Putative Immunogenicity Expression Profiling Using Human Pluripotent Stem Cells and Derivatives

JASON P. AWE, " ERIC H. GSCHWENG, B, C AGUSTIN VEGA-CRESPO, B JON VOUTILA, B MARY H. WILLIAMSON, A BRIAN TRUONG, A DONALD B. KOHN, B, C, D NORIYUKI KASAHARA, B, F JAMES A. BYRNE, A, C

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ABSTRACT

Autologous human induced pluripotent stem cells (hiPSCs) should allow cellular therapeutics without an associated immune response. This concept has been controversial since the original report that syngeneic mouse iPSCs elicited an immune response after transplantation. However, an investigatory analysis of any potential acute immune responses in hiPSCs and their derivatives has yet to be conducted. In the present study, we used correlative gene expression analysis of two putative “immunogenicity” genes, ZG16 and HORMAD1, to assay their human homologous expression levels in human pluripotent stem cells and their derivatives. We found that ZG16 expression is heterogeneous across multiple human embryonic stem cell and hiPSC-derived cell types. Additionally, ectopic expression of ZG16 in antigen-presenting cells is insufficient to trigger a detectable response in a peripheral blood mononuclear cell coculture assay. Neither of the previous immunogenicity-associated genes in the mouse currently appears to be relevant in a human context.

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INTRODUCTION

Human induced pluripotent stem cells (hiPSCs) provide a viable alternative to human embryonic stem cells (hESCs) as an autologous stem cell source, ideally eliciting no detectable immune response in a transplant setting [1]. Multiple groups, however, have provided evidence of differences manifested in the transcriptome, epigenome, genomic imprinting, somatic mutations, and differentiation efficiencies between ESCs and iPSCs [2–12]. Furthermore, it was reported that syngeneic transplanted mouse iPSCs, but not ESCs, were capable of eliciting an immune response on teratoma formation [13]. Follow-up studies in the mouse have produced variable and contradictory results [14, 15]. Only one group has investigated the immunogenicity of hPSCs, although differences in HORMAD1 and ZG16 between lines have suggested no significant variation that would manifest as a possible immunogenic difference between hiPSCs and hESCs [16]. Expanding on that study, we present a comprehensive analysis of the expression of the human homologs of mouse HORMAD1 and ZG16 in both undifferentiated hPSCs and varying degrees of differentiated derivatives. We also used a modified peripheral blood mononuclear cell (PBMC) coculture assay to test for an in vitro-mediated acute immune response.

MATERIALS AND METHODS

Ethics Statement

Written approval and informed consent regarding human skin biopsy procedures and human fibroblast derivation, culture, and experimental use are detailed elsewhere [17].

