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Predicted indirectly recognizable HLA epitopes are not associated with clinical outcomes after haploidentical hematopoietic stem cell transplantation

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ABSTRACT

Haploidentical stem cell transplantation (haplo-SCT) provides an alternative method to cure patients with malignant and nonmalignant hematologic diseases who lack a human leukocyte antigen (HLA) matched related or unrelated donor. HLA disparity between donor and patient was the main reason causing lots of clinical immune response. The aim of this study was to investigate whether indirect recognition of mismatched HLA could predict the clinical outcomes in haplo-SCT. The probability of indirect recognition was predicted by the Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE) model. 577 patients with acute leukemia or myelodysplastic syndrome receiving haplo-SCT were enrolled in the study. Patients were divided into 4 quartiles according to PIRCHE-I or PIRCHE-II. Although the cumulative incidences of chronic graft-versus-host disease (GVHD) were significantly different among the 4 PIRCHE-Igroups, with 20.4% for group 0–6, 40.5% for group > 6–11, 26.1% for group > 11–19 and 23.9% for group > 19 ($P = .007$), PIRCHE-I was not significantly associated with chronic GVHD in multivariate models (RR, 0.993; 95% CI, 0.858–1.149; $P = .926$). And no significant associations were observed between PIRCHE-I or PIRCHE-II and other clinical outcomes. In summary, PIRCHE did not correlate with clinical outcomes and could not predict haplo-SCT outcomes.

1. Introduction

Related haploidentical donor is an alternative option for patients without matching human leukocyte antigen (HLA) related or unrelated donors. HLA mismatches were associated with increased risk of graft-versus-host disease (GVHD) after unrelated stem cell transplantation, leading to increased risk of mortality [1–3]. However, a number of studies found that greater HLA disparity did not appear to worsen the overall outcome after haploidentical stem cell transplantation (haplo-SCT) [4–8]. Massive immunosuppressive agent (antithymocyte globulin or high-dose posttransplant cyclophosphamide) was used in the haplo-SCT, leading to the weakening effect of HLA disparity on transplant outcomes, but this did not mean that HLA mismatch was not important in the haplo-SCT. On the contrary, we needed to further investigate the function of HLA and provided a new strategy to predict the clinical outcomes.

On the basis of the indirect T-cell recognition [9], Spierings et al. have proposed a method called Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE) which can predict indirect recognition in silico and studied the effect of the numbers of PIRCHE presented on HLA class I and II (PIRCHE-I and -II) in pediatric patients [10–12]. They found that above median PIRCHE-I were associated with reduced relapse risk after cord blood transplantation [11]. In adult unrelated SCT, both PIRCHE-I and -II were related to graft-versus-host disease (GVHD) development [13]. However, there were no reports about the effect of PIRCHE on outcomes after haplo-SCT. To address this question, we investigate the Association between PIRCHE and clinical outcomes in 577 patients with acute leukemia or myelodysplastic syndrome after haplo-SCT.

Abbreviations: haplo-SCT, Haploidentical stem cell transplantation; HLA, human leukocyte antigen; PIRCHE, Predicted Indirectly ReCognizable HLA Epitopes; GVHD, chronic graft-versus-host disease; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; MDS, myelodysplastic syndrome; rhG-CSF, recombinant human granulocyte colony-stimulating factor; MNC, mononuclear cell count; PBSC, peripheral blood stem cells; CSA, cyclosporine A; MTX, methotrexate; MMF, mycophenolate mofetil; PCR, polymerase chain reaction; TRM, Transplant-related mortality; OS, overall survival; DFS, disease free survival; TCR, T cell receptor; mHag, minor histocompatibility antigens

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Table 1
Characteristics of patients by PIRCHE-Iquartile.

Variable	PIRCHE-I Quartile				*P*
	0–6	> 6–11	> 11–19	> 19	
Number of patients	155	126	143	133	
Median patient age, range, y	26 (2 − 56)	26 (3 − 57)	26 (2 − 67)	25 (4 − 58)	.931
Median donor age, range, y	42 (12–63)	39 (13–64)	10 (13–63)	40 (13–63)	.098
Disease type, n (%)					.743
Acute myeloid leukemia	79 (51)	63 (50)	73 (51)	55 (41.4)	
Acute lymphoblastic leukemia	64 (41.3)	53 (42.1)	53 (37.1)	68 (51.1)	
Myelodysplastic syndrome	12 (7.7)	10 (7.9)	17 (11.9)	10 (7.5)	
Disease state, n (%)					.951
Standard risk	122 (78.7)	102 (81)	116 (81.1)	106 (79.7)	
High risk	33 (21.3)	24 (19)	27 (18.9)	27 (20.3)	
Donor-recipient gender, n (%)					.313
Male-male	58 (37.4)	55 (43.7)	59 (41.3)	53 39.8)	
Male-female	33 (21.3)	30 (23.8)	37 (25.9)	35 (26.3)	
Female-male	33 (12.3)	25 (19.8)	26 (18.2)	29 (21.8)	
Female-female	31 (20)	16 (12.7)	21 (14.7)	16 (12)	
Donor-recipient blood type, n (%)					.011
Match	83 (53.5)	74 (58.7)	95 (66.4)	66 (49.6)	
Major mismatch	33 (21.3)	21 (16.7)	17 (11.9)	27 (20.3)	
Minor mismatch	30 (19.4)	24 (19)	27 (18.9)	30 (22.6)	
Major + minor	9 (5.8)	7 (5.6)	4 (2.8)	10 (7.5)	
Match degree, n (%)					< .0001
10/10	5 (3.2)	0	0	0	
9/10	9 (5.8)	3 (2.4)	1 (0.7)	1 (0.8)	
8/10	9 (5.8)	3 (2.4)	7 (4.9)	1 (0.8)	
7/10	26 (16.8)	21 (16.7)	18 (12.6)	11 (8.3)	
6/10	26 (16.8)	22 (17.5)	32 (22.4)	23 (17.3)	
5/10	80 (51.6)	77 (61.1)	85 (59.4)	97 (72.9)	

