Analysis of KIR Genes in HLA-Identical Sibling Hematopoietic Stem Cell Transplantation in Thai Patients with Leukemia

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Background: *Killer cell immunoglobulin-like receptors (KIRs) are members of a group of regulatory molecules found on the natural killer (NK) cells that regulate NK cells function by interacting with the human leukocyte antigen (HLA) class I molecules or ligands. The effects of KIR genes on the outcome of hematopoietic stem cell transplantation (HSCT) are still controversial.*
Objective: *To investigate the distribution of KIR genes in HLA-identical sibling and the effect of KIR genes on the outcome of HSCT.*
Material and Method: *The present study included 74 patients and their HLA-identical sibling donors. KIR genes and HLA ligands typing were determined by polymerase chain reaction-sequence specific primer (PCR-SSP). A retrospective study was carried out to analyze the outcomes of the recipients.*
Results: *There was no effect of KIR gene mismatch and missing ligand on the outcome regarding graft-versus host disease (GVHD), relapse, and overall survival (OS) ($p > 0.05$). However, the presence of donor activating KIR2DS5 was associated with decreased aGVHD ($p = 0.01$).*
Conclusion: *Our findings suggest an important role of donor activating KIR in identical sibling HSCT.*

Keywords: *Killer cell immunoglobulin-like receptors, KIR, Hematopoietic stem cell transplantation, HSCT, Thai*

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Natural killer (NK) cells are key components of the innate immune response and possess the ability to kill cell that are undergoing tumorigenesis or infection by viruses or internal pathogens without previous stimulation. The killer immunoglobulin-like receptors (KIR) are cell surface receptors of the immunoglobulin superfamily that are expressed on natural killer cells and subsets of activated or memory T lymphocytes in humans. KIR can be divided into inhibitory (L) and stimulatory (S) based on functional and structural features. Several of KIR receptors use HLA molecules as their ligand. The ligand for KIR2DL1 is the C2 group (HLA-Cw with asparagine at position 77 and lysine at position 80). The ligand for KIR2DL2 and 2DL3 is the C1 group (HLA-Cw alleles with serine at position 77 and asparagine at position 80). The ligand for KIR3DL1 is Bw4. The effects of KIRs on host immune responses are mediated by specific interaction of these receptors and HLA class I ligands, which result in either activating or inhibition of NK cell cytotoxicity. KIR are encoded by a family of highly polymorphic genes clustered in the leukocyte receptor complex on chromosome 19q13.4. KIR exhibits extensive genotypic diversity due to gene number and component[1].

In hematopoietic stem cell transplantation (HSCT), donor-derived NK cells enhance antileukemia effect, promote bone marrow engraftment, and prevent graft-versus-host disease (GVHD)[2]. NK cell alloreactivity can be predicted by missing ligand model in identical sibling HSCT[3], where at least one of the donor KIR does not recognize a HLA allele in the recipient ligand. However, the effect of KIR genes and ligands in HLA identical sibling HSCT is still inconclusive[3-5]. In addition to inhibitory KIRs and HLA class I ligand, studies evaluating the role of activating KIRs for which the ligands are not well identified are conflicting[6-11]. The aim of the present

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study was to analyze KIR genes in HLA-identical siblings and evaluate the effect of KIR genotype mismatch, missing KIR ligands and individual activating KIR on the outcome of HSCT in a Thai population.

Material and Method

Patients

Seventy-four DNA samples from patients with leukemia who received non-T cell depleted hematopoietic stem cell transplants (HSCT) from HLA-identical siblings between 1999 and 2008 in Siriraj Hospital were included in the present study. Sibling donors were selected by haplotype analysis based on low resolution of HLA class I and high resolution typing for class II loci. Fifty-one patients with complete clinical data were analyzed for the outcome. All patients received myeloblative conditioning and received GVHD prophylaxis with cyclosporine A and methotrexate. Clinical information was obtained by the use of medical records. The present study was approved by the ethics committee of Siriraj Hospital, Mahidol University, Thailand.

HLA and KIR genotyping

Patients and donors were typed for HLA-A, HLA-B by microlymphocytotoxicity test. HLA-DR DQ were typed at high resolution by sequencing specific priming (PCR-SSP) kit (MicroSSP, One-Lambda Inc., California, USA). Presence of HLA-C1 and C2 groups was determined by a sequence specific oligonucleotide probe (SSOP) approach[12]. The presence or absence of KIR genes was detected by using PCR-SSP (polymerase chain reaction with sequence specific primer)[13,14]. Typing method and the correction of results were validated by IHWC[15] and external QC program. In brief, the reactions of 10 ul were set up to include 0.1 ug test DNA, buffer IV, 0.2 mM dNTP, 1.08 mM magnesium chloride, 0.3 U Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany) and 0.5 uM specific primer mix (except for 3DL1, 2DS4 which were at final concentration of 1 uM). Internal controls (5'-CAGTGCCTTCCCAACCATTCCCTTA-3', r 5'-ATCCACTCACGGATTTCTGTTGTGTTTC-3') specific for a 485 basepair human growth hormone fragment were included at 0.067 uM in each reaction. All amplifications were performed in duplicate in Perkin Elmer 9700 (PE Biosystem, California, USA) under thermal cycling conditions as follows 5 min denaturing step at 94°C, 10 cycles of 94°C 10 s, 65°C 60 s, then 20 cycles of 94°C 10 s, 61°C 50s, 72°C 30 s. The products were photographed from standard 1% agarose electrophoresis gels containing ethidium bromide.

