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Vascular Endothelial Growth Factor Gene Polymorphisms May Predict the Risk of Acute Graft-versus-Host Disease following Allogeneic Transplantation: Preventive Effect of Vascular Endothelial Growth Factor Gene on Acute Graft-versus-Host Disease

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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 +405, 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.

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KEY WORDS: Vascular endothelial growth factor, Single nucleotide polymorphism, Graft-versus-host disease, Allogeneic stem cell transplantation

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 An association between these inflammatory reactions and angiogenesis is well established.

Recently, endothelialitis and subsequent microvessel injury were found to be involved in the pathogenesis of GVHD. One study found strikingly higher microvessel densities in skin samples in healthy normal donors than in patients with acute GVHD (aGVHD) or chronic GVHD (cGVHD). Accordingly, 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.

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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 *VEGFA* 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 5́-untranslated region (+405C>G [rs2010963]) and its 3́-untranslated region (+936C>T [rs3025039]) 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>T (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 conformed

Table 1. Patient Characteristics and Transplantation Procedures

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

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.