Title

Humanized Mice Reveal Differential Immunogenicity of Cells Derived from Autologous Induced Pluripotent Stem Cells.

Journal

Cell stem cell, 17(3)

ISSN

1934-5909

Authors

Zhao, Tongbiao
Zhang, Zhen-ning
Westenskow, Peter D
et al.

Publication Date

2015-09-01

DOI

10.1016/j.stem.2015.07.021

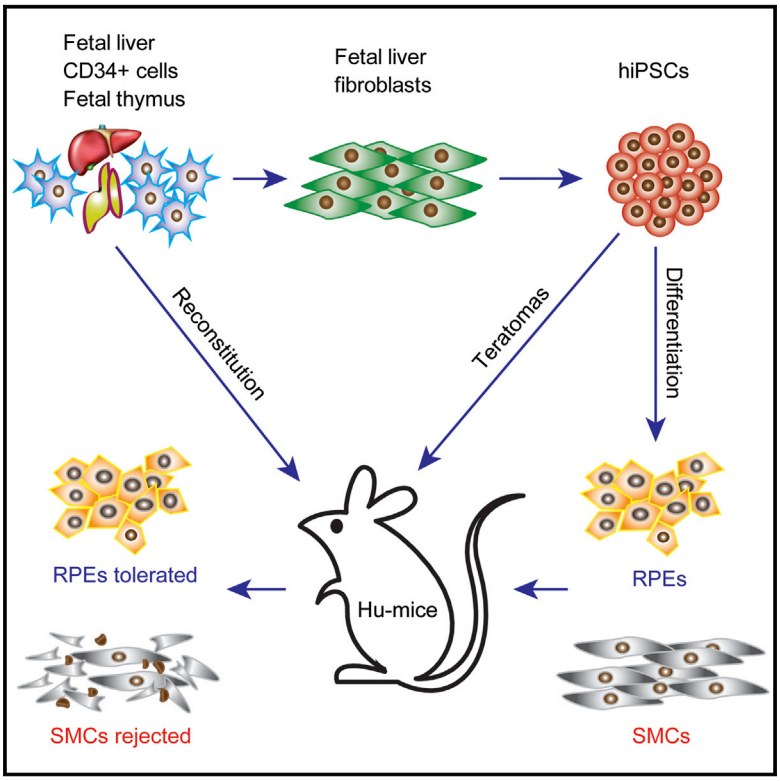
Peer reviewed

Short Article

Cell Stem Cell

Humanized Mice Reveal Differential Immunogenicity of Cells Derived from Autologous Induced Pluripotent Stem Cells

Graphical Abstract



Authors

Tongbiao Zhao, Zhen-ning Zhang, Peter D. Westenskow, ..., Kun Zhang, Martin Friedlander, Yang Xu

Correspondence

yangxu@ucsd.edu

In Brief

Patient iPSCs have potential as a renewable source for autologous cell therapy that avoids immune rejection. Using a humanized mouse model with a functional human immune system, Zhao et al. observe differential immune responses to various autologous hiPSC derivatives, including rejection of smooth muscle cells and tolerance to retinal pigmented epithelium.

Highlights

- Hu-mice offer a model to study immune responses to autologous hiPSC derivatives
- Hu-mice reveal differential immune responses to hiPSC-derived SMCs and RPEs
- Misexpression of immunogenic antigens in hiPSC-derived SMCs leads to T cell response
- hiPSC-RPEs are tolerated even in non-ocular sites, supporting their clinical use

Zhao et al., 2015, Cell Stem Cell *17*, 353–359
September 3, 2015
http://dx.doi.org/10.1016/j.stem.2015.07.021

Cell Stem Cell
Short Article

Humanized Mice Reveal Differential Immunogenicity of Cells Derived from Autologous Induced Pluripotent Stem Cells

Tongbiao Zhao,[1,2,10] Zhen-ning Zhang,[1,10] Peter D. Westenskow,[3,10] Dilyana Todorova,[1] Zheng Hu,[4] Tongxiang Lin,[5] Zhili Rong,[6] Jinchul Kim,[6] Jingjin He,[1,6] Meiyan Wang,[1] Dennis O. Clegg,[7] Yong-guang Yang,[4,8] Kun Zhang,[9] Martin Friedlander,[3] and Yang Xu[1,6,*]

[1]Division of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 920932, USA
[2]State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[3]Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037, USA
[4]First Hospital of Jilin University, Changchun, Jilin 130021, China
[5]The Second Clinical Hospital of Guangzhou University of Traditional Chinese Medicine, Guangzhou, Guangdong 510006, China
[6]Cancer Research Institute, Southern Medical University, Guangzhou, Guangdong 510515, China
[7]Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA 93106, USA
[8]Center for Translational Immunology, Columbia University Medical Center, New York, NY 10032, USA
[9]Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
[10]Co-first author
*Correspondence: yangxu@ucsd.edu
http://dx.doi.org/10.1016/j.stem.2015.07.021

SUMMARY

The breakthrough of induced pluripotent stem cell (iPSC) technology has raised the possibility that patient-specific iPSCs may become a renewable source of autologous cells for cell therapy without the concern of immune rejection. However, the immunogenicity of autologous human iPSC (hiPSC)-derived cells is not well understood. Using a humanized mouse model (denoted Hu-mice) reconstituted with a functional human immune system, we demonstrate that most teratomas formed by autologous integration-free hiPSCs exhibit local infiltration of antigen-specific T cells and associated tissue necrosis, indicating immune rejection of certain hiPSC-derived cells. In this context, autologous hiPSC-derived smooth muscle cells (SMCs) appear to be highly immunogenic, while autologous hiPSC-derived retinal pigment epithelial (RPE) cells are immune tolerated even in non-ocular locations. This differential immunogenicity is due in part to abnormal expression of immunogenic antigens in hiPSC-derived SMCs, but not in hiPSC-derived RPEs. These findings support the feasibility of developing hiPSC-derived RPEs for treating macular degeneration.

