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The Impact of HLA-E Polymorphisms in Graft-*versus*-Host Disease following HLA-E Matched Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

The non-classical MHC class-I mainly involves in the regulation of innate immune responses where HLA-E plays a significant role in the cell identification by natural killer cells. HLA-E is a main regulatory ligand for natural killer cells and given the importance of these effector cells in hematopoietic stem cell transplantation, we investigated the effect of HLA-E polymorphisms on post-hematopoietic stem cell transplantation outcomes.

The study group included 56 donor-patient pairs with underlying malignant hematological disorders undergoing HLA-E matched allogeneic hematopoietic stem cell transplantation. They were genotyped for HLA-E locus using a sequence specific primer-polymerase chain reaction. The median follow-up was 20.6 months (range 0.2-114.8) and the parameters assessed were acute and chronic graft-*versus*-host disease and overall survival.

We showed a lower frequency of acute graft-*versus*-host disease (grade II or more; p=0.02) and chronic graft-*versus*-host disease (extensive; p=0.04) in the patients with HLA-E*0103/0103 genotype compared to other genotypes of HLA-E. There was also an association between HLA-E*0103/0103 and improved overall survival (p=0.001).

Conclusively, our results suggest a protective role for HLA-E*0103/0103 genotype against acute graft-*versus*-host disease (grade II or more) and chronic graft-*versus*-host disease (extensive) as well as an association between this genotype and a better overall survival after HLA-E matched allogeneic hematopoietic stem cell transplantation.

Keywords: Graft-*versus*-host disease; Hematopoietic stem cell transplantation; HLA-E polymorphisms; Overall survival

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is complicated by histocompatibility dependent immune responses such as graft rejection and graft-*versus*-host disease (GvHD),[1] the most frequent complication and also a major cause of morbidity and mortality after allogeneic HSCT. Several lines of evidence suggest an important role for non-classical MHC class-I molecule, HLA-E in HSCT.[2]

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The interaction of HLA-E with NKG2A, its main receptor on natural killer (NK) cells, modulates cell mediated cytotoxicity and cytokine production,[3-5] the phenomenon highlighting HLA-E importance in the innate immune responses.[6]

Replacement substitution changes an arginine (HLA-E^R) to a glycine (HLA-E^G) at position 107 on the α_2 domain of HLA-E heavy chain, identifying two HLA-E*0101 and HLA-E*0103 alleles respectively at the HLA-E locus. Various combination of these two alleles presents three possible genotypes of HLA-E*0101/0101, HLA-E*0103/0103 and HLA-E*0101/0103. The two alleles of HLA-E are different in cell surface expression where HLA-E*0103 has been shown to have a higher cell surface expression compared to HLA-E*0101. It has been suggested that allelic variation may influence the signaling pathway of their counter receptor, NKG2/CD94, through the affecting of ligand interaction, the phenomenon which might describe functional differences between these molecules.[7-10]

The polymorphisms of HLA-E is also a matter of interest for several studies where describing an association between a distinct genotype of HLA-E and outcomes in various situations and diseases. The association of HLA-E*0101/0101 genotype with increased rate of recurrent spontaneous abortion in Indian women[7] and type I diabetes mellitus,[8] the protective effect of HLA-E*0103/0103 genotype on HIV-1 infection in Zimbabwean women,[9] the significant increase in the frequency of HLA-E*0103 allele in the patients with Behcet's disease (a chronic inflammatory disorder)[10] as well as increased frequencies of HLA-E*0103 allele and HLA-E*0103/0103 genotype in the patients with nasopharyngeal carcinoma (a tumor of epithelial lining of nasopharynx)[11,12] are some examples of the association between HLA-E polymorphisms and clinical outcomes.

There are also few studies suggesting the effects of different genotypes of HLA-E on HSCT outcomes. They reported a decreased incidence of acute GvHD (aGvHD) and an improved survival in the patients with HLA-E*0103/0103 genotype after either HLA-identical sibling bone marrow transplantation (BMT)[13] or HLA-matched allogeneic HSCT.[14] However, the association of HLA-E polymorphisms with chronic GvHD (cGvHD) has not been reported yet. To further evaluate the effects of different genotypes of HLA-E on acute and chronic GvHD in addition to overall survival (OS), the study presented here attempts to examine HLA-E polymorphisms in the patients with hematological malignancies following HLA-E matched allogeneic HSCT.

