FAS Promoter Polymorphism: Outcome of Childhood Acute Myeloid Leukemia. A Children’s Oncology Group Report

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Abstract

Purpose: FAS is a cell surface receptor involved in apoptotic signal transmission. Deregulation of this pathway results in down-regulation of apoptosis and subsequent persistence of a malignant clone. A single nucleotide polymorphism resulting in guanine-to-adenine transition in the FAS promoter region (position -1377) is thought to reduce stimulatory protein 1 transcription factor binding and decrease FAS expression. Previous work has shown increased risk of developing acute myeloid leukemia (AML) in adult patients with a variant allele at this site. The same authors have shown that the presence of an adenine residue rather than a guanine residue at -1,377 bp significantly attenuates transcription factor stimulatory protein 1 binding and may contribute to a reduction in FAS expression and ultimately to the enrichment of apoptosis-resistant clones in AML. We hypothesized that FAS genotype by altering susceptibility to apoptosis might affect outcome of childhood AML therapy.

Experimental Design: Four hundred forty-four children treated for de novo AML on a uniform protocol were genotyped for FAS 1377.

Results: There were no significant differences in overall survival, event-free survival, treatment-related mortality, or relapse rate between patients with FAS 1377GG genotype versus 1377GA/1377AA genotypes.

Conclusions: FAS 1377 genotype does not alter outcome of de novo AML in children.

Human FAS (TNFRSF6/CD95/APO-1) protein is a cell surface receptor, belonging to the family of tumor necrosis factor receptors, and is involved in apoptotic signal transmission (1, 2). It is a 48-kDa type I membrane glycoprotein composed of three domains: an extracellular domain comprising three cysteine-rich motif subdomains characteristic of the superfamily, a transmembrane domain, and a highly conserved intracellular domain known as a death domain (3). Binding to the receptor by the FAS ligand (CD95L) triggers receptor trimerization and subsequent assembly of the death-inducing signaling complex (4, 5). Germ-line mutations or deletions within FAS, resulting in a loss or a reduction in receptor function, have been shown to cause autoimmune lymphoproliferative syndrome as well as an overall increased risk of hematologic malignancies (6). Dysregulation of this pathway is believed to result in down-regulation of apoptosis, allowing subsequent persistence of a malignant clone.

FAS expression levels may also be affected by mutations or polymorphisms in the promoter region of FAS, particularly when they affect the transcription binding sites. A single nucleotide polymorphism resulting in guanine-to-adenine transition in the FAS promoter region occurs at position -1,377, affecting a stimulatory protein 1 transcription factor binding site. An adenine residue at this position significantly reduces stimulatory protein 1 binding compared with guanine residue, causing a decrease in FAS expression. Functional germ-line and somatic mutations in the FAS gene and perhaps also in the FAS ligand gene that lead to decreased expression of FAS and/or increased expression of FAS ligand favors malignant transformation and progression (4) by impairing apoptotic signal transduction and are associated with an increased risk of cancer (7–10). A recent case-control study in adults indicated increased risk of developing acute myeloid leukemia (AML) in patients with variant allele (A) at this site (11). In addition, reduced expression of Fas-associated protein with death domain, the main adaptor for transmission of FAS signaling, is associated with decreased response to chemotherapy in AML cells (12). We hypothesized that FAS -1377 polymorphism by altering susceptibility to apoptosis might affect outcome of AML therapy.
Translational Relevance

Available response of children to chemotherapy for AML remains a challenge to the cure of this difficult disease. The development of personalized medicine allows access to potential avenues to the development of the individualized therapy and optimize outcomes for each individual child. In this study, we looked at a polymorphic locus and its influence on outcome and describe the relevance of variation at the FAS locus to chemotherapy response for childhood AML. In the future, we anticipate that chemotherapy regimens will be individualized to the patient’s genotype. We anticipate that further candidate studies such as the one presented here will help individualize therapy for children with AML.