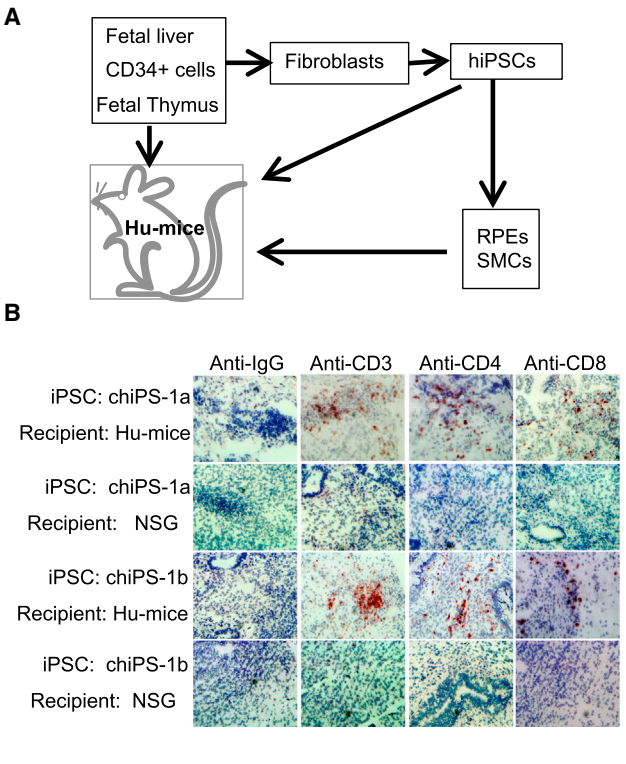
INTRODUCTION

The recent breakthrough in the generation of induced pluripotent stem cells (iPSCs) by reprogramming somatic cells with defined factors has raised the hope that iPSCs, which are identical to human embryonic stem cells (hESCs) in the context of pluripotency, could become a renewable source of autologous cells for transplantation into human patients (Lewitzky and Yamanaka, 2007). In addition, the disease-specific iPSCs could provide the unique opportunity to develop the much needed disease models in drug discovery. While it has been assumed that the immune rejection problem challenging hESCs could be mitigated by the development of patient-specific hiPSCs without the concern of immune rejection (Park et al., 2008; Takahashi et al., 2007; Yu et al., 2007), recent studies have shown that certain cell types derived from mouse iPSCs such as cardiomyocytes are immunogenic in syngeneic recipients, and other immunogenic cell types such as endothelial cells are immune tolerated by expressing high levels of immune-suppressive cytokines such as IL-10 (Araki et al., 2013; de Almeida et al., 2014; Zhao et al., 2011). Therefore, as an integral part of the effort to develop hiPSCs into human cell therapy, it is important to evaluate the immunogenicity of hiPSC-derived cells in the context of an autologous human immune system. To address this bottleneck, we established humanized mouse models that are efficiently reconstituted with both T and B cells as well as macrophages and dendritic cells required for antigen presentation (Figure S1A). As we recently published (Rong et al., 2014), these Hu-mice can mount vigorous immune rejection of allogeneic cells derived from hESCs (Figures S1B–S1E). Therefore, the Hu-mice provide a unique opportunity to examine the immunogenicity of autologous cells derived from hiPSCs.

RESULTS

Teratomas Formed by hiPSCs Are Immunogenic to Autologous Human T Cells

To generate autologous integration-free hiPSCs, fibroblasts were derived from the same human fetal liver used to reconstitute the human immune system in Hu-mice (Figure 1A). These cells were reprogrammed into integration-free hiPSCs with either the episomal approaches we previously described (Zhao et al.,



C

Survival rate and T cell infiltration in the teratomas formed by hiPSCs in Hu-mice

iPS line	Teratoma	CD3 infiltration
chiPS-1a	4/4	4/4
chiPS-1b	2/2	2/2
chiPS-2a	22/24	22/22
chiPS-2b	8/8	8/8
chiPS-3a	3/3	2/3*
chiPS-4a	2/2	2/2
chiPS-4b	4/4	1/1
chiPS-5a	3/3	2/2
chiPS-5b	4/4	3/3
Zg16- chiPS-5a	0/2	-

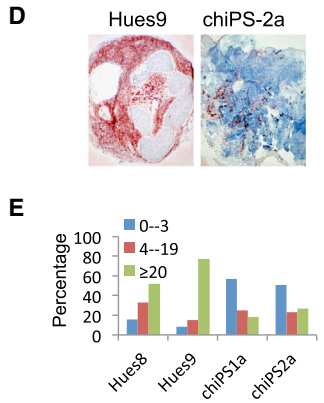


Figure 1. Cells within Teratomas Formed by hiPSCs Can Be Immunogenic in Hu-Mice Reconstituted with Autologous Immune System

(A) Strategy to study the immunogenicity of hiPSC derivatives. Human fetal liver/thymus and autologous CD34$^+$ fetal liver cells were transplanted into NSG mice to generate Hu-mice. Fibroblasts were isolated from the same fetal liver and reprogrammed into autologous integration-free hiPSCs, which were either directly transplanted into the Hu-mice or differentiated into mature cell types before being transplanted into Hu-mice.