PATIETNS AND METHODS

Study Cohort

The study group included 56 donors and 56 patients undergoing allogeneic HSCT. The median follow-up of the patients was 20.6 months (range 0.2 to 114.8). Written consent was obtained from all patients and donors. The patients with underlying malignant hematological disorders all received peripheral blood stem cells. Donor selection was performed using molecular typing for HLA-A, -B, -Cw, -DRB1, -DQ and -DP. The transplants included HLA-identical sibling HSCT (n=33, 59%), matched unrelated donor HSCT (n=12, 21.4%) all matched for HLA-A, -B, -C, -DR and -DQ, and HLA-haploidentical killer Ig-like receptor mismatched HSCT (n=11, 19.6%). All donor-patient pairs were matched for HLA-E alleles. Characteristics of the study cohort are given in table 1.

The parameters assessed in this study were HLA-E allele and genotype frequencies, aGvHD, cGvHD and OS. The standard conditioning regimen was total body irradiation (TBI) based and the reduced-intensity conditioning regimen was mostly fludarabine and melphalan without irradiation. Either Cyclosporine A (CsA) alone, CsA combined with methotrexate/ mycophenolate or in vitro $CD34^+$ selection/T cell depletion (for the haploidentical HSCTs) was used as aGvHD prophylaxis. This study has been reviewed and approved by the Ethics Committee at the Alfred Hospital (Project 30/07), Monash University, Melbourne, Australia.

HLA-E Genotyping

All patient-donor pairs were genotyped for HLA-E locus using a sequence specific primer-polymerase chain reaction (SSP-PCR) strategy. Amplification was performed using the PC-960G Gradient Thermal Cycler machine (Corbett, Australia).

In this method, 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 used to distinguish HLA-E*0101 and HLA-E*0103 alleles. All oligonucleotides used in the project were synthesized by Monash Micromon Oligonucleotide Synthesis Facility in Microbiology, Monash University, Melbourne, Australia.

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)
Positive CMV serology	34 (61)
ABO incompatibility	23 (41)
Transplantation	
Source	
Peripheral blood stem cells	56 (100)
HLA matching	
HLA-identical sibling	33 (59)
MUD	12 (21.4)
Killer Ig-like receptor mismatched	11 (19.6)
Conditioning regimen	
TBI based	22 (39.3)
Fludarabine + melphalan + others	34 (60.7)
GvHD prophylaxis	
CsA alone	16 (28.6)
CsA + methotrexate	14 (25)
CsA + mycophenolate	15 (26.8)
$CD34^+$ selection/T cell depletion	11 (19.6)

Acute lymphoblastic leukemia, ALL; Acute Myeloid Leukemia, AML; Cytomegalovirus, CMV; Complete Remission, CR; Cyclosporine A, CsA; Graft-*versus*-host Disease, GvHD; Matched Unrelated Donor, MUD; Total Body Irradiation, TBI

Statistical Analysis

Univariate and multivariate proportional hazard regression models were performed to recognize the probability of OS using the Kaplan-Meier (significance was estimated by the log rank test) and Cox-regression analyses, respectively. The probability of acute and chronic GvHD was identified using univariate and multivariate regression analyses. The statistical significance of the allele and genotype frequencies in this study was determined using the Chi-square test. Analyzed variables such as relation between donor and recipient, transplant type, donor-recipient gender, age and cytomegalovirus (CMV) serology, ABO incompatibility, underlying diagnosis, disease status, conditioning regimen and GvHD prophylaxis in addition to HLA-E genotypes were considered in logistic regression model. All tests were two-sided and the p value <0.05 was considered as statistically significant. Statistical analyses were performed using SPSS 11.5 software package.

