Microvessel injury is associated with the development of graft-versus-host disease (GVHD), whereas high levels of posttransplantation vascular endothelial growth factor (VEGF) have a protective effect on severe acute GVHD (aGVHD) and transplantation-related mortality. The current study aimed to determine the impact of VEGFA gene single-nucleotide polymorphisms (SNPs) on the risk of aGVHD after allogeneic stem cell transplantation (SCT). Using polymerase chain reaction and restriction fragment length polymorphism, 4 VEGFA SNPs—-2578 C>A (rs699947), -460 T>C (rs833061), +405 G>C (rs2010963), and +936 C>T (rs3025039)—were analyzed in 98 recipients. Strong linkage disequilibrium was noted among loci -2578, -460, and 1405, but not among these loci and locus +936. Accordingly, 4 haplotypes were generated based on the genotypes of -2578, -460, and +405: CTC (47.9%), CTG (26.7%), ACG (24.2%), and CCC (1.0%). The group with low VEGF production (ie, +936CT genotype and 2 copies of the ACG haplotype) had a higher incidence of aGVHD. Significant associations were found between the risk of grade 2-4 aGVHD and the +936 CT (P = .006), -2578 AA (P = .003), and -460 CC (P = .002) genotypes and the ACG haplotype (P = .003). No association between the VEGFA SNPs and chronic GVHD was observed. The VEGFA SNPs might predict a lower risk of aGVHD. Our findings suggest that VEGF may have a protective role in the pathogenesis of aGVHD.

**INTRODUCTION**

The pathogenesis of graft-versus-host disease (GVHD) has yet to be fully elucidated, although it is generally accepted that alloreactive T cell cytotoxicity is a central mediator. Alloreactive T cells recognize the recipients’ target tissues as nonself and evoke GVHD. The final step in the development of GVHD occurs in targeted tissues, in which inflammation develops due to interactions between these tissues and cytotoxic T cells. It has been suggested that the endothelium is targeted during GVHD and that microvessel injury is a consequence of perivascular inflammation and endothelial cell death, which results in progressive microvessel loss and consequent tissue ischemia and stimulates the production of VEGF [1]. Accordingly, angiogenesis also is involved in the pathogenesis of GVHD.
VEGF, a soluble 34- to 46-kDa heparin-binding glycoprotein dimer, is a potent angiogenic peptide with diverse biological activities that include angiogenesis in both physiological and pathological situations [2]. VEGF gene (VEGFA) expression is regulated by various growth factors, cytokines, and hormones, as well as by hypoxia [3]. VEGF can be produced by numerous cells, including lymphocytes, macrophages, vascular smooth muscle cells, fibroblasts, keratinocytes, megakaryocytes, neutrophils, basophils, and mast cells. Moreover, previous investigations have suggested that type 2 cytokine stimulates VEGF production [4,5].

Interestingly, higher VEGF levels at day 14 or 15 posttransplantation have been suggested to protect against the development of severe GVHD [4,6]. The first study to investigate this concept found an association between high VEGF levels and a lower incidence of nonrelapse-related mortality (NRM) (23% vs 4%), along with an inverse correlation between VEGF levels at day 14 posttransplantation with the severity of aGVHD [4]. Moreover, patients with severe grade 3-4 aGVHD had significantly lower log-transformed VEGF levels than those with or without grade 1-2 aGVHD [4]. Another study similarly reported improved survival in patients with higher VEGF levels at day 15 posttransplantation [6]. These 2 studies suggest that VEGF protects against severe aGVHD.

Recent investigations have demonstrated that VEGF polymorphisms contribute to interindividual variations in VEGF expression. The VEGFA gene is located on chromosome 6p21 and consists of 8 exons and 7 introns [7,8]. Furthermore, polymorphisms in its promoter region (loci -2578C>A [rs699947] and -460T>C [rs833061]), its 5'-untranslated region (+405C>G [rs2010963]) and its 3'-untranslated region (+936C>G [rs2010963]) have been associated with different levels of VEGF expression [9-14]. Accordingly, in the present study, we investigated the impact of VEGFA polymorphisms on the development of aGVHD on outcome after allogeneic stem cell transplantation (SCT).

