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Biology: Genetic Markers

Analysis of a Genetic Polymorphism in the Costimulatory Molecule *TNFSF4* with Hematopoietic Stem Cell Transplant Outcomes

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A B S T R A C T

Despite stringent procedures to secure the best HLA matching between donors and recipients, life-threatening complications continue to occur after hematopoietic stem cell transplantation (HSCT). Studying single nucleotide polymorphism (SNP) in genes encoding costimulatory molecules could help identify patients at risk for post-HSCT complications. In a stepwise approach we selected SNPs in key costimulatory molecules including CD274, CD40, CD154, CD28, and TNFSF4 and systematically analyzed their association with post-HSCT outcomes. Our discovery cohort analysis of 1157 HLA-A, -B, -C, -DRB1, and -DQB1 matched cases found that patients with donors homozygous for the C variant of rs10912564 in *TNFSF4* (48%) had better disease-free survival ($P = .029$) and overall survival ($P = .009$) with less treatment-related mortality ($P = .006$). Our data demonstrate the *TNFSF4*C variant had a higher affinity for the nuclear transcription factor Myb and increased percentage of *TNFSF4*-positive B cells after stimulation compared with CT or TT genotypes. However, these associations were not validated in a more recent cohort, potentially because of changes in standard of practice or absence of a true association. Given the discovery cohort, functional data, and importance of TNFSF4 in infection clearance, *TNFSF4C* may associate with outcomes and warrants future studies.

INTRODUCTION

Although hematopoietic stem cell transplantation (HSCT) has become the gold standard therapy for hematologic disorders and malignancies, the full therapeutic potential of HSCT has been limited because of its complications. Although the standardization of pretransplant donor–recipient matching for HLAs has greatly improved post-HSCT outcomes, the mortality rate still remains twice that of the general population even in patients who survived 2 or more years after allogenic HSCT [1]. This excess mortality is caused by a number of complications post-transplantation such as graft-versus-host disease (GVHD), infection, and relapse of the primary disease [1,2].

There is growing evidence to support the importance of genetic variability outside the HLA system that is contributing to the heterogeneity in HSCT outcomes [3-5]. Responses to alloantigen, tumor surveillance, and infectious complications post-HSCT rely heavily on a functional immune system. Costimulatory molecules represent an essential regulatory component of the immune system, which may be functionally affected by gene polymorphisms [5,6]. For T cell activation 2 activation signals are required. The first signal occurs after the T cell receptor and a coreceptor interact with the antigen peptide presented on an MHC

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molecule by an antigen-presenting cell. The second positive costimulatory signal occurs when 1 or more T cell surface receptors engage with their specific ligands on antigen-presenting cells [7-10]. Conversely, negative costimulatory molecules decrease T cell proliferation and cytokine production, promote T cell anergy or apoptosis, and induce the activity of regulatory T cells [11].

For this study we selected single nucleotide polymorphisms (SNPs) based on a stepwise criteria from costimulatory genes, which have an established association in human and animal HSCT outcomes [12-14]. SNPs needed to have a strong linkage disequilibrium to additional tagged SNPs within their gene to maximize a subset of informative SNPs. Each SNP required an adequate minor allelic frequency of polymorphism above 5% to ensure the least common variant was present in our population. Finally, every SNP required enough subjects in our population to detect a significant association at 80% power. From these guidelines we created a panel of SNPs with a high likelihood of discovering an association in our HSCT population. Determining novel predictors could help develop a pretransplant matching system to identify patients at risk and modify immunosuppressive regimens based on their risk assessment.

The tumor necrosis factor ligand superfamily member 4 (TNFSF4) and tumor necrosis factor receptor superfamily member 4 (TNFRSF4) pathway represent key positive costimulatory signals required for cell activation. TNFRSF4 is present on both activated $CD4^+$ and $CD8^+$ T cells and its cognate ligand, TNFSF4, is expressed on dendritic cells, B cells, and activated endothelial cells [15]. Signaling through the TNFSF4/TNFRSF4 pathway facilitates Th 2 differentiation, enhances effector $CD8^+$ T cell memory commitment and promotes cytokine production [16,17]. Gene polymorphisms in *TNFSF4* have been associated with atherosclerosis and systemic lupus erythematosus [18-20]. These studies postulate that TNFSF4 is a major component in the T cell–antigen-presenting cell interaction, leading to activation of immune cells to produce proinflammatory cytokines and chemokines, resulting in active disease. The role of *TNFSF4* in determining the post-HSCT outcomes remains to be explored.

Infectious complications are a contributing source of severe morbidity and nonrelapse-related mortality in unrelated donor allogeneic HSCT [21]. They account for a higher percentage of mortality compared with GVHD in both HLA-identical sibling and unrelated donor transplants studied over a 5-year period [22]. Although the early prophylactic regimens reduce the incidence of early infection, the risk of late infection remains [23]. Deficiencies in the function of immunoregulatory genes that activate the cellular and humoral immune responses can be the underlying cause of an increased risk of infection. As part of the immune system's response to infection, activation of T cells through TNFSF4 costimulation has been shown to effectively clear pathogens [15,24]. Genetic variation may influence the timing and strength of TNFSF4 signaling to effectively respond to infectious pathogens.