Tissue Culture Maintenance of Primary Human Skin Cells

The human skin-derived primary cell line used in our study was derived and cultured as previously described [17]. Additionally, two other fibroblast lines, MGM2 and LAVIV (azficel-T, part no. DR01; Fibrocell Science, Exton, PA, http://www.fibrocellscience.com), were used in the present study as previously described [17]. LAVIV adult human skin-derived dermal fibroblasts were obtained from a 4-mm skin punch biopsy, as described in the Isolagen Standardized Manufacturing Process EX-GTR-110, version 00 (Fibrocell Science). All three fibroblast lines were cultured in standard fibroblast media conditions, as detailed previously [17]. In brief, the fibroblast lines were cultured in complete Dulbecco’s modified Eagle’s medium (DMEM) nutrient mixture/F-12 (DMEM/F-12) supplemented with fetal bovine serum (FBS), 1X nonessential amino acids, 1X GlutaMAX, and 100 IU/ml penicillin-streptomycin (Invitrogen/Gibco, Carlsbad, CA, USA).
Embryonic stem cells (hESCs) lines 1 and 9 were procured from the WiCell Research Institute (Madison, WI, http://www.wicell.org). UCLA embryonic stem cell lines 2, 3, and 6 were procured from the Eli and Edythe Broad Stem Cell Research Center, Stem Cell Core, University of California, Los Angeles (UCLA) (Los Angeles, CA, http://www.stemcell.ucla.edu). hESC lines 1, 2, 3, 6, and 9 are hereafter referred to as E51 through E55, respectively. Multiple integration iPSCs were derived as previously reported [18]. mRNA, adult pre- and postexcision hiPSCs, and MGM 2.19, 6.7, and 13.1.0 hiPSCs were derived from patient-derived fibroblasts using standard skin biopsy procedures. hiPSCs were derived using the stem cell cassette, lentiviral-based reprogramming method [17, 19, 20]. The pre- and postexcision hiPSCs (genetically identical lines) are hereafter referred to as iP51 and iP52, respectively. The mRNA-derived line is hereafter referred to as iP53. MGM 2.19, 6.7, and 13.1.0 are hereafter referred to as iP54, iP55, and iP56, respectively. The multiple integration line is hereafter referred to as iP57. All hESC lines were originally plated on mouse embryonic fibroblasts and maintained in hESC media as previously described [17]. The colonies were subsequently passaged into feeder-free conditions using an 18-gauge needle (Fisher Scientific) onto reduced supplements as indicated by the protocol for a 5-day period with 0.05% trypsin (Invitrogen) and rinsed with DMEM/F-12 (Invitrogen). The cells were regularly passaged with either 0.05% trypsin (Invitrogen) and resuspended in ice-cold Matrigel diluted at 1:2 in DMEM to a total volume of 50 μl. Each 10-cm dish was split into two (e.g., 7.5 million cells per injection site). For the testicular injections, both testes in a severe combined immunodeficient (SCID) adult male beige mouse were injected with 50 μl of the cell/Matrigel slurry. For subcutaneous injections, 7.5 million cells were injected into the subcutaneous space in each hind leg of the SCID beige mouse. For both testicular and subcutaneous injections, the mice were anesthetized; this was used for the nonsurgical subcutaneous injections to ensure the cells were not immediately dispersed on movement and an adequate interval for Matrigel solidification could occur. The teratomas were harvested at 7 weeks for both testicular and subcutaneous teratomas by surgery. Immediately, one half of the teratoma was sectioned with a scalpel into 10 pieces and placed into RNAlater buffer (Qiagen, Valencia, CA, http://www.qiagen.com). The other half of the teratomas were fixed in 4% formaldehyde, and the sections were embedded in paraffin and stained with hematoxylin and eosin for histological analysis at the UCLA Translational Pathology Laboratory. All animal experiments were performed in accordance with the UCLA Animal Research Committee and Division of Laboratory Animal Medicine.

Non-directed Embryoid Body Differentiation

Embryoid bodies (EBs) were made by taking 95% confluent 10-cm dishes of hESCs or hiPSCs and washing them once with 1× phosphate-buffered saline (PBS) (Invitrogen). This was followed by incubation for 5 minutes with StemPro Accutase (Life Technologies) to form a single cell suspension. The plate was rinsed twice with nonsupplemented DMEM/F-12 and spun down at 300g for 5 minutes. This pellet was then resuspended in AggreWell Medium (STEMCELL Technologies) with rho-associated protein kinase inhibitor. Different EB sizes were created by changing the number of input hES or hiPS single cells from 100 cells per EB, 1,000 cells per EB, and 10,000 cells per EB into AggreWell 400 or AggreWell 800 plates (STEMCELL Technologies). The EBs were placed in ultra-low attachment multiwall plates (Sigma-Aldrich, St. Louis, MO, http://www.sigma-aldrich.com) for 24 hours and then underwent one media change with AggreWell medium (STEMCELL Technologies) for 24 hours and then plated onto 0.2%-coated gelatin wells in a 6-well plate in standard fibroblast-containing media until harvested at the designated points. The media were changed every 2 days for the duration of the experiment.