MATERIALS AND METHODS

The objective of the present study was to investigate an association between VEGFA polymorphisms and the development of aGVHD or cGVHD after allogeneic SCT.

Patient Characteristics and Transplantation Procedure

Ninety-eight consecutive patients who had received an HLA-matched sibling transplant at the Kyungpook National University Hospital, Daegu, Korea between August 1998 and June 2005 were included in this retrospective study. Detailed information is provided in Table 1. The conditioning regimens consisted of busulfan/cyclophosphamide (n = 57; 58%), fludarabine-based regimens (n = 31; 32%), and cyclophosphamide/antithymocyte globulin (ATG) (n = 10; 10%). All 98 patients received peripheral blood stem cells (PBSCs), as described previously [15]. GVHD prophylaxis included cyclosporin A (CSA) plus methotrexate (MTX) in 86 patients (88%) and CSA alone or FK506/MTX in 6 patients each (6%/6%). Treatment for aGVHD and cGVHD was provided according to a standard protocol, as described previously [16].

Genotyping of VEGFA and Genotype Analysis

For VEGFA genotyping, genomic DNA was extracted from peripheral blood using the Wizard genomic DNA purification kit (Promega, Madison, WI). The VEGF -2578C>A (rs699947), -460T>C (rs833061), +405C>G (rs2010963), and +936C>G (rs3025039) genotypes were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism, as described previously [17-20]. To confirm genotyping results, selected PCR-amplified DNA samples (n = 2 for each genotype) were examined by DNA sequencing [17]. The study design was approved by the Kyungpook National University Hospital Institutional Research Board and confirmed

Table 1. Patient Characteristics and Transplantation Procedures

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of pts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipients</td>
<td></td>
</tr>
<tr>
<td>Sex, female/male, n (%)</td>
<td>34/64 (35/65)</td>
</tr>
<tr>
<td>Age, years, median (range)</td>
<td>33 (16 to 58)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>AML/ALL</td>
<td>50/11 (51/11)</td>
</tr>
<tr>
<td>CML/MDS</td>
<td>14/4 (14/4)</td>
</tr>
<tr>
<td>SAA/NHL</td>
<td>10/8 (10/8)</td>
</tr>
<tr>
<td>Solid tumor*</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Advanced disease</td>
<td>48 (49)</td>
</tr>
<tr>
<td>Donors</td>
<td></td>
</tr>
<tr>
<td>Sex, female/male, n (%)</td>
<td>34/64 (35/65)</td>
</tr>
<tr>
<td>Age, years, median (range)</td>
<td>34 (15 to 65)</td>
</tr>
<tr>
<td>Conditioning, n (%)</td>
<td></td>
</tr>
<tr>
<td>BuCy</td>
<td>57 (58)</td>
</tr>
<tr>
<td>CyATG</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Fludarabine-based RIST</td>
<td>31 (32)</td>
</tr>
<tr>
<td>Infused cell</td>
<td></td>
</tr>
<tr>
<td>dose, median MNCs, 10^6/kg</td>
<td>6.75</td>
</tr>
<tr>
<td>CD34+ cells, 10^6/kg</td>
<td>6.32</td>
</tr>
<tr>
<td>CD3+ cells, 10^6/kg</td>
<td>1.97</td>
</tr>
<tr>
<td>GVHD prophylaxis, n (%)</td>
<td></td>
</tr>
<tr>
<td>CSA/MTX</td>
<td>86 (88)</td>
</tr>
<tr>
<td>CSA</td>
<td>6 (6)</td>
</tr>
<tr>
<td>FK506/MTX</td>
<td>6 (6)</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; SAA, severe aplastic anemia; NHL, non-Hodgkin lymphoma; BuCy, busulfan/cyclophosphamide; CyATG, cyclophosphamide/ATG; RIST, reduced-intensity conditioning stem cell transplantation; MNC, mononuclear cell; sMTX, short-term MTX. *Metastatic colorectal carcinoma.
to the Helsinki Declaration. Each patient provided written informed consent.

