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CORD BLOOD

Risk of HLA Homozygous Cord Blood Transplantation: Implications for Induced Pluripotent Stem Cell Banking and Transplantation

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ABSTRACT

Clinical application of induced pluripotent stem cells (iPS) in autologous settings has just begun. To overcome the high time and cost barriers in the individual production of autologous iPS, the use of allogeneic iPS with a homozygous human leukocyte antigen (HLA) haplotype (HLA-homo HP) has been proposed. Cord blood transplantation (CBT) is a suitable model for evaluating the allogeneic immunogenicity of iPS transplantation from HLA-homo donors. We analyzed 1,374 Japanese single cord blood transplant pairs who were retrospectively typed as HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1. Among these, six pairs with donor HLA homo—patient-HLA hetero (homo-hetero) were found, all of which showed favorable neutrophil engraftment. Multivariate analysis revealed a significantly elevated engraftment risk (HR = 1.59) compared with hetero-hetero pairs with HLA 1-2 locus mismatch (789 pts) and comparative risk (HR = 1.23) compared with hetero-hetero pairs with 0 mismatch (104 pts). These results for CBT with HLA-homo HP cord blood carry an important implication, namely the possibility that HLA-homo iPS transplantation results in favorable engraftment. Furthermore, we obtained detailed information on HLA alleles and haplotypes of HLA-homo. All donor HLA-homo HPs had a common specific ethnicity and high conservation of the HLA region, and one of two patient heterogeneous HPs invariably shared the same HP as donor HLA-homo HP, and another non-shared patient HP was mismatched with 1 to 4 HLA alleles of HLA-A, -B, -C, and -DRB1 loci in the GVH direction. These findings indicate that patients possessing a single common HLA haplotype have a higher chance of yielding HLA-homo iPS. STEM CELLS TRANSLATIONAL MEDICINE *2018;7:173–179*

SIGNIFICANCE STATEMENT

Clinical outcome of cord blood transplantation transplanted with human leukocyte antigen (HLA) homozygous haplotype (HP) yielded a number of important implications, namely the possibility of favorable engraftment in HLA homo induced pluripotent stem cells (iPS) transplantation and detailed information of HLA alleles and haplotypes of HLA-homozygous HPs. Accumulation of such immunological genetic data for established HLA-homo iPSs and for pairs in upcoming clinical iPS transplantation is essential to ensuring the safety and consistent success of iPS transplantation.

INTRODUCTION

Induced pluripotent stem cells (iPS), prepared by the introduction of four specific genes encoding the transcription factors Oct4, Sox2, Klt4, and c-Myc, have the capacity to generate various tissues/organs [1]. Clinical application in autologous settings in humans has just begun, such as in retinal regeneration [2–5]. However, autologous transplantation is hampered by the high time and cost required for the individual production of iPS. To solve these issues, allogeneic iPS transplantation from iPS banking has been proposed [6, 7]. One candidate for this is iPS induced from individuals with a homozygous human leucocyte antigen (HLA) haplotype (HLA-homo) [8–10], on the basis that HLA-homo iPS might not be rejected by HLA haplotype-matched patients. At present, HLA-homo volunteer donors in Japan are recruited from among donors with available HLA data in HLA-matched platelet transfusion registries, bone marrow donor registries, and cord blood banks.

However, concerns have been raised over whether allogeneic iPS transplantation from HLA-

homo iPS induces immunogenicity in clinical settings [11–13]. In vitro assay and animal model studies to experimentally verify the risk of HLA-homo iPS transplantation have been extensively reported. Among these, Sugita et al. showed a lack of T cell response by iPSC-derived retinal pigment epithelial cells [14]; Suzuki et al. showed natural killer cell activity against iPS-derived HLA-knockout megakaryocytes [15]; Mizukami et al. analyzed immune response in pigs [16], and Sun et al. showed the insensitivity of human iPS in a hind limb ischemia mouse model [17]. To date, however, no clinical data assessing the immunological reactivity of allogeneic iPS transplantation have yet been reported.