Directed Trilineage Differentiation

Directed differentiation into mesoderm was performed as previously published [21]. In brief, hESCs and hiPSCs were routinely passaged at high confluence onto Matrigel (BD Biosciences) with daily media changes. After 48 hours, the stem cell media were replaced with basal differentiation media (STEMdiff APEL, STEMCELL Technologies) supplemented with 5-μM GSKi (CHIR99021; Stemgent) for 24 hours and further differentiated in APEL media supplemented with 25 ng/ml human recombinant bone morphogenetic protein 4 (PeproTech, Rocky Hill, NJ, http://www.peprotech.com) for 24 hours. The hESCs and hiPSCs were differentiated into ectoderm by following the manufacture’s protocol (STEMCELL Technologies). In brief, the stem cell colonies were made into a single cell suspension as detailed previously using StemPro Accutase (Life Technologies). The cells were plated onto Matrigel-coated plates or glass cover slips (BD Biosciences) overnight and then rinsed with DMEM/F-12. Appropriate volumes of STEMdiff Neural Induction Media (STEMCELL Technologies) were placed onto the cells for a 10-day period with daily media changes. The hESCs and hiPSCs were differentiated into endoderm by following the manufacturer’s protocol (STEMCELL Technologies). In brief, the stem cell colonies were made into a single cell suspension as detailed and plated onto Matrigel-coated wells or glass cover slips (BD Biosciences) overnight and then rinsed with DMEM/F-12. The cells were then incubated with the specific media and supplements as indicated by the protocol for a 5-day period with daily media changes.

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Directed Differentiation Into Cardiomyocytes, Oligodendrocyte Progenitor Cells, and Hepatocytes

For cardiomyocyte differentiation, the hESCs and hiPSCs were differentiated as previously published [22, 23]. Specifically, 1 million cells originally plated onto Matrigel-coated wells (BD Biosciences) were found to be the optimal cell density for the hESC and hiPSC lines. The oligodendrocyte progenitor cells (OPCs) were differentiated as previously published [24]. Specifically, EBs were made with 5,000 cells per EB, because it has been shown that larger EBs tend to result in better neural lineages [25]. Hepatocytes were differentiated as previously published [17, 26]. Human fetal cardiomyocytes, hepatocytes, oligodendrocyte progenitor cells, and adult cardiomyocytes were purchased and used as controls (ScienCell Research Laboratories, Carlsbad, CA, http://www.sciencellonline.com). Adult hepatocytes were also used as a control (Triangle Research Laboratories, Charlotteville, VA, http://triangleresearchlabs.net).

RNA Isolation and Reverse Transcription Polymerase Chain Reaction

Total RNA was isolated using a High Pure RNA Isolation Kit as per the manufacturer’s recommendations (Roche Diagnostics USA, Indianapolis, IN, http://www.roche-diagnostics.us). cDNA was synthesized using 1,000 ng/μl total RNA using the Transcriptor First Strand cDNA Synthesis Kit and both anchored-oligo(dt)18 and random hexamer primers (Roche Diagnostics USA) in accordance with the manufacturer’s recommendations. Reverse transcription polymerase chain reactions (PCRs) were performed as previously described [17]. The primer sequences were as follows: oligodendrocyte transcriptions factor 2, forward, 5'-CTCTCTGATGGGCTCCAG-3', reverse, 5'-ACTTCTCTGCCTTTTGTTGGAGG-3'; platelet-derived growth factor receptor-α, forward, 5'-CTCTGAAAAGGGTCAGAAGGA-3', reverse, 5'-GTTGGTGAATGCACAACCCTG-3'; NKX homeobox 2, forward, 5'-GGGCAGAGCAACCACAGCACA-3'; myelin-associated glycoprotein, forward, 5'-TATGATTTCAGGCTATGG-3', reverse, 5'-AAGAC-GCTT-GCTTGAATGGC-3'; GATA binding protein, forward, 5'-ACGGGTTGTTGTCGATCTTC-3', reverse, 5'-ACTTCTCGCTTTTGGTCTTT-3'; phospholamban, forward, 5'-GCCGAATAGCTGAGCTCCAAAAC-3', reverse, 5'-GCGCAGTCAGCCAAAGAC-3'; platelet-derived growth factor receptor-β, forward, 5'-CCACCCTG-3', reverse, 5'-CTTGGAGAGCTTCCCAAC-3'; amyloid, forward, 5'-ACCCCCAAGTGCCTCAACACAAAGGAG-3'; albumin, forward, 5'-ACCCCAATGCTCAGCGTCC-3'; α1-antitrypsin, forward, 5'-AAGGACACCGAGGAAAGGAGG-3'; reverse, 5'-TTGCCAGAAGGATCC-3'.
Enzyme-Linked Immunosorbent Assay

