Ecotropic Virus Integration Site-1 Gene Preferentially Expressed in Post-Myelodysplasia Acute Myeloid Leukemia: Possible Association With GATA-1, GATA-2, and Stem Cell Leukemia Gene Expression

By Junko H. Ohyashiki, Kazuma Ohyashiki, Takashi Shimamoto, Ken Kawakubo, Toshikatsu Fujimura, Shinpei Nakazawa, and Keisuke Toyama

We investigated expression of the human ecotropic virus integration site-1 (EVI1) gene in patients with leukemia and myelodysplastic syndrome (MDS) using the reverse transcriptase-polymerase chain reaction (RT-PCR) method. The EVI1 transcripts were detected in 5 (10.0%) of 50 patients with de novo acute myeloid leukemia (AML), including two AML patients with trilineage myelodysplasia, and in 8 (34.8%) of 23 patients with post-mydelyodysplastic syndrome AML (post-MDS AML). EVI1 expression was also detected in 6 (35.3%) of 17 MDS patients and three of six patients with chronic myeloid leukemia (CML) in myelomakaryoblastic crisis. No EVI1 transcripts were detected in patients with acute lymphoid leukemia (n = 15) or CML in lymphoid blast crisis (n = 4). Chromosomal abnormalities at the 3q26 region, where the EVI1 gene is located, were found in one patient with MDS and two patients with CML myelomakaryoblastic crisis who had EVI1 expression. Our results showed that EVI1 expression was frequent in patients with post-MDS AML and AML with trilineage myelodysplasia, regardless of the presence or absence of 3q26 abnormalities. EVI1 expression was accompanied by expression of GATA-1 and GATA-2, and often by stem cell leukemia (SCL) gene expression. In patients with post-MDS AML, EVI1 expression was not always associated with a 3q26 abnormality, whereas EVI1 expression in CML myelomakaryoblast crisis was often linked to a 3q26 abnormality. Our results suggest that the leukemogenic role of EVI1 expression may differ between post-MDS AML and leukemia, with EVI1 expression associated with a 3q26 abnormality.

THE GENETIC HETEROGENEITY of leukemia has been demonstrated at the molecular level.1-3 Gene activation at the transcriptional level is important in the ultimate development of cell-specific phenotypes in normal cellular differentiation and in leukemia.4-6 Expression of GATA-1 and the stem cell leukemia (SCL) gene is found in the normal development process of erythroidcytic and megakaryocytic cells,7,8 and GATA-1 may affect expression of the SCL gene. Thus, both factors may be essential to promote differentiation of these hematopoietic cells.9,10 These transcription factors bind to GATA motif in the promoter region for target genes and control their expression.9,9 In leukemia cells, expression of transcription factors in a cell-specific manner has also been confirmed.6,11 Moreover, aberration of the transcription factor caused by a specific chromosomal translocation has been linked to leukemogenesis.9,11,12 Recently, we have shown that the expression patterns of GATA-1, GATA-2, and the SCL gene, lineage-specific transcription factors, may help to define the distinct phenotype of leukemia cells.13 This indicates that the pattern of expression of transcription factors in leukemia cells correlates with the differentiation level of hematopoietic cells.

The ecotropic virus integration site-1 (EVI1) gene was identified as a gene associated with retrovirally induced myeloid leukemias in mice; it encodes a nuclear DNA-binding protein that contains two domains of Cys2His2 zinc fingers.14-17 The EVI1 gene shows a restricted pattern of expression and is not normally found in nonfractioned bone marrow or peripheral blood cells.18 The human EVI1 gene is transcriptionally activated in acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and myelodysplastic syndrome (MDS) by translocations or inversions involving the chromosomal band at 3q26, where the gene is located.19-21 In hematologic neoplasia with t(3;21) (q26;22), several genes at the 3q26 region, including the EVI1 gene, fuse the AML-1 gene resulting in novel transcripts.22,23 In AML with inv(3) (q21q26), breakpoints in a 400-kb region distal to the EVI1 locus were detected.24 These findings clearly indicate that ectopic expression of the EVI1 gene is closely related to chromosomal aberrations at the 3q26 region.