RESULTS

HLA-E Allele and Genotype Frequencies

All donors and patients were matched for HLA-E genotypes. HLA-E*0101/0103 [25/56 (44.6%)] was more frequent than HLA-E*0101/0101 [17/56 (30.4%); p=0.08] and HLA-E*0103/0103 [14/56 (25%); p=0.01] whereas HLA-E*0101 and HLA-E*0103 alleles were found at comparable frequencies [59/112 (52.7%) and 53/112 (47.3%) respectively; p=0.4]. Our observed HLA-E allele frequencies were similar to those previously reported in the literatures.

Association of HLA-E Polymorphisms with post-HSCT Outcomes

For this study, applied univariate and multivariate analyses did not show any significant impact of the transplant types on outcomes. Furthermore, because the patients with HLA-E*0101/0101 and HLA-E*0101/0103 genotypes did not exhibit any significant difference in the analyzed outcomes, they were combined in one group termed “others”. The analyses were then performed with these two groups “others” group *versus* HLA-E*0103/0103 group. Table 2 shows the incidence of post-allogeneic HSCT outcomes in patients with different genotypes of HLA-E.

aGvHD

In this study, 31 (55.3%) out of 56 patients had aGvHD of any grade. Of those, 7 (22.6%) patients experienced aGvHD of grade I and 24 (77.4%) patients developed aGvHD of grade II or more.

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

HSCT outcomes	n (%)	E*0101/0101 n (%)	E*0101/0103 n (%)	E*0103/0103 n (%)	*p* value
aGvHD (grade I)	7/56 (12.5%)	3/17 (17.6%)	2/25 (8%)	2/14 (14.3%)	ns
aGvHD (grade II/more)	24/56 (43%)	8/17 (47.1%)	14/25 (56%)	2/14 (14.3%)	0.02
cGvHD (limited)	6/56 (10.7%)	2/17 (11.8%)	3/25 (12%)	1/14 (7.1%)	ns
cGvHD (extensive)	19/56 (34%)	8/17 (47.1%)	10/25 (40%)	1/14 (7.1%)	0.04
OS	23/56 (41%)	6/17 (35.3%)	6/25 (24%)	11/14 (78.6%)	0.001

Acute Graft-*versus*-host Disease, aGvHD; Chronic Graft-*versus*-host Disease, cGvHD; Hematopoietic Stem Cell Transplantation, HSCT; Overall Survival, OS

p values show statistical differences between HLA-E*0103/0103 group and "others" group. $p>0.05$; not significant (ns).

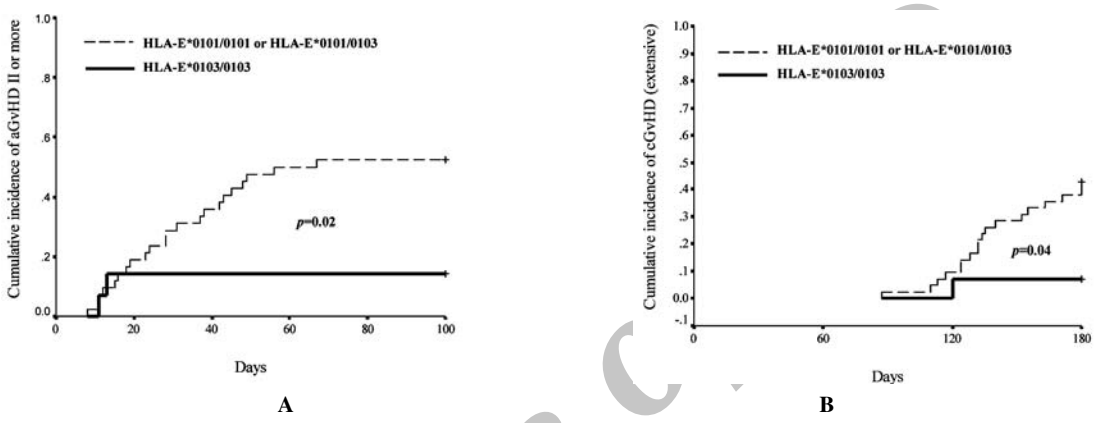

Figure 1. Cumulative incidence of aGvHD and cGvHD after HLA-E matched allogeneic HSCT.

