Comparison of Human Induced Pluripotent and Embryonic Stem Cells: Fraternal or Identical Twins?

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Human induced pluripotent stem cells (hiPSCs) have been hailed as an effective replacement for human embryonic stem cells (hESCs) and a prime candidate cell source for regenerative medicine aims. Both hESCs and hiPSCs share the important properties of self-renewal and pluripotency; that is, they are theoretically capable of generating unlimited amounts of any differentiated cell in the human body. However, accumulating reports of gene expression differences between hESCs and hiPSCs have led many to question the equivalence of these two promising cell types. Seemingly random variation in the differentiation propensity of hiPSCs to neural,[1] cardiovascular,[2] and hemangioblastic lineages[3] has frustrated investigators hoping to better exploit their potential for disease modeling and cell replacement therapies. In light of these somewhat dispiriting results, the recent publication of genome-wide reference "scorecards" for monitoring the quality and utility of 32 human pluripotent stem cell lines is a welcome advance.[4] Such advances are crucial to aiding our ability to predict a cell line's differentiation propensity in a high-throughput fashion.

hESCs are derived from the inner cell mass of fresh or frozen embryos at the blastocyst stage of development[5] (**Figure 1a**). Most importantly, hESCs self-renew to allow for indefinite maintenance of the undifferentiated state *in vitro* and thereby retain the ability to differentiate into derivatives of the three embryonic germ layers that subsequently form all the tissues of a developing fetus. Consequently, hESCs are a promising candidate cell source for the generation of differentiated cells for use in cell replacement therapies, as well as a valuable tool for disease modeling and drug screening applications. Unfortunately, however, hESC derivation remains ethically controversial in the United States and somewhat challenging logistically because of a limited supply of donor human embryos. Therefore, the landmark discovery that hiPSCs with remarkable similarity to hESCs could be derived relatively easily from somatic tissues was hailed as a significant advance.[6,7] In contrast to hESCs, hiPSCs are derived by "reprogramming" of somatic cells to a pluripotent state through the overexpression of a key set of transcription factors (**Figure 1b**). This process does not require the destruction of human embryos *ex utero*, thereby circumventing much of the ethical debate surrounding hESC derivation. In addition, because the techniques for hiPSC derivation are easily applicable to adult somatic cell types, cell lines can be easily derived from a variety of genetic backgrounds. This allows not only for the creation of patient-specific hiPSCs that are theoretically secure against immune rejection but also for novel studies of heritable genetic disorders in their human cell types.[8]

hiPSCs are similar to hESCs in terms of their morphology, feeder dependence, surface marker expression, and *in vivo* teratoma formation capacity.[6,7] Despite these similarities, reports of variability in the *in vitro* differentiation potential of hiPSCs with respect to hESCs have called into question the functional and molecular equivalence of the two cell types (**Table 1**). For instance, a reduced and more variable yield of neural[1] and cardiovascular progeny[2] has been observed in hiPSCs, irrespective of the presence of reprogramming transgenes in the hiPSC genome. In addition, hiPSC-derived early blood progenitor and endothelial cells appear to undergo premature senescence.[2,3] What underlies these differences in yield of useful differentiated cell types? Such results are perhaps unsurprising in light of the fact that murine iPSC lines differ from one another in terms of their developmental potency when tested in the definitive tetraploid blastocyst complementation assay.[9-11] Namely, only certain murine iPSC lines are capable of generating "all-iPSC" mice upon injection into tetraploid blastocysts. Yet because embryo-based assays of pluripotency are not feasible using human cell types, the need for a better understanding of the molecular surrogates of pluripotency has become critical to our comprehension of these seemingly random variations in developmental potential.

Microarray-based analysis of global gene expression profiles has been an invaluable tool in the characterization of the transcriptional state of cells and the identification of key differences between cell types. Although global gene expression profiles of hESCs and hiPSCs are largely similar,[12] subtle differences in the expression of messenger RNAs (mRNAs)[13] and micro RNAs[14] have been reported. Importantly, residual transgene expression[15] and genetic background[16] have both been found to perturb the global gene expression profile of human pluripotent stem cells (PSCs), and such effects cannot be excluded in the above-mentioned -omic studies.[13,14] Fortunately, the confounding effect of residual transgene expression can be overcome through the use of newly developed transgene-free hiPSCs. However, it bears mentioning that even transgene-free hiPSCs have displayed transcriptional differences from their hESC counterparts in one report.[17] On the other hand, controlling for genetic background by comparing genetically matched hESCs and

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Table 1 Comparisons of iPSCs with ESCs and their somatic cells of origin

