Mutations of the E2F4 gene in hematological malignancies having microsatellite instability

Naoki Komatsu, Seiho Takeuchi, Takayuki Ikezoe, Taizo Tasaka, Yoshihiro Hatta, Hisanori Machida, Ian K. Williamson, Claus R. Bartram, H. Phillip Koeffler, and Hirokuni Taguchi

Mutations of coding repeats within the E2F4, TGF-βRII, BAX, IGFIIR, and hMSH3 are critical targets of microsatellite instability (MSI) in many kinds of cancers. We analyzed 9 childhood acute lymphoblastic leukemia (ALL) samples, 5 acute myelocytic leukemia (AML) samples, and 10 adult T-cell leukemia (ATL) samples having MSI to determine whether they had mutations of the E2F4, TGF-βRII, BAX, IGFIIR, and hMSH3 genes. Frameshift mutations were found at trinucleotide repeats within a coding exon of the E2F4 gene in 2 of 10 (20%) ATL samples and 1 of 9 (11%) childhood ALL samples. No mutations were found in the TGF-βRII, BAX, IGFIIR, and hMSH3 genes. E2F4 is a transcription factor that influences the cell-cycle progression. These results suggest that mutations of the E2F4 gene, presumably caused by an abnormality of one of the DNA repair genes, may play an important role in development of ATL and childhood ALL. (Blood. 2000;95:1509-1510)

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Introduction

Microsatellite instability (MSI), representing either an expansion or a reduction of (C-A), repeats, has been reported in many kinds of human malignancies.1,3 MSI appears to reflect multiple replication errors because of a defective mismatch repair gene, including hMSH2, hMLH1, hPMS1, and hPMS2.4,5 Cancers with MSI show exaggerated genomic instability at simple repeat sequences that generate somatic frameshift mutations in genes containing these repeat sequences. Somatic frameshift mutations of coding repeats within the E2F4, TGF-βRII, BAX, IGFIIR, and hMSH3 are recognized as critical targets of MSI in many kinds of cancers.6-10 We previously found that MSI is present in childhood acute lymphoblastic leukemia (ALL), acute myelocytic leukemia (AML), and adult T-cell leukemia (ATL).11,12 However, the genes that are the target for mutation in hematological malignancies with MSI are not known. In this study, we analyzed 24 hematological malignancies having MSI to determine whether mutations occurred at coding repeats of 5 target genes: E2F4, TGF-βRII, BAX, IGFIIR, and hMSH3.

Study design

We previously analyzed for MSI using 48 childhood ALL, 17 AML, and 22 ATL samples.11-13 MSI was present in 9 (19%) childhood ALL samples, 5 (29%) AML samples, and 10 (45%) ATL samples. These 24 samples with MSI were used in this study. Informed consent was obtained from the patients, their parents, or both, as appropriate. Mutation analysis of the E2F4, TGF-βRII, BAX, IGFIIR, and hMSH3 genes were performed as reported previously.6,10 A trinucleotide (AGC) repeat spanning codons 306-321, which normally encodes 13 serine residues of the E2F4 gene, was studied.6 For the TGF-βRII gene, the (A) sequence (nucleotides 709-718) was analyzed.7 For the BAX gene, the (G) sequence in the third coding exon was analyzed.4 A fragment containing the deoxyguanine repeat (nucleotides 4030-4140) was investigated in the IGFIIR gene.5 For the hMSH3 gene, the track of 8 deoxyadenine in exon 7 was assessed.10

Results and discussion

Three (13%) samples contained mutations of the poly (AGC) tract within E2F4 (Table 1). One sample contained an E2F4 allele that was 6 codons (18 nucleotides) shorter than the normal sequence. The 2 other samples contained E2F4 alleles that were 3 codons (9 nucleotides) longer than the normal sequence (Figure 1). By leukemia subtype, 1 of 9 (11%) childhood ALL samples and 2 of 10 (20%) ATL samples contained E2F4 mutations. The ALL patient with an E2F4 mutation had a T-cell phenotype; 2 ATL patients with E2F4 mutations had acute-type ATL. All 3 samples with E2F4 mutations had MSI at 1 or 2 loci. None of the 5 AML samples had mutations within E2F4. No mutations were found within the repeat sequence of TGF-βRII, BAX, IGFIIR, and hMSH3 genes (data not shown).

The present study has found for the first time that mutations within the important cell cycle gene E2F4 were present in hematological malignancies with MSI. E2F is a family of transcription factors, the activity of which influences the progression through the G1-S transition of the cell cycle.14 E2F consensus binding sites have been identified in the promotors of several growth-regulatory genes, including c-myc, cyclin-dependent kinases, and cyclin D1.15,17 E2F-mediated transcripational activation is repressed through a physical association between E2F2 and proteins of the retinoblastoma family (pRB, p107, p130). Five
different E2Fs have been identified: E2F1, E2F2, and E2F3 interact preferentially with pRB;18 E2F5 with p130;19 and E2F4 associates with all 3 of these proteins, especially p107 and p130 in a cell cycle–dependent manner.14,18,20,21 The p107 and p130 proteins inhibit the ability of E2F4 to transactivate genes whose promoter binding region of the retinoblastoma family of proteins. This serine (AGC) tract of E2F4 lies between the “marked box” region and the binding region of the retinoblastoma family of proteins. This serine repeat domain may induce transcriptional target genes.6 Even though a functional effect of a mutation of this region has not been studied, changes in this region might potentiate transcriptions of growth-stimulatory E2F4 target genes.

In this study, mutations were found only at the trinucleotide repeat sequence within the E2F4 gene, and no mutations were found at the single nucleotide repeat within the TGF-RII, BAX, IGFIIIR, and hMSH3 genes. This finding is congruent with the previous findings that no mutations of the TGF-RII and BAX genes were found in childhood ALL.13,24 Moreover, Kaneko et al25 reported that mutations of the TGF-RII gene were not found in MDS patients with MSI. Taken together, these findings suggest that the mechanisms underlying the occurrence of such trinucleotide replication errors could differ from those of the single nucleotide frameshifts in hematological malignancies.

Acknowledgment
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References
18. Ikeda MA, Jakob L, Nevins JR. A unique role for the Rb protein in controlling E2F accumula

Table 1. Summary of clinicopathologic data, MSI status of patients with E2F4 mutations

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>MSI Status</th>
<th>E2F4 (AGC)</th>
<th>TGF-RII (A)</th>
<th>BAX (G)</th>
<th>IGFIIIR (A)</th>
<th>hMSH3</th>
</tr>
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<tr>
<td>54</td>
<td>T-ALL</td>
<td>+ Deletion (7)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Acute ATL</td>
<td>+ Insertion (16)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>
| 18         | Acute ATL | + + Insertion (16) | N | N | N | N | MSi indicates microsatellite instability; +, MSI at 1 locus; ++, MSI at 2 loci; N, negative for mutation. *The number in parentheses on the E2F4 gene indicates total number of AGC repeats observed in the E2F4 gene in each of the samples.
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