Previous studies have shown that aberrant EVI1 expression in a myeloid progenitor cell line may block granulocytic differentiation,20,22 Furthermore, inappropriate expression of EVI1 also blocks erythropoiesis by suppressing the transcription of a GATA-1 target gene.20 These findings allow us to speculate that EVI1 expression may induce a differentiation block of, at least, granulocyte and erythrocyte lineages. Thus, the EVI1 gene may play an important role in impaired hematopoesis.

To understand the possible role of transcription factors in human leukemia and to obtain further insight into the expression of transcription factors in differentiating impairment of hematopoietic cells, we analyzed EVI1 expression in various types of human leukemia, because a subset of leukemia has actually been shown to have differentiation block. We further investigated an association between EVI1 expression and expression of other transcription factors.

MATERIALS AND METHODS

Patients. We studied 115 samples from 108 consecutive patients with hematologic neoplasia: 73 adults with AML, including 23 with post-myelodysplastic syndrome AML (post-MDS AML); 17 patients from the Ministry of Education, Science, and Culture, Japan. Address reprint requests to Junko H. Ohyashiki, MD, First Department of Internal Medicine, Tokyo Medical College, Tokyo; and the Department of Pediatrics, Yamanashi Medical School, Yamanashi, Japan.

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with MDS; 15 patients with acute lymphoblastic leukemia (ALL); and 10 patients with CML in blast crisis (CML-BC). Seven patients with MDS were analyzed at both MDS and AML phases. One patient with MDS had t(3;4)(q26;q21), and two patients with CML-BC had inv(3)(q21q26) and t(3; 12;2O)(q26;q12;q13), respectively, in addition to the Philadelphia translocation.27,28

Diagnosis of de novo AML was performed using the French-American-British (FAB) criteria.29 AML with trilineage myelodysplasia (AML-TMDS) was identified based on the previously described criteria. Diagnosis of AML-TMDS was made in two patients of 43 assessable de novo AML. Patients with post-MDS AML were identified based on the FAB criteria for acute leukemia; when greater than 30% of blasts were detected in the bone marrow or peripheral blood, transformation of MDS was considered to have occurred. All bone marrow and peripheral blood samples were acquired after informed consent was obtained from patients. We used two cell lines as controls: YS9;22, a myeloid cell line with dic(3;3)(q26;~12),“ and one patient with t(3;4)(q26;q21). Of the other three patients, one patient with t(3;4)(q26;q21) was included in a group of patients whose disease characteristics were similar to those of the other two patients. Of the remaining two patients, one patient had M1, M2, and M4 morphology (Table 2). In contrast, EVII transcripts were detected in 8 (34.8%) of the 23 patients with post-MDS AML, including one patient with t(3;4)(q26;q21). Of 17 samples from MDS patients, six (35.3%) had EVII expression; they consisted of three patients with refractory anemia (RA) and three

### RESULTS

Frequency of EVII expression in human leukemia cells.

None of the bone marrow cells obtained from healthy volunteers showed EVII expression, confirming that EVII gene is not expressed in nonfractionated normal bone marrow cells. Among 50 patients with de novo AML, only five patients (10.0%) had EVII expression; however, none showed a detectable chromosomal change at 3q26. Two of the five AML patients showing EVII expression had AML-TMDS, and the other three patients had M1, M2, and M4 morphology (Table 2). In contrast, EVII transcripts were detected in 8 (34.8%) of the 23 patients with post-MDS AML, including one patient with t(3;4)(q26;q21). Of 17 samples from MDS patients, six (35.3%) had EVII expression; they consisted of three patients with refractory anemia (RA) and three
with RA with excess of blasts (RAEB). The incidence of MDS patients with EVII expression did not depend on the diagnosis of MDS, because 37.5% of RA and 42.9% of MDS patients with EVII expression did not show EVII expression at the MDS and AML phases; however, the other three patients (42.9%) had EVII expression both before and after leukemic transformation. No MDS patients who showed appearance or disappearance of EVII expression were associated with disease progression of MDS. EVII transcripts were also detected in three of six patients with CML myeloidemakaryoblastic crisis, two of whom had chromosomal changes at 3q26, that is, inv(3)(q21q26) and t(3;12;20)(q26;q12;q13). In contrast, four patients with CML lymphoid blast crisis did not have EVII transcripts under the RT-PCR condition. Similarly, none of the 15 patients with ALL had EVII expression (Table 2).

