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ORIGINAL ARTICLE

Overcoming the challenges of scalable iPSC generation in translation medicine

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Abstract

Background: The potential of induced pluripotent stem cells (iPSCs) in revolutionizing regenerative medicine cannot be overstated. iPSCs offer a profound opportunity for therapies involving cell replacement, disease modeling, and cell transplantation. However, the widespread application of iPSC cellular therapy faces hurdles, including the imperative to regulate iPSC differentiation rigorously and the inherent genetic disparities among individuals. To address these challenges, the concept of iPSC super donors emerges, holding exceptional genetic attributes and advantageous traits. These super donors serve as a wellspring of standardized, high-quality cell sources, mitigating inter-individual variations and augmenting the efficacy of therapy.

Methods: In pursuit of this goal, our study embarked on the establishment of iPSC cell lines specifically sourced from donors possessing the HLA type (A33:03-B58:01-DRB1*03:01). The reprogramming process was meticulously executed, resulting in the successful generation of iPSC lines from these carefully selected donors. Subsequently, an extensive characterization was conducted to comprehensively understand the features and attributes of these iPSC lines.

Results: The outcomes of our research were highly promising. The reprogramming efforts culminated in the generation of iPSC lines from donors with the specified HLA type. These iPSC lines displayed a range of distinctive characteristics that were thoroughly examined and documented. This successful generation of iPSC lines from super donors possessing advantageous genetic traits represents a significant stride towards the realization of their potential in therapeutic applications.

Conclusion: In summary, our study marks a crucial milestone in the realm of regenerative medicine. The establishment of iPSC lines from super donors with specific HLA types signifies a paradigm shift in addressing challenges related to iPSC cellular therapy. The standardized and high-quality cell sources derived from these super donors hold immense potential for various therapeutic applications. As we move forward, these findings provide a solid foundation for further research and development, ultimately propelling the field of regenerative medicine toward new horizons of efficacy and accessibility.

Keywords: Cell-based therapy; HLA type; Induced pluripotent stem cells; Regenerative medicine, Super donor

1. INTRODUCTION

Stem cells possess remarkable characteristics such as self-renewal and the ability to differentiate into various cell lineages. Among the different types of stem cells utilized for therapeutic purposes, embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells have gained significant attention. ESCs, derived from the inner cell mass of blastocysts, exhibit pluripotency and the potential to generate cell types from all three germ layers. However, ethical concerns regarding the use of germ cells and the destruction of human embryos limit the clinical application of ESCs.[1]

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The groundbreaking technology of iPSCs was first reported in 2006 using mouse cells and later successfully achieved with human cells in 2007.[2,3] iPSCs share similar characteristics to ESCs and have demonstrated immense potential for cell therapy.[4–6] iPSC differentiation has diverse applications in cellular therapy. First, iPSCs can be directed to replace damaged cells by differentiating into specific cell types, such as cardiomyocytes or pancreatic β-cells, restoring normal function in diseases like heart disease or diabetes. Second, iPSCs serve as disease model cells to study disease mechanisms and develop drugs, as they can be derived from patients and differentiated into patient-specific cell types. Lastly, iPSC differentiation facilitates cell transplantation therapy by generating transplantable cell types for treating conditions like spinal cord injuries or heart disease. Overall, iPSC differentiation offers cell replacement, disease modeling, and cell transplantation possibilities in therapeutic approaches.

Despite the immense potential of iPSC cellular therapy, there are still certain bottlenecks that hinder its application in the field. First, the differentiation process of iPSCs requires strict regulation to ensure their differentiation into the desired cell types with normal functionality and maturity.[7] This process still faces technical challenges, including low efficiency, uncertain differentiation pathways, and difficulty in controlling cell quality.[8] These limitations restrict the scalable application and clinical translation of iPSC cellular therapy. Second, genetic variations among individuals present challenges to the application of iPSC cellular therapy. As each person's genome is unique, utilizing patient-specific iPSCs may require individualized handling and differentiation, increasing complexity and costs.[9] Additionally, inter-individual genetic variations may also impact the stability, therapeutic efficacy, and safety of the cells, necessitating further research and evaluation.[9]

The concept of iPSC super donors offers a potential solution to the challenges mentioned above.[7–9] By utilizing specific super donors, iPSCs with superior genetic characteristics or specific advantageous traits can be obtained, providing standardized and high-quality cell sources to replace patient-specific iPSCs.[10] This approach eliminates the limitations posed by inter-individual differences in iPSC therapy and reduces the need for individualized handling. Additionally, iPSC super donors can offer a more stable and consistent cell source, enhancing the scalability of cellular therapy and clinical translation.[11] The superior genetic features or specific advantageous traits of iPSC super donors can potentially improve the effectiveness and safety of the therapy, enabling their broader applications in cellular therapy, personalized medicine, and drug discovery.[9]