Reference	Cell lines	Level of analysis	Key findings
Marchetto *et al.* (2009)[17]	2 hiPSCs + 2 hESCs	Transcriptome via microarray	Distinct gene expression signature of hiPSCs
Chin *et al.* (2009)[13]	4 hESCs + 5 hiPSCs + 3 somatic	Transcriptome via microarray MicroRNA-ome via microarray Histone methylation via ChIP	Distinct gene expression signature of hiPSCs
Ghosh *et al.* (2010)[20]	4 hiPSCs + 4 somatic	Transcriptome via microarray	Transcriptional memory of somatic cell of origin
Guenther *et al.* (2010)[12]	6 hiPSCs + 6 hESCs	Histone methylation via ChIP-seq Transcriptome via microarray	Inconsistent hiPSC vs. hESC differences Lab-specific gene expression differences
Newman *et al.* (2010)[27]	17 hESCs + 67 hiPSCs	Meta-analysis of microarray data	Lab-specific gene expression signatures
Feng *et al.* (2010)[3]	6 hiPSCs + 14 hESCs	Hemangioblastic differentiation propensity Endothelial cell differentiation propensity	Early senescence of hiPSC progeny
Hu *et al.* (2010)[1]	5 hESCs + 12 hiPSCs	Neural differentiation propensity	Variable yield of neural progeny
Polo *et al.* (2010)[22]	12 Murine iPSCs	mRNA transcripts via qPCR DNA methylome via HELP Histone modification via ChIP	Epigenetic memory abrogated by extended passaging
Kim *et al.* (2010)[21]	31 Murine iPSCs + 14 murine ESCs + somatic	DNA methylome via CHARM Hematopoietic differentiation potential Osteogenic differentiation potential	Epigenetic memory of somatic cell of origin
Narsinh *et al.* (2011)[2]	3 hESCs + 4 hiPSCs	mRNA transcripts via single-cell qPCR Cardiovascular differentiation propensity	Single-cell heterogeneity of hiPSCs Variable yield of cardiovascular progeny
Lister *et al.* (2011)[23]	2 hESCs + 5 hiPSCs	DNA methylome via methylC-seq Histone methylation via ChIP-seq Transcriptome via RNA-Seq	Hot spots of aberrant methylation
Bock *et al.* (2011)[4]	20 hESCs + 12 hiPSCs	Transcriptome via microarray DNA methylome via RRBS mRNA transcripts via fluorescent counting	Bioinformatic analysis predicts differentiation propensity
Laurent *et al.* (2011)[28]	69 hESCs + 37 hiPSCs + somatic	Genomic stability via SNP genotyping	CNV in hiPSCs and hESCs

Selective inclusion of studies using mouse cells for completeness.

CHARM, comprehensive high-throughput array-based relative methylation; ChIP-seq, chromatin immunoprecipitation followed by sequencing; CNV, copy number variation; HELP, HpaII tiny fragment enrichment by ligation-mediated polymerase chain reaction; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; methylC-seq, cytosine methylome sequencing; mRNA, messenger RNA; qPCR, quantitative polymerase chain reaction; RRBS, reduced-representation bisulfite sequencing; SNP, single-nucleotide polymorphism.

hiPSCs has not yet been undertaken. However, genetically matched comparison of murine iPSCs and ESCs recently revealed consistent differences in the expression of the *Dlk1-Dio3* imprinted gene cluster.[18,19] Specifically, expression of the *Dlk1-Dio3* locus served as a marker of "fully pluripotent" murine iPSC lines that were capable of forming viable "all-iPSC" offspring in the tetraploid blastocyst complementation assay. Validation of this imprinted region as a potential marker for "full pluripotency" across hiPSC lines is ongoing.

Some of the differences between hiPSCs and hESCs appear to be related to the hiPSC's somatic cell of origin in the form of an "epigenetic memory," a term that refers to persisting epigenetic marks from the cell type of origin in the resulting hiPSC that continue to affect gene expression. Gene expression differences indicative of an epigenetic memory have been demonstrated in hiPSCs derived from fibroblasts, adipose tissue, and keratinocytes[20] as well as in murine iPSCs.[21,22] Notably, continuous passaging[22] or treatment with chromatin-modifying drugs[21] seems to abrogate transcriptional differences attributable to epigenetic memory in murine iPSCs, indicating that this phenomenon may affect differentiation propensity only transiently.