Definitions

The clinical outcome in the present study included acute graft-versus-host-disease (aGVHD), defined as development of grade II-IV GVHD during the first 100 days post transplantation, chronic GVHD (cGVHD), defined as GVHD occurring in the patients after day 100 post transplantation and relapse, defined by morphologic or cytogenetic evidence either in peripheral blood or bone marrow. Overall survival (OS), defined as time to death from any cause. For missing ligand algorithm, patient-donor pairs were divided in two groups based on the presence or absence of recipient HLA ligand for donor inhibitory KIR (KIR2DL1, KIR2DL2, KIR2DL3, and KIR3DL1). This approach has previously been described[3].

Statistical analysis

Comparison for incidence of aGVHD, cGVHD, and relapse between two groups was tested by Chi-square tests or Fisher's exact testes when appropriated. Overall survival were estimated by Kaplan-Meier method and compared with the log-rank test. The calculation was done by SPSS version 11.5. $P < 0.05$ was considered to be statistically significant.

Results

The distribution of KIR genes in the donors and patients are shown in Fig. 1. All were typed positive for the framework genes (KIR2DL4, KIR3DL2, KIR3DL3, and KIR3DP1). Twenty-two different genotypes could be found. Forty-one pairs (55.4%) of

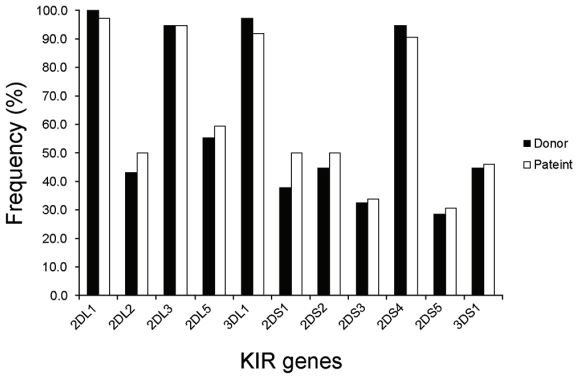

Fig. 1 Frequencies of KIR genes in donors and patients

HLA identical sibling donor-recipients showed KIR mismatch.

KIR ligand typing in the patients showed that 39.2% had both C1C2, 60.8% are homozygotes for C1 alleles. There were no homozygotes for C2. Forty-nine percent of the patients were Bw4 positive and 81.1% of the patients lack HLA ligand for donor inhibitory KIR. The patient and donor characteristics are shown in Table 1. When 51 patients with complete clinical data were analyzed, 27.5% (14/51) had aGVHD, 49% (25/51) had cGVHD, and 19.6% (10/51) had relapse. The effects of KIR genes on the outcome of transplant are shown in Table 2. There was no difference in the incidence of aGVHD (38.1% vs. 20%, p = 0.2) cGVHD (57.1% vs. 43.3%, p = 0.3) and relapse (9.5% vs. 26.7%, p = 0.2) between KIR match and mismatch group. In addition, there was no difference in the incidence of aGVHD (24.4% vs. 50%, p = 0.3) cGVHD (48.9% vs. 50%, p = 1.0) and relapse (20% vs. 16.7%, p = 1.0) between the patients with missing ligand and those with all ligands.

When the effect of each donor-activating KIR receptor (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, and KIR3DS1) on transplantation outcome were analyzed according to the presence or absence of donor-activating KIR, the presence of KIR2DS5 was significantly associated with decreased aGVHD. (0% vs. 36.8%, p = 0.01). An increasing of relapse rate was found but not significant (30.8% vs. 15.8%, p = 0.3). However, there was no effect for KIR2DS5 on cGVHD (46.2% vs. 50%, p = 0.8). When overall survival were analyzed, no effect were seen from KIR genotype mismatch, missing KIR ligand and the presence of donor KIR 2DS5 or other activating KIR (data not shown).