At present, South Korea and Japan have established clinical-grade iPSC haplobanks,[12,13] and similar efforts are underway in countries like Australia.[14] In this study, our objective was to establish and characterize Taiwan High-Coverage HLA-A, -B, and -DRB1 Haplotype iPSC Lines. To achieve this, we collected blood samples from donors and performed analyses to determine their HLA-A, -B, and -DRB1 haplotypes. Based on the results, we selectively chose donors with high-coverage haplotypes and generated iPSC lines from their cells. These iPSC lines underwent further characterization to evaluate their unique stem cell properties.

2. METHODS

2.1. Establishment of Human iPSCs

Human iPSCs were derived from peripheral blood mononuclear cells (PBMCs) of human donors. PBMCs of 5×10^5 were seeded on a 24-well plate and were maintained in StemPro®-34 medium (Gibco) supplemented with 100 ng/ml SCF, 100 ng/ml FLT3, 20 ng/ml IL-3, and 20 ng/ml IL-6 for 4 days. Cells were then reprogrammed by the infection with the Sendai virus which expresses the four Yamanaka factors: OCT4, SOX2, KLF4, and c-MYC using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher, A16518) following the manufacturer's instruction. 24 hours later, PBMCs were harvested and cultured in 1ml of fresh PBMC complete medium for 2 days, then transferred to MEF feeders and maintained in StemPro-34 medium without cytokines. The medium was refreshed once every 2 days. Seven days after infection, the culture medium was replaced with human ESCs medium which contains DMEM/F12 with 20% Knock-out Serum Replacement, 1 mM L-glutamine, 0.1 mM non-essential amino acids, 55 μM 2-mercaptoethanol and 10 ng/ml bFGF, and were incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. The medium was refreshed daily. Multiple colonies of iPSCs were generated and examined.

The iPSCs were formerly established in our lab. The novel iPSCs THH7 were newly generated as described above. iPSCs were maintained in StemFlex Medium Kit (ThermoFisher, A3349401). The culture dishes were coated with Geltrex matrix (ThermoFisher, A1413301) diluted with cold DMEM/F12 (1X) (ThermoFisher, Cat. no. 10565-018) and incubated in a humidified incubator at 37°C and 5% CO_2 in the incubator with a humidified atmosphere of 95% air and 5% CO_2 at 37°C for at least 1 hour. The cells were sub-cultured using Versene solution (ThermoFisher, 15040066).

2.2. Alkaline phosphatase staining

Cells were washed in PBS twice, fixed with 80% alcohol for at least 2 hours at 4°C, followed by soaking in dd H2O for 2–3 min, and 100 mM Tris-HCl (pH 8.2–8.5) for 5 min. Alkaline phosphatase (ALP) substrate working solution (Vector Laboratories, Burlingame, CA, USA) was added for 1 hour and stained colonies were visualized under the light microscope.

2.3. Immunofluorescence

Initiate the process by seeding the iPSCs onto 6-well plates and allowing them to fully grow by incubating at 37°C with 5% CO_2. Remove the medium and wash the cells with PBS, then fix them with 4% paraformaldehyde (P6148, Sigma-Aldrich, St. Louis, MO, USA) for 20 minutes. Discard the paraformaldehyde solution and wash the cells three times with PBS for 10 minutes at room temperature. Proceed to permeabilize the cells for 10 minutes with 0.1% Triton X-100 and suspend them in a blocking buffer (0.3% BSA in PBST) at room temperature for 30 minutes. Add primary antibodies (OCT4, NANOG, SOX2, SSEA4, TRA-1-60) to the cells at a dilution of 1:100 in 5% FBS in PBS and keep them overnight (≥12 hours) at 4°C.

Wash the cells three times with PBS before adding secondary antibodies (Alexa 488 NE goat anti-mouse IgG, Alexa 594 NE goat anti-mouse IgG, Alexa 488 goat anti-rabbit IgG, and Alexa 594 goat anti-rabbit IgG) at a dilution of 1:200 in 5% FBS in PBS. Incubate the cells for 1 hour in the dark before washing them three times with PBS. Stain the nuclei with DAPI (Sigma-Aldrich, diluent 1:10 000) in 5% FBS in PBS for 10 minutes in the dark, followed by another three washes with PBS. Finally, place the cells on a slide with the mounting solution and seal them with nail polish. Observe the slides through a microscope (Olympus America, Melville, NY, USA).