(B) T cell infiltration was detected in the majority of teratomas formed by hiPSCs in Hu-mice with autologous human immune system. T cells are revealed by their dark brown color.

(C) The summary of teratoma formation and T cell infiltration into teratomas after subcutaneous implantation of nine independent hiPSC lines reprogrammed from five different donors as well as Zg16-hiPSCs in Hu-mice reconstituted with corresponding autologous immune system.

(D) Low magnification (4×) images show the apparent difference in T cell infiltration between hESC-derived teratoma and autologous iPSC-derived teratoma.

(E) Frequency of T cells infiltrating each teratoma. Sections of teratomas formed by Hues8, Hues9, chiPS1a, and chiPS2a in Hu-mice were stained with anti-CD3 antibody. Five teratomas from each line were analyzed. At least 50 frames (20×) from each teratoma were randomly chosen for analysis. The numbers of T cells detected were categorized into three groups: 0–3, 4–19, or ≥20 T cells per frame.

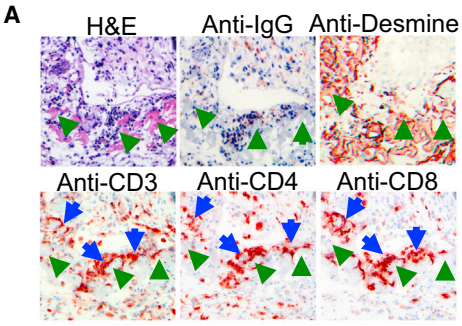
2011) or those developed by the Yamanaka group (Okita et al., 2011). The hiPSCs are positive for alkaline phosphatase, express pluripotency markers to the same levels as hESCs, have normal karyotypes, and can form teratomas containing cells derived from each of the three germ layers in immunodeficient mice, confirming their pluripotency (Figures S2A–S2D). In addition, Southern blotting analysis with probes covering the entire episomal vector further confirmed that hiPSCs are integration free (Figure S2E). These integration-free hiPSCs were transplanted subcutaneously into Hu-mice to form teratomas, allowing the simultaneous evaluation of the immunogenicity of various cell types differentiated from hiPSCs.

Serial section analysis of the teratomas formed by hiPSCs in Hu-mice reconstituted with autologous immune system indicated that the majority of these teratomas contained regions that were infiltrated with human CD4$^+$ and CD8$^+$ T cells and exhibited tissue necrosis (Figures 1B and 1C; Figure S1K). However, the immunogenicity of autologous hiPSC-derived teratomas in Hu-mice appeared to be much weaker than allogeneic hESC-derived teratomas that were extensively infiltrated with T cells (Figures 1D and 1E). In this context, after examining sequential sections of one teratoma formed by autologous hiPSCs in Hu-mice, we did not detect any T cell infiltration (Figure S1L). Therefore, certain, but not all, cells derived from hiPSCs are immunogenic to the autologous human immune system.

T Cell Response to Specific Cell Types within Autologous Teratomas

To identify the potential immunogenic tissues inside the teratoma, we analyzed the histology of a large number of teratomas infiltrated with T cells. We found that the autologous hiPSC-derived Desmin$^+$ smooth muscle cells (SMCs) were frequently surrounded by infiltrating T cells in the teratomas (Figures 2A and 2C). In contrast, the majority of autologous hiPSC-derived retinal pigment epithelium (RPE) cells within the teratomas were not surrounded by T cells (Figures 2B and 2C).

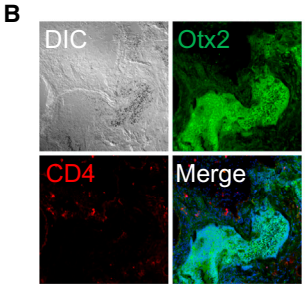
To further characterize the T cell responses to the teratomas formed by autologous hiPSCs, we performed deep sequencing to analyze the T cell receptor (TCR) repertoire of the T cells that had infiltrated into the teratomas formed by either allogeneic



C

T cell infiltration into the tissues within teratomas formed by hiPSCs in Hu-mice

Tissues	CD3 infiltration
Muscle	13/15
RPE	3/14



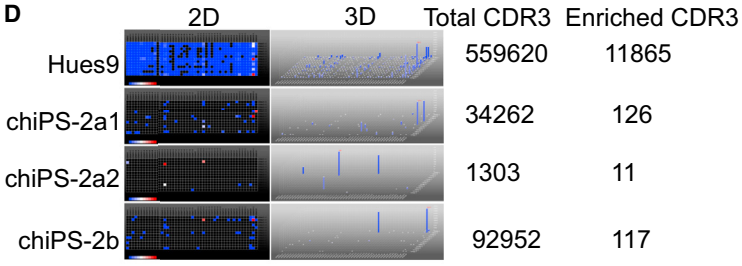


Figure 2. Characterization of the Immune Responses to the Teratomas Formed by hiPSCs in Hu-Mice Reconstituted with Autologous Immune System

(A) Smooth muscle cells (SMCs) within the teratomas formed by hiPSCs in Hu-mice are frequently infiltrated with T cells (green arrow: smooth muscle cells; blue arrow: T cells).

(B) The retinal pigment epithelium (RPE) cells within the teratomas formed by hiPSCs are immune tolerated.

(C) Summary of the T cell infiltration into SMCs and RPEs within the teratomas formed by hiPSCs in Hu-mice reconstituted with autologous immune system.