In this study we carefully chose candidate SNPs found within a group of extensively studied costimulatory molecules that might associate with HSCT outcomes. We analyzed genetic data from discovery (n = 1157) and validation (n = 1188) cohorts using HLA-matched (at the HLA-A, -B, -C, -DRB1, and -DQB1 loci) HSCT recipients and their respective donors and then searched for associations with important transplant outcomes.

METHODS

Patient Population

A discovery cohort of 1157 and a validation cohort of 1188 recipient–donor pairs from unrelated HLA-A, -B, -C, -DRB1, and -DQB1 allele-matched transplantations facilitated by the National Marrow Donor Program (NMDP) were included in the study. A detailed description can be found in Supplementary Methods. Patient data were acquired from the Center for International Blood and Marrow Transplant Research, a research affiliation between the Medical College of Wisconsin, and the NMDP. Observational studies conducted by the Center for International Blood and Marrow Transplant Research were performed in compliance with the Privacy Rule under the Health Insurance Portability and Accountability Act of 1966, as a Public Health Authority, and in compliance with all applicable federal regulations pertaining to the protection of human research participants and the Declaration of Helsinki as determined by continuous review of the Institutional Review Board of the NMDP.

Definition of Outcome

The primary endpoints analyzed in the study were overall survival (OS), disease-free survival (DFS), treatment-related mortality (TRM), relapse, acute GVHD (aGVHD) grades II to IV and III to IV occurring within the first 100 days post-transplant, and chronic GVHD (cGVHD). Our analysis of OS treated death from any cause as the event, and surviving patients were censored at the date of last contact. For analysis of DFS, failures were relapse or death from any cause with patients who were alive and in complete remission censored at time of last follow-up. TRM was defined as death during a continuous complete remission. Relapse was defined as clinical or hematologic relapse of primary disease with death without evidence of disease as a competing risk. For chronic myeloid leukemia patients our definition of relapse included cytogenetic, molecular, and hematologic relapse as an event. Assessment of aGVHD grades II to IV and III to IV were defined using the Glucksberg scale [25], and extensive cGVHD was defined according to the Seattle criteria [26].

Genotyping

We genotyped 9 SNPs located in 5 immunoregulatory genes: *CD274*, *CD40*, *CD154*, *CD28*, and *TNFSF4* (Supplementary Table 1). These SNPs were selected based on strong linkage disequilibrium to tagged SNPs within their gene and power calculations suggested in our population a high likelihood of discovering an association. For the discovery set, genomic DNA was isolated from cryopreserved leukocytes from donor and recipient blood samples provided by the NMDP Research Sample Repository following the manufacturer's protocol (Promega, MAdison, WI). Isolated DNA was quantitated using a Nanodrop (Thermo Scientific, Waltham, MA) and whole genome amplified with Phi29 DNA polymerase. For the validation set, genomic DNA was isolated from frozen blood samples using the QIAmp 96 DNA Blood Kit and the manufacturer's protocol (Qiagen, Valencia, CA). Genotyping was then performed using a Taqman SNP genotyping assay (Applied Biosystems, Foster City, CA) and automated genotype calling software. Samples in the discovery (n = 213) and validation (n = 12) cohorts where a SNP genotype was not obtained due to DNA degradation or minimal isolated genomic DNA were excluded from further analysis.

SNP Selection and Identification of Tag SNPs

SNPs were investigated using the Hapmap Genome Browser Phase 1 and 2 full dataset (International Hapmap Project, http://www.hapmap.org/). Gene SNP data from the white population was imported into Haploview (http://www.broad.mit.edu/mpg/haploview/) using the Hapmap format function. The uploaded SNP data were further modeled by setting the following Haploview parameters: Hardy-Weinberg *P* cutoff of .001, a minimum genotype percent of 80, a maximum number Mendel error of 1, and a minimum minor allele frequency of .05. The minor allele frequency cutoff of 5% was selected for compatibility with HapMap, which includes only those SNPs with allele frequencies greater than .05. The tagger function in Haploview was performed using pair-wise tagging only with an r^2 equal to .8. Identified tag SNPs and the corresponding predicted common SNPs were compiled.

Statistical Analysis

Discovery set

Univariate probabilities of DFS and OS were calculated using the Kaplan-Meier method. The log-rank test was used for comparing survival curves. Probabilities of TRM, relapse, aGVHD, and cGVHD were calculated using cumulative incidence estimates. The cumulative incidence calculated for aGVHD and cGVHD treated death as a competing risk [27]. Relapse was treated as a competing risk for TRM and vice versa.