2.4. Teratoma formation assay

To initiate teratoma formation, iPSCs were harvested and suspended in a mixture of Matrigel and growth medium. Subsequently, the iPSC-Matrigel mixture was injected subcutaneously into immunodeficient mice. After a predetermined incubation period, the mice were euthanized, and the resulting teratomas were excised and fixed in formalin. Paraffin-embedded

tissue sections were prepared, and hematoxylin and eosin staining were performed to visualize the presence of differentiated cell types from all three germ layers. The sections were then examined under a microscope, and representative images were captured for analysis.

3. RESULTS

3.1. Comparison of HLA-A, -B, and -DRB1 haplotype frequencies between Taiwanese and Japanese populations

To investigate the differences in HLA-A, -B, and -DRB1 haplotype frequencies between the Taiwanese and Japanese populations, we compared the existing data from the Allele Frequency Net Database (AFND). The HLA-A, -B, and -DRB1 haplotype frequencies specific to the Taiwanese and the Japanese populations are shown in Tables 1 and 2, respectively. The comparative analysis of these haplotype frequencies provides insights into the genetic variations between the two populations, shedding light on their distinct HLA profiles. The total numbers of the three-locus haplotypes with frequencies greater than 0.05% were 341 in the Taiwanese, respectively. The top 20 three-locus haplotypes based on occurring frequencies in Taiwanese are shown in Table 1. For Taiwanese, the two haplotypes with frequencies greater than 2% were A*3303-B*5801- DRB1*0301(8.361%) and A*0207-B*4601-DRB1*0901(4.430%). The top 20 three-locus haplotypes based on occurring frequencies in the Japanese population database are shown in Table 2. Japanese, there are four haplotypes with frequencies greater than 2%, the most frequent haplotype is A*2402-B*5201-DRB1*1502(8.370%), followed by A*3303-B*4403- DRB1*1302(4.473%) in second place. Among the top 20 most common haplotypes in either group, only two haplotypes (A*0207-B*4601-DRB1*0803 and A*1101-B*1501-DRB1*0406) were shared by both groups; The (A*0207-B*4601-DRB1* 0803) haplotype frequency was similar in Taiwanese (1.26%) and in Japanese (1.86%). The (A*1101-B*1501-DRB1*0406) haplotypes in Taiwanese were 0.81% and in Japanese were 1.34%, respectively. However, the occurring frequencies were quite different. Collectively, these differences in the aforementioned haplotype frequencies between the Taiwanese and Japanese populations indicate that HLA genetic diversity exists between the two populations.

3.2. Study workflow

The workflow of this study is depicted in Fig. 1. The study commenced with the initial phase of conducting HLA typing to determine the human leukocyte antigen (HLA) profile of potential donors. Based on the compatibility of their HLA profiles, super donors were carefully selected. PBMCs were subsequently collected from the chosen super donors. A reprogramming process was employed to induce the conversion of PBMCs into iPSCs. These iPSC lines were further characterized to assess their unique features and suitability for various applications.

3.3. Identification of HLA-homozygous donors

We screened a total of 500 individuals and collected peripheral blood from the subjects with specific haplotypes, including the most prevalent A3303-B5801-DRB10301 haplotype (8.361%) among Taiwanese HLA haplotypes. Through this screening, we successfully identified patients who exhibited HLA-homozygous genotypes for A3303-B5801-DRB10301, meeting the criteria for being potential "super donors." These findings highlight the feasibility of identifying individuals with desirable genetic profiles and their potential to serve as valuable resources for transplantation. Comparison with Taiwan Biobank revealed that out of 1104 healthy individuals, 150 shared the same HLA type (A33:03-B*58:01-DRB1*03:01), accounting for 13.58% of the total population. This proportion was consistent with the 13.5% observed in our screening samples, and in a six-site HLA typing analysis, it was found in 92 out of 1104 people (8.33%). In contrast, Japan's first HLA typing (A*24:02-B*52:01-DRB1*15:02) was found in only 24 out of 1104 individuals (2.2%), while the HLA type (*A*24:02-B*52:01-C*12:02-DPB*09:01-DQB*06:01-DRB1*15:02) was found in only one out of 1104 individuals (0.09%).