Allogeneic hematopoietic stem cell transplantation (HSCT) is characterized as the regenerative transplantation of hematopoietic progenitor cells, and has yielded practical clinical applications for the last 50 years [18]. HSCT, especially cord blood transplantation (CBT) [19, 20], might be a suitable model for evaluating the allogeneic immunogenicity of iPS transplantation from HLA-homo donors. HLA mismatch CBT from donors with less than two HLA loci mismatches is common but carries a high engraftment failure rate, with a range of 10% to 20%. Further, cord blood cells are reported to be good source for the production of iPS [21–25].

Here, as part of our efforts to improve HLA-homo iPS banking and transplantation, we report an analysis of allogeneic immunogenicity which focuses on engraftment of unrelated transplantation from HLA-homo cord blood.

Materials and Methods

Study Population

This analysis examined cord blood transplant pairs ($n = 1,374$) obtained from the collected samples of seven cord blood banks in Japan. To be included in the analysis, samples had to meet the following criteria: (a) transplantation pairs retyped for HLA-A, -B, and -DRB1 alleles and newly typed for HLA-C, -DQB1, and -DPB1 alleles, as described below; (b) first transplantation; (c) single-unit cord blood; (d) HLA-A, -B, and -DRB1 antigen level matching within 2 mismatches, which is the standard criterion for cord blood donor selection in Japan; (e) no use of anti-thymocyte globulin for graft-versus-host disease (GVHD) prophylaxis; (f) Japanese pairs; and (g) survival for more than 7 days after transplantation. Patient and transplant characteristics are shown in Table 3. Patient age ranged from 0 to 88 years (median 44). Disease status and classification of patients at the time of transplantation were as follows. Standard-risk leukemia was defined as acute myeloblastic leukemia in the first or second complete remission (CR), acute lymphoblastic leukemia in the first CR, chronic myelocytic leukemia in the first chronic phase, and myelodysplastic syndrome in refractory anemia or in refractory anemia with ringed sideroblasts. High-risk leukemia was defined as transplantation at a more advanced stage than with standard-risk leukemia.

All cord bloods were selected through the Japan Cord Blood Bank Network [22]. The final clinical survey of patients was completed by 2013, and informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Aichi Cancer Center and each participating cord blood bank.

Outcome Definition

Neutrophil engraftment was defined as more than 500 cells per μl in peripheral blood at three consecutive measurements within 100 days after transplantation. Platelet engraftment was defined as more than 50,000 cells per μl without transfusion support. Mortality was defined as death from any causes that occurred after the day of transplantation. Clinical grading of acute GVHD was performed according to established criteria [23].

HLA Typing and the Definition of HLA Homozygosity

All donor-patient pairs were retrospectively genotyped at the Japanese Red Cross Kanto-Koshinetsu Block Blood Center for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles at the field 1 and field 2 level of the 2010 World Health Organization Nomenclature for factors for the HLA system [26]. All samples were examined using the PCR-SSO method, with the PCR-SBT method used to confirm any rare or new alleles. An HLA-locus mismatch in the GVH direction among the donor-recipient pairs was scored when the recipient's HLA alleles were not shared by the donor, while an HLA-locus mismatch in the HVG direction was scored when the donor's HLA alleles were not shared by the recipient.

HLA homozygosity of HLA-A, -B, -C, -DRB1, and -DQB1 in allele level was defined when only one allele was observed in each of these five HLA loci, and HLA heterozygosity was defined when two alleles were observed in any of these loci. The combination of donor HLA homozygote (homo) and patient HLA heterozygote (hetero) was simplified as homo-hetero; donor hetero and patient homo as hetero-homo; donor homo and patient homo as homo-homo; and donor hetero and patient hetero as hetero-hetero. Hetero-hetero combination was further subdivided into three groups by the number of HLA-A, -B, and -C mismatched loci in the HVG direction at the allele level, namely hetero-hetero (0) and hetero-hetero (1–2) and (3). As the risk of mismatch of HLA-DRB1, -DQB1, and -DPB1 was not observed (Supporting Information Table 2), HLA-A, -B, and -C matching was selected for analysis of the number of HLA locus mismatches, and the risk of the number of HLA-A, -B, and -C mismatches is shown in Supporting Information Table 3. As the risk of 1 locus mismatch and 2 loci mismatch had the same hazard ratio of 0.76, 1, and 2 locus mismatches were lumped together. KIR2DL ligand specificity of HLA-C antigen was determined according to the HLA-C allele. The epitope of HLA-Cw3 group (C1 specificity) consists of Asn80, and that of the HLA-Cw4 group (C2 specificity) consists of Lys80. KIR ligand mismatch in the GVH direction was scored when the donor KIR2DL epitope of HLA-C was not shared by the patient epitope. This mismatch occurred when KIR2DL2/3- or KIR2DL1-positive effector cells were activated without expression of the corresponding HLA-C ligand (C1 or C2, respectively), on the patient target cells. KIR ligand mismatch in the HVG direction was scored when the patient KIR2DL epitope of HLA-C was not shared by the donor. This mismatch occurred when patient KIR2DL2/3- or KIR2DL1-positive effector cells were activated without expression of the corresponding HLA-C ligand (C1 or C2, respectively) on donor cells.

