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Distinguishing Between Mouse and Human Pluripotent Stem Cell Regulation: The Best Laid Plans of Mice and Men

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ABSTRACT

Pluripotent stem cells (PSCs) have been derived from the embryos of mice and humans, representing the two major sources of PSCs. These cells are universally defined by their developmental properties, specifically their self-renewal capacity and differentiation potential which are regulated in mice and humans by complex transcriptional networks orchestrated by conserved transcription factors. However, significant differences exist in the transcriptional networks and signaling pathways that control mouse and human PSC self-renewal and lineage development. To distinguish between universally applicable and species-specific features, we collated and compared the molecular and cellular descriptions of mouse and human PSCs. Here we compare and contrast the response to signals dictated by the transcriptome and epigenome of mouse and human PSCs that will hopefully act as a critical resource to the field. These analyses underscore the importance of accounting for species differences when designing strategies to capitalize on the clinical potential of human PSCs. STEM CELLS *2010;28:419–430*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Both hope and hype surround the potential to generate abundant sources of differentiated cells for cell replacement therapies from human pluripotent stem cells (PSCs), and although progress has been made in the last decade, there is still more work to be done before achieving this ultimate goal [1–3]. The clinical promise of PSCs versus other sources of somatic stem cells is based on two defining characteristics: 1) robust self-renewal capacity in vitro [4], and 2) multilineage differentiation to derivatives of the three embryonic germ layers and subsequent lineages [5]. PSCs represent a unique class of developmentally plastic cells that have been derived from several sources in mice and humans exemplified by: fetal gonadal ridges and mesenteries (primordial germ cells) [6, 7], preimplantation blastocysts (embryonic stem cells; [ESCs]) [8–11], postimplantation mouse embryos (epiblast stem cells; EpiSCs) [12, 13], and, recently, induced pluripotent stem cells (iPS cells) [14–21]. Of these, human and mouse ESCs represent the most prevalently used and studied PSCs to date, and serve as the comparative benchmark for other sources of PSCs, including reprogrammed somatic cells.

Dissection of the molecular basis of multilineage differentiation and self-renewal in mouse and human PSCs may provide insight into the current paucity of clinically useful cells arising from all sources of human PSCs. Much of our current understanding of molecular networks involved in pluripotency and stem cell maintenance was initially derived from the murine system. The discovery of a conserved pluripotency network established by the transcription factors Oct4, Sox2, and Nanog lends credence to the continued use of the mouse model for understanding human PSCs. However, fundamental differences in the global molecular signatures [22, 23] and signaling pathways [24] that maintain mouse and human PSCs exist. In addition, differences in colony shape, growth rate, surface markers, and developmental potential between mESC and hESC cultures further demonstrate that distinct cellular and molecular mechanisms define mouse versus human PSCs.

Currently, hundreds of reports have described the molecular basis of self-renewal and differentiation in mouse and human, yet a thorough collation and comparison of this information is lacking. Here we provide a collective resource comparing the core transcriptional networks and the emerging roles of microRNAs (miRNAs) and epigenetics in orchestrating signals that govern self-renewal and differentiation, and clearly reveal convergent and divergent pathways that maintain mouse and human PSCs.

CONTRASTING SIGNALING PATHWAYS IN MOUSE AND HUMAN PSCS

Our understanding of the transcriptional networks associated with self-renewal and pluripotency of mouse and human