Patients and Methods

Patients. The study population included 440 children with de novo AML treated on Children’s Cancer Group (CCG) therapeutic studies CCG-2941 (n = 36) and CCG-2961 (n = 404) between 1995 and 2002. Clinical data, including age, sex, WBC at diagnosis, race, presence of chloroma, presence of central nervous system disease, and immunophenotype, were collected prospectively (Table 1). Cases were classified based on criteria established and revised by the French-American-British Cooperative Study Group by central pathology review. All French-American-British categories except acute promyelocytic leukemia (AML M3) were eligible for enrollment and were treated with the same chemotherapy regimens.

Chemotherapy treatment regimen. CCG-2961 study was a randomized phase III trial of intensively timed induction, consolidation, and intensification therapy for pediatric patients with previously untreated AML or MDS (13). The study was conducted between August 1996 and December 2002. CCG-2941 was a feasibility pilot of the same chemotherapy regimen that preceded the randomized study. Induction included five drugs (idarubicin, etoposide, dexamethasone, cytarabine, and 6-thioguanine) given on days 0 to 3 followed by five drugs (daunorubicin, etoposide, dexamethasone, cytarabine, and 6-thioguanine) given on days 10 to 13 (14). On recovery of WBC and platelet counts, patients were randomly assigned to consolidation therapy consisting of the same sequence of drugs or to fludarabine/cytarabine/idarubicin. Intrathecal cytarabine was used for central nervous system prophylaxis. Patients with matched-related donors were assigned to allogeneic marrow transplant intensification. Pretransplant cytoreduction was busulfan and cyclophosphamide. Patients without a related donor received high-dose cytarabine/l-asparaginase (Capizzi II) and additional intrathecal cytarabine. After recovery from chemotherapy, patients were randomized again to either receive interleukin-2 or standard follow-up care. Transplanted patients were not eligible for randomization to interleukin-2.

FAS genotyping. DNA extracted from diagnostic marrow samples using standard methods was normalized to 10 ng/μL. Primers were synthesized based on the sequence of the Apo-1/FAS gene reported in GenBank (X87625).

For each sample testing, two PCRs in different wells were done; one reaction detected the wild-type allele and the other detected the mutant allele using mismatch amplification assay coupled with real-time PCR (MAMA assay). This assay is based on the concept that two mismatched nucleotides instead of a single mismatch at the 3' end of the primer effectively abrogates PCR amplification efficiency. For each reaction, a 20 ng DNA template was added to the reaction mixture containing a final concentration of 0.2 μmol/L of each primer-specific forward primer (F1 or F2) and the common reverse primer and SYBR Green Master Mix (Applied Biosystems). Reverse transcription-PCR was done using an ABI 7700 Thermocycler for 40 cycles, with annealing temperature of 62°C and a total of 50 μL reaction volume.