Four genotypes were evaluated using the $\chi^2$ test to determine whether they conformed with the Hardy-Weinberg equilibrium (HWE). Genotype frequencies were determined using Haplovlew software (available at http://www.broad.mit.edu/mpg/haplovlew). Additive, dominant, and recessive models were used to investigate associations between each single-nucleotide polymorphism (SNP) and transplantation outcomes. Haplotype analysis for deviation from the HWE was conducted, and haplotype frequencies were estimated using linkage disequilibrium (LD) coefficients, $D'$. Individual haplotypes were determined with a Bayesian algorithm using the Phase program (available at http://www.stat.washington.edu/stephens/phase.html) [21].

As in our previous study [20], here a VEGF risk score model was generated based on the genotype at locus $+936$ and the copy number of the ACG haplotype at loci $-2578/-405/+460$. A score of 1 was assigned to risk alleles (ie, $+936$ CT or TT genotypes, or 2 copies of the ACG haplotype), and a score of 0 was assigned to other alleles (ie, the $+936$ CC genotype, or 0 or 1 copy of the ACG haplotype). The scores were summed, and 2 risk groups were defined: high risk (composite score, 2 or 1) and low risk (composite score, 0).

**Definition and Endpoints**

The day of stem cell infusion was defined as day 0. Engraftment was confirmed by peripheral blood counts, that is, a peripheral absolute neutrophil count of $> 0.5 \times 10^9/L$ and a peripheral platelet count of $> 20 \times 10^9/L$ for at least 3 consecutive days without requiring transfusion. Overall survival (OS) was defined as the time from transplantation until death from any cause. aGVHD and cGVHD were diagnosed and graded based on established criteria [22,23].

**Statistical Analysis**

The data were analyzed according to information available on July 2005. The clinical characteristics and transplantation outcomes of patients were compared using the $\chi^2$ test, Fisher’s exact test, or the Mann-Whitney $U$ test for different $VEGFA$ genotypes.

Probabilities of OS were calculated and plotted using the Kaplan-Meier method. The incidences of aGVHD, cGVHD, NRM, and recurrence were estimated using the cumulative incidence method considering competing risks [24]. During single-marker analyses, the OSs of $VEGFA$ SNPs were compared using additive, dominant, and recessive models through the log-rank test, whereas the incidences of aGVHD, cGVHD, NRM, and recurrence for different $VEGFA$ SNPs were compared using Gray’s test.

During multivariate analyses using Cox proportional hazard models, clinical factors and significant genotypes were considered as covariates for each event. Because our analysis was confined to HLA-matched sibling PBSCT transplants, HLA disparity, donor relationship, and stem cell source were not included in the multivariate analysis. Before introducing potential time-dependent covariates, such as aGVHD or cGVHD, into the time-dependent Cox proportional hazard model, we investigated the appropriateness of using a non–time-dependent hazard model with either aGVHD or cGVHD. In univariate analyses, the $P$ values of the omnibus test (which indicates a model’s appropriateness in a non–time-dependent Cox model) were $< .001$ for aGVHD and .238 for cGVHD. Based on this result, we applied cGVHD only as time-dependent covariate in the model.