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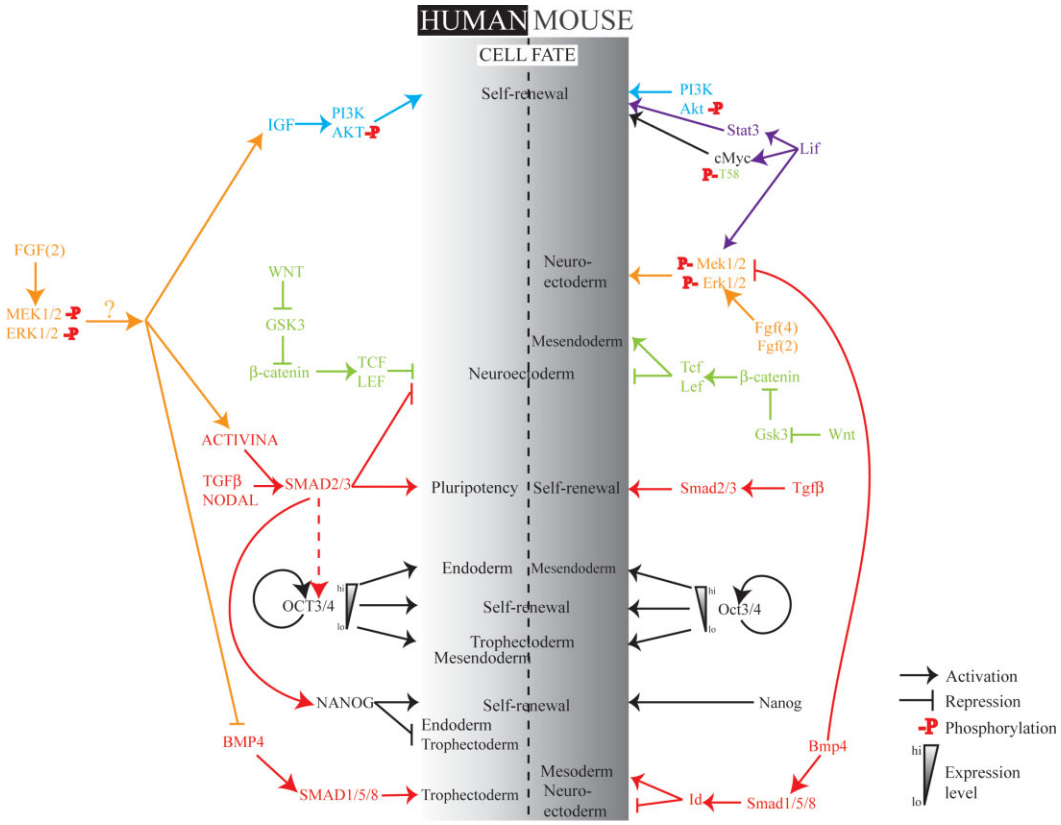

Figure 1. Human and mouse embryonic stem cell (ESC) identity is sustained by mainly distinct signaling networks. Both FGF and IGF pathways are central mediators in the maintenance of undifferentiated hESCs, likely through MEK/ERK [117–119] and PI3K/Akt activation. FGF2 has been reported to induce the expression of hESC maintenance factors such as transforming growth factor beta (summarized in the review article by Stewart et al. [120]). SMAD2/3 indirectly regulates OCT-4 hESCs via Activin A signaling which is mediated by SMAD2/3 [121] (indirect regulation shown by dashed arrows). In contrast, LIF/Stat3 is required for maintaining the undifferentiated state in mESCs [122]. As long as the balance remains in favor of Stat3, self-renewal is promoted at the expense of differentiation (MEK/ERK signaling pathway) [123, 124]. BMP4 can inhibit the MEK/ERK differentiation pathway resulting in mESC self-renewal [125]. Under specific chemical inhibition GSK3, FGF, and ERK signaling STAT3 is not required for mouse ES self-renewal [116] providing a groundstate for self-renewal, however, for the purposes of this review, we chose to display the canonical signaling pathways of self-renewal in ES cells. Abbreviations: ERK, extra-cellular-signal-related kinase; FGF, fibroblast growth factor; IGF, insulin-like growth factor; MEK, MAPK/ERK kinase.

