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Vascular Endothelial Growth Factor Gene Polymorphism Is Associated With Long-term Kidney Allograft Outcomes

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Introduction: Vascular endothelial growth factor (VEGF) regulates vasculogenesis in physiological and pathological states. We evaluated the role of VEGF single-nucleotide polymorphisms (SNPs) −1154 G/A, −2578 C/A, +936 C/T, and −2549 Ins/Del in chronic allograft nephropathy.

Methods: Blood samples were collected before renal transplantation, and DNA was extracted. Genotyping of VEGF SNPs −1154 G/A (rs1570360), −2578 C/A (rs699947), +936 C/T (rs112005313), and −2549 Ins/Del (18bpindel) polymorphisms were carried out. Relative quantification of VEGF-A mRNA expression for 4 VEGF SNPs were quantified by the $2^{-\Delta\Delta Ct}$ algorithm. Kidney allografts were categorized into graft loss (n = 98) and normally functioning (n = 174) groups. Genotype frequencies were calculated using additive, dominant, and recessive models. Hardy–Weinberg Equilibrium was assessed between outcome groups by standard procedure using χ^2 analysis. The cumulative allograft survival was estimated by Kaplan–Meier analysis and compared among VEGF genotypes by the log-rank test. Study limitations were the lack of VEGF serum levels, donor-specific antigens, and protocol biopsies.

Results: There was an association of AA (hazard ratio = 2.42, $P = 0.0001$) and CA (hazard ratio = 1.83, $P = 0.009$) genotypes of −2578 C/A SNP with graft loss. After adjustment for transplant-related covariates, associations of VEGF SNPs −2578 C/A and −2549 Ins/Del with graft failure were found to be significant. There was prolonged graft survival for cases with the CC genotype of VEGF −2578 C/A SNP. The carrier −2578*CC, −1154*GG, and +936*CC genotypes were shown to have a strongly protective association. There was no association with posttransplantation lymphomas.

Discussion: Recipients of kidney allografts possessing low-producing VEGF genotypes are associated with less prolonged graft survival.

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The management of chronic allograft dysfunction (CAD) continues to be a challenge. The cause of CAD is multi-factorial, and may include a combination of immunological and nonimmunological factors.[1] Renal microvascular injury has been a prominent feature, possibly due to a decrease in pro-angiogenic survival factors.[2]

Vascular endothelial growth factor (VEGF) is a central regulator of vasculogenesis both in physiological and in pathological states. It induces endothelial fenestration and maintains vascular permeability.[3–5] VEGF also supports vascular survival by preventing endothelial apoptosis. VEGF has been shown to repair the interstitial tubule compartment in cyclosporine nephrotoxicity, whereas VEGF mRNA level has been up-regulated in tubules in hypoxic states.[6,7] Although VEGF is expressed constitutively, its function in pathological states is less clearly defined. VEGF mRNA and protein are increased in pathological conditions associated with a macrophage inflammatory infiltrate.

Previous studies have shown an association of VEGF-A polymorphism with end-stage renal disease[8]

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and early acute rejection.[9] Our hypothesis is that VEGF may contribute to the pathogenesis of CAD. Herein, we report the results of a prospective, single-center study seeking an association of 4 functionally relevant VEGF SNPs (VEGF −1154 G/A, −2578 C/A, +936 C/T, and −2549 Ins/Del) with long-term kidney allograft outcomes.