Characteristics of leukemia cells with EVII expression. We focused on 16 leukemia patients with EVII expression shown that GATA-I, GATA-2, and SCL expression is re-

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Age/Sex</th>
<th>Sample (%)</th>
<th>Dysmeg</th>
<th>CD41</th>
<th>CD42</th>
<th>GATA-1</th>
<th>GATA-2</th>
<th>SCL</th>
<th>Cytochemistry [no. of cells]</th>
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<tbody>
<tr>
<td>1</td>
<td>AML-M1</td>
<td>67/F</td>
<td>PB (94)</td>
<td>-</td>
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<td>+</td>
<td>+</td>
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<td>-</td>
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<td>2</td>
<td>AML-M2</td>
<td>54/F</td>
<td>BM (58)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>3</td>
<td>AML-M2*</td>
<td>24/F</td>
<td>PB (31)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td>4</td>
<td>AML-M4</td>
<td>45/F</td>
<td>BM (96)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,XX,del(11)(q23)[20]</td>
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<td>AML-M6*</td>
<td>56/M</td>
<td>BM (90)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>46,XY[20]</td>
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<tr>
<td>6</td>
<td>MDS-RAEB</td>
<td>53/M</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,XY,[t(3;4)(q26;q21)][21]/46,XY[11]</td>
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<td>7</td>
<td>Post-MDS AML</td>
<td>31/M</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,idem[20]</td>
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<tr>
<td>8</td>
<td>MDS-RAEB</td>
<td>67/M</td>
<td>PB (46)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,XY dup(1)[q24][28]/29, idemx2(8)/184, idemx4[1]</td>
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<tr>
<td>9</td>
<td>Post-MDS AML</td>
<td>72/F</td>
<td>BM (70)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>43-X,Y,t(3;4)(p21;q22),hur(6)(q27)add(6)(q27),-7,hsr(11)(q23)add(11)(q23),-20,add(22)(p11)[20]</td>
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<td>+</td>
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<td>BM (76)</td>
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<td>+</td>
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<td>Post-MDS AML</td>
<td>75/M</td>
<td>PB (68)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>PB (71)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>PB (41)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>PB (54)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>46,XX,t(9;22)(q34;q22)[19]</td>
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<tr>
<td>16</td>
<td>CML-MyMeg</td>
<td>40/M</td>
<td>PB (42)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>48,XY,t(9;22)(q34;q22),t(3;12;20)[q26;q12;q13][20]</td>
</tr>
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Abbreviations: Dysmeg, dysmegakaryopoiesis; MyMeg-BC, myeloidemakaryoblastic crisis; PB, peripheral blood; BM, bone marrow.
* De novo AML with trilineage dysplasia.
† Blasts were positive for platelet peroxidase.
‡ No EVII expression.
§ EVII expression-positive.
GATA-1 and SCL genes are commonly expressed in AML-M6 and -M7, and also in leukemias bearing the platelet-associated antigens.13 We also found that AML cells with GATA-1, but not SCL, expression may be derived from early myeloid progenitors.13 To determine an association between EVII expression and other lineage-specific transcription factors, we also examined GATA-1, GATA-2, and SCL expression in the 16 patients with EVII expression (Table 3 and Fig 1).

GATA-1 transcripts were detectable in all patients with EVII expression and in YS9;22 cells, but no GATA-1 message was apparent in normal bone marrow cells, HAL-01 cells, and ALL cells, indicating that the GATA-1 message likely represents characteristics of leukemia cells rather than a contaminating erythroid component. Expression of GATA-2 was usually associated with GATA-1 expression, except for one AML-M4 patient (case 4), although GATA-2 expression was also seen in normal bone marrow cells and was widely detected in leukemia cells with a nonlymphoid nature, as reported previously.13 Expression of SCL was not seen in cells obtained from normal bone marrow. SCL transcripts were detected in 11 of the 16 patients with EVII expression and most of them showed dysmegakaryopoiesis, the presence of platelet-associated antigens, or both. In the present study, we detected eight patients with SCL expression in 73 AML patients examined, and all of them had EVII transcripts. Patient 4 had SCL expression without any evidence of megakaryocytic or erythrocytic lineage involvement. Of the 60 AML patients without EVII expression, we detected GATA-1 expression in 31 (51.7%), GATA-2 in 53 (88.3%), and SCL in only one patient (1.7%). These findings indicate that EVII expression is always associated with GATA-1 expression and is often associated with SCL expression; 67% of AML and 100% of CML-BC patients expressing EVII had SCL transcripts (Table 3).