(D) TCR repertoire distributions in four teratomas formed by Hues9 and two independent hiPSC lines (chiPS-2a and chiPS-2b) in Hu-mice. 2D: two-dimensional map; dot: different TCR, with the change of color from blue to red representing the enrichment of the specific TCR; 3D: three-dimensionL map; blue column: enrichment of the specific TCR. The enrichment of several monoclonal TCRS in hiPSC-derived teratomas suggests the antigen-specific T cell response to cells within the teratomas formed by autologous iPSCs.

hESCs or autologous hiPSCs (Figure 2D). Intriguingly, in contrast to the highly polyclonal nature of the TCR repertoire of T cells infiltrated into the allogeneic hESC-derived teratomas, the TCR repertoire of T cells infiltrating into the teratomas formed by autologous hiPSCs in Hu-mice was oligoclonal, indicating antigen-specific T cell responses to the autologous hiPSC-derived cells within the teratomas.

Differential Immunogenicity of SMCs and RPEs Derived from Autologous hiPSCs

To further evaluate the immunogenicity of hiPSC-derived RPE cells, we differentiated the autologous hiPSCs into RPE cells in vitro with a high efficiency protocol as previously described (Krohne et al., 2012). As a positive control, allogeneic hESCs were also differentiated into RPE cells. hiPSC-derived RPEs were tightly coupled, positive for Mitf and Bestrophin, exhibited vectorial transport of water and ions, phagocytosed photoreceptor outer segments (POSs), could be incorporated into diseased retinas in a polarized monolayer, and rescued photoreceptors in an animal model (RCS rats) of RPE-mediated retinal degeneration (Figures S3A–S3G). Furthermore, the global gene expression analysis demonstrated a high similarity between hiPSC-derived RPEs and the human primary RPEs (Figures 4A and 4B). These findings validated the identity and function of hiPSC-derived RPEs. The autologous hiPSC-derived RPEs and the control allogeneic hESC-derived RPEs were transplanted into the eye of Hu-mice. Since the eye is considered an immune-privileged site, and thus, could mask the immunogenicity of the hiPSC-derived RPE cells, we also implanted these RPEs into the hindleg skeletal muscle of Hu-mice. In support of this concept, even the allogeneic hESC-derived RPE cells survived in the eye of Hu-mice without apparent immune rejection. When injected into the skeletal muscle of Hu-mice, the allogeneic hESC-derived RPEs were infiltrated with T cells, consistent with T-dependent immune rejection (Figures 3A, 3C, 3D, and 3G). In contrast, autologous hiPSC-derived RPEs implanted in the muscle of the same Hu-mice were mostly protected from T cells (Figures 3B–3D and 3G). Therefore, we conclude that hiPSC-derived RPEs have low immunogenicity.

The studies of teratomas formed by hiPSCs in Hu-mice suggest that SMCs derived from hiPSCs might be immunogenic to the autologous immune system. To further investigate the immunogenicity of SMCs differentiated from autologous hiPSCs, we efficiently derived SMCs from hiPSCs using established protocols (Huang et al., 2006). The hiPSC-derived SMCs expressed SMC-specific markers and were associated with vascular networks in vitro and in vivo, validating their functionality as SMCs (Figures S4H–S4K). In addition, the profile of the global gene expression showed high similarity between hiPSC-derived SMCs and primary human SMCs, further confirming the identity