Table 1

The top 20 three-locus haplotypes based on occurring frequencies in Taiwanese dataset

Rank	Haplotypes	Frequency (%)	Sample size	Population
1	A*33:03~B*58:01~DRB1*03:01	8.3610	46,628	Taiwan
2	A*02:07~B*46:01~DRB1*09:01	4.4300	46,628	Taiwan
3	A*11:01~B*15:02~DRB1*12:02	1.5000	46,628	Taiwan
4	A*02:03~B*38:02~DRB1*16:02	1.4950	46,628	Taiwan
5	A*30:01~B*13:02~DRB1*07:01	1.3310	46,628	Taiwan
6	A*02:07~B*46:01~DRB1*08:03	1.2620	46,628	Taiwan
7	A*02:01~B*40:01~DRB1*11:01	1.1050	46,628	Taiwan
8	A*11:02~B*27:04~DRB1*12:02	0.9990	46,628	Taiwan
9	A*11:01~B*40:01~DRB1*09:01	0.9290	46,628	Taiwan
10	A*11:01~B*13:01~DRB1*15:01	0.9270	46,628	Taiwan
11	A*11:01~B*46:01~DRB1*09:01	0.8700	46,628	Taiwan
12	A*11:01~B*15:01~DRB1*04:06	0.8110	46,628	Taiwan
13	A*11:01~B*40:01~DRB1*08:03	0.7670	46,628	Taiwan
14	A*11:01~B*40:01~DRB1*11:01	0.7560	46,628	Taiwan
15	A*11:01~B*40:01~DRB1*04:05	0.6770	46,628	Taiwan
16	A*02:03~B*38:02~DRB1*08:03	0.6620	46,628	Taiwan
17	A*11:01~B*13:01~DRB1*16:02	0.6360	46,628	Taiwan
18	A*11:01~B*40:01~DRB1*12:01	0.6340	46,628	Taiwan
19	A*24:02~B*40:01~DRB1*15:01	0.6330	46,628	Taiwan
20	A*24:02~B*40:01~DRB1*09:01	0.5750	46,628	Taiwan

Table 2

The top 20 three-locus haplotypes based on occurring frequencies in the Japanese population database

Rank	Haplotypes	Frequency (%)	Sample size	Population
1	A*24:02~B*52:01~C*12:02~DRB1*15:02	8.3770	18,604	Japan
2	A*33:03~B*44:03~C*14:03~DRB1*13:02	4.4730	18,604	Japan
3	A*24:02~B*07:02~C*07:02~DRB1*01:01	3.7220	18,604	Japan
4	A*24:02~B*54:01~C*01:02~DRB1*04:05	2.5390	18,604	Japan
5	A*02:07~B*46:01~C*01:02~DRB1*08:03	1.8660	18,604	Japan
6	A*11:01~B*15:01~C*04:01~DRB1*04:06	1.3450	18,604	Japan
7	A*24:02~B*59:01~C*01:02~DRB1*04:05	1.0580	18,604	Japan
8	A*11:01~B*54:01~C*01:02~DRB1*04:05	1.0010	18,604	Japan
9	A*26:01~B*40:02~C*03:04~DRB1*09:01	0.7460	18,604	Japan
10	A*24:02~B*40:06~C*08:01~DRB1*09:01	0.7090	18,604	Japan
11	A*24:02~B*51:01~C*14:02~DRB1*09:01	0.6520	18,604	Japan
12	A*31:01~B*51:01~C*14:02~DRB1*08:02	0.5790	18,604	Japan
13	A*33:03~B*44:03~C*14:03~DRB1*08:03	0.5470	18,604	Japan
14	A*26:02~B*40:06~C*08:01~DRB1*09:01	0.5420	18,604	Japan
15	A*02:01~B*13:01~C*03:04~DRB1*12:02	0.5320	18,604	Japan
16	A*24:02~B*46:01~C*01:02~DRB1*08:03	0.5320	18,604	Japan
17	A*02:06~B*40:06~C*08:01~DRB1*09:01	0.4640	18,604	Japan
18	A*11:01~B*39:01~C*07:02~DRB1*08:03	0.4330	18,604	Japan
19	A*26:01~B*40:02~C*03:04~DRB1*08:02	0.4280	18,604	Japan
20	A*02:06~B*35:01~C*03:03~DRB1*15:01	0.4220	18,604	Japan