DNA from normal controls was extracted using standard techniques and genotyped as described for cases. Genotyping results were duplicated in 10% of samples; concordance between repeats was 100%. Furthermore, 10% of the samples were also genotyped using direct sequencing; concordance with MAMA genotyping was 100%.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GG (n = 347), n (%)</th>
<th>GA (n = 51), n (%)</th>
<th>AA (n = 12), n (%)</th>
<th>GG vs GA, P</th>
<th>GA vs AA, P</th>
<th>GG vs AA, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median and range (y)</td>
<td>9.4 (0.01-20.9)</td>
<td>11.5 (0.47-19.0)</td>
<td>10.1 (1.64-16.2)</td>
<td>0.12</td>
<td>0.99</td>
<td>0.50</td>
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<tr>
<td>WBC, median and range</td>
<td>20,600 (1,000-860,000)</td>
<td>18,400 (300-373,300)</td>
<td>17,700 (1,500-848,000)</td>
<td>0.67</td>
<td>0.52</td>
<td>0.38</td>
</tr>
<tr>
<td>Study</td>
<td>CCG-2941</td>
<td>30 (9)</td>
<td>6 (7)</td>
<td>0 (0)</td>
<td>0.89</td>
<td>1.00</td>
</tr>
<tr>
<td>Gender</td>
<td>CCG-2961</td>
<td>317 (91)</td>
<td>75 (93)</td>
<td>12 (100)</td>
<td></td>
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<tr>
<td>Male</td>
<td>189 (55)</td>
<td>49 (61)</td>
<td>6 (50)</td>
<td>0.39</td>
<td>0.54</td>
<td>0.99</td>
</tr>
<tr>
<td>Female</td>
<td>158 (45)</td>
<td>32 (39)</td>
<td>6 (50)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Race</td>
<td>White</td>
<td>246 (71)</td>
<td>54 (67)</td>
<td>6 (50)</td>
<td>0.52</td>
<td>0.34</td>
</tr>
<tr>
<td>Black</td>
<td>31 (9)</td>
<td>4 (5)</td>
<td>1 (8)</td>
<td>0.34</td>
<td>0.51</td>
<td>1.00</td>
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<tr>
<td>Hispanic</td>
<td>49 (14)</td>
<td>18 (22)</td>
<td>1 (8)</td>
<td>0.10</td>
<td>0.45</td>
<td>1.00</td>
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<tr>
<td>Asian</td>
<td>5 (1)</td>
<td>4 (5)</td>
<td>4 (33)</td>
<td>0.07</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Other</td>
<td>15 (4)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0.33</td>
<td>1.00</td>
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<td>Unknown</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Response at end of first course</td>
<td>Remission</td>
<td>290 (87)</td>
<td>71 (90)</td>
<td>12 (100)</td>
<td>0.55</td>
<td>0.59</td>
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<tr>
<td>Progressive disease</td>
<td>23 (7)</td>
<td>7 (9)</td>
<td>0 (0)</td>
<td>0.71</td>
<td>0.59</td>
<td>1.00</td>
</tr>
<tr>
<td>Death</td>
<td>22 (7)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0.10</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Withdrawal or unevaluable</td>
<td>12</td>
<td>2</td>
<td>0</td>
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</table>
Statistical analysis. Data were analyzed from CCG-2941 and CCG-2961 through April 2005 and October 2006, respectively. The significance of observed differences in proportions was tested using the χ2 test and Fisher’s exact test when data were sparse. The Mann-Whitney test was used to determine the significance between differences in medians (15). The Kaplan-Meier method was used to calculate estimates of overall survival, event-free survival, and disease-free survival (16). Estimates are reported with their Greenwood SEs (17). Differences in these estimates were tested for significance using the log-rank statistic (18). Overall survival is defined as time from study entry to death from any cause. Event-free survival is defined as time from study entry to failure at the end of two courses, relapse or death from any cause. Disease-free survival is defined as time from the end of one course of therapy to failure at the end of two courses, relapse or death from any cause. Cumulative incidence estimates were used to determine relapse rate and treatment-related mortality. Relapse rate is defined as time from the end of one course of therapy to failure at the end of two courses, relapse or death from progressive disease where deaths from nonprogressive disease were competing events. Treatment-related mortality is defined as time from study entry to death from nonprogressive disease where failures at the end of two courses, relapses and deaths from progressive disease, were competing events. Differences between relapse rate and treatment-related mortality estimates were tested for significance using Gray’s test (19). Children lost to follow-up were censored at their date of last known contact or at a cutoff 6 months before April 2005 (CCG-2941) or October 2006 (CCG-2961). Cox regression was used for multivariate models that looked at differences between groups adjusting for study assignment, age, gender, race, and WBC. Reported P values represent comparisons between patients with FAS 1377GG genotype versus those with FAS 1377GA or 1377AA genotypes.

Results

FAS genotype and outcome. There were no significant differences in overall survival from study entry between patients with FAS 1377GG, 1377GA, or 1377AA genotypes (50 ± 6% versus 57 ± 11% versus 75 ± 25%, respectively at 5 years; P = 0.21, log-rank). Although point estimate for overall survival for AA genotype was 75% compared with 50% for GG genotype, the AA group included only small number of patients (n = 12; Fig. 1; Table 2). Analysis of event-free survival from study entry in different genotypes also showed similar estimates, 40 ± 5% in FAS 1377GG versus 44 ± 11% in 1377GA versus 67 ± 27% in 1377AA patients at 5 years (P = 0.36, log-rank).