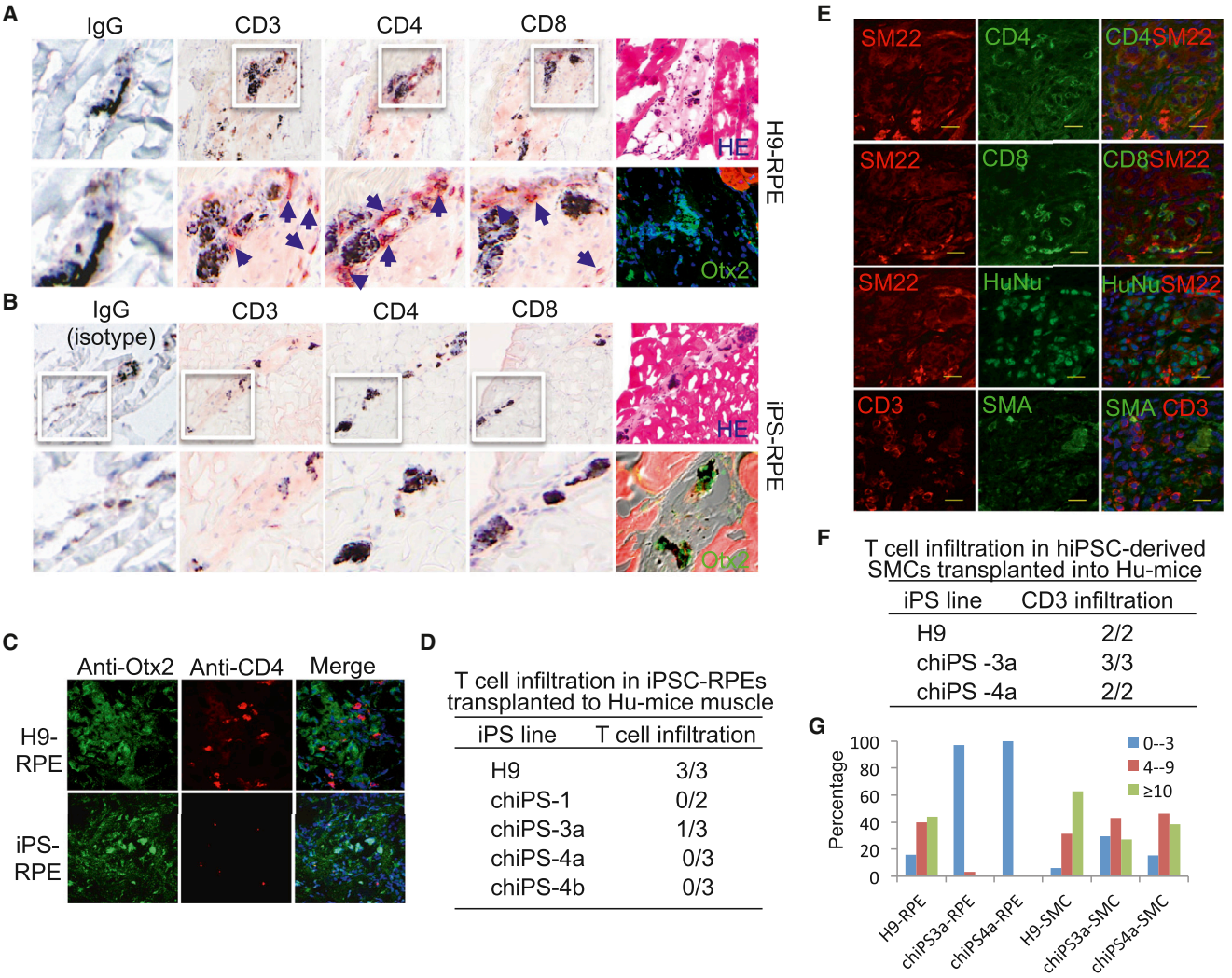


Figure 3. The Immunogenicity of RPE Cells and SMCs Differentiated from hiPSCs in Hu-Mice Reconstituted with Autologous Immune System

(A) The RPEs differentiated from H9 hESCs were infiltrated with T cells 5 weeks after being transplanted into the hindleg muscle of the Hu-mice. Blue arrow indicates the infiltrated T cells. H&E staining of H9-derived RPE cells transplanted into the hindleg muscle of the Hu-mice is shown. Otx2 staining shows the well-differentiated RPE cells (green: Otx2; blue: DAPI) transplanted into the hindleg muscle of Hu-mice.

(B) The hiPSC-derived RPEs are not infiltrated with T cells 5 weeks after being transplanted into the hindleg muscle of the Hu-mice reconstituted with autologous immune system. H&E staining of hiPSC-derived RPEs transplanted into the hindleg muscle of Hu-mice is shown. Otx2 staining shows hiPSC-derived RPEs; red: mouse muscle.

(C) The co-staining of anti-Otx2 and anti-CD4 antibodies confirmed the status of T cell infiltration into the allogenic hESC-derived and autologous hiPSC-derived RPEs transplanted into the Hu-mice. Green: Otx2; red: CD4; blue: DAPI.

(D) Summary of T cell infiltration into RPE cells differentiated from four independent hiPSCs reprogrammed from three donors after being transplanted into Hu-mice reconstituted with autologous human immune system.

(E) SMCs differentiated from hiPSCs in vitro were immunogenic in Hu-mice reconstituted with autologous immune system. hiPSC-derived SMCs were transplanted into hindleg muscle of Hu-mice reconstituted with autologous human immune system. Five weeks later, the transplants were harvested and stained with indicated antibodies, showing infiltration of T cells into the SMC grafts. SM22 and SMA, smooth muscle markers; HuNu, human nuclei. Cell nuclei were counterstained with DAPI.

(F) Summary of T cell infiltration into SMCs differentiated from two independent hiPSCs reprogrammed from two donors after being transplanted into Hu-mice reconstituted with autologous human immune system.

(G) Frequency of T cell infiltration into the grafts of hiPSC-RPEs and hiPSC-SMCs in Hu-mice with autologous immune system. Sections of grafts were stained with anti-CD3 antibody. Three sections from each graft were analyzed. At least 10 frames (20×) from each slide were randomly chosen for analysis. The numbers of T cells detected were categorized into three groups: 0–3, 4–9, or ≥10 T cells per frame.