**DISCUSSION**

Acute leukemia may result from an impairment of cellular differentiation. A previous study showed that expression of EVII blocks myeloid differentiation in response to granulocyte colony-stimulating factor,15 suggesting that EVII expression may play a role in cellular differentiation in AML cells. Although the biologic characteristics of de novo AML have been extensively investigated, little is known about post-MDS AML. In de novo AML, the impairment of cellular differentiation is usually restricted to granulocytic or monocytic series; impaired erythropoiesis and megakaryopoiesis are uncommon. In contrast, the impairment of differentiation in multilineage hematopoiesis is a characteristic feature in post-MDS AML and AML-TMDS. In the current study, EVII expression was frequently observed in patients with MDS, post-MDS AML, and AML-TMDS. Regardless of detectable chromosomal changes at 3q26, Russell et al19 also reported that 14% of 23 MDS patients expressed the EVII gene. We did not perform pulse-field gel electrophoresis using the EVII genomic probe. We performed Southern blot analysis. However, EcoRI- and BamHI-digested DNA from the 16 patients with EVII expression and YS9;22 cells did not show any rearrangements. Therefore, it is still unclear
whether or not ectopic EVII expression in our patients without 3q26 abnormalities resulted from rearrangements around the EVII locus. However, chromosomal abnormality at 3q26 is not frequently detected in MDS. Thus, it is natural to consider that EVII expression in our patients may not be related to rearrangements around the EVII gene. The high incidence of ectopic EVII expression might represent multilineage impaired hematopoiesis, because patients with EVII expression were clustered in post-MDS and AML-TMDS; this finding was also noted in MDS patients. Moreover, in some MDS patients, EVII expression was not related to disease progression, which allows the conclusion that EVII expression without detectable 3q26 abnormalities represents impaired hematopoiesis.

EVII expression was frequently associated with the expression of erythrocyte- and megakaryocyte-specific transcription factors, namely, GATA-1, GATA-2, and SCL. Kreider et al reported that impaired erythropoiesis may result from inappropriate expression of the EVII gene, because EVII expression may block erythropoiesis by repressing the transcription of a subset of GATA-1 target genes. Delwel et al reported that four of the seven zinc fingers of the EVII gene are required for sequence-specific binding to GATCA/GATAA sequence. More recently, it has been suggested that GATA-1 normally suppresses GATA-2 expression during erythropoiesis and that SCL regulates GATA-1 expression in erythrocytic differentiation. The coordination of several transcription factors thus may play a key role in the development of normal hematopoiesis. In contrast, most leukemia patients with EVII expression coexpressed the GATA-1 and GATA-2 and often the SCL gene. Inappropriate expression of these transcription factors may represent the neoplastic nature of hematopoietic cells and may be linked to inappropriate differentiation of hematopoietic cells.

We have proposed that telomeres, the repeated sequence of the chromosome terminus, shorten as a result of genomic instability in MDS patients. Genomic instability may induce not only deletion or mutation of certain gene(s) but also alteration of transcriptional activation of certain genes. In contrast, a high incidence of EVII expression in patients with CML in myelomegakaryoblast crisis may reflect chromosomal instability that is associated with disease progression rather than genomic instability. EVII expression in CML is usually associated with 3q26 changes found as additional chromosomal abnormalities. Thus, EVII expression may contribute to disease evolution in a subset of CML with 3q26 abnormalities, although it is still uncertain why EVII expression is restricted to hematologic neoplasias expressing myeloidmegakaryoblastic phenotypes.

Proliferation and differentiation of hematopoietic cells is regulated by coordination of many genes. Our results show that the EVII gene is preferentially expressed in leukemia with impaired hematopoiesis in two or more lineages. EVII expression is also associated with GATA-1, GATA-2, or SCL expression, and inappropriate expression of these transcription factors may represent multilineage impairment of hematopoietic cells. Based on our results, we consider that the EVII gene may be activated by at least two pathways. One pathway is related to 3q26 abnormalities and expression of megakaryocytic phenotype, whereas the other is linked to multilineage impairment of hematopoietic cells, which usually do not show 3q26 abnormalities.

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Ecotropic virus integration site-1 gene preferentially expressed in post-myelodysplasia acute myeloid leukemia: possible association with GATA-1, GATA-2, and stem cell leukemia gene expression

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