In model 1, the following covariates were included in transplantation outcome (ie, OS, NRM, or relapse) analysis: cGVHD (time-dependent covariate), age ($< 40$ years vs $\geq 40$ years), the development of aGVHD (grade 0-2 vs grade 3-4), disease risk (high risk vs standard risk), conditioning regimen (myeloablative vs reduced intensity), $VEGFA +936 C>T$ genotype (CT vs the CC genotype), and $VEGFA$ haplotype (2 copies vs 0 or 1 copy of the ACG haplotype). In model 2, VEGF risk score was adopted instead of $VEGFA +936$ C>T genotype and $VEGFA$ haplotype, to confirm the VEGF risk score as a surrogate for GVHD risk. The covariates for aGVHD grade 2-4 or grade 3-4 included age ($< 40$ years vs $\geq 40$ years), disease risk (high risk vs standard risk), conditioning regimen (myeloablative vs reduced intensity), $VEGFA +936 C>T$ genotype (CT vs the CC genotype), and $VEGFA$ haplotype (2 copies vs 0 or 1 copy of the ACG haplotype), whereas those for cGVHD included age ($< 40$ years vs $\geq 40$ years), development of aGVHD (grade 0-2 vs grade 3-4), disease risk (high risk vs standard risk), conditioning regimen (myeloablative vs reduced intensity), $VEGFA +936$ C>T genotype (CT vs CC genotype), and $VEGFA$ haplotype (2 copies vs 0 or 1 copy of the ACG haplotype). Multivariate analyses using time-dependent or non–time-dependent Cox proportional hazard models were conducted using backward-stepwise modeling and a $P$ value $> .05$ for the likelihood ratio test. Hazard ratios (HRs) and 95% confidence intervals (CIs) also were estimated.

Statistical significance was accepted for $P$ values $< .05$. Statistical data were obtained using SPSS version 13.0 (SPSS Inc, Chicago, IL), NCSS version 4.0 (NCSS, Kaysville, UT), and the R package (version 2.4.1, available at http://CRAN.R-project.org).

**RESULTS**

**Overall Transplantation Outcomes**

With a median follow-up of 29.5 months post-transplantation (range, 0.5 to 74.5 months), 33 patients
(34%) progressed and 53 patients (54%) succumbed to primary disease progression \((n = 15)\) or NRM \((n = 38)\). The 2-year OS was \(44.3% \pm 5.3\%\); cumulative incidences of NRM were \(10.8\% \pm 3.6\%\) at 100 days and \(24.3\% \pm 5.1\%\) at 2 years, and the cumulative incidence of recurrence at 2 years was \(33.4\% \pm 5.0\%\). The cumulative incidences of grade 1-4, grade 2-4, and grade 3-4 severe aGVHD were \(81.6\% \pm 0.2\%, 72.2\% \pm 0.3\%, \) and \(39.3\% \pm 0.5\%, \) respectively, whereas those of cGVHD were \(58.1\% \pm 5.8\%\) at 6 months and \(68.9\% \pm 5.5\%\) at 2 years.

### Genotype Frequencies of the VEGFA Polymorphisms

The genotype frequencies of the VEGFA polymorphisms are summarized in Table 2. All polymorphisms complied with the HWE (Table 2). The LDs of VEGFA polymorphisms at loci -2578/-460/+405/+936 are shown in Figure 1. Strong LDs can be seen between loci -460 and +405 \((D = 0.94)\), between loci -2578 and +460 \((D = 1.00)\), and between loci -2578 and +405 \((D = 1.00)\); however, linkages of locus +936 with -2578 \((D = 0.07)\), -460 \((D = 0.09)\), or +405 \((D = 0.13)\) are weak \((D < 0.5)\). Accordingly, we generated haplotypes of the VEGFA polymorphisms based on 3 genotypes at loci -2578, -460, and +405. The frequencies of these haplotypes at loci -2578/-460/+405 were \(47.9\%\) for CTC, \(26.7\%\) for CTG, \(24.2\%\) for ACG, and \(1.0\%\) for CCC.

### Univariate Analyses for Factors Associated with Transplantation Outcomes, Especially aGVHD

On single-marker analysis, the incidence of grade 2-4 aGVHD was higher in patients with the +936 CT genotype (88%) than in those with the CC genotype \((64\%; \ P = .006; \ \text{Figure 2A})\), higher in patients with the -2578 AA genotype (100%) than in those with the CA \((71\%\); P = .003) or CC genotype \((73\%\); P = .002). On haplotype analysis, the incidence of grade 2-4 aGVHD (100%) was higher in patients with 2 copies of the ACG haplotype than in those with 0 or 1 copy of this haplotype \((68\%; \ P = .003; \ \text{Figure 2B})\). No association was found between VEGFA SNPs and the incidence of cGVHD.