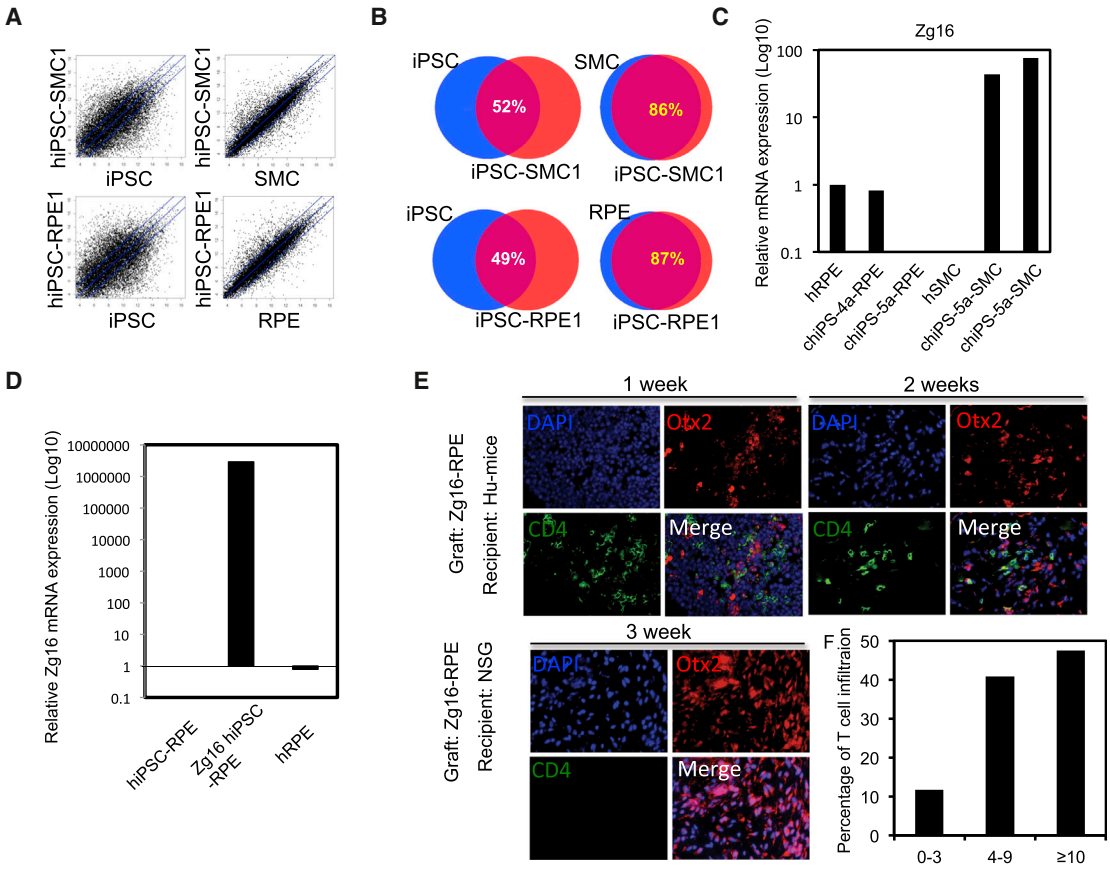


Figure 4. Abnormal Expression of Immunogenic Antigens Contributes to the Differential Immunogenicity of hiPSC-Derived SMCs and RPEs

(A) The global gene expression was profiled by oligonucleotide DNA microarrays and compared between hiPSCs and hiPSC-derived SMCs (hiPSC-SMCs), human primary SMCs and hiPSC-SMCs, hiPSCs and hiPSC-derived RPEs (hiPSC-RPEs), human primary RPEs and hiPSC-RPEs. The blue lines indicate the diagonal and 3-fold changes between the two samples.

(B–D) The gene expression similarity between various samples (B). The overexpression of the Zg16 gene in hiPSC-SMCs (C) and RPEs differentiated from Zg16-hiPSCs (D) was confirmed by qPCR.

(E) The RPE cells derived from Zg16-hiPSCs are immunogenic in Hu-mice with autologous immune system. The Zg16-RPE grafts were extensively infiltrated by T cells and mostly rejected 2 weeks after being transplantated into the hindleg muscle of Hu-mice with autologous immune system. As a control, the same batch of Zg16-RPEs were injected into the hindleg muscle of NSG mice, and they survived 3 weeks after transplantation.

(F) Frequency of T cell infiltration into the grafts of RPEs derived from Zg16-hiPSCs in Hu-mice as quantified in Figure 2G.

of hiPSC-derived SMCs. The hiPSC-derived SMCs were transplanted into the skeletal muscle of Hu-mice reconstituted with autologous human immune system. When analyzed by serial sections, the hiPSC-derived SMC grafts were uniformly infiltrated with T cells, confirming that hiPSC-derived SMCs are immunogenic to autologous human immune system (Figures 3E–3G).

Misexpression of Immunogenic Antigens Induces T Cell Responses to hiPSC-Derived SMCs

The overexpression of IL-10 has been shown to induce immune tolerance of mouse iPSC-derived endothelial cells in syngeneic recipients (de Almeida et al., 2014). However, the immune tolerance of hiPSC-derived RPEs is not due to the lack of HLA expression or the overexpression of immune-suppressive cytokines such as IL-10 (Figure S3). To understand the mechanism underlying the differential immunogenicity of hiPSC-derived RPEs and SMCs, we screened these cells for the expression of potential immunogenic antigens. While the global gene expression of hiPSC-derived SMCs and RPEs exhibited close to 90% similarity to their primary counterparts, we found that the expression of Zg16, an immunogenic antigen, was greatly increased in hiPSC-derived SMCs, but not in hiPSC-derived RPEs (Figure 4C). In addition, another immunogenic antigen, Hormad1, was also overexpressed in the hiPSC-derived SMCs (Figure S4G). To determine whether the abnormal expression of immunogenic antigens induces the immunogenicity of hiPSC-derived cells, we ectopically expressed Zg16 in iPSCs (Zg16-hiPSCs). This blocked the teratoma formation of Zg16-hiPSCs in Hu-mice with autologous immune system, suggesting that Zg16-hiPSCs are immune rejected (Figure 1C). More importantly, in contrast to hiPSC-RPEs that are immune protected from autologous immune system, RPEs differentiated from Zg16-hiPSCs expressed Zg16 and were immune rejected by autologous immune system (Figures 4D and 4E). Together with previous findings that the abnormal expression of these

immunogenic antigens (Zg16 and Hormad1) can directly induce antigen-specific T cell responses to mouse iPSC-derived cells transplanted in syngeneic mice (Zhao et al., 2011), these data support the notion that the abnormal expression of immunogenic antigens in hiPSC-derived SMCs induces their immunogenicity to autologous human immune system.