Statistical Analysis

The hazard ratio of neutrophil engraftment was assessed using multivariable competing risk regression analysis [27]. Potential confounders considered in the multivariate study were transfused mononuclear cell number per patient weight (kg), patient age (linear), disease, disease stage (standard or high risk), GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other regimen), conditioning regimen (myeloablative or non-myeloablative/reduced intensity), and period of transplantation. Impact by the factor of interest was assessed using the log-rank test, and impact

Table 1. Clinical data of donor HLA-homo and patient HLA-hetero pairs

No.	Disease	Leukemia risk	Patient age (y.o.)	TNC/kg[a] ($\times 10^7$)	Neutrophil eng.[b] (days)	Platelet eng.[c] (days)	Acute GVHD (grade)	Alive/Dead (days)
1	AML	High	66	4.27	Y (25)	N (43)	0	Dead (43)
2	ALL	High	62	3.19	Y (17)	N (41)	III	Dead (41)
3	AML	Standard	60	3.88	Y (15)	Y (41)	II	Alive (726)
4	CML	High	37	3.11	Y (23)	Y (51)	0	Alive (1,250)
5	MDS	Standard	47	2.92	Y (20)	Y (75)	0	Alive (1,455)
6	MDS	High	66	2.50	Y (30)	Y (101)	0	Dead (400)

Abbreviations: AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelocytic leukemia; MDS, myelodysplastic syndrome.
[a]Total nuclear cell number of cord blood per patient's weight (kg).
[b]Y: Engraftment of neutrophil. Days of neutrophil number reached to 500/microliter.
[c]Y: Engraftment of platelet. Days of platelet number reached to 50,000/microliter.

on overall survival was evaluated using Cox's proportional hazards regression model [26]. The cumulative incidence curve of engraftment was assessed using the competing risk regression method adjusted with the confounders described above. Overall survival was calculated using the Kaplan-Meier method. All outcomes were assessed in patients who survived more than 7 days after transplantation. We assessed acute GVHD, neutrophil engraftment, and platelet engraftment at 100 days. *p* values of less than .05 were considered significant. All analyses were conducted using STATA version 12 (Stata Corp.; College Station, TX).

Results

Clinical Outcome of Donor HLA-Homo and Patient HLA-Hetero Pairs

Six pairs with donor HLA homo-patient HLA hetero (homo-hetero) were found, all of which showed favorable neutrophil engraftment between 15 and 30 days after transplantation (Table 1). Platelets were engrafted in all 4 patients, excluding 2 patients with early death, and no secondary engraftment failure (rejection) occurred in any pair. Grade III and grade II acute GVHD did occur in pair no. 2 and 3, respectively, while no acute GVHD was seen in the other four pairs (Table 1).

HLA Haplotype and Alleles in Donor HLA-Homo and Patient HLA-Hetero Combination

Donor and patient HLA alleles and HLA haplotypes are shown in Table 2. Patient HLA alleles not shared with the donor are in bold character style. All 6 pairs had the common haplotypes (HP-1, 3 pairs; HP-2, 2 pairs; and HP-3, 1 pair). Five donor HPs, but not pair no. 5, had the same HLA-DPB1 allele, consistent with reports that these common HLA-HP show a long-stretch homogeneous region from HLA-A to HLA-DPB1 [28]. HPs with high to low frequency were found in patient non-shared HPs, some of which showed the possibility of recombination between HLA loci, such as no. 1 and no. 4.