aGvHD of grade II or more (A) and extensive cGvHD (B) are less represented in the patients having HLA-E*0103/0103 genotype with *p* values 0.02 and 0.04 respectively. Acute graft-*versus*-host disease, aGvHD; chronic graft-*versus*-host disease, cGvHD; hematopoietic stem cell transplantation, HSCT

Univariate analysis of data did not support a significant effect of HLA-E polymorphisms on the development of grade I aGvHD in the patients [Hazard Ratio (HR)=0.83; 95% confidence interval (CI)=0.28-2.4; $p=0.7$] – HLA-E*0101/0101 [3/17 (17.6%)], HLA-E*0101/0103 [2/25 (8%)] and HLA-E*0103/0103 [2/14 (14.3%)]. Amongst the patients developed aGvHD of grade II or more, no significant difference ($p=0.7$) was observed between HLA-E*0101/0101 [8/17 (47.1%)] and HLA-E*0101/0103 [14/25 (56%)] genotypes whereas figure 1A demonstrates aGvHD of grade II or more was less frequent in HLA-E*0103/0103 group (14.3%; HR=0.2; 95% CI=0.03-0.7; $p=0.02$) compared to "others" group (52.4%; HR=6.6; 95% CI=1.3-33.1; $p=0.02$).

Furthermore, using multivariate regression analysis, adjusted for confounding variables, two factors were shown to significantly increase the risk of aGvHD (grade II or more): HLA-E genotype (HLA-E*0101/0101 or HLA-E*0101/0103: HR=1.2; 95% CI=1.03-1.5; $p=0.02$) and CMV status of the patient (HR=6.03; 95% CI=1.13-32.3; $p=0.04$).

cGvHD

Longer follow-up of the patients also identified 25 (44.6%) out of 56 patients developed cGvHD. Of these, 6 (24%) patients experienced limited cGvHD which had no association with the genotypes of HLA-E (HR=0.8; 95% CI=0.25-2.5; $p=0.7$) – HLA-E*0101/0101 [2/17 (11.8%)], HLA-E*0101/0103 [3/25 (12%)] and HLA-E*0103/0103 [1/14 (7.1%)], while 19 (76%) patients developed extensive status of cGvHD.

Figure 1B shows extensive cGvHD was less frequent in HLA-E*0103/0103 group (7.1%; HR=0.1; 95% CI=0.01-0.85; $p=0.04$) compared to "others" group (42.8%; HR=1.3; 95% CI=1.01-1.6; $p=0.04$),

whereas there was no significant difference (p=0.7) between HLA-E*0101/0101 [8/17 (47.1%)] and HLA-E*0101/0103 [10/25 (40%)]. Multivariate regression analysis, adjusted for confounding variables, exhibited a lower risk of cGvHD (extensive) in the patients with HLA-E*0103/0103 genotype (p=0.04).

OS

The median follow-up of the patients was 20.6 months (range 0.2 to 114.8) and the estimate of OS was 41% (23/56). Using Kaplan-Meier analysis, there was a significant difference on OS concerning HLA-E genotypes (p=0.01). No significant difference (p=0.4) was observed between HLA-E*0101/0101 [6/17 (35.3%)] and HLA-E*0101/0103 [6/25 (24%)] genotypes. However a better OS shown to be associated with HLA-E*0103/0103 group [11/14 (78.6%)] compared to "others" group [12/42 (28.6%); p=0.001; Figure 2).

In a multivariate Cox-regression analysis, adjusted for confounding variables, two factors of HLA-E genotype (HLA-E*0103/0103: HR=0.3; 95% CI=0.08-0.9; p=0.03) and underlying diagnosis for patients [acute myeloid leukemia (AML); HR=2.9; 95% CI=1.3-6.4; p=0.01] were shown to significantly affect the OS rate − HLA-E*0103/0103 was a prognostic factor for a better OS (about 3.3 times) whereas a diagnosis of AML resulted in an increased risk of mortality (2.9 times).