Evaluation of interferon-γ (IFN-γ) release was determined using the Human IFN-γ ELISA Ready-SET-Go! kit per the manufacturer’s instructions (Affymetrix; eBioscience, San Diego, CA, http://www.ebioscience.com). The plates were read on a Tecan Infinite M1000 (Tecan, San Jose, CA, http://www.tecan.com). The 450-nm optical density (OD) was measured using the Infinite 200 multimode microplate reader provided with the Tecan-i-Control Plate reader analysis software (Tecan). A separate analysis was performed to normalize the result to a total protein quantity that
was measured using the Bio-Rad protein assay kit (no. 500-0001; Bio-Rad Laboratories, Hercules, CA, http://www.bio-rad.com), according to the manufacturer’s instructions. The OD595 was measured using the Infinite 200 multimode microplate reader provided with the Tecan-i-Control Plate reader analysis software (Tecan).

**HORMAD1 AND ZG16 LENTIVIRAL PRODUCTION AND TRANSDUCTION INTO HUMAN FIBROBLASTS**

**Lentiviral Vector Construction and Production**

ZG16 was cloned as a PCR product from human hepatocyte cDNA and inserted into a third-generation lentiviral vector with a murine stem cell virus promoter for PBMC transduction. Vesicular stomatitis virus G pseudotyped lentivirus preparations were produced by cotransfection of packaging plasmids pMD2.G, pMDLg/pRRE, pRSV-Rev, and transfer vector into 293T cells (American Type Culture Collection, Manassas, VA, http://www.atcc.org) with jetPRIME reagent (Polyplus Transfection, New York, NY, http://www.polypus-transfection.com). Forty-eight hours later, virus-containing supernatant was collected, filtered, and concentrated by ultracentrifugation. The virus titers were determined by p24 ELISA, performed by the UCLA Virology Core (Los Angeles, CA), and quantitative PCR of transduced genomic DNA.

**PBMC Transduction**

The cells were thawed and stimulated for 72 hours with Dynabeads Human T-Activator CD3/CD28 (Life Technologies) per the manufacturer’s instructions. The cells were isolated from beads and transduced with viruses, as described, at a vector concentration of 1 × 10⁶ transduction units per milliliter for 48 hours. The cells were subsequently cocultured at a 1:1 ratio with freshly thawed PBMCs for 5 and 15 days before the medium was collected for ELISA (described above).

**Western Blot**

Jurkat cells and PBMCs were transduced as described above. At 1 week after transduction, whole cell lysate was generated using RIPA buffer (Thermo Fisher Scientific) and HALT Protease Inhibitor (Thermo Fisher Scientific). Next, 12 μg of lysate was loaded and run on a 4%–12% bis-tris gradient gel (Invitrogen, Life Technologies). The separated gels were loaded onto polyvinylidene difluoride membranes (Innogen, Life Technologies), and blocked with 5% milk in PBS with Tween (PBST) (0.05%) for 1 hour at RT. The blots were probed using ZG16 antibody (Proteintech Group, Inc., Chicago, IL, http://www.ptglab.com) at 1:500 in blocking buffer overnight at 4°C. The separated gels were loaded onto polyvinylidene difluoride membranes (Innogen, Life Technologies), and blocked with 5% milk in PBS with Tween (PBST) (0.05%) for 1 hour at RT. The blots were probed using ZG16 antibody (Proteintech Group, Inc., Chicago, IL, http://www.ptglab.com) at 1:500 in blocking buffer overnight at 4°C. The membranes were washed three times with PBST, probed with secondary anti-mouse and anti-rabbit peroxidase (Sigma-Aldrich) at 1:1,000 in blocking buffer overnight at 4°C. The membranes were washed three times with PBST. Peroxidase activity was detected using ECL-PLUS (Thermo Fisher Scientific) and Typhoon FLA 9000 (GE Healthcare).

**Statistical Analysis**

The results are presented as the mean ± SD and mean ± SE. The statistical significance of the differences for all gene expression analyses was evaluated using SPSS, version 21 (IBM Corp., Armonk, NY, http://www-01.ibm.com/software/analytics/spss/). The results from analysis of variance, the t test for independent samples, Levene’s homogeneity of variance test, and the Mann-Whitney and Kruskal-Wallis nonparametrical one-way analysis of variance tests were considered statistically significant at p < .05.