### VEGF Risk Score Model Predicting the Risk of aGVHD

Based on the +936 C>T genotype and VEGFA haplotype information, we scored VEGFA SNPs based on the genotype at locus +936 and copy number of the ACG haplotype at loci -2578/-460/+405/+936. A score of 1 was assigned to risk alleles (ie, +936 CT or TT genotypes, or 2 copies of the ACG haplotype), and a score of 0 was assigned to other alleles (ie, +936 CC genotype, or 0 or 1 copy of the ACG haplotype). After summing scores, 2 risk groups were defined: high risk \((\text{composite score 2 or 1; } n = 37)\) and low risk \((\text{composite score 0; } n = 56)\). Significant correlations were found between the incidence of grade 1-4 aGVHD or grade 2-4 aGVHD and the VEGFA SNP score model (Figure 3A and B). The incidence of grade 2-4 aGVHD was \(89\%\) in the high-risk patients \((\text{score 2 or 1; } \text{ie, } +936 \text{ CT/TT genotype or 2 copies of ACG haplotype})\) and \(62\%\) in the low-risk patients \((\text{score 0; } \text{ie, } +936 \text{ CC genotype and 0 or 1 copy of ACG haplotype})\) \((P = .001)\). No differences were seen between the high-risk and low-risk patients in terms of OS, NRM, or recurrence (Table 3).
Organ-Specific Development of aGVHD According to VEGFA SNPs

We examined the relationships between organ-specific onset of aGVHD and the VEGFA SNPs. The VEGFA SNPs, especially the -2578 and -460 genotypes, were found to be closely associated with the development of gut GVHD (Figure 4), and a higher risk of gut GVHD was found to be associated with the -2578 AA genotype ($P = 0.04$, adjusted $P = 0.08$; Figure 4A) or a non-C carrier ($P = 0.02$, adjusted $P = 0.05$), as well as with the -460 TT genotype ($P = 0.05$, adjusted $P = 0.07$; Figure 4B) or a non-T carrier ($P = 0.02$, adjusted $P = 0.05$). Adjustment was performed for age, conditioning regimen, and disease risk.

Multivariate Analysis

Multivariate analysis for the risk of grade 2-4 aGVHD identified 2 risk factors in model 1 (Table 4): the +936 CT genotype ($P = 0.03$; HR = 1.79; 95% CI = 1.07 to 2.99) and the ACG VEGFA haplotype ($P = 0.01$; HR = 3.09; 95% CI = 1.27 to 7.52). In model 2, the high-risk group (VEGFA risk score 1 or 2) had a significantly greater risk of aGVHD grade 2-4 ($P = 0.001$; HR = 2.32; 95% CI = 1.41 to 3.81). The only independent risk factor for grade 3-4 severe aGVHD was the ACG haplotype ($P = 0.02$; HR = 4.46; 95% CI = 1.29 to 15.38). Other clinical factors were not found to be associated with the risk of aGVHD, perhaps because our cohort included only HLA-matched sibling PBSCT recipients.

VEGFA SNPs were not found to be associated with the risk of cGVHD, although a history of a previous episode of grade 3-4 aGVHD was found to be significantly associated with a greater risk of cGVHD ($P < 0.001$; HR = 3.70; 95% CI = 1.96 to 6.99). In terms of overall survival, grade 3-4 aGVHD ($P < 0.001$; HR = 40.31; 95% CI = 9.97 to 162.89) and high risk of

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**Figure 2.** VEGFA genotype/haplotype and its association with the risk of aGVHD. Higher incidences of grade 2-4 aGVHD were observed in patients with the +936 CT genotype (A) or the ACG haplotype for loci -2578/-460/+405 (B).

