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The impact of HLA-E polymorphisms on relapse following allogeneic hematopoietic stem cell transplantation

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ABSTRACT

Since relapse following allogeneic hematopoietic stem cell transplantation (HSCT) can be due to the escape of the residual malignant cells from the graft-*versus*-leukemia (GvL) effect and given the role of NK cells in GvL and the importance of HLA-E in the modulation of NK cell function, we investigated whether polymorphisms of HLA-E molecule could impact on the incidence of relapse and the improvement of Disease-free Survival (DFS) after allogeneic HSCT. The study group included 56 pairs of donors and patients with malignant hematological disorders undergoing HLA-E matched allogeneic HSCT. The median follow-up was 43.6 (range 20.5–113.1) months. They were genotyped for HLA-E locus using a sequence-specific primer (SSP)-PCR. We found a lower incidence of relapse ($p = 0.02$) in the patients with HLA-E*0103/0103 genotype compared to those with other genotypes of HLA-E. We also showed an association between HLA-E*0103/0103 genotype and a better DFS ($p = 0.001$). Our results suggest a protective role for HLA-E*0103/0103 genotype against relapse and an association between this genotype and an improved DFS following HLA-E matched allogeneic HSCT.

1. Introduction

Recent studies suggest a role for HLA-E in allogeneic hematopoietic stem cell transplantation (HSCT) outcomes [1]. However, the mechanism by which this molecule can modulate HSCT is poorly understood. Non-classical MHC class I molecules are mainly involved in the regulation of the innate immune responses [2], of which *HLA-E* plays an important role in cell recognition by NK cells [3]. *HLA-E* is expressed in association with peptides derived from the other molecules [4,5]. The binding of MHC class I peptides to *HLA-E* allows NK cells to indirectly monitor the overall cell surface expression of other MHC class I molecules *via* its main receptor NKG2A/CD94 [6,7]. Abnormal expression of MHC class I molecules can stimulate NK cells to eradicate infected or transformed cells through the cytolytic activity and cytokine production – a phenomenon that highlights the importance of HLA-E and NK cell surveillance in innate immune responses [7–9].

Substitution of an arginine with a glycine at position 107 on the α_2 domain of HLA-E heavy chain creates two alleles, HLA-E*0101 and HLA-E*0103, at the HLA-E locus. Each of these alleles induces different levels of cell surface expression of HLA-E molecules which might be caused by their different peptide affinity and thermal stability. The allelic variation has also been shown to alter affinity to their counter receptor on NK cells. In fact, the combination of these two mechanisms – different level cell surface expression and distinct affinities of HLA-E – might explain functional differences between the mentioned HLA-E alleles [10,11].

There are three possible genotypes of HLA-E alleles – HLA-E*0101/0101, HLA-E*0103/0103 and HLA-E*0101/0103. Several lines of evidence have shown that HLA-E polymorphisms may affect clinical outcomes in different diseases. For instance, the association of HLA-E*0101/0101 genotype with the higher rate of recurrent spontaneous abortion in Indian women [12] and type I diabetes mellitus has been reported [13]. Other studies also exhibited the protective impact of HLA-E*0103/0103 genotype on HIV-1 infection in Zimbabwean women [14], a higher frequency of *HLA-E*0103* allele in the patients with Behcet's disease [15] and nasopharyngeal carcinoma [16,17].

In addition, there are a few studies that suggest transplant outcomes may be affected by HLA-E polymorphisms, where a lower incidence of acute graft-*versus*-host disease (aGvHD), transplant-related mortality (TRM; is defined as death due to causes unrelated

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to the underlying disease) and a better overall survival were reported to be associated with HLA-E*0103/0103 genotype in the patients who underwent HLA-identical sibling bone marrow transplantation [4] or HLA-matched allogeneic HSCT [5]. These data are consistent with another report that identified HLA-E*0101/0101 genotype as a risk factor for early severe bacterial infections and TRM after unrelated-donor bone marrow transplantation [18]. However, no association between different genotypes of HLA-E and relapse has been reported to date [4,5].

Relapse is the major cause of mortality following allogeneic HSCT [19] and can be caused by the escape of residual malignant cells from the graft-versus-leukemia (GvL) effect which is often crucial for the eradication of malignant cells that have survived the conditioning regimen prior to HSCT. A growing body of evidence has shown an important role for NK cells in the GvL effect. This is a finding that suggests early reconstitution of these cells after HSCT may improve the immune responses leading to a successful transplant [20–23]. Given the importance of HLA-E molecule in modulation of NK cell function and the regulation of the innate immune responses, in this study, we examined the impact of HLA-E polymorphisms on the incidence of relapse and the improvement of Disease-free Survival (DFS) following HLA-E matched allogeneic HSCT.