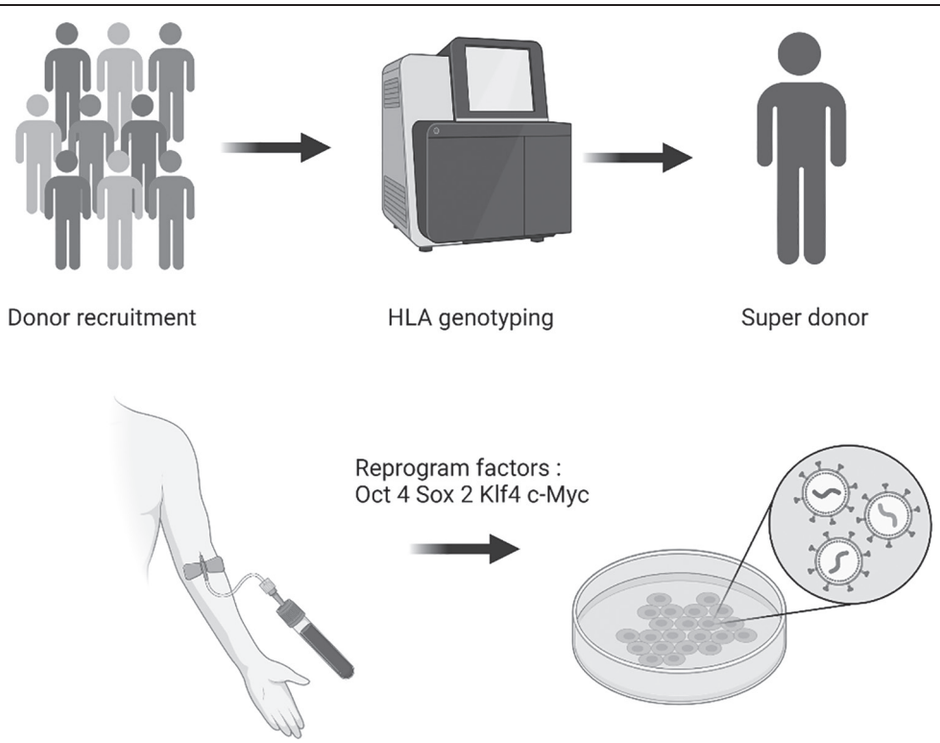

Fig. 1 Schematic overview of the project This figure illustrates the step-by-step process of the project, starting from HLA typing to the generation of induced pluripotent stem cells (iPSCs) from selected super donors. (1) HLA typing: Donors are subjected to HLA typing to identify their human leukocyte antigen (HLA) profile. (2) Super donor selection: Based on the HLA typing results, super donors with compatible HLA profiles are identified and selected. (3) Blood collection: Peripheral blood mononuclear cells (PBMCs) are obtained from the selected super donors through blood collection. (4) iPSC generation: PBMCs are reprogrammed using induced pluripotency techniques to generate iPSCs. Created with BioRender.com

3.4. Establishment of super donor iPSCs lines

We used a Sendai virus delivery method to reprogram PBMCs from a super donor by expressing Oct4, Sox2, Klf4, and c-Myc. To ensure the quality of our iPSC colonies, we first examined their morphology under a light microscope. The super donor iPSCs displayed typical features of pluripotent stem cells, such as small, tightly packed cells, a high nucleus-to-cytoplasm ratio, and compact colonies with clear borders and well-defined edges (as shown in Fig. 2). To assess pluripotency in stem cells, it is common to examine the expression of ALP.

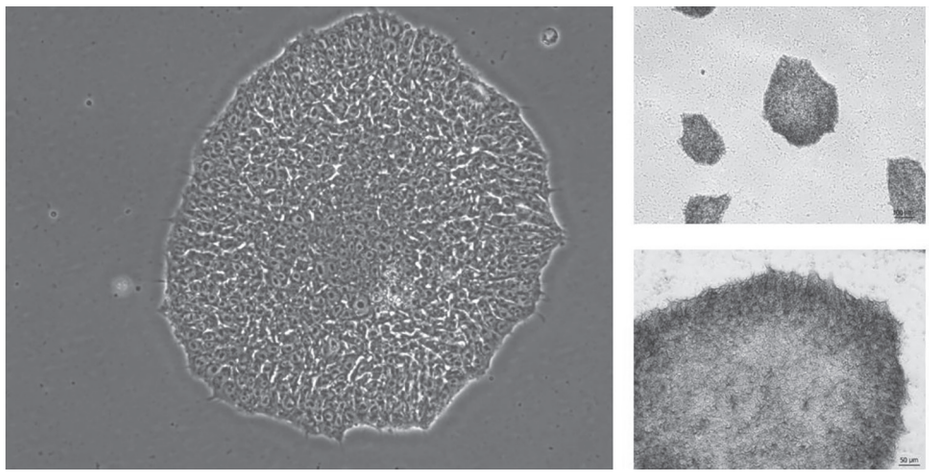

Fig. 2 Alkaline phosphatase staining of induced pluripotent stem cell (iPSC) colonies. Scale bars indicate 50 μm (bars = 50 μm).

This glycoprotein is bound to the cell membrane and catalyzes the hydrolysis of phosphate monoesters in alkaline conditions. ALP expression is exceptionally high in iPSCs, making it a reliable biomarker. In our study, we analyzed ALP expression in our cell lines and observed apparent positive ALP activity in the iPSCs (Fig. 2), as expected. We have confirmed the presence of common pluripotency markers like NANOG, and SOX2 in our iPSCs through immunofluorescence testing, as seen in Fig. 3. The generated super donor iPSCs were successful and displayed typical traits of pluripotent stem cells. Teratoma formation assay was conducted to assess the pluripotency and tumorigenic potential of the iPSC lines (Fig. 4). The excised teratomas were subjected to tissue staining and histological analysis. The staining results revealed the presence of cell types representing all three germ layers: endoderm, mesoderm, and ectoderm. This observation confirmed the multilineage differentiation capacity of the iPSCs, indicating their ability to differentiate into diverse cell types. The teratoma formation assay provided strong evidence of the iPSCs' pluripotency, supporting their potential applications in regenerative medicine and disease modeling.