**Table 3.** Transplantation Outcome According to the VEGFA Genotype at Position +936 C>T, the VEGFA ACG Haplotype for Loci -2578/-460/+405, and the VEGFA Risk Score

<table>
<thead>
<tr>
<th>Overall Patients</th>
<th>VEGFA +936 C&gt;T Genotype</th>
<th>VEGFA ACG Haplotype at -2578/-460/+405</th>
<th>VEGFA Risk Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT Genotype (n=62)</td>
<td>CC Genotype (n=36)</td>
<td>0/1 Copy (n=92)</td>
</tr>
<tr>
<td>Follow-up, months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluable patients</td>
<td>93 (81)</td>
<td>59 (76)</td>
<td>29 (88)</td>
</tr>
<tr>
<td>Overall</td>
<td>75 (81)</td>
<td>45 (76)</td>
<td>30 (88)</td>
</tr>
<tr>
<td>Grade 2-4</td>
<td>63 (68)</td>
<td>34 (58)</td>
<td>29 (85)</td>
</tr>
<tr>
<td>Grade 3,4</td>
<td>26 (28)</td>
<td>16 (27)</td>
<td>10 (29)</td>
</tr>
<tr>
<td>cGVHD, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluable patients</td>
<td>74 (73)</td>
<td>43 (70)</td>
<td>31 (77)</td>
</tr>
<tr>
<td>Overall cGVHD</td>
<td>54 (73)</td>
<td>30 (70)</td>
<td>24 (77)</td>
</tr>
<tr>
<td>Extensive cGVHD</td>
<td>30 (41)</td>
<td>16 (37)</td>
<td>14 (45)</td>
</tr>
<tr>
<td>Survival, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td>33 (34)</td>
<td>22 (36)</td>
<td>11 (31)</td>
</tr>
<tr>
<td>Death</td>
<td>53 (54)</td>
<td>37 (60)</td>
<td>16 (45)</td>
</tr>
<tr>
<td>NRM</td>
<td>38 (39)</td>
<td>27 (44)</td>
<td>11 (31)</td>
</tr>
</tbody>
</table>

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disease \( (P = .001; \text{HR} = 6.86; 95\% \text{ CI} = 2.15 \text{ to } 21.86) \)
were found to be independent risk factors. With re-
spect to other transplantation outcomes, such as
NRM and relapse, grade 3-4 aGVHD was found to
be significantly associated with a greater risk of
NRM \( (P < .001; \text{HR} = 74.02; 95\% \text{ CI} = 16.58 \text{ to }
826.21) \), as was \text{VEGFA} -936C>T genotype \( (P =
.01; \text{HR} = 29.43; 95\% \text{ CI} = 1.99 \text{ to } 435.24) \).
High-
risk status \( (P = .002; \text{HR} = 3.46; 95\% \text{ CI} = 1.59 \text{ to }
7.53) \) and a previous episode of grade 3-4 aGVHD \( (P =
.05; \text{HR} = 0.23; 95\% \text{ CI} = 0.05 \text{ to } 0.97) \) were
found to be independent predictors of relapse after allogeneic
transplantation.

**DISCUSSION**

The results of the present study suggest an associ-
tion between \text{VEGFA} SNPs and a lower risk of
aGVHD after allogeneic SCT (especially gut
gVHD), as well as a protective role for VEGF in
the pathogenesis of aGVHD. VEGF exerts 2 actions in
this respect: a proinflammatory effect that may pro-
voke inflammatory reactions in target tissues [25] and
an angiogenic effect that may facilitate tissue reper-
fusion and regeneration in inflamed tissues [26]. Conse-
quently, VEGF may have a protective or augmentative
role in the pathogenesis of GVHD in opposite direc-
tions; elevated VEGF could either increase the severity
of GVHD by promoting inflammation or reduce the
severity of GVHD by stimulating tissue perfusion.