Table 1. Patient and donor characteristics

Characteristics	All patients (n = 74)
Patient age, median (range)	37.85 (17-57)
Patient/donor sex	
Male/male	20 (27.0%)
Male/female	24 (32.4%)
Female/female	12 (16.2%)
Female/male	18 (24.3%)
Diagnosis	
AML	25 (33.8%)
CML	42 (56.8%)
ALL	7 (9.5%)
Type of condition	
Bu/Cy	65 (87.8%)
Bu/melphalan	1 (1.4%)
Cy/TBI	4 (5.4%)
Unknown	4 (5.4%)

Discussion

The distribution of KIR gene frequencies in the donors is similar to the normal control in the Thai population as previously described[15,16]. In HLA identical sibling, the probability of KIR genotype mismatch is high. The effects of KIR mismatch and missing ligand for inhibitory KIR on the outcome of transplant were not found in the present study. This may be from non-T-depleted transplantation. It was shown that the presence of T cells and immuno-suppressants both affect NK reconstitution after HSCT and this may negate the beneficial effect of

Table 2. The effect of KIR genes on the clinical outcomes of transplants in 51 patients

	aGVHD (n = 14)	cGVHDa (n = 25)	Relapse (n = 10)
KIR genotype, n (%)			
Match	8/21 (38.1)	12/21 (57.1)	2/21 (9.5)
Mismatch	6/30 (20.0)	13/30 (43.3)	8/30 (26.7)
KIR ligand, n (%)			
Present	3/6 (50.0)	3/6 (50.0)	1/6 (16.7)
Missing	11/45 (24.4)	22/45 (48.9)	9/45 (20.0)
Donor KIR2DS5, n (%)			
Positive	0/13 (0)	6/13 (46.2)	4/13 (30.8)
Negative	14/38 (36.8)*	19/38 (50.0)	6/38 (15.8)

* p = 0.01

NK alloreactivity[17,18]. The effect of missing ligand on the outcome could not be concluded in the present study, because the number of patients with ligand matched was very low to compare. This might be from a high frequency of C1 homozygotes in the Thai population, whereas in a Caucasian population, the frequency of matched ligand was higher than in Asian population[15]. In other Asian populations, no effect of missing ligand in HLA identical non-T-depleted sibling donor HSCT was seen[19].

The role of activating KIR in the outcome of HSCT is still controversial. In the present study, only the presence of KIR2DS5 was associated with decreased aGVHD. There was no effect on cGVHD and overall survival in the presence of KIR 2DS5 in donors. The increase of relapse was also observed, but not significant. However, the number of relapse cases in the present study was rather small. For KIR2DS5, the function and ligand has not been identified yet. In the previous studies, KIR2DS5 was found to be associated with higher relapse rate after HLA-identical HSCT[9,10]. The effects of other activating KIR genes on aGVHD were also found in other previous studies. KIR2DS3 was found to be associated with increased aGVHD[6] however, KIR3DS1 was associated with decreased aGVHD[11].

The effects of KIR genes and ligands on clinical outcome varied between different studies, which might be from several factors. First, there were differences in transplant protocols that included conditioning regimen, the application of T-cell depleted grafts. Second, there were differences in the characteristic of the patients such as the disease types, disease stages, age of both patients and donors, and matching for gender. Third, the frequencies of KIR genes and ligands are different between populations.

Although the mechanism of activating KIR in HSCT is not elucidated yet, the findings in this preliminary study might suggest the important role of individual activating KIR in HSCT. Further studies in larger populations are needed to confirm and studies in the biologic roles of activating KIR are required.

In summary, individual activating KIR gene might be important in the outcome of HLA-identical sibling hematopoietic stem cell transplantation.

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Potential conflicts of interest

None.

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การวิเคราะห์จีน KIR ในผู้ป่วยไทยโรคลิวคีเมียที่ได้รับการปลูกถ่ายไขกระดูกจากพี่น้องที่มี HLA เหมือนกัน

ณัฐา วงศ์วุฒิสาโรช, ศศิจิต เวชแพศย์, วิโรจน์ จงกลวัฒนา, สุรพล อิสรไกรศีล

ภูมิหลัง: *จีน KIR เป็นสมาชิกของกลุ่มโมเลกุลที่พบบน NK cell ซึ่งควบคุมการทำหน้าที่ของ NK cell โดยทำปฏิกิริยากับโมเลกุล HLA class I ผลของจีน KIR ต่อผลลัพธ์ของการปลูกถ่ายไขกระดูกยังไม่ชัดเจน*

จุดประสงค์: *เพื่อดูการกระจายของจีน KIR ในพี่น้องที่มี HLA เหมือนกัน และผลของจีน KIR ต่อการปลูกถ่ายไขกระดูก*

วัสดุและวิธีการ: *ประกอบด้วยผู้ป่วยและพี่น้องที่มี HLA เหมือนกัน 74 ราย การตรวจจีน KIR และ HLA ligand ทำโดยวิธี PCR-SSP และได้มีการศึกษาย้อนหลังเพื่อวิเคราะห์ผลลัพธ์ของการปลูกถ่ายไขกระดูก*

ผลการศึกษา: *ไม่พบผลของจีน KIR mismatch และ missing ligand ต่อผลลัพธ์ได้แก่ GVHD, relapse และ overall survival ($p > 0.05$) อย่างไรก็ตาม KIR 2DS5 ที่พบในผู้บริจาคมีความสัมพันธ์กับการเกิด aGVHD ($p = 0.01$)*

สรุป: *การศึกษานี้แสดงบทบาทที่สำคัญของจีน activating KIR ในผู้บริจาคในการปลูกถ่ายไขกระดูก จากพี่น้องที่มี HLA เหมือนกัน*