4. DISCUSSION

The potential of iPSC in regenerative medicine is remarkable, as they possess the ability to self-renew and differentiate into

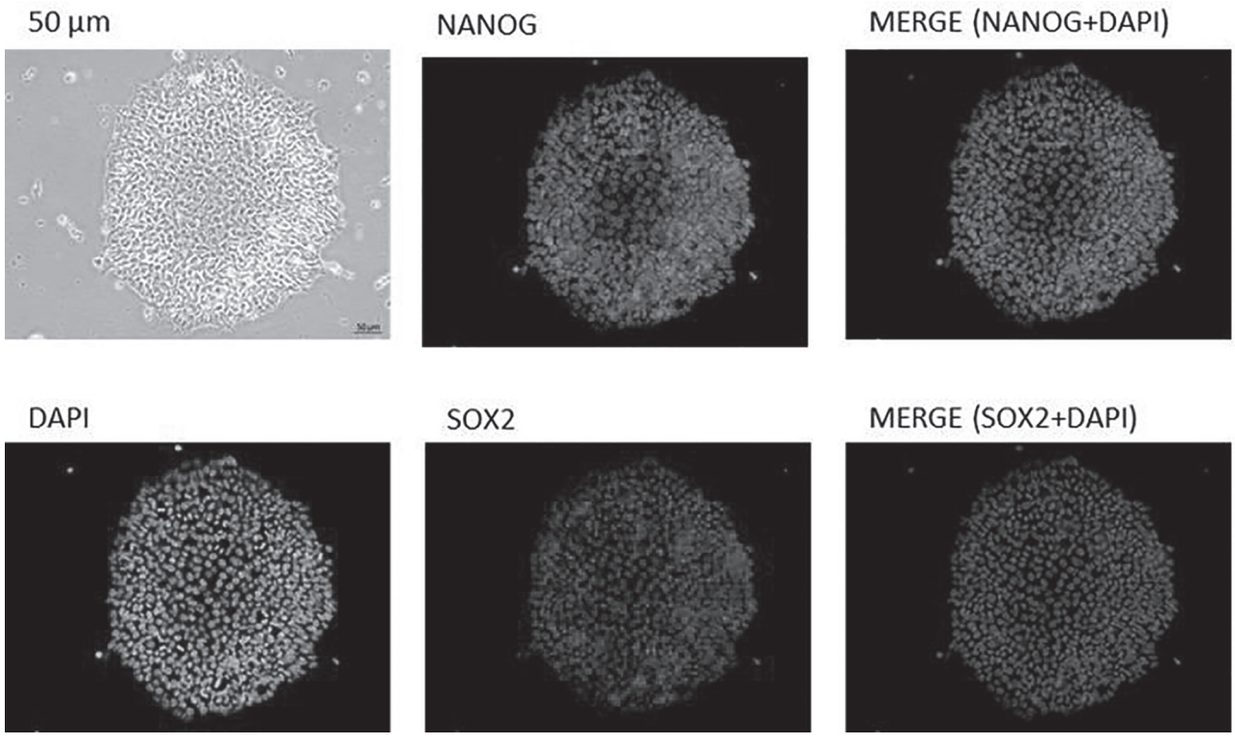

Fig. 3 Characterization of HLA-homozygous hiPSC lines. Immunolabeling of DAPI (blue) and pluripotent markers SOX2(red), NANOG. Scale bars indicate 50 μm (bars = 50 μm).