DISCUSSION

Using inbred mouse strains, recent studies have revealed the immunogenicity of the cells derived from mouse iPSCs (Araki et al., 2013; de Almeida et al., 2014; Zhao et al., 2011). While the debate on the levels of immunogenicity of cells derived from mouse iPSCs in syngeneic recipients is ongoing, these mouse studies support the notion that certain cells such as cardiomyocytes derived from mouse iPSCs in vitro are immunogenic in syngeneic recipients (Araki et al., 2013; de Almeida et al., 2014; Guha et al., 2013; Zhao et al., 2011). Therefore, to achieve the potential of hiPSCs in human cell therapy, it is important to evaluate the immunogenicity of the cells differentiated from hiPSCs. We demonstrated that certain hiPSC-derived cells such as RPE cells are much less immunogenic than other hiPSC-derived cell types such as SMCs. The mechanisms underlying the differential immunogenicity can be very complex, depending on the intrinsic immune characteristics of the cell types and the impact of epigenetic abnormality of hiPSCs on differentiated cell types as previously noted (Bar-Nur et al., 2011; Bock et al., 2011; Kim et al., 2010, 2011; Polo et al., 2010; Ruiz et al., 2012). In support of this notion, we demonstrate that the abnormal expression of immunogenic antigens in some hiPSC-derived cells, but not in others, contributes to the differential immunogenicity. Consistent with this finding, immunogenic antigens are also overexpressed during the differentiation of mouse iPSCs, but not during the differentiation of mouse ESCs, leading to antigen-specific T cell responses to iPSC-derived cells in syngeneic recipients (Araki et al., 2013; de Almeida et al., 2014; Zhao et al., 2011). While SMCs differentiated from hiPSCs exhibit functionalities and global gene expression profiles highly similar to those of normal human counterparts, the finding that the immunogenic antigens expressed in hiPSC-derived SMCs are not expressed by normal human SMCs suggests that further improvement of the robustness of the differentiation process of hiPSCs could help to reduce the immunogenicity of hiPSC-derived cells. Finally, our findings of the lack of immunogenicity of hiPSC-derived RPEs support the feasibility of developing hiPSC-derived RPE cells for treating atrophic age-related macular degeneration.

EXPERIMENTAL PROCEDURES

Reconstitution of Humanized Mice

The NOD/SCID/γ null (NSG) mice were purchased from the Jackson Laboratories at 7–10 weeks of age. Human fetal thymus and liver tissues at age 17–20 weeks were obtained from Advanced Bioscience Resource. All protocols used for human tissues and animal manipulation have been approved by University of California San Diego Research Committees on human subjects and research animal care.

Hu-mice were generated as previously described (Lan et al., 2006). Briefly, the NSG mice were first treated with sublethal irradiation (2 Gy), and human fetal thymus and liver pieces (1 mm^3) were transplanted under the kidney capsule of the irradiated NSG mice. The human $CD34^+$ cells were simultaneously purified from the same human fetal liver using a MACS separation system with anti-human $CD34^+$ antibody (Miltenyi Biotec), and 1 to 5 × 10^5 human $CD34^+$ cells were intravenously transfused into the same mice.

Generation of Integration-free hiPSCs

The fibroblasts derived from human fetal liver were reprogrammed into hiPSCs with episomal approaches as previously described (Okita et al., 2011; Zhao et al., 2011) After puromycin selection, the transfected cells were plated on feeder layer, and the hiPSC colonies were mechanically picked, dissected into small pieces, and plated onto the feeder layer in a 24-well plate. Southern blotting analysis or real-time PCR was used to confirm that the hiPSCs were integration-free as described.

Histological Analysis

Tissues were harvested and frozen in OCT (for immunohistochemical and fluorescent staining) or fixed in 10% buffered formalin and embedded in paraffin (for H7&E staining). Antibodies include polyclonal rabbit anti-human CD3 antibody (DAKO), mouse anti–human CD4 mAb (RPA-T4, BD PharMingen), mouse anti-human CD8 mAb (RPA-T8, BD PharMingen), and mouse anti-human CD123 mAb (6H6; eBioscience).

TCR Repertoire Analysis of Infiltrating T Cells

Total RNA was extracted from explanted grafts using QIAGEN RNA extraction kits (QIAGEN). TCRβ repertoires were amplified and sequenced using Illumina MiSeq by iRepertoire Inc. (Huntsville). Data analysis was performed using the website provided by iRepertoire Inc. (http://www.irepertoire.com).

Differentiation of hiPSC/hESCs into RPEs and SMCs In Vitro

The differentiation of hiPSCs/hESCs into RPEs and subsequent expansion were performed as previously described (Krohne et al., 2012). Briefly, hiPSCs/hESCs were maintained in serum-free differentiation media consisting of Knockout DMEM (Invitrogen), 2mM glutamine (Invitrogen), 0.1mM NEAA (Invitrogen), 0.1mM β-mercaptoethanol (Sigma-Aldrich), 100 U/ml penicillin (Invitrogen), 100 μg/ml streptomycin (Invitrogen), and 20% Knockout Serum Replacement (Invitrogen). Pigmented colonies began appearing after about 6 weeks. Once they reached adequate size, the colonies were manually excised and placed onto plates coated with Matrigel (Becton Dickinson). Only very low-passage cells (P1–P3) were used in these experiments.

To enable expression of Zg16 in the hiPSC-derived RPEs, the human Zg16 cDNA was inserted downstream of the CAG promoter of an episomal vector we used for iPSC reprogramming (Zhao et al., 2011) and electroporated into hiPSC lines. The empty vector was used as a control. The hiPSC lines with stable Zg16 expression were differentiated into Zg16-expressing RPEs.

Subretinal Injections

Subretinal injections were performed as described previously (Krohne et al., 2012). Briefly, the eyes from deeply anaesthetized mice were pierced with a sharp 30G needle (Becton Dickinson) just below the limbus. A blunt-tipped 33G Hamilton syringe (Hamilton) was used to deliver roughly 100,000 cells in suspension into the subretinal space using a transscleral route through the diametrically opposed retina.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes four figures and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2015.07.021.

