To the editor:

Mutations of Chk2 in primary hematopoietic neoplasms

Chk2 is a novel checkpoint kinase isolated as a human homologue of yeast Cds1/Rad53.1 Recent analyses have revealed that it is among key molecules signaling DNA damage via the ATM protein kinase to p53.1,2 Of great interest is the report that germ line mutations of the Chk2 gene are found in a fraction of Li-Fraumeni syndrome (LFS),3 a hereditary cancer-susceptibility syndrome originally linked with germ line p53 mutations, suggesting that Chk2 is a tumor suppressor gene whose functional deficit will lead to development of human cancers. Given that the p53 and ATM genes are inactive in leukemias and lymphomas, it is intriguing to investigate whether or not somatic mutations of Chk2 are also responsible for leukemias and lymphomas.

To address this point, we screened for mutations of Chk2 in a variety of human hematopoietic neoplasms. A total of 109 tumor specimens of hematopoietic malignant disorders were examined for mutations of Chk2 using reverse transcriptase–polymerase chain reaction/single strand conformational polymorphism (RT-PCR/SSCP) analysis. Numbers and diagnoses of these patients are listed in Table 1. Two samples showed abnormally migrating bands on RT-PCR/SSCP analysis of the Chk2 transcripts (patient 1375 and patient 154), and the nucleotide alterations were further confirmed by sequencing analysis in both cases (Figure 1 and Table 2).

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Table 1. Frequency of Chk2, p53, and p16 alterations in primary hematopoietic neoplasms

| Diagnosis | No. of patients | Chk2 | p53 | p16 |
|-----------|----------------|------|-----|-----|
| ALL       | 14             | 0    | 0   | 5   |
| AML       | 55             | 1    | 5   | 1   |
| CML       | 12             | 0    | 0   | 0   |
| CLL       | 5              | 0    | 1   | 1   |
| PLL       | 2              | 1    | 0   | 0   |
| NHL       | 7              | 1    | 0   | 1   |
| ATL       | 4              | 0    | 0   | 1   |
| MM        | 2              | 0    | 0   | 0   |
| MDS       | 8              | 0    | 0   | 0   |
| Total     | 109            | 2    | 7   | 9   |

ALL indicates acute lymphocytic leukemia; AML, acute myelocytic leukemia; CML, chronic myelocytic leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; MDS, myelodysplastic syndrome.

Table 2. Summary of Chk2 alterations in primary hematopoietic neoplasms

| Patient | Diagnosis | DNA position | Alteration | Transcript |
|---------|-----------|--------------|------------|------------|
| 1375    | AML(M1)   | intron 10    | A > G transition | exon 10 and 11 stop codon at 424 |
|         | NHL       | 223-237      | 15-bp deletion | 5 amino acids' deletion |
| 154     | NHL       | exon 11      | 8-bp before exon 11 |

Patient 1375 was diagnosed with acute myeloid leukemia (AML), French-American-British subtype M1, and had a 7-bp insertion at the boundary of exons 10 and 11 of Chk2 (Figure 1A and Table 2), which caused a frameshift of the coding sequence and resulted in premature truncation of the protein at codon 424. Sequencing analysis of the corresponding genomic sequence revealed an A>G substitution at the splicing acceptor site of the intron 10, 8 bp before exon 11, suggesting that the mutation created an alternative splicing acceptor site 7 bp upstream from the original one and resulted in the 7-bp insertion between exons 10 and 11. Because a DNA sample from his normal skin showed an A/A genotype at this position, this is really a somatic mutation (Figure 1A). Because the RT-PCR/SSCP analysis showed exclusively abnormally migrating bands, function of Chk2 is expected to be lost in patient 1375.

The other patient, patient 154, was diagnosed with non-Hodgkin lymphoma (NHL), with mantle cell morphology. Direct sequencing of the abnormal bands on the SSCP analysis revealed a 15-bp deletion between codons 75 and 79. The 15-bp deletion resulted in loss of 5 amino acids as shown in Figure 1B and Table 2. The deleted 15 nucleotides are a half of the two 15-bp repeats between codons 75 and 84. Because genomic sequences of both tumor and normal samples also had the 15-bp deletion, this deletion was most likely to be a germ-line mutation. In this case, normally migrated bands were also detected. But because this sample was apparently contaminated by normal bone marrow cells, we could not determine whether it represented a residual allele in tumor cells or it was derived from the contaminated normal cells and the tumor cells themselves lacked a wild-type allele.

We compared the mutation rate of Chk2 with those of other well-known tumor-suppressor genes in the same panel of 109 hematopoietic neoplasms. p53 was mutated in 7 samples (6.4%), while homozygous deletion of p16 was identified in 9 samples (8.3%). There appeared to be a tendency that more p53 mutations were found in AML and p16 deletions occurred preferentially in acute lymphoid leukemia (ALL). Distributions of these mutations are summarized in Table 1. There were no overlapping mutations of Chk2, p53, and p16, except for in patient 1375, in whom, in addition to the Chk2 mutation described above, a missense mutation (TGT>TAT, Cys→Tyr) at codon 238 in p53 existed. On the other hand, patient 154 also had an additional genetic alteration that may affect cell-cycle regulation. Because her lymphoma cells had a t(11;14)(q13;q32) translocation with rearrangement between the cyclin D1 gene and the JH region of immunoglobulin heavy chain causing overexpression of cyclin D1.

It is noteworthy that both Chk2 mutations were compounded with other genetic alterations that were presumed to disrupt the G1 checkpoint mechanism. The first patient (patient 1375) had a point mutation in p53, and the second (patient 154) carried a t(11;14)(q13;q32) translocation with overexpression of cyclin D1. In this context, it may be worth mentioning that the other case of Chk2 mutation thus far reported in a case with small-cell lung cancer also carried mutation of p53. In these cases, both G1 and G2 checkpoint regulations are simultaneously abrogated; p53 mutation and overexpression of cyclin D1 will affect G1 regulation, and the Chk2 mutations will be associated with compromised G2 checkpoint. The Chk2 mutation associated with mantle cell lymphoma (MCL) carrying t(11;14)(q13;q32) may be in parallel with a recent observation that ATM, an upstream regulator of Chk2 kinase, is frequently inactivated in MCL. Mouse null for both p53 and ATM genes show accelerated tumor growth as compared with mice null only for either p53 or ATM alone. Thus compounded G1 and G2 checkpoint abnormalities might confer more proliferative or anti-apoptotic advantages upon the tumor cells.

In conclusion, sporadic mutation of Chk2 is rare in hematopoietic neoplasms. Recently Hofmann et al also reported a similar observation in myelodysplastic syndrome (MDS) and AML, where...
only one MDS case had a Chk2 mutation.\textsuperscript{7} Chk2 is rarely mutated in sporadic cases of small-cell lung cancers and tumor-derived cell lines.\textsuperscript{3,4} While germ-line mutations of Chk2 predispose to several cancers in LFS, our and others’ observations indicate that Chk2 belongs to a tumor suppressor gene of a “caretaker” type, just like hMLH1 and BRCA1.\textsuperscript{8} Inactivation of Chk2 itself may not be sufficient for tumorigenesis but could induce a kind of genetic instability, which will facilitate the oncogenic processes in pathogenesis of sporadic cancers, including hematopoietic neoplasms.

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