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RESULTS

Teratoma-Based Immunogenicity Gene Expression Profiling

To duplicate the teratoma-based iPSC-specific immune response assay, teratomas were created from a variety of hiPSCs and hESCs that encompass a spectrum of hPSCs and therefore should represent the inherent variability among stem cell lines and any putative differences in hESCs and hiPSCs. All tested hESC and hiPSC lines formed intratesticular teratomas consisting of all three germ layers (Fig. 1A; supplemental online Fig. 1A). Because of their rich tissue diversity, teratomas were sectioned into 10 pieces and analyzed via quantitative PCR (QPCR) for both HORMAD1 and ZG16. After averaging the 10 pieces, HORMAD1 expression was found to be very low, although heterogeneous, with high QPCR-based cycle threshold values present across all hPSC lines analyzed (Fig. 1B, top). Concordantly, although ZG16 had much higher expression, it too exhibited heterogeneous expression across all hPSC lines (Fig. 1B, bottom). Statistically significant gene expression differences were observed between the hESC lines and hiPSC lines. We classified hESC and hiPSC differences between specific hESC and hiPSC lines as “interexperimental variation.” Statistically significant differences were also observed when comparing individual hiPSC lines, or individual hESC lines, with other hiPSC, or hESC, lines, respectively. We classified these differences as “intraexperimental variation.” The immunogenicity gene expression variation previously observed proved inconsistent between the hiPSC and hESC inter- and intraexperimental lines. This inconsistent variation provides evidence that interline variation (i.e., between hiPSC and hESC lines) does not provide any more variation than the intraline differences observed in hESCs or hiPSC lines.

We hypothesized that the gene expression differences in testicular teratomas had resulted from intrinsic differences between the different hESC and hiPSC lines, manifesting in differential responses to niche differentiation factors. We tested this potential inherent variability of teratoma formation by injecting cells into the same mouse; we transplanted ES1 or iPS2 cells into the left and right hind legs of an immunocompromised mouse (Fig. 1C) and again found low overall expression of HORMAD1. In addition, only the iPS2 cells showed a significant difference between the left and right hind leg teratomas (Fig. 1D, top left). We again noted that ZG16 expression was higher than HORMAD1 expression. Both lines
alyzed displayed a significant difference between the left and right hind leg teratomas, indicating induced differences despite forming in the same mouse and injection site location (Fig. 1D, bottom left). When the subcutaneous and testicular teratomas from the ES1 and iPSC2 lines were compared, a significant difference was found in relation to HORMAD1 expression between the ES1 teratomas, but not the iPSC2 teratomas (Fig. 1D, top right). However, not only was ZG16 found to differ between the ES1 and iPSC2 subcutaneous- and testicular-derived teratomas, but a difference was also exhibited between ES1 and iPSC2 subcutaneous teratomas (Fig. 1D, bottom right). Notwithstanding, because of the variation previously observed between the left and right hind leg teratomas, we have concluded that the differences between the subcutaneous and testicular teratomas in both lines for HORMAD1 and ZG16 display variable expression, likely owing to line-to-line and clonal variation.

Putative Immunogenicity Gene Expression Across hPSC Derivatives

To maintain clinical applicability, our investigation focused on the expression patterns of HORMAD1 and ZG16 across varying levels of nondirected, partially directed, and fully directed differentiation of hPSCs. We hypothesized that, owing to the gene expression heterogeneity observed in teratomas, we would again see broad expression variance owing to hPSC line variation and differential amenability to differentiation protocols. Therefore, the hESC and hiPSC lines were first differentiated in a nondirected manner by EB formation (Fig. 2A; supplemental online Fig. 1B).