AUTHOR CONTRIBUTIONS

T.Z. and Y.X. designed the experiments; T.Z., Z.-N.Z., T.L., Z.R., D.T., and M.W. executed the experiments related to hiPSC generation, characterization, transplantation, and gene expression analysis of iPSC-derived cells; H.Z., Z.R., D.T., T.Z., and Y.-G.Y. established the Hu-mice; and P.W., D.T., D.O.C., and M.F. performed the in vitro differentiation of RPEs/SMCs and their characterization and transplantation studies. T.Z. and Y.X were responsible for

the initial draft of the manuscript while the other authors contributed to the final edited versions.

ACKNOWLEDGMENTS

We thank UCSD Cancer Center pathologic core for help with histologic analysis and Ms. Shengyun Zhu for technical support. This work was supported by a grant from National Natural Science Foundation of China (81430032), grants from Chinese Ministry of Science and Technology (2013CB966900 and 2013ZX10002008-002), the Strategic Priority Research Program of the Chinese Academy of Sciences to T.Z. (XDA01040108), an NIH Ruth L. Kirschstein National Research Service Award to P.D.W. (EY021416), grants to M.F. from the California Institute of Regenerative Medicine (TR1-01219) and the National Eye Institute of the National Institutes of Health (EY11254), and an Early Translational Award from California Institute for Regenerative Medicine to Y.X. (TR1-01277).

Received: July 1, 2014
Revised: April 1, 2015
Accepted: July 27, 2015
Published: August 20, 2015

REFERENCES

Araki, R., Uda, M., Hoki, Y., Sunayama, M., Nakamura, M., Ando, S., Sugiura, M., Ideno, H., Shimada, A., Nifuji, A., and Abe, M. (2013). Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. Nature *494*, 100–104.

Bar-Nur, O., Russ, H.A., Efrat, S., and Benvenisty, N. (2011). Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell *9*, 17–23.

Bock, C., Kiskinis, E., Verstappen, G., Gu, H., Boulting, G., Smith, Z.D., Ziller, M., Croft, G.F., Amoroso, M.W., Oakley, D.H., et al. (2011). Reference Maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell *144*, 439–452.

de Almeida, P.E., Meyer, E.H., Kooreman, N.G., Diecke, S., Dey, D., Sanchez-Freire, V., Hu, S., Ebert, A., Odegaard, J., Mordwinkin, N.M., et al. (2014). Transplanted terminally differentiated induced pluripotent stem cells are accepted by immune mechanisms similar to self-tolerance. Nat. Commun. *5*, 3903.

Guha, P., Morgan, J.W., Mostoslavsky, G., Rodrigues, N.P., and Boyd, A.S. (2013). Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. Cell Stem Cell *12*, 407–412.

Huang, H., Zhao, X., Chen, L., Xu, C., Yao, X., Lu, Y., Dai, L., and Zhang, M. (2006). Differentiation of human embryonic stem cells into smooth muscle cells in adherent monolayer culture. Biochem. Biophys. Res. Commun. *351*, 321–327.

Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I.R., et al. (2010). Epigenetic memory in induced pluripotent stem cells. Nature *467*, 285–290.

Kim, K., Zhao, R., Doi, A., Ng, K., Unternaehrer, J., Cahan, P., Huo, H., Loh, Y.-H., Aryee, M.J., Lensch, M.W., et al. (2011). Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nat. Biotechnol. *29*, 1117–1119.

Krohne, T.U., Westenskow, P.D., Kurihara, T., Friedlander, D.F., Lehmann, M., Dorsey, A.L., Li, W., Zhu, S., Schultz, A., Wang, J., et al. (2012). Generation of retinal pigment epithelial cells from small molecules and OCT4 reprogrammed human induced pluripotent stem cells. Stem Cells Transl. Med. *1*, 96–109.

Lan, P., Tonomura, N., Shimizu, A., Wang, S., and Yang, Y.-G. (2006). Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. Blood *108*, 487–492.

Lewitzky, M., and Yamanaka, S. (2007). Reprogramming somatic cells towards pluripotency by defined factors. Curr. Opin. Biotechnol. *18*, 467–473.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K., et al. (2011). A more efficient method to generate integration-free human iPS cells. Nat. Methods *8*, 409–412.

Park, I.H., Zhao, R., West, J.A., Yabuuchi, A., Huo, H., Ince, T.A., Lerou, P.H., Lensch, M.W., and Daley, G.Q. (2008). Reprogramming of human somatic cells to pluripotency with defined factors. Nature *451*, 141–146.

Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T., et al. (2010). Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat. Biotechnol. *28*, 848–855.

Rong, Z., Wang, M., Hu, Z., Stradner, M., Zhu, S., Kong, H., Yi, H., Goldrath, A., Yang, Y.-G., Xu, Y., and Fu, X. (2014). An effective approach to prevent immune rejection of human ESC-derived allografts. Cell Stem Cell *14*, 121–130.

Ruiz, S., Diep, D., Gore, A., Panopoulos, A.D., Montserrat, N., Plongthongkum, N., Kumar, S., Fung, H.-L., Giorgetti, A., Bilic, J., et al. (2012). Identification of a specific reprogramming-associated epigenetic signature in human induced pluripotent stem cells. Proc. Natl. Acad. Sci. USA *109*, 16196–16201.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell *131*, 861–872.

Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science *318*, 1917–1920.

Zhao, T., Zhang, Z.-N., Rong, Z., and Xu, Y. (2011). Immunogenicity of induced pluripotent stem cells. Nature *474*, 212–215.