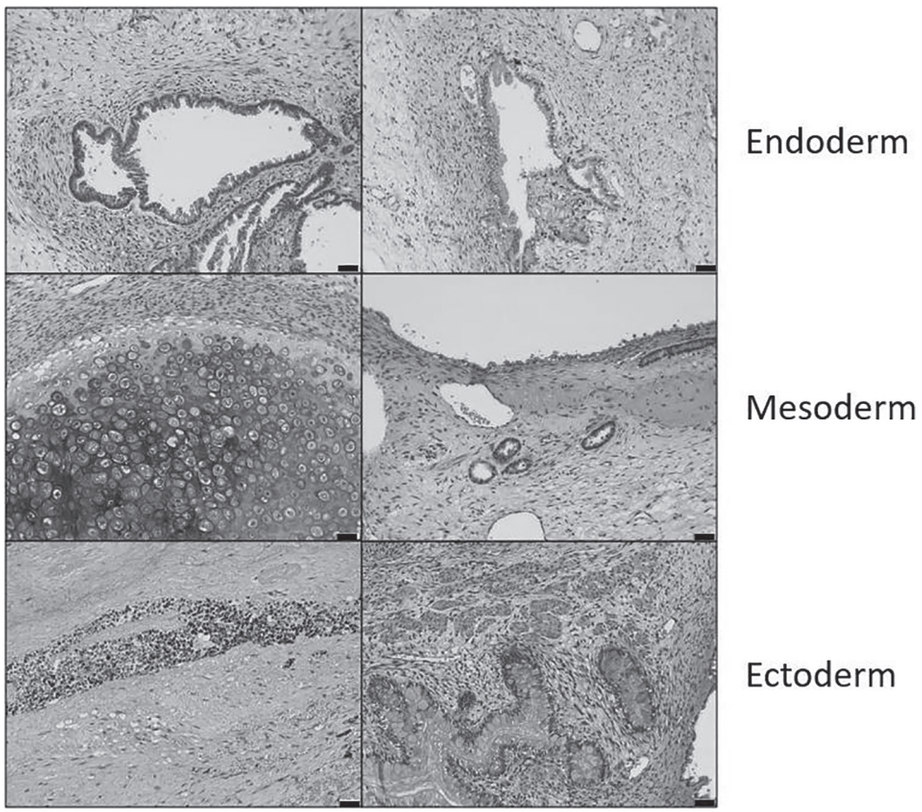

Fig. 4 Hematoxylin and eosin staining of paraffin-embedded tissue sections from a mouse iPSC teratoma. The staining reveals the presence of cells from all three germ layers (endoderm, mesoderm, and ectoderm), confirming the pluripotent nature of the iPSCs. Hematoxylin stains the nuclei blue, while eosin stains the cytoplasm and extracellular matrix pink. Scale bars indicate 50 μm (bars = 50 μm).

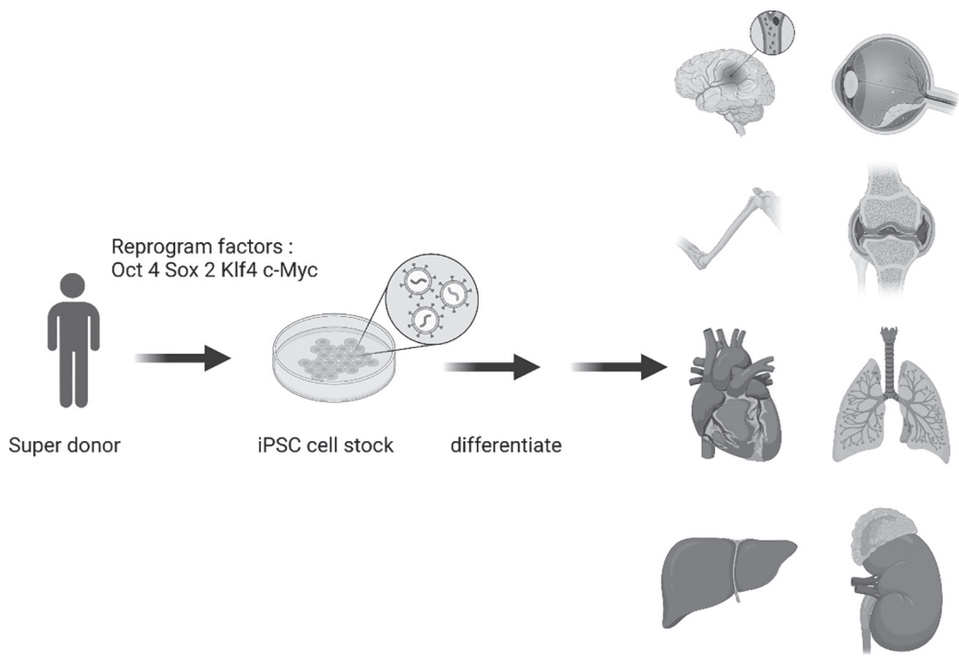

Fig. 5 Schematic diagram of iPSC super donor cell therapy. The figure illustrates the potential applications of super donor iPSCs in differentiated cell types for clinical therapy. iPSCs derived from super donors can be directed to differentiate into various cell lineages, as depicted by the arrows. This schematic diagram demonstrates the versatility of super donor iPSCs in differentiating into diverse cell types, offering promising prospects for their utilization in regenerative medicine and clinical therapy.

various cell types within the human body. One key advantage of iPSC over ESCs is their ethical acceptability and potential for autologous cell replacement therapies.[15,16] Extensive research has focused on developing safe iPSCs and establishing differentiation protocols to generate clinically relevant cells for therapeutic applications.