The KIR2DL1 epitope was donor C1/C1 and patient C1/C1 in all six pairs, making the KIR2DL ligand match combination in both the HVG and GVH vectors.

Statistical Analysis for Risk of Neutrophil Engraftment by HLA Homozygosity

Combinations of HLA-homo and HLA-hetero between cord blood and patient were grouped. The hetero-hetero group was further subdivided according to the number of HLA-A, -B, and -C mismatch loci in the HVG direction at the allele level, and analyzed by

Table 2. HLA haplotype and its alleles in donor HLA-homo and patient HLA-hetero pairs

		HLA-haplotype[a]	A	C	B	DRB1[a]	DQB1	DPB1[a]
1	Donor	HP-1	24:02	12:02	52:01	15:02	06:01	09:01
	Patient	HP-1/HP-16	24:02	12:02	52:01	15:02/**09:01**	06:01/**0303**	09:01/**0501**
2	Donor	HP-1	24:02	12:02	52:01	15:02	06:01	09:01
	Patient	HP-1/HP-3	24:02	12:02/**07:02**	52:01/**07:02**	15:02/**0101**	06:01/**0501**	09:01/**0402**
3	Donor	HP-1	24:02	12:02	52:01	15:02	06:01	09:01
	Patient	HP-1/HP-136	24:02	12:02/**07:02**	52:01/**40:01**	15:02/**08:03**	06:01	09:01/**02:01**
4	Donor	HP-2	33:03	14:03	44:03	13:02	06:04	04:01
	Patient	HP-2/HP-X	33:03/**0201**	14:03	44:03	13:02	06:04	04:01
5	Donor	HP-2	33:03	14:03	44:03	13:02	06:04	04:01/**06:01**
	Patient	HP-2/HP-Y	33:03	14:03/**07:02**	44:03/**39:02**	13:02/**0901**	06:04/**03:03**	04:01/**02:01**
6	Donor	HP-3	24:02	07:02	07:02	01:01	05:01	04:02
	Patient	HP-3/HP-Z	24:02	07:02/**14:02**	07:02/**51:01**	01:01/**04:04**	05:01/**04:02**	04:02/**02:01**

Patient HLA alleles not shared with the donor are in bold character style.
[a]Numbered individual haplotypes in order of frequency. X, Y, and Z indicate nongrouped rare haplotypes.

Table 3. Statistical analysis for risk of neutrophil engraftment by HLA homozygosity and other clinical variables

Variable	Patient no.	Hazard ratio	[95% conf. Interval]	*p* value
HLA matching (HLA mismatch locus[a] *no.)*				
hetero-hetero (1–2)	789	1.00		
hetero-hetero (3)	455	0.85	0.75–0.97	.016
hetero-hetero (0)	104	1.25	1.00–1.55	.047
homo-hetero	6	1.59	1.00–2.54	.049
hetero-homo	13	0.50	0.24–1.03	.062
homo-homo	7	1.21	0.51–2.88	.660
Transfused nuclear cell no./weight (kg) $\times 10^7$				
< 3.0	851	1.00		
$3.0 \leq -5.0 <$	371	1.20	1.05–1.37	.007
$5 \leq$	124	1.68	1.34–2.12	$<.001$
Unknown	28	1.29	0.78–2.14	.328
Patient age, yrs, median (range): 44 (0–88)				
linear	1,374	1.00001	0.996–1.004	.996
Disease				
AML	272	1.00		
ALL	664	0.82	0.71–0.96	.012
MDS	148	0.83	0.67–1.03	.097
Others	290	0.85	0.69–1.04	.113
Transplanted year				
1999–2006	398	1.00		
2007–2009	581	1.14	0.99–1.31	.069
2010–2012	395	1.26	1.06–1.49	.008
GVHD prophlaxis				
Cyclosporine based	574	1.00		
Tacroliumus based	787	1.04	0.92–1.17	.537
Others	13	0.75	0.34–1.62	.461
Disease stage				
Standard risk	470	1.00		
High risk	769	0.78	0.69–0.89	$<.001$
Others	135	0.83	0.64–1.09	.182
Conditioning				
Non-myeloablative	457	1.00		
Myeloablative	619	0.85	0.73–0.99	.033
Unknown	298	0.82	0.68–0.98	.030