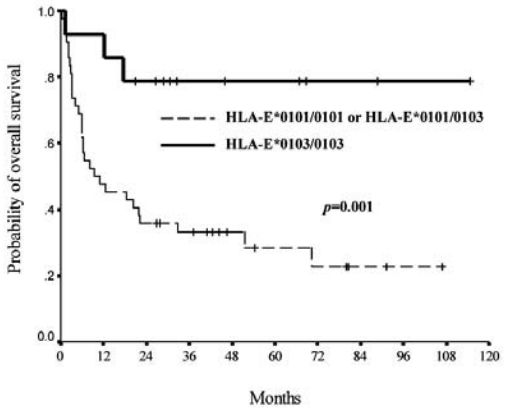

Figure 2. Probability of OS after HLA-E matched allogeneic HSCT. A better OS is associated with HLA-E*0103/0103 genotype with p value 0.001. Hematopoietic stem cell transplantation, HSCT; overall survival, OS

DISCUSSION

Our study found comparable frequencies for the HLA-E*0101 and HLA-E*0103 alleles, the finding which was in line with other studies in Caucasian, African-American and Hispanic populations. However, in Japanese and Chinese populations, the frequency of HLA-E*0103 was shown to be higher than HLA-E*0101 allele. Furthermore, in our study group, HLA-E*0101/0103 genotype was more frequent than HLA-E*0101/0101 and HLA-E*0103/0103 in the donors and patients, the observation that was also consistent with those previously reported by other groups.[2,13-17]

The association of different genotypes of HLA-E with the clinical conditions has been an area of interest for several studies.[7-12,18] Amongst those reports which have noted such associations, there are just few studies suggesting a role for HLA-E polymorphisms in outcomes after bone marrow and stem cell transplantation.[13,14] To further support and investigate whether HLA-E polymorphisms can influence the clinical outcomes after HSCT, we evaluated the probability of OS and the occurrence of acute and chronic GvHD in the patients with different HLA-E genotypes. Our results revealed the presence of HLA-E*0103/0103 genotype was associated with a lower incidence of moderate to severe (grade II or more) aGvHD after HLA-E matched allogeneic HSCT. In addition, we found an improved OS in the patients with HLA-E*0103/0103 genotype. These data confirmed other groups' observations that already showed the association of homozygous status for HLA-E*0103 allele with a lower incidence of aGvHD and a better survival in either HLA-genoidentical BMT[13] or HLA matched related and unrelated HSCT.[14]

The possible mechanism of the aforementioned associations could be related to the importance of endothelium in transplant rejection where the up-regulation of HLA-E molecule on endothelial cells of vessels by cytokines released in the inflammatory circumstances after transplant involves this immunoregulatory molecule in vascular homeostasis.[23] Given that, due to lower cell surface expression of HLA-E*0101 compared to HLA-E*0103, the endothelial cells of vessels having HLA-E*0101 allele may be more susceptible to the destruction mediated by NK and T cells leading to a higher frequency of aGvHD in the patients with HLA-E*0101/0101 genotype.[14] Conversely, higher cell surface expression

of HLA-E*0103 compared to HLA-E*0101 allele in the healthy host cells of the patients with HLA-E*0103/0103 genotype may be better protect those cells from tissue damage mediated by NK cells resulting in lower incidence of aGvHD.

The study here also showed a lower frequency of extensive cGvHD in the patients with HLA-E*0103/0103 genotype compared to those with the other genotypes. To our knowledge, this is the first report implicating the association of HLA-E polymorphisms and extensive cGvHD following HLA-E matched allogeneic HSCT. The mechanism of the lower incidence of extensive cGvHD for this genotype may be similar to that for aGvHD. Nevertheless, lack of any association between limited cGvHD and HLA-E polymorphisms in these patients is the observation that may suggest the involvement of other conditions and or mechanisms which may need further investigation.

In conclusion, our data suggest a protective role for HLA-E*0103/0103 genotype against aGvHD (grade II or more) and cGvHD (extensive), the finding which was also in consistent with a better OS in the patients with this genotype. However, whether the co-occurrence of lower incidence of GvHD and improved OS is mechanistically relevant, requires further investigation in which other risk factors involved in transplant outcomes should be also considered.

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