Brief report

Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis

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As many as 10% of infants with Down syndrome (DS) present with transient myeloproliferative disorder (TMD) at or shortly after birth. TMD is characterized by an abundance of blasts within the peripheral blood and liver, and notably undergoes spontaneous remission in the majority of cases. TMD may be a precursor to acute megakaryoblastic leukemia (AMKL), with an estimated 30% of TMD patients developing AMKL within 3 years. We recently reported that mutations in the transcription factor GATA1 are associated with DS-AMKL. To determine whether the acquisition of GATA1 mutations is a late event restricted to acute leukemia, we analyzed GATA1 in DNA from TMD patients. Here we report that GATA1 is mutated in the TMD blasts from every infant examined. These results demonstrate that GATA1 is likely to play a critical role in the etiology of TMD, and mutagenesis of GATA1 represents a very early event in DS myeloid leukemogenesis.

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Study design

Patient samples

Cryopreserved peripheral blood samples from infants with TMD, who were enrolled on the prospective Children’s Oncology Group (COG) trial, A2971, for children with TMD or AML, were provided by the Children’s Oncology Group. All clinical samples were obtained with informed consent and used with approval from the University of Chicago Institutional Review Board. DNA was extracted by means of standard methods.

SSCP and sequence analysis

DNA samples were screened for mutations in GATA1 by means of the single-strand polymorphism assay (SSCP) as previously described, as well as by direct sequencing of polymerase chain reaction (PCR)–amplified DNA. SSCP assays were performed on exons 2 and 3 of GATA1. Primer sequences are available upon request.

Results and discussion

DNAs extracted from peripheral blood of 7 infants with TMD were assayed for the presence of GATA1 mutations by SSCP. All...
harbored a functional alteration in sequencing of Sequencing of the excised SSCP products, as well as direct detection in DNAs from healthy individuals are similar to those detected in DS-AMKL.4 as previously demonstrated, each sample harbored a functional alteration in GATA1. In all cases, the mutations occurred within exon 2, which encodes the N-terminal transactivation domain (Figure 1B). Each of the mutations is a small insertion or deletion that alters the reading frame of GATA-1s, which leads to the production of GATA-1s.

7 patient samples displayed abnormal migrating PCR products, indicative of a mutation in GATA1 (Figure 1A, TMD samples 1-7). As previously demonstrated, DNAs from healthy individuals generated a single SSCP product (Figure 1A, controls 1-5). Sequencing of the excised SSCP products, as well as direct sequencing of GATA1 in the samples, confirmed that each sample harbored a functional alteration in GATA1. In all cases, the mutations occurred within exon 2, which encodes the N-terminal transactivation domain (Figure 1B). Each of the mutations is a small insertion or deletion that alters the reading frame of GATA-1 and introduces a premature stop codon (Table 1). These mutations are similar to those detected in DS-AMKL.4

We previously demonstrated that a short isoform of GATA-1, named GATA1s, is produced in the leukemic blasts of an individual with DS-AMKL and in the cell line CMK, which was derived from the malignant cells of a child with Down syndrome and AMKL.4 GATA1s is initiated at Met84, which lies in exon 3, downstream of each of the patient mutations (Figure 1B). GATA1s lacks the N-terminal transactivation domain and, consequently, exhibits a much lower transcriptional activation potential than wild-type GATA-1 (Wechsler et al.5). While it has not been established whether GATA1s is an oncogenic factor in these myeloid disorders, the finding that all GATA1 mutations identified to date in both DS-AMKL and TMD can potentially produce GATA1s strongly supports the hypothesis that the short isoform of GATA-1 has an active oncogenic role.

The observation that GATA1 is mutated in the abnormal cells of every TMD patient examined indicates that the gene is affected in as many as 10% of infants with Down syndrome. Furthermore, the finding that the TMD blasts from patient TMD-7, which were harvested from peripheral blood the day of birth, indicates that the acquisition of GATA1 mutations can occur in utero. It remains to be determined whether this high rate of mutation is a consequence of Down syndrome or representative of an extreme growth advantage of megakaryoblasts that harbor trisomy 21 and a truncating GATA1 mutation. While we are unable to confirm that these GATA1 mutations are not germ line, owing to the unavailability of DNA from nondiseased tissue, it is very unlikely that these GATA1 mutations are constitutional. First, the GATA1 mutations in DS-AMKL are somatically acquired.4 Second, the known rare inherited mutations within GATA1 result in chronic anemia (or thalassemia) and thrombocytopenia,5-8 a feature that is not observed in individuals with Down syndrome. Finally, GATA1 mutations were not previously identified in DNA samples from 21 healthy individuals, 75 patients with AML unrelated to DS-AMKL, or a patient with DS who had acute lymphocytic leukemia (ALL).4

Mutagenesis of GATA1, in conjunction with trisomy 21, may be sufficient to promote the transient expansion of immature megakaryoblasts seen in TMD. However, the acquisition of additional mutations or chromosomal alterations are likely to be necessary for leukemic transformation. For example, alterations in TP53 may be involved in the evolution of this malignancy, as one report described TP53 mutations in 2 of 3 patients with DS-AMKL but no mutations in 7 patients with TMD.9 A detailed study of the status of other genes that are commonly mutated in AML, such as FLT3 and RAS,10 is warranted. Furthermore, comparing the type of GATA1 mutations found in the AMKL blasts with the TMD blasts from the same individual will provide key insights into the relationship between TMD and AMKL.

**Acknowledgments**

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**Table 1. Individuals with transient myeloproliferative disorder have GATA1 mutations**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, days</th>
<th>Trisomy 21</th>
<th>Hepatomegaly</th>
<th>WBC count, in PB</th>
<th>Blasts, %</th>
<th>Mutation</th>
<th>Final GATA-1 residue†</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMD-1</td>
<td>Male</td>
<td>3</td>
<td>C</td>
<td>yes</td>
<td>116 400</td>
<td>55</td>
<td>159-160ins20</td>
<td>Ala53</td>
</tr>
<tr>
<td>TMD-2</td>
<td>Female</td>
<td>13</td>
<td>M</td>
<td>no</td>
<td>11 000</td>
<td>25</td>
<td>174-175ins14</td>
<td>Ala57</td>
</tr>
<tr>
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<td>Male</td>
<td>1</td>
<td>C</td>
<td>no</td>
<td>62 300</td>
<td>62</td>
<td>127-128ins1</td>
<td>Asp42</td>
</tr>
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<td>40 600</td>
<td>26</td>
<td>173-174ins16</td>
<td>Ala58</td>
</tr>
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<td>3</td>
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<td>164 100</td>
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<td>146-180del</td>
<td>Thr48</td>
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<td>4</td>
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<td>49</td>
<td>170-171ins14</td>
<td>Ala57</td>
</tr>
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<td>0</td>
<td>C</td>
<td>yes</td>
<td>34 400</td>
<td>64</td>
<td>205-218del</td>
<td>Ala68</td>
</tr>
</tbody>
</table>

WBC indicates white blood cell; PB, peripheral blood; C, constitutional trisomy 21; M, mosaic trisomy 21.

*Percentage of blasts in the peripheral blood.

†The final GATA-1 residue encoded in exon 2 prior to the frameshift.

2. Gamis AS, Hilden JM. Transient myeloproliferative disorder, a disorder with too few data and many unanswered questions: does it contain an important piece of the puzzle to understanding hematopoiesis and acute myelogenous leukemia? J Pediatr Hematol Oncol. 2002;24:2-5.