A recent comparison of the DNA methylome in hiPSCs versus hESCs at single-base resolution revealed further insight into the epigenomics of reprogramming.[23] Predominantly, DNA methylation patterns between hiPSCs and hESCs were similar, but differentially methylated regions were identified. Approximately 45% of these differentially methylated regions were attributed to a failure to reprogram the somatic cell epigenome (epigenetic memory), whereas ~55% were found to be specific to hiPSCs (not found in the somatic cell of origin or in hESCs). Their results suggest that aberrant methylation patterns dissimilar to the start and end points of reprogramming are frequently generated in susceptible "hotspot" regions of the genome. However, the reference standard consisted of only two to four hESC lines, which may limit the ability to generalize these results in light of the known variability among hESC lines.[24] Continued study of a wider variety of hESC and hiPSC lines will be required to fully understand the appropriate range of variability and better define the "gold standard."

To this end, the recent comprehensive characterization of a large number of hESC and hiPSC lines warrants special mention.[4] Bock *et al.* established reference maps of variation in the transcriptome and DNA methylome of 20 representative hESC lines. By comparing 12 well-characterized hiPSC lines to this reference standard, they were able to make "deviation scorecards" and arrive at several interesting conclusions. First, although most genes exhibit similar degrees of variation in hiPSC and hESC lines, a small number of genes exhibited substantially increased deviation from the

hESC reference standard in hiPSCs. Interestingly, only a very small fraction of this gene expression variation was attributable to epigenetic memory of the somatic cell of origin (fibroblasts). Second, as suggested by previous studies, an hiPSC-specific gene expression and DNA methylation signature was able to distinguish most, but not all, hiPSC lines from hESC lines. Thus, hESCs and hiPSCs can be thought of as two overlapping clouds in which some, but not all, hiPSCs can be distinguished from hESCs. However, no unique epigenetic or transcriptional deviation was found to be shared by all tested hiPSC lines. Interestingly, expression of MEG3, which has been proposed as a surrogate marker of developmental potency and is located in the aforementioned *Dlk1-Dio3* imprinted region, was not found to correlate with the quality or utility of hESC or hiPSC lines for biomedical research applications such as *in vitro* differentiation.

In light of such variability, it seems likely that different cell lines will ultimately be best suited for different applications. Undoubtedly, the teratoma formation assay does not provide the necessary speed or detail to comprehensively predict differentiation propensity in a high-throughput fashion. To aid in the ability to prospectively identify cell lines with enhanced differentiation potential toward a particular lineage, Bock *et al*. created a "lineage scorecard" based on quantitative expression profiling of 500 lineage-related genes in differentiating embryoid bodies.[4] Remarkably, the scorecard prediction of neural lineage differentiation propensity was highly correlated with the observed efficiency of differentiation to motor neurons (Pearson's $r = 0.87$). With continued validation, these assays could serve as more efficient and informative measures of a newly derived hiPSC line's pluripotent quality and differentiation potential. Streamlining the process of selecting, monitoring, and predicting the quality and utility of newly derived PSC lines is a welcome advance to the field.

Despite the excellent performance of the lineage scorecard in predicting neural differentiation propensity, several potential confounding factors warrant mention. Practically speaking, the lineage scorecard assay was demonstrated using fluorescent mRNA counting technology that is not yet widely available.[25] Next, heterogeneity among single cells in hESC populations *in vitro* has previously been shown to underlie important cell fate decisions, with initial evidence suggesting an increased degree of heterogeneity among single hiPSCs than among single hESCs.[2] To what degree could heterogeneity in the cell population contribute to observed variations in yield of differentiated progeny? Also, correlation of lineage scorecard predictions between biological replicates performed by two different researchers in different labs was modest (Pearson's $r = 0.59$). In light of these results, to what extent are the observed differences between cell lines attributable to experimental variables such as physical handling, media renewal, or passage number? Finally, the 12 hiPSC lines used in the study did not vary in terms of the originating somatic cell type (fibroblasts) or the reprogramming technique (retroviral transgenesis).[26] Because the influence of such parameters on differentiation propensity has been demonstrated in alternative settings,[15,20] future studies may warrant inclusion of transgene-free hiPSCs derived from a greater variety of somatic cell types.