The utilization of iPSCs in clinical applications presents various challenges and concerns. These encompass the potential for tumorigenicity due to insufficient differentiation, the accumulation of genetic mutations, epigenetic abnormalities, and the expression of reprogramming factors if iPSCs were created using integrated plasmids or viral vectors. Additionally, long-term in vitro culture can result in immunogenicity, while the heterogeneity within and between different iPSC clones further hampers their clinical utility.[6,17–19]

To address these challenges, various methods have been developed for generating iPSCs. Exogenous gene delivery approaches, such as retroviral or lentiviral vectors, allow for the introduction of reprogramming factors into somatic cells, promoting their reprogramming into iPSCs.[10–15] However, the integration of exogenous genes into the host genome raises concerns about the stability and safety of iPSCs. Non-integrating methods, on the other hand, utilize techniques such as mRNA, episomal vectors, or proteins to deliver reprogramming factors without genomic integration, minimizing the risk of genetic alterations.[16] These non-integrating methods offer a safer approach for generating iPSCs with reduced tumorigenic potential and improved clinical applicability. The other method for generating IPSCs are selecting multiple somatic cell sources and small compounds. These techniques aim to enhance the efficiency of the reprogramming process.[20]

Notably, a significant milestone was reached in 2015 when the first successful clinical trial in Japan utilized iPSC-derived retinal pigmented epithelial cells to treat a patient with age-related macular degeneration (RIKEN trial).[21] However, the cost and time-consuming nature of iPSC production has posed challenges to their widespread use. Consequently, the concept of employing clinically matched iPSC with matching HLA-A, HLA-B, and HLA-DRB1 alleles as an allogeneic treatment has gained considerable attention.[22]

In the context of allogeneic transplantation, MHC (major histocompatibility complex) class I and II molecules have the potential to elicit an immune response. Among these molecules, HLA-A, HLA-B, and HLA-DRB1 are recognized as the primary determinants of rejection.[23] Extensive research has provided substantial evidence regarding the impact of HLA-A, HLA-B, and HLA-DRB1 mismatches on the rejection of solid organ transplants.[24] It is worth noting that HLA matching in solid organ transplantation primarily focuses on the HLA-A, HLA-B, and HLA-DRB1 loci at a low-resolution level.[25] However, for kidney transplants, the concept of "beneficial match" is taken into account, allowing for various degrees of compatibility. This can range from a single mismatch in HLA-A or HLA-B to matching HLA-DRB1 alone.[26] Currently, high-resolution sequencing of five HLA loci using next generation sequencing is commonly employed for typing in hematopoietic progenitor transplantation.[27]

Extensive discussions have taken place regarding the importance and strictness of HLA matching in the context of iPSC-derived cells for clinical transplantation.[28] Unlike transplantation of hematopoietic progenitor cells, iPSC derivatives lack contaminating immune cells. Consequently, the required level of compatibility is primarily assessed based on the host vs graft relationship, as the graft cannot mount an immune response against host HLA antigens that may be mismatched. Studies have demonstrated that matching HLA-A, HLA-B, and HLA-DRB1 alleles provide a significant advantage over completely allogeneic transplantation, with varying degrees of immunological responses observed, emphasizing the necessity of immunosuppressive measures.[29]

At present, South Korea and Japan have established clinical-grade iPSC haplobanks,[12,13] and similar efforts are underway in countries like Australia.[14] To maximize the effectiveness of this approach, global collaboration is essential for the sharing of plotlines. This collaborative effort necessitates standardized production and quality control procedures across different banks.[30,31]

In this study, our primary aim was to establish iPSC cell lines from donors carrying the HLA type (A33:03-B58:01-DRB1*03:01). Through the reprogramming process, we successfully generated iPSC lines from the selected donors and extensively characterized their features. Moreover, the successful establishment of super-donor iPSCs opens up numerous possibilities for clinical applications. Fig. 5 illustrates the potential applications of these iPSCs in differentiated cell types for clinical therapy. Controlled differentiation processes can be employed to generate specific cell lineages from super donor iPSCs. For instance, iPSCs can be differentiated into cardiomyocytes, providing potential treatment options for heart diseases.[32] Similarly, iPSCs can be directed to differentiate into pancreatic β-cells, holding promise for the management of diabetes.[33] Additionally, iPSCs can be guided to differentiate into specific cell lineages to address conditions such as spinal cord injuries or neurodegenerative diseases.[34] The versatility of super donor iPSCs in differentiating into diverse cell types paves the way for their potential utilization in various regenerative medicine approaches.

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