Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis

Running Title: GATA1 mutations in transient myeloproliferative disorder

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Brief Report/ Neoplasia
Abstract

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 three 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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Introduction

Children with Down syndrome have a 10-20-fold increased risk of developing leukemia, in particular acute megakaryoblastic leukemia (AMKL)\(^1\). DS children are also predisposed to a related myeloid disorder, termed transient myeloproliferative disorder (TMD)\(^2\). As many as 10% of DS infants develop TMD, in which immature megakaryoblasts accumulate in the peripheral blood and liver. TMD spontaneously resolves in most cases, without therapeutic intervention. However, severe and sometimes fatal forms of TMD do occur, with hepatic fibrosis and liver dysfunction. Based on the liver infiltration and the spontaneous remission, it has been speculated that TMD may arise from fetal liver hematopoietic progenitors\(^2\). Of note, approximately 30% of DS infants with TMD develop AMKL within three years. TMD blasts are morphologically indistinguishable from those observed in AMKL, contributing to the hypothesis that the second disease is derived from the first\(^1,3\). It is likely that AMKL results from the acquisition of additional genetic mutations following remission of TMD.

We recently reported that mutations in the essential X-linked hematopoietic transcription factor gene \textit{GATA1} are tightly associated with AMKL in Down syndrome\(^4\). We detected mutations in \textit{GATA1} in six out of six DS-AMKL samples, but did not find mutations in \textit{GATA1} in leukemic cells of DS patients with other types of acute leukemia, or in other patients with AMKL who did not have DS. Furthermore, we did not detect \textit{GATA1} mutations in DNAs from over 75 other patients with acute leukemia or from 21 healthy individuals. Finally, we established that these mutations are somatically acquired, as remission samples from patients did not harbor \textit{GATA1} mutations. Based on these observations, we hypothesized that disruption of normal GATA-1 function is an essential step in the initiation or progression of megakaryoblastic leukemia in DS.
To determine whether GATA1 mutations represent a late event that contributes to the acute phase of DS myeloid leukemia, we assayed DNA samples from the peripheral blood of infants with TMD for GATA1 mutations. Here we report that GATA1 is mutated in every case of TMD examined. These findings demonstrate that the development of a GATA1 mutation is an early event in DS myeloid leukemogenesis and contributes to both TMD and AMKL.

**Patients, materials, and methods**

_Compound Samples_

Cryopreserved peripheral blood samples from infants with TMD, who were enrolled on the prospective 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 using standard methods.

**SSCP and Sequence Analysis**

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

DNAs extracted from peripheral blood of seven infants with TMD were assayed for the presence of GATA1 mutations by SSCP. All seven 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.

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. GATA-1s is initiated at Met 84, which lies in exon 3, downstream of each of the patient mutations (Figure 1B). GATA-1s lacks the N-terminal transactivation domain and, consequently exhibits a much lower transcriptional activation potential than wild-type GATA-1 (ref. 4). While it has not been established whether GATA-1s 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 GATA-1s 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 germline, due to the unavailability of DNA from non-diseased tissue, it is very unlikely that these $GATA1$ mutations are constitutional. First, the $GATA1$ mutations in DS-AMKL are somatically acquired\textsuperscript{4}. Second, the known rare inherited mutations within $GATA1$ result in chronic anemia (or thalassemia) and thrombocytopenia\textsuperscript{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, nor in a DS patient with ALL\textsuperscript{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 two of three patients with DS-AMKL, but no mutations in seven patients with TMD\textsuperscript{9}. A detailed study of the status of other genes that are commonly mutated in AML, such as $FLT3$ and $RAS$,\textsuperscript{10} is warranted. Furthermore, a
comparison of the type of \textit{GATA1} mutations found in the AMKL blasts to the TMD blasts from the same individual will provide key insights into the relationship between TMD and AMKL.

\textbf{Acknowledgments}

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References


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Table 1: Individuals with Transient Myeloproliferative Disorder have \textit{GATA1} mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (days)</th>
<th>Trisomy 21(^a)</th>
<th>Hepatomegaly</th>
<th>WBC (PB)</th>
<th>Blasts(^b) (%)</th>
<th>Mutation</th>
<th>Final GATA-1 residue(^c)</th>
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<tbody>
<tr>
<td>TMD-1</td>
<td>Male</td>
<td>3</td>
<td>C yes</td>
<td></td>
<td>116,400</td>
<td>55</td>
<td>159-160 ins 20 bp</td>
<td>Ala53</td>
</tr>
<tr>
<td>TMD-2</td>
<td>Female</td>
<td>13</td>
<td>M no</td>
<td></td>
<td>11,000</td>
<td>25</td>
<td>174-175 ins 14 bp</td>
<td>Ala57</td>
</tr>
<tr>
<td>TMD-3</td>
<td>Male</td>
<td>1</td>
<td>C no</td>
<td></td>
<td>62,300</td>
<td>62</td>
<td>127-128 ins 1 bp</td>
<td>Ala57</td>
</tr>
<tr>
<td>TMD-4</td>
<td>Male</td>
<td>2</td>
<td>C yes</td>
<td></td>
<td>40,600</td>
<td>26</td>
<td>173-174 ins 16 bp</td>
<td>Ala58</td>
</tr>
<tr>
<td>TMD-5</td>
<td>Male</td>
<td>3</td>
<td>C yes</td>
<td></td>
<td>164,100</td>
<td>68</td>
<td>146-180 del</td>
<td>Thr48</td>
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<tr>
<td>TMD-6</td>
<td>Male</td>
<td>4</td>
<td>C no</td>
<td></td>
<td>47,200</td>
<td>49</td>
<td>170-171 ins 14 bp</td>
<td>Ala57</td>
</tr>
<tr>
<td>TMD-7</td>
<td>Male</td>
<td>0</td>
<td>C yes</td>
<td></td>
<td>34,400</td>
<td>64</td>
<td>205-218 del</td>
<td>Ala68</td>
</tr>
</tbody>
</table>

\(^a\)M-Mosaic Trisomy 21; C-Constitutional Trisomy 21
\(^b\)Percentage of blasts in the peripheral blood
\(^c\)Represents the final GATA-1 residue encoded in exon 2 prior to the frameshift
Figure Legend

**Figure 1. GATA1 is mutated in TMD** (A) Single strand polymorphism (SSCP) analysis of the second exon of GATA1 revealed the presence of mutated conformational GATA1 alleles in DNA from each of seven TMD patients. In contrast, GATA1 mutations were not detected in DNAs from five healthy individuals. A longer exposure of the SSCP gel revealed a mutant allele in DNA from TMD-2 (arrow, TMD-2* lane). (B) Schematic illustration of the functional domains of GATA-1. AD, activation domain; Nf, N terminal zinc finger; Cf, C-terminal zinc finger. The asterisks indicate the positions of the mutations in the seven individuals with TMD (numbered 1 through 7). Met 84 is an alternate translation initiation codon, which leads to the production of GATA-1s.
Figure 1
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