In light of the aforementioned studies, it seems that a complex cell state such as pluripotency may not be adequately characterized by the assessment of a half-dozen molecular markers. Although initial comparisons on a global scale revealed considerable similarity between hESCs and hiPSCs, closer inspection at finer resolution reveals differences, for instance, at the single-base-pair[23] or single-cell[2] level. If related cell types are thought of as analogous to siblings, hESCs and hiPSCs can perhaps be thought of as twins—but are they fraternal or identical? Fraternal twins often look considerably alike. Identical twins are much more difficult to distinguish from each other, but there are appreciable differences upon closer inspection. For hESCs and hiPSCs, whether these differences are functionally consequential or simply related to the scale of analysis remains largely unknown. Perhaps the epigenetic marks that set hiPSCs apart from hESCs carry an unfairly negative connotation, because epigenetic memory can be used judiciously to bias hiPSCs toward a cell fate of interest. An investigator desiring large quantities of blood cells, for example, may opt to use blood-derived hiPSCs so as to enhance yield for this application.[21] The ability to prime PSCs selectively toward the desired cell lineage—using epigenetic

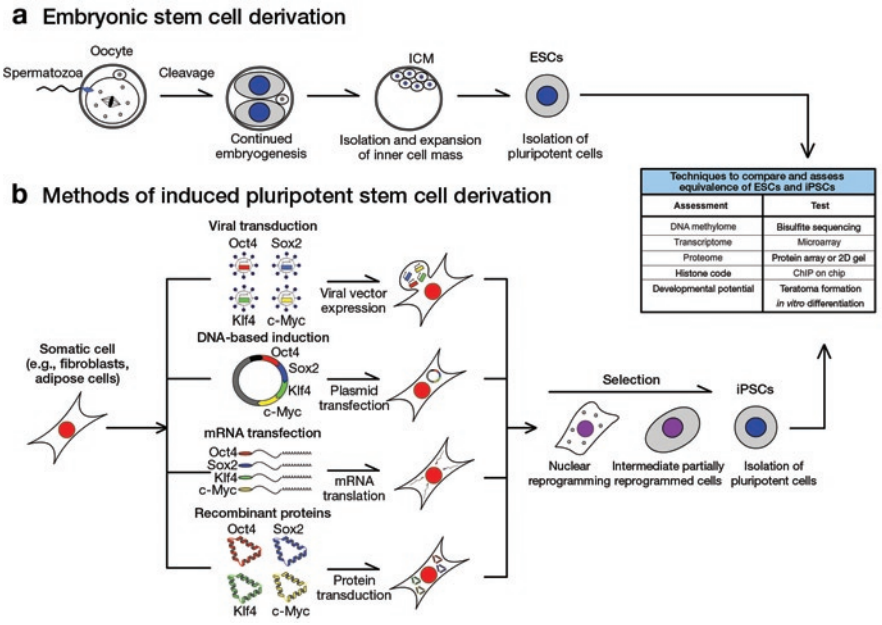

Figure 1 Schematic of human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) derivation protocols. (**a**) ESCs are derived from the inner cell mass (ICM) of the blastocyst, whereas (**b**) iPSCs can be derived from a variety of somatic cell types using a variety of reprogramming techniques. Commonly used assays to determine the equivalence of hESCs and hiPSCs at the molecular and functional levels are listed in the table on the right.

memory, cytokines, genetic modification, small molecules, or otherwise—may therefore occupy increasing interest in the future. Undoubtedly, monitoring the effect of any perturbation on the global hiPSC transcriptome and epigenome in a high-throughput fashion will aid in our ability to derive PSC lines that faithfully serve their intended purpose.

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Will Engineered T Cells Expressing CD20 scFv Eradicate Melanoma?

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Melanomas account for ~4% of all dermatological cancers and for 80% of deaths from skin cancers.[1] Although many primary melanomas can be cured through surgery, treatment of metastatic melanomas remains challenging.[1] Melanoma patients undergoing chemotherapy or even targeted therapy with small-molecule inhibitors aimed at blocking the most frequently mutated oncogene ($BRAF^{V600E}$) are known to develop drug resistance and tumor recurrence.[1–4] Even though some of the molecular mechanisms underlying acquired drug resistance have recently been described,[4–7] recurrence of initially responsive melanomas can also be due in part to the presence and potential enrichment of tumor subpopulations that are inherently resistant to therapy. Schmidt *et al.* recently reported that by targeting a small subset (~2%) or subpopulation of tumor cells expressing CD20, a cell surface marker typically associated with B cells,[8] long-lasting tumor regression can be achieved in an experimental immunodeficient mouse tumor model,[9] whereas targeting of other tumor subpopulations had only minimal effects on tumor regression.

As is the case with other malignancies, melanoma is a highly heterogeneous neoplasia, composed of distinct subpopulations of tumor cells.[10–13] These subpopulations provide the cellular basis for the complex biology of the disease, including phenomena such as self-renewal, differentiation, tumor initiation, progression and maintenance, and therapy resistance. Several phenotypically distinct subpopulations—some with stem cell–like characteristics—have been described in melanoma, including one previously

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