Homo-hetero: combination of donor with HLA-homo and patient with HLA-hetero. hetero-hetero (1–2): combination of donor with HLA-hetero and patient with HLA-hetero and 1 or 2 locus mismatches in HLA-A, -B, and -C loci at the allele level.
Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; MDS, myelodysplastic syndrome.
[a]HLA-A, -B and -C locus mismatch in HVG direction.

competing risk regression analysis with adjustment for other cofounders, as listed in the Materials and Methods section above.

These six donor homo-patient hetero (homo-hetero) pairs revealed significantly higher engraftment risk (HR = 1.59) than the hetero-hetero group with HLA 1–2 mismatch (789 pts) ($p = .047$) (Table 3), and tended to show a comparatively higher risk (HR = 1.23) than hetero-hetero with 0 mismatches (104 pts) ($p = .337$) (Supporting Information Table 1). The competing risk regression curve of neutrophil engraftment is shown in Figure 1. These data indicated that HLA-homo cord blood produced favorable engraftment.

The donor hetero-patient homo group (hetero-homo) consisted of 13 patients, who revealed a tendency to a lower engraftment ratio (HR = 0.50) than the hetero-hetero group with HLA 1–2 mismatch (789 pts) ($p = .062$) (Table 3)

With hetero-hetero combination, the risk of engraftment decreased as the number of HLA mismatch loci in HLA-A, -B, and -C increased (Table 3).

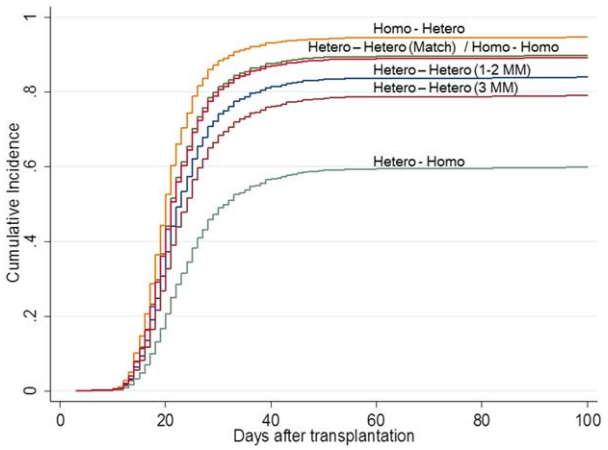

Figure 1. Neutrophil engraftment curve by the combination of HLA-haplotypes. Cumulative incidence curve was assessed using the competing risk regression method with adjustment for the confounders described in Table 3. Homo-hetero, combination of donor with HLA-homo and patient with HLA-hetero; hetero-hetero (1–2), combination of donor with HLA-hetero and patient with HLA-hetero and 1 or 2 locus mismatches in HLA-A, -B, and -C loci at the allele level. The other lines are described in detail in the Materials and Methods section.

Risk of variables other than HLA are also listed in Table 3. Transplanted nuclear cell number per patient weight, disease, transplanted year, disease stage, and conditioning regimen were found to be significant factors.

Statistical Analysis for Risk of Mortality by HLA Homozygosity

The six donor homo-patient hetero (homo-hetero) pairs revealed no significant mortality risk (HR = 0.81) compared with the hetero-hetero group with HLA 1–2 mismatch (817 pts) ($p = .72$) (Table 4), or mortality risk (HR = 0.85) compared with hetero-hetero with 0 mismatches (108 pts) ($p = .72$) (Supporting Information Table 4). The Kaplan-Meier curve of mortality is shown in Supporting Information Figure 1.

Discussion

In this study, we identified 6 CBT pairs with unrelated HLA-homo cord blood to HLA-hetero patient transplantation among 1,374 CBT pairs. All six pairs showed favorable neutrophil engraftment. Statistical analysis demonstrated a possibly compatible engraftment ratio compared with CBTs from hetero cord blood to hetero patients with HLA-match, and a better engraftment ratio than in CBTs from hetero cord blood to hetero patients with HLA-one locus mismatch. These findings and the HLA haplotype information carry a number of implications for HLA-homo iPS banking and transplantation.