2. Method

2.1. Patients

We enrolled patients with acute myeloid leukemia (AML), acute lymphoid leukemia (ALL) and myelodysplastic syndrome (MDS) who were suitable for transplantation and had no related or unrelated HLA-identical donors. 913 patients received haplo-SCT between January 2010 and February 2014. A total of 557 donor-pairs for which DNA were available for retrospective HLA-C and -DQB1 sequencing were included in the analysis. Written informed consent was obtained from all of the patients and donors before their entry into the study, in accordance with Declaration of Helsinki. Both the patients and their donors provided written informed consent, and the Institutional Review Board of the Peking University Institute of Hematology approved this study. Subjects were categorized as standard-risk if they were in first or second complete remission. Others were classified as high risk. The characteristics of the 557 patients are presented in Table 1.

2.2. Transplant protocol

Transplant procedures were described in our previous reports [14]. All patients received myeloablative regimens including a combination of cytosine arabinoside (4 g/m^2 × 2 days, on days −10 and −9), Busulfan (12 mg/kg administered in 12 doses over 3 days, on days −8, −7 and −6), cyclophosphamide (1.8 g/m^2 × 2 days, on days −5 and −4), semustine (MeCCNU) (250 mg/m^2, on day −3), and rabbit anti-human thymocyte immunoglobulin (thymoglobulin [Sangstat Medical], 2.5 mg/kg, on days −5 to −2).

Donors received 5 μg/kg recombinant human granulocyte colony-stimulating factor (rhG-CSF) (filgrastim) once daily for 5–6 days. The target mononuclear cell count (MNC) was 4–6 × 10^8 cells/kg recipient weight. On the fourth day, bone marrow cells were harvested. On the fifth day, peripheral blood stem cells (PBSC) were collected with a COBE Blood Cell Separator (Spectra LRS; COBE BCT, Inc., Lakewood, CO, USA) from a total blood volume of 10 L. The fresh and unmanipulated bone marrow and peripheral blood progenitor cells were infused into the recipients on the day of collection.

Prophylaxis for GVHD included cyclosporine A (CSA) and short-term methotrexate (MTX) with mycophenolate mofetil (MMF). Cyclosporine was started intravenously on day −9, at dosage of 2.5 mg/kg, and switched to oral formulation as soon as the patient was able to take medication after engraftment. The dosage was adjusted to keep blood levels between 150 and 300 ng/mL. MMF was administered orally, 0.5 g every 12 h, from day −9 before transplantation to day 30 after transplantation, then 0.25 g twice a day for 1–2 months. The dosage of MTX was 15 mg/m^2, administered i.v. on day 1, and 10 mg/m^2 on days 3, 6, and 11 after transplantation. Filgrastim (G-CSF) 5 μg/kg per day was given to all recipients subcutaneously from day 6 after transplantation until the neutrophil count reached 0.5 × 10^9 cells/L for three consecutive days. Bone marrow aspiration and cytogenetic studies were performed at 1–3 months after transplantation to assess engraftment. Polymerase chain reaction (PCR) DNA fingerprinting (short tandem repeat) were used for donor chimeric detection.

2.3. HLA typing

HLA-A, HLA-B and HLA-DRB1 were typed by the polymerase chain reaction-sequence specific oligonucleotide probes followed the recommendations of the manufacturer (LAB○RType, One lambda Inc., Canoga Park, CA) before transplantation. HLA-C and HLA-DQB1 were typed by the polymerase chain reaction-sequencing based typing (SeCore○R, invitrogen, Brown Deer, WI, USA) using available DNA retrospectively.

2.4. PIRCHE

PIRCHE were determined as described previously [10,12]. In short, PIRCHE-I were identified in 2 steps. First, proteasome-mediated cleavage and transportation via the TAP (transporter associated with antigen processing) channel were predicted for all donor and patient HLA molecules using NetChop C term 3.0 (http://www.cbs.dtu.dk/services/).