METHODS

Patient Selection and Follow-up

A total of 272 renal allograft recipients who underwent renal transplantation at our institution between 2005 and 2011 were included in the study. All of the patients were followed up after renal transplantation until the end of the study period (December 2015). Clinical events of graft dysfunction, allograft biopsy for cause, infections, hospitalization, graft failure, initiation of dialysis therapy, malignancies, and death were recorded prospectively. Renal allograft biopsy was performed for an unexplained acute rise of serum creatinine by $>$ 25% of baseline, a progressive rise of creatinine, new-onset or increase in pre-existing proteinuria, CAD without an apparent cause, or whenever a specific diagnosis was considered. The updated Banff 2007 nomenclature was used to score histologic findings and to classify diagnostic categories.[10] Graft failure was defined as estimated glomerular filtration rate (eGFR, calculated from the 4-variable Modification of Diet in Renal Disease [MDRD] Study equation) of $<$ 15 ml/min per 1.73 m^2 or initiation of dialysis. The study was approved by the institutional ethics committee of the Sanjay Gandhi Postgraduate Institute of Medical Sciences and Department of Biotechnology, Government of India, New Delhi, India. Informed consent was obtained from all individuals, and the study was performed according to the principles of the Declaration of Helsinki.

DNA Extraction and VEGF Genotyping

Blood samples were collected before renal transplantation, and DNA was extracted using a QIAmp DNA Blood Mini Kit (Brand GMbH and Co KG, Cat. No. 51104; Qiagen, Valencia, CA). Genotyping of VEGF SNPs −1154 G/A (rs1570360), −2578 C/A (rs699947), +936 C/T (rs112005313), and −2549 Ins/Del (18bp indel) polymorphisms were carried out as previously reported.[8]

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from whole blood using Trireagent (Invitrogen, Carlsbad, CA). Complementary DNA (cDNA) was prepared from 5 μg of total RNA using oligo (dT) primers and Moloney murine leukemia virus reverse transcriptase (Agilent Technologies, Santa Clara, CA). The real-time reaction was performed at 42°C for 50 minutes. Real-time polymerase chain reaction amplification was carried out using 4 VEGF-A SNP-specific primers. In addition, real-time polymerase chain reaction amplification of GAPDH (endogenous control) was carried out using primers to estimate the amount of RNA in all samples. Relative quantification of VEGF-A mRNA expression for 4 VEGF SNPs were quantified by the $2^{-\Delta\Delta Ct}$ algorithm with GAPDH as the housekeeping gene and a commercial human cDNA as the universal reference.

Statistical Analysis

Data were expressed as percentages or as mean ± SD and compared using the χ^2 test or analysis of variance as appropriate. Genotype frequencies were calculated using additive, dominant, and recessive models. Hardy−Weinberg equilibrium was assessed between outcome groups by standard procedure using χ^2 analysis. Variables included patient age, patient gender, diabetic status, donor age, donor gender, donor glomerular filtration rate (GFR), number of human leukocyte antigen (HLA) mismatches, and acute rejection episodes. These were adjusted when significance of VEGF SNPs with graft loss was found. Graft loss (GFR $<$ 15 ml/min per 1.73 m^2 or initiation on dialysis) was considered as an event. Cumulative allograft survival was estimated by Kaplan−Meier analysis and compared among VEGF genotypes by the log-rank test. Unadjusted and adjusted hazard ratios for graft loss were calculated for various genotype models by using Cox univariate and multivariate regression, respectively. For all analyses, a value of $P \leq 0.05$ was considered significant. All the analyses were performed with SPSS 16.0 software (SPSS Inc, Chicago, IL).

RESULTS

Recipient Characteristics and Outcomes

Baseline demographic and clinical characteristics were shown in Table 1. The mean duration of follow up was 48.1 ± 28.7 months (range, 3−126 months). During the follow-up period, 98 grafts (36%) were lost. Histologic diagnosis was available for 86 patients. Causes of graft failure were acute rejection (n = 10), vascular causes (n = 3), and surgical complications (n = 6), chronic antibody-mediated rejection including transplant glomerulopathy (n = 25), chronic calcineurin toxicity (n = 14), recurrence of primary disease (n = 8), *de novo* glomerulonephritis (n = 8), BK polyoma virus nephropathy (n = 3), and chronic pyelonephritis (n = 2). Nonspecific interstitial fibrosis and tubular atrophy (IF/TA) was found in 19 renal biopsy samples. Of the 98 patients with graft loss, 64 patients died (61 due to cardiovascular events and 3 due to infection), and 174