First, only 6 HLA-homo individuals were identified among this large cohort of 1,374 CBT donors, and all 6 possessed common HLA haplotypes. Therefore, it is practical to recruit HLA haplotype-homo individuals with common HLA haplotypes for iPS banking. The chance of encountering an HLA-homo individual has been calculated in diverse populations, including Japanese [24, 29, 30], Chinese [31], British [32, 33], and Brazilian [28]. Results showed that the chance of finding an HLA-homo HP markedly differs among countries, and depends on the diversity of the ethnic population. Nakatsuji et al. calculated that 15,000 individuals would be required to find top 50 HLA-homo individuals at the HLA -A, -B, and -DR antigen level, which covers 82.2% of the Japanese population [30]. Okita et al. also calculated the chance of finding HLA-homo by HLA allele level [24]. Efforts are now underway in Japan

Table 4. Statistical analysis for the risk of mortality by HLA homozygosity and other clinical variables

Variable	Patient no.	Hazard ratio	[95% conf. Interval]	*p* value
HLA matching (HLA mismatch locus[a] no.)				
hetero-hetero (1–2)	817	1.00		
hetero-hetero (3)	476	1.05	0.90–1.22	.559
hetero-hetero (0)	108	0.95	0.71–1.28	.738
homo-hetero	6	0.81	0.26–2.54	.72
hetero-homo	13	1.02	0.50–2.06	.96
homo-homo	7	1.79	0.66–4.85	.249
Transfused nuclear cell no./weight (kg) $\times 10^7$				
3.0<	883	1.00		
3.0≥–5.0<	386	1.20	1.02–1.41	.026
5.0≥	125	1.17	0.83–1.64	.377
Unknown	33	1.65	1.04–2.60	.032
Patient age, yrs, median (range): 44 (0–88)				
linear	1,427	1.02	1.02–1.03	<.001
Disease				
AML	281	1.00		
ALL	690	0.92	0.74–1.14	.450
MDS	151	0.67	0.50–0.91	.010
Others	305	0.77	0.58–1.03	.076
Transplanted year				
1999–2006	424	1.00		
2007–2009	604	0.76	0.64–0.91	.003
2010–2012	399	0.84	0.68–1.04	.118
GVHD prophlaxis				
Cyclosporine based	595	1.00		
Tacroliumus based	813	1.01	0.88–1.18	.844
Others	19	1.05	0.56–1.97	.887
Disease stage				
Standard risk	483	1.00		
High risk	799	2.52	2.10–3.01	<.001
Others	145	3.46	2.49–4.82	<.001
Conditioning				
Non-myeloablative	479	1.00		
Myeloablative	639	0.94	0.78–1.13	.526
Unknown	309	1.05	0.85–1.30	.620

Homo-hetero: combination of donor with HLA-homo and patient with HLA-hetero. hetero-hetero (1–2): combination of donor with HLA-hetero and patient with HLA-hetero and 1 or 2 locus mismatches in HLA-A, -B and -C loci at the allele level.
Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; MDS, myelodysplastic syndrome.
[a]HLA-A, -B, and -C locus mismatch in HVG direction.

to identify such individuals among already-HLA-typed donors in public cord blood banks, the Japan Marrow Donor Program, and the platelet donor registry of the Japanese Red Cross Blood Center.

Second, HPs with high frequency are considered to represent a conserved extended haplotype (CEH), and to be specific to the particular ethnic population. In the present study, HLA-homo HPs of cord blood were most frequently the best three CEHs, namely HP-1, HP-2, and HP-3, which revealed highly conserved MHC blocks through the entire HLA region, as we reported previously [28]. The most common extended HLA haplotype in northern European populations is 8.1 HP, with HLA-A1-Cw3-B8-DR3-DQ2 and showing remarkable conservation [34]. These common CEHs can be distinguished from each another at many levels of genetic diversity, including coding regions of complex genes such as HLA, and noncoding forms of variation. Thus, it is important to recognize that not only alleles of classical HLA loci, such as HLA-A, -B, -C, and -DRB1, but also those of other HLA genes, as well as non-HLA genes within HLA regions and noncoding regions of these HPs, are common, either entirely or partly.

