no. 2), PCR products obtained using the Ipsogen kit were subject to agarose gel electrophoresis. Both samples had single prominent bands which were the same size as control samples (data not shown). These results are consistent the mutant probe not binding to the mutant allele in both patients and are consistent with 617V and 618 mutations being on the same allele.

In conclusion, the Ipsogen kit failed to identify 2 samples with JAK2 617V>F mutations which had additional mutations. The Ipsogen kit would likely miss other 617V>F samples which have a second mutation and other exon 14 mutations.5-8 Since the JAK2 617V>F mutation (or other functionally similar mutations) are part of the revised WHO criteria for myeloproliferative disorders,1 failure to detect this mutation may result in misdiagnosis.

These findings highlight the broader issue patents impose upon clinical laboratories and the potential for inferior diagnostic testing and missed mutations. This issue came to a forefront on May 12, 2009, when the American Civil Liberties Union filed a lawsuit against Myriad Genetics, which has exclusive rights to perform BRCA1 and BRCA2 mutation testing.9 The lawsuit claims patents on these genes are unconstitutional and invalid. Moreover, BRCA1/2 mutations have been missed.10 We evaluated the Ipsogen kit because it was cost-prohibitive to pay the substantial upfront fee and additional royalty on test sales using a “home-brew” assay. It is unfortunate that such restrictions can compromise patient care, and it will be interesting to see the outcome of the Myriad Genetics lawsuit and whether this paves the way to prevention of monopologies created by gene patenting.

Figure 1. Melt-curve, sequence, and Ipsogen MutaScreen kit results. Results of heterozygous control (top) and patients no. 1 (middle) and no. 2 (bottom). Melting temperatures (Tm) of wild-type and JAK2 mutations are indicated. Sequence analysis of 617V>F heterozygote compared with patients no. 1 (homozygous 617V>F/618C>R; 1849G>T, 1851C>T, 1852T>C) and no. 2 (heterozygous 617V>F/618C>F; 1849G>T, 1853G>T). Using the Ipsogen kit, no signal is obtained in patient no. 1, who is homozygous mutant, due to failure of the mutant probe to bind to the mutant allele. Patient no. 2, who is heterozygous, genotypes as wild-type because the mutant probe does not bind to the mutant allele.

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The role of CBFβ in AML1-ETO’s activity

Identification of interacting proteins essential for oncogenic functions of leukemia-associated transcription factors is important for understanding the underlying transformation mechanisms and designing effective cancer therapeutics. We have recently found that homo-oligomerization property, but not its interaction with core-binding factor beta subunit (CBFβ), is critical for AML1-ETO-mediated transformation of primary hematopoietic cells. Strikingly, the conclusion on CBFβ requirement contradicts another study by Roudaia et al, which reported an essential function of CBFβ interaction for AML1-ETO activity based on an AML1-ETO double-point mutant (Y113A/T161A). One possible explanation for these discrepancies is the use of different point mutants in these studies. To this end, we have generated an identical AML1-ETO Y113A/T161A mutation used in the study of Roudaia et al and compared it with our M106V point mutant in the transformation assay. In contrast to cells transduced with empty vector or the AML1-ETO DNA binding mutant that rapidly lost their proliferative capacity, cells transduced with the CBFβ defective mutants including Y113A/T161A could still form significant numbers of third and subsequent rounds of colonies in the serial replating assay (Figure 1A). Despite reduced number (an average of 12 different experiments), the resultant colonies exhibited very similar morphology and immunophenotypes as wild-type AML1-ETO transformed cells (Figure 1B-C and supplemental Figure 1, available on the Blood website; see the Supplemental Materials link at the top of the online article). This is in stark contrast to the results by Roudaia et al, where Y113A/T161A mutant when transduced into 5FU-treated bone marrow cells failed to give third-round colonies. It is possible that 5FU treatment may have depleted certain AML1-ETO target cells that are responsible for the observed phenotype in the assay using positively selected c-kit cells. We also note that the transformation data in Roudaia and colleagues’ study had not been normalized with the number of plated cells; that is, colony number in the second and third platings were derived from 10 times more cells (10^4) number of plated cells; that is, colony number in the second and third platings were derived from 10 times more cells (10^4) compared with the first plating (10^3). If presented as normalized data, the results would look quite significantly different, and the difference between the wild-type and Y113A/T161A mutant would be more modest.

In addition, one must be cautious when interpreting mutagenesis data, as it is almost impossible to engineer absolutely specific mutations that will affect only a single property of the mutated protein. Thus it is critical to have an alternative approach targeting CBFβ expression without altering the structure of AML1-ETO. Consistent with our point mutant data, we further demonstrated that 2 independent shRNAs that effectively knocked down more than 95% CBFβ expression at protein level in primary hematopoietic cells did not compromise AML1-ETO-mediated transformation. Together, these results indicate that a significant reduction of CBFβ activity has only modest effect on AML1-ETO-mediated transformation. However, a decisive experiment to determine whether there is an absolute CBFβ dependence is to assess the behavior of AML1-ETO in a complete absence of CBFβ using genetic approaches such as conditional knockout mice that will be instrumental to this issue.
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