EBs are three-dimensional multicellular aggregates that not only mimic early embryogenesis (e.g., can differentiate into mesoderm, endoderm, and ectoderm), but also differentiate spontaneously in suspension culture and in basic serum-containing media [27, 28]. Additionally, EB size and measurement points can bias EBs and cause different and/or multiple differentiation trajectories. We chose to make EBs with 100, 1,000, and 10,000 cells per EB and then transferred these EBs onto gelatin-coated plates for 1, 2, and 3 weeks of growth before harvesting [25, 29]. HORMAD1 expression was again extremely low, with expression levels that varied across the hESC and hiPSC lines (Fig. 2B, top). We observed high ZG16 expression compared with HORMAD1, although still at relatively low levels compared with teratoma expression, which varied significantly between the inter- and intraexperimental hESC and hiPSC lines (Fig. 2B, bottom). Statistically significant variance was observed for both HORMAD1 and ZG16, although the levels were inconsistent and no specific correlation between the different cell numbers and time points across all lines tested was observed.

Understanding that different tissues present an array of potentially immunogenic targets, the hPSCs were subjected to directed differentiation into specific early representatives of the three embryonic germ layers characterized by typical gene expression patterns (supplemental online Fig. 2) and protein expression (Fig. 3A; supplemental online Fig. 3) [30, 31]. Again, we found a low expression of HORMAD1, at comparable levels to EBs, that varied across the inter- and intraexperimental hESC and hiPSC lines (Fig. 3B, top). The variable expression of HORMAD1 did not demonstrate any pattern from the uninduced to induced cells.

Figure 5. ZG16 expression increased significantly on differentiation of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) into clinically relevant cell derivatives. (A): HORMAD1 expression in hESC and hiPSC derivatives was very low and heterogeneous (top). ZG16 expression analysis resulted in a significant increase across all four lines on differentiation (bottom). *, p < .05 for HORMAD1 expression for fibroblast-2 line compared with all other PSC derivatives. **, p < .05 for human fetal OPCs compared with other OPCs derived from all lines. ***, p < .05 for each PSC line undifferentiated compared with differentiated. Error bars represent ±SD. The y-axis is a logarithmic scale, with display units of 100. All polymerase chain reactions were run in triplicate. (B): Gene expression analysis for ZG16 was assessed in hiPSC1- and hiPSC3-derived hepatocytes. The significant increase in ZG16 expression seen during hepatocyte differentiation from control iPSCs is reproducible across both iPS1 and iPS3 lines. A, B, and C listed in the cell line annotations indicate the duplicate differentiations across three replicates. *, p < .05 for iPS3-A, -B, and -C differentiated replicates compared with iPS3-A, -B, and -C differentiated replicates; **, p < .05 for iPS3-D compared with iPS3-A, -B, and -C differentiated replicates; and ***, p < .05 for iPS3-D compared with iPS-D. ZG16 y-values are in units of hundreds. Error bars represent ±SD. Abbreviations: Card., cardiomyocytes; Card.-A, adult cardiomyocytes; dif, differentiation; ES, human embryonic stem cell line; Hep., hepatocytes; Hep.-A, adult hepatocytes; OPCs, oligodendrocyte progenitor cell; PSC, pluripotent stem cell.
expression. Although this revealed overall low and heterogeneous expression differences on induction would also be observed with an immune response or interact with other molecules that could potentially cause an acute immune response. Owing to the required activation step of PBMCs to allow successful viral transduction, the PBMCs in coculture with their parental donor PBMCs led to a basal level of self-reactivity that was somewhat decreased from days 5 to 15. No indication was observed that these differences between the cell lines arose during the differentiation protocols, as confirmed via Western blot analysis for protein expression. We subsequently examined HORMAD1 expression for HORMAD1, we noted a trend that differentiation increased expression across all cell types for all derivative cell types. We postulated that because the adult fibroblast line had a significant increase in HORMAD1 expression that was higher than that in the hPSC derivatives (Fig. 5A, top), HORMAD1 would be unlikely to be involved in causing an immune response. Statistical analysis indicated that ZG16 expression was significantly increased with differentiation of every cell line tested into the specific derivatives; the human fetal cells had higher expression than all other OPC derivatives (Fig. 5A, top). HORMAD1 would be unlikely to be involved in causing an immune response. Statistical analysis indicated that ZG16 expression was significantly increased with differentiation of every cell line tested into the specific derivatives; the human fetal cells had higher expression than all other OPC derivatives (Fig. 5A, top). HORMAD1 would be unlikely to be involved in causing an immune response. Statistical analysis indicated that ZG16 expression was significantly increased with differentiation of every cell line tested into the specific derivatives; the human fetal cells had higher expression than all other OPC derivatives (Fig. 5A, top).
compared with the undifferentiated iPSc1 cells. The iPSc3-differentiated line also had a significant increase across all replicates compared with the undifferentiated iPSc3 cells (Fig. 5B). The iPSc1 replicates, when averaged together, were again found to be significantly lower than the iPSc3-differentiated line when averaged across all three replicates. However, significant variability was also seen in the iPSc3-A and iPSc3-B replicates, between both themselves and that of the original iPSc3 differentiation, thus indicating a high level of variability between the hPSC lines and the intraexperimental replicates (Fig. 5B). Thus, although ZG16 expression is definitively and reproducibly increased with differentiation, our data suggest that the inherent intra- and interline variability and, possibly, clonality issues with hPSC thawing and seeding would preclude this as a useful immunogenicity screening tool (Fig. 5B).