Third, one of the two patient heterogeneous HPs was invariably shared with the same HP as the cord blood HLA-homo HP, while another non-shared patient HP was mismatched with 1 to 4 HLA alleles among HLA-A, -B, -C, and -DRB1 loci in the GVH direction. Conversely, patients possessing one common HLA haplotype have a higher chance of finding the HLA-homo iPS.

Fourth, because all six pairs of HLA-homo cord blood and HLA-hetero patient were KIR2DL ligand-matched, the risk of KIR ligand mismatch remains to be elucidated. As the frequency of the C2 epitope among Japanese is low (7%), the chance of KIR2DL ligand mismatch for HLA-homo iPS, that is, a pair with cord C2 and patient C1C2, is rare. Among the 10 most frequent Japanese HPs, only the eighth-most frequent HP has HLA-C*04:01 (C2 epitope). Nevertheless, KIR ligand mismatch should be kept in mind, given our previous report that KIR ligand mismatch in the HVG direction induces a poorer engraftment rate than match in unrelated HSCT [35] and KIR incompatibility in the HVG direction ameliorated engraftment in ALL CBT patients [36]. A mechanism termed "hybrid resistance" in a mouse model [37] might be evoked in HLA-homo iPS situations. An understanding of the expression of HLA molecules on iPS cells is important. For example, when iPS differentiate to a committed tissue progenitor cell, these cells might not express HLA-class I molecule, making them eligible for targeting by NK cells [15, 16]. HLA mismatch combination in the present six homo to hetero pairs was also found to be KIR ligand-matched in the HLA-A, B locus.

As the present results for CBT were obtained using immunosuppressants, including calcineurin inhibitors, the question of whether iPS transplantation requires immunosuppression to suppress the immunogenicity of HLA-homo iPS remains unclear.

Our analysis also provides other HLA-homozygosity results for engraftment. The wide range of hazard ratio for the seven pairs with the cord HLA-homo and patient HLA-homo combination failed to provide evidence. Thirteen pairs with the combination of cord HLA-hetero and patient homo showed a significantly poorer engraftment ratio, which might have been due to HVG mismatch combination and/or possible donor specific antibodies (data not shown). With an increase in HLA locus mismatch at the allele level of HLA-A, -B, and -C, a lower engraftment ratio was observed, confirming that the rejection mechanism in this CBT study involved HLA disparity.

Acute GVHD occurred in two of six pairs with HLA-homo HPs, or namely mismatch combination in the GVH direction. This situation might be kept in mind when cytotoxic T cell-iPS specific to some tissues is induced in allogeneic HLA-homo donor settings. Engrafted cells with HLA-homo iPS might induce hyperacute GVHD mimicking post transfusion GVHD, or induce T-cell anergy [38].

Several limitations of our study warrant mention. First, the number of homo-hetero pairs was small, which may have affected the accuracy of the results. Recruitment of more CBTs with HLA allele typing is required. Second, data were only available for the three major donor HLA-homo HPs, and the risk of other HLA haplotypes among Japanese needs to be explored. Also, data for other ethnic populations with a more diverse HLA haplotype are lacking. Addressing this situation will require international collaboration and a very large database.

Summary

The clinical outcome of CBT with HLA-homo HP yields a number of important implications, namely the possibility of favorable engraftment in HLA homo iPS transplantation and detailed information of HLA alleles and haplotypes of HLA-homo HPs. Accumulation of such immunological genetic data for established HLA-homo iPSs and for pairs in upcoming clinical iPS transplantation is essential to ensuring the safety and consistent success of iPS transplantation.

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Author Contributions

Y.M., S.M., M.S., M.T., and T.Y. conception and design; F.A., K.N., and K. Kashiwase performed the histocompatibility analysis; K.M., T.O., H.Y., S.K., K. Kato, S.K., and T.M. collection and/or assembly of data and samples for transplantation; Y.M., S.M., and T.Y. statistical data analysis; Y.M. and S.M. analysis and manuscript writing; Y.M., F.A., K. Kashiwase, K.M., T.O., H.Y., S.K., K. Kato, S.K., T.M. K.N., S.M., M.S., M.T., and T.Y.: final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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