Our findings suggest that VEGF may protect
against the development of severe GVHD, in agree-
ment with previous reports associating elevated
VEGF levels on day 14 or 15 posttransplantation
with decreased risk of severe aGVHD and NRM
[4,6] Whether VEGF up-regulation is a consequence of
tissue hypoxia in GVHD or whether it acts as a me-
diator of endothelial regeneration by inhibiting irre-
versible fibrosis caused by cytotoxic T lymphocytes is
unclear, however. Regardless, it is apparent that high
VEGF level may be a surrogate marker of a decreased risk for severe aGVHD or NRM after allogeneic SCT. On the other hand, a study in a murine model concluded that VEGF has a proinflammatory function in the alloimmunity setting [27]. In the murine renal allograft model used in that study, anti-VEGF antibody was found to markedly inhibit T cell infiltration into allografts and acute rejection, and thus it was concluded that VEGF exerts robust proinflammatory activity in the alloimmunity setting [27]. But the renal allograft environment in a murine model likely differs greatly from that in a human GVHD setting. Accordingly, further studies are warranted to clarify this issue, and to determine whether VEGF is a driver or passenger in the development of GVHD.

Similarly, studies of VEGF in inflammatory bowel disease (IBD) suggest that VEGF has proinflammatory activity, with VEGF expression found to be higher in patients with IBD than in healthy controls and to be positively correlated with disease activity [28,29]. These results are at odds with our finding that lower VEGF production is associated with a higher risk of gut GVHD. But microvessel densities are attenuated in GVHD tissues [1] but elevated in IBD [30], and thus we postulate that although gut GVHD and IBD share similar pathogenetic mechanisms and pathological findings, VEGF’s proinflammatory activity is more prominent during the pathogenesis of IBD, whereas its angiogenic activity is more significant in GVHD.

Genetic variations in the genes encoding these molecules may affect transcription and translation or may modulate the functions of the gene products. Growing evidence supports the notion that cytokine gene polymorphisms are important predictors of transplantation-related complications, including aGVHD and cGVHD, and transplantation outcomes [15,31-33]. For example, it is generally accepted that the SNPs of proinflammatory cytokines, such as interleukin (IL)-1, IL-2, IL-6, interferon-γ, or tumor necrosis factor-α, and of anti-inflammatory cytokines, such as IL-10, affect transplantation outcomes, including aGVHD and cGVHD and transplantation-related mortality [15].

In the present study, patients with the +936 CT genotype were found to have a higher risk of grade 2-4 aGVHD than patients with the CC genotype (88% vs 64%; \( P = .006 \); Figure 2A). In addition, patients with 2 copies of the ACG haplotype were found to have a higher risk of grade 2-4 aGVHD than patients with 0 or 1 copy of the ACG haplotype (100% vs 68%; \( P = .003 \); Figure 2B). Furthermore, according to our risk score model, the incidence of grade 2-4 aGVHD was 89% in the high-risk patients (score 1-2, ie, +936 CT genotype or any ACG haplotype), compared with 62% in the low-risk patients (\( P = .001 \); Figure 3B). However, a limitation of the current study is the very low number of high-risk patients (ie, 6). Accordingly, further studies are needed with larger numbers of patients to enable a clear conclusion on this issue.

In terms of the specific effects of the VEGFA genotypes, the +936 CT genotype and the ACG haplotype
(for loci -2578/-460/+405) appeared to reduce VEGF production. In our previous study of patients with acute myeloid leukemia (AML), those patients with the +936 CC genotype and CTG haplotype had a higher risk of relapse, and this was associated with VEGF up-regulation. Conversely, the +936 CT genotype and the ACG haplotype were associated with VEGF down-regulation. Previous studies also have found an association between the +936T allele in the +936C>T genotype and VEGF down-regulation [10,34]. VEGF expression differences caused by VEGFA SNPs may be due to (1) a loss of a potential binding with the transcription factor AP-4 in the presence of the C-to-T transition; (2) LD of the polymorphism with another, as-yet unidentified polymorphism; or (3) modification of the mRNA structure [10]. In the present study, we did not perform promoter assays to evaluate the functional role of VEGFA haplotypes; however, a previous study in a Korean population with the TG haplotype at loci -460/+405 suggested an association between the TG haplotype at loci -460/+405 and VEGF up-regulation [17].

In conclusion, our findings support the notion that VEGF protects against aGVHD, especially against the development of severe aGVHD. They also suggest that VEGFA polymorphisms can be used to predict the risk of aGVHD.

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REFERENCE