There was no difference in treatment-related mortality from study entry between different genotypes (18 ± 4% in GG versus 14 ± 8% in GA versus 17 ± 22% in AA patients) at 5 years (P = 0.42). Relapse rates from end of one course of therapy for patients in remission were also similar: 41 ± 6% in FAS 1377GG versus 38 ± 12% in 1377GA versus 19 ± 24% in 1377AA patients at 5 years (P = 0.47).

Multivariate analyses that adjusted for study assignment, age, gender, race, and WBC also suggested that FAS genotype did not modify overall survival or event-free survival. Thus, FAS genotype was not significantly associated with either resistant disease or treatment-related toxicity.

Discussion

In this pediatric study, we found that FAS 1377 genotype does not alter outcome of de novo AML in children. Role of FAS variants in leukemogenesis is supported by studies that report increased risk of lymphoproliferative disease and certain hematologic malignancies associated with defects in the FAS pathway. Down-regulation of apoptosis prolongs the normal cellular lifespan, allows cells to acquire mutations, and facilitates tumor progression; for example, BCL2 transgenic mice with targeted expression in myeloid cells develop myeloproliferation similar to human chronic myelomonocytic leukemia; however, they rarely go on to develop acute leukemia. Interestingly, when mice constitutively expressing BCL2 are crossed onto a FAS−/− background, ~15% develop AML, implicating FAS-mediated apoptosis in the pathogenesis of AML (20).

In humans, germ-line mutations or deletions within FAS have been shown to cause autoimmune lymphoproliferative syndrome, a condition associated with generalized lymphoproliferation and systemic autoimmunity, as well as an overall increased risk of hematologic malignancies (6). Also, aberrant apoptosis is known to be important in the

<table>
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<tr>
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<th>FAS 1377GG (%)</th>
<th>FAS 1377GA (%)</th>
<th>FAS 1377AA (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 y overall survival</td>
<td>50 ± 6</td>
<td>57 ± 11</td>
<td>75 ± 25</td>
<td>0.21</td>
</tr>
<tr>
<td>5 y event-free survival</td>
<td>40 ± 5</td>
<td>44 ± 11</td>
<td>67 ± 27</td>
<td>0.36</td>
</tr>
<tr>
<td>5 y disease-free survival</td>
<td>46 ± 6</td>
<td>50 ± 12</td>
<td>67 ± 27</td>
<td>0.63</td>
</tr>
<tr>
<td>5 y treatment-related mortality</td>
<td>18 ± 4</td>
<td>14 ± 8</td>
<td>17 ± 22</td>
<td>0.42</td>
</tr>
<tr>
<td>5 y relapse rate</td>
<td>41 ± 6</td>
<td>38 ± 12</td>
<td>19 ± 24</td>
<td>0.47</td>
</tr>
</tbody>
</table>
pathogenesis of AML (6) because up-regulation of the antiapoptotic protein BCL2 is a common feature of leukemic blasts and may be a critical event in myeloid transformation (21). Myeloblasts are known to express high levels of FAS, and functional deficiencies of FAS signaling have been shown to be important in several subtypes of AML, providing further evidence for FAS-mediated apoptosis in the etiology of AML (22).

These same mechanisms potentially make these AML cells more resistant to therapy. As shown by Sibley et al. (11), the presence of an adenine residue rather than a guanine residue at -1,377 bp significantly attenuates transcription factor stimulatory protein 1 binding and may contribute to a reduction in FAS expression and ultimately to the enrichment of apoptosis-resistant clones in AML. However, our study did not show significant effect of FAS genotype on outcome of childhood AML. It is possible that this FAS variant may be of functional importance in children with AML when combined with polymorphic alleles of other genes in the apoptosis pathway. Mechanistic studies further defining the functionality of FAS polymorphisms will help clarify the importance of variants at this site.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**

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