2. Patients and methods

2.1. Study cohort

Our study group included 56 patients and their donors who underwent allogeneic HSCT between 2001 and 2009. All patients had a malignant hematological disorder. The patients all received peripheral blood stem cells as the stem cell source. The median follow-up of patients was 43.6 (range 20.5–113.1) months. Written consent was obtained from all patients and donors. Donor selection was performed using molecular typing for HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1. All donor–patient pairs were matched for HLA-E alleles. The primary outcomes assessed in this study were relapse incidence and DFS. Characteristics of the study cohort including transplant type, aGvHD prophylaxis and the conditioning regimen administrated for the patients are given in Table 1. This study has been reviewed and approved by the Ethics Committee at the Alfred Hospital (Project 30/07), Monash University, Melbourne, Australia.

2.2. HLA-E genotyping

All the patients and donors were genotyped for HLA-E locus using a sequence specific primer-(SSP)-PCR strategy. Amplification was carried out using the PC-960G Gradient Thermal Cycler machine (Corbett, Australia). To distinguish HLA-E alleles, the forward primers of E*0101F (5-GGCTCGAGCTGGGGCCCGCCA-3) and E*0103F (5-GGCTCGAGCTGGGGCCCGCCG-3) in combination with a common reverse primer (5-AGCCTGTGGACCCTCTT-3) were applied. All oligonucleotides used in the project were synthesized by Monash Micromon Oligonucleotide Synthesis Facility at Microbiology, Monash University in Melbourne, Australia.

2.3. Statistical analysis

Clinical variables that were analyzed included in donor-recipient gender, age and CMV serology, relation between donor and recipient, transplant type, underlying diagnosis, disease status, GvHD prophylaxis and conditioning regimen as well as HLA-E genotypes. Disease-free Survival curve was calculated by the Kaplan–Meier method and groups were compared using the Log-Rank test statistic. A multivariable Cox proportional hazards model with backward selection method for identifying variables associated with DFS was constructed. The proportional hazards (PH) assumption was checked using chi-square test of correlation coefficient between transformed survival time and the scaled Schoenfeld residuals [24]. The Survival package was used to check the proportional hazards assumption [25]. Cumulative incidence function was used to estimate the relapse incidence as an endpoint in a competing risks setting. The competing risks regression (crr) function was carried out with cmprsk package for multivariable regression model in a competing risks setting. Death without relapse was considered as a competing event for relapse. Groups were compared by the Gray test method in the competing risks setting. R software was applied for Survival and cmprsk analyses. The rest analyses were performed using software SPSS version 15.0 for windows (SPSS Inc., Chicago, IL) [26]. All p-values less than or equal to 0.05 were considered statistically significant.

Table 1
Characteristics of the study cohort.

Characteristics	n (%) or median (range)
Patients	56
Median age at transplantation (years) (range)	48.5 (23–64)
Male	35 (62.5)
Positive CMV serology	43 (77)
Underlying diagnosis	
AML	33 (59)
ALL	13 (23)
Other malignant disorders	10 (18)
Disease status	
CR1	23 (41)
CR2	10 (18)
Relapse 1	5 (9)
Relapse 2	8 (14)
Others	10 (18)
Donors	56
Median age (years) (range)	44 (11–64)
Male	32 (57)
Related	42 (75%)
Unrelated	14 (25%)
Positive CMV serology	34 (61)
ABO incompatibility	23 (41)
HLA compatibility	45 (80.4)
Transplantation	
Haploidentical HSCT	11 (19.6%)
Allogeneic HSCT	22 (39.3%)
Mini-allogeneic HSCT	23 (41.1%)
Source	
Peripheral blood stem cells	56 (100)
Conditioning regimen	
TBI based	22 (39.3)
Fludarabine + melphalan + others	34 (60.7)
GvHD prophylaxis	
CsA alone	16 (28.6)
CsA + methotrexate/mycophenolate	29 (51.7%)
$CD34^+$ selection/T cell depletion	11 (19.6)

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMV, cytomegalovirus; CR, complete remission; CsA, cyclosporine A; GvHD, graft-*versus*-host disease; HSCT, hematopoietic stem cell transplantation; TBI, total body irradiation.

3. Results

Association between HLA-E polymorphisms and post-HSCT outcomes

Given that no significant differences in the analyzed outcomes after allogeneic HSCT were observed between patients with HLA-E*0101/0101 and HLA-E*0101/0103 genotypes, they were combined and named "Others". The analyses were then carried out with two groups – "Others" *versus* HLA-E*0103/0103. Table 2 shows the incidence of post-allogeneic HSCT outcomes in patients with different genotypes of HLA-E.

3.1. DFS

DFS is defined as the probability of being alive free of disease at any point in time. Thus, death or disease relapse are treated as events and patients alive and free of disease at their last follow-up

Table 2
The incidence of post-allogeneic HSCT outcomes in patients with different genotypes of HLA-E.

HSCT outcomes	n (%)	"Others" n (%)	HLA-E*0103/0103 n (%)	p value
Relapse	18/56 (32.1%)	17/42 (40.5%)	1/14 (7.1%)	0.02
TRM	17/56 (30.4%)	15/42 (35.7%)	2/14 (14.2%)	0.02

HSCT, hematopoietic stem cell transplantation; TRM, transplant-related mortality. p values show statistical differences between HLA-E*0103/0103 group and "Others" group, a combination of HLA-E*0101/0101 and HLA-E*0101/0103 genotypes.