These data, thus far, have made it difficult to classify ZG16, because human fetal oligodendrocytes and hepatocytes yielded high levels of ZG16, similar to the levels expressed across the various differentiated hPSC lines, indicating that ZG16 could be a fetally associated antigen. These findings are in contrast to the high level of ZG16 expression from adult hepatocytes, thereby indicating that adult cells are capable of expressing this antigen and eliminating ZG16 as a candidate exclusive fetal antigen.

Testing for an In Vitro Acute Immune Response

To investigate a functional link between ZG16 expression and an immune response in a human model, ZG16 was transduced into PBMCs and used in a modified PBMC coculture assay with IFN-γ secretion. This assay was then used as a metric of whether PBMCs and used in a modified PBMC coculture assay with IFN-γ.

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Author Contributions

J.P.A.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; E.H.G.: conception and design, collection and analyze data, data analysis and interpretation, manuscript writing; A.V.-C.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing; J.V.: conception and design, collection and analyze data, manuscript writing; M.H.W. and B.T.: collection and/or assembly of data, data analysis and interpretation; D.B.K. and N.K.: conception and design, data analysis and interpretation, manuscript review; J.A.B.: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

J.A.B. has multiple financial relationships with Fibrocell Science, Inc. (consultant, sponsored research agreement, royalties), but they do not affect the content of this article. A.V.-C. has compensated research funding from Fibrocell Science, Inc. The other authors indicated no potential conflicts of interest.

In the present study, we performed gene expression analysis across a variety of hESC and hiPSC derivatives with varying levels of differentiation using two putative immunogenicity genes that were previously correlated to an in vivo T-cell-mediated immune response in the mouse [13]. Gene expression differences between the hESCs and hiPSCs, in both their undifferentiated form and a variety of clinically relevant derivatives, indicate that no consistent or specific differences inherent to hESCs or hiPSCs exist. This observation carried through a variety of differentiation representative of the different stages of cell maturation during development. The modified PBMC coculture assay tested for an acute immune response with what we propose to be the only relevant gene capable of potentiating an immune response, ZG16, and was found incapable of producing a significant IFN-γ-based response in PBMCs. Therefore, we conclude that the levels of two putative immunogenicity-related antigens presented in human cells are not capable of producing a significant in vitro acute immunological response.

Conclusion

In the present study, we performed gene expression analysis across a variety of hESC and hiPSC derivatives with varying levels of differentiation using two putative immunogenicity genes that were previously correlated to an in vivo T-cell-mediated immune response in the mouse [13]. Gene expression differences between the hESCs and hiPSCs, in both their undifferentiated form and a variety of clinically relevant derivatives, indicate that no consistent or specific differences inherent to hESCs or hiPSCs exist. This observation carried through a variety of differentiation representative of the different stages of cell maturation during development. The modified PBMC coculture assay tested for an acute immune response with what we propose to be the only relevant gene capable of potentiating an immune response, ZG16, and was found incapable of producing a significant IFN-γ-based response in PBMCs. Therefore, we conclude that the levels of two putative immunogenicity-related antigens presented in human cells are not capable of producing a significant in vitro acute immunological response.

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