ESCs has increased significantly in recent years; however, very little is known about the crosstalk between pathways. This is largely obscured by the complexity of ESC cultures, which may be, in part, owing to their heterogeneity [25, 26]. The intersections between the core signaling pathways in mouse and human ESCs, with emphasis on species differences, have been compiled in Figure 1. In addition, the expression of key components of these signaling pathways and their functions has been summarized in Table 1. Questions as to whether the dissimilarity between mouse and human ESC signaling pathways are the result of genuine species-specific, developmental stage-specific [27], or epigenetic variations have arisen. It has been reported that mouse EpiSCs, derived from the postimplantation epiblast, exhibit characteristics similar to hESCs at multiple levels (culture requirements, expression profiles, transcriptional networks, and epigenetic status) [12, 13]. Interestingly, an ~7-fold greater overlap in Oct4 targets exists between hESCs and EpiSCs compared with mESCs [13]. This suggests that EpiSCs and hESCs represent a similar developmental stage; however, progress toward modeling human differentiation in EpiSCs has yet to be reported. The derivation of iPS [28–30] and the potential to model and treat human diseases using patient-derived iPS [31–34] has de-emphasized the focus on using EpiSCs to understand human pluripotent stem cell-isms. Regardless, consistent differences in the signaling pathways controlling the stem cell state and fate exist between mouse and human PSCs, thus devising improved differentiation strategies, for instance, small molecule delivery [35], should be informed by and validated in human PSC lines.

A Common Transcriptional Hub of Pluripotency?

The consistent requirement of the three transcription factors: Oct4, Sox2, and Nanog, for the maintenance of both mouse and human ESCs form the foundation of mammalian pluripotency. In spite of the conservation of this "transcriptional hub," the gene targets and functional effects following modulation of these factors appear to be species-specific. The functional effects of gene expression changes and a summary of the gene targets of Oct4/Sox2/Nanog in hESCs and Oct4 in mESCs are discussed below in detail and presented in supporting Table S1.

Oct4, the POU-family homeobox transcription factor, is exclusively expressed in pluripotent cells of the developing human and mouse embryo (inner cell mass [ICM] and early germ cells), or its in vitro counterparts (ESCs and embryonic

Table 1. Signaling pathways in mouse and human embryonic stem cells

Pathway	Gene	Expression		In Vivo/In Vitro Embryonic Phenotypes
		Human ESCs	Mouse ESCs	
Mouse Embryonic Stem Cells				
LIF	LIF	Low or no	Yes	Required for blastocyst implantation
	LIFR	Variable[a]	Yes	Dispensable in vivo except for Stat3[b]
	GP130	Low or no	Yes	Indispensable in vitro for mESCs
	STAT3	Low or no	Yes	Dispensable in vitro for hESCs
	JAK	Low or no	Yes	Embryonic lethal
BMP	BMP4	Variable[a]	Variable[a]	Dispensable in vivo[b]
	BMP2, BMP7	Yes	Low or no	Supportive in vitro for mESCs
	GDF3	Variable[a]	Yes	Suppression of BMP signaling appears beneficial for hESCs in vitro
	Id1, Id2, Id3, Id4	Variable[a]	Yes	n/a
	SMAD1/5/8	Low	Yes	Embryonic lethal
	Acvrl1/Bmpr1a/ Bmpr1b	Yes	Yes	Embryonic lethal; lack mesoderm
	Bmpr2	Yes	Yes	Embryonic lethal; lack mesoderm
	Noggin/Chordin	Yes	Yes	Defective embryogenesis; lethality at birth/embryonic lethal
	Follistatin	Yes	Low or no	Perinatial lethal
	SMAD4/6/7	Yes	Yes	Lethal prior to gastrulation/prenatal lethality
Human Embryonic Stem Cells				
FGF	FGF2 (bFGF)	Yes	Variable[a]	Dispensable in vitro for mESCs. However, FGF4 mouse null mutants display impaired proliferation of ICM cells. Indispensable in vitro for hESCs (FGF2).
	FGF4	Variable[a]	Yes	
	FGFR1	Yes	Yes	
	FGFR2, FGFR3, FGFR4	Yes	Variable[a]	Dispensable in vivo[b] and in vitro for mESCs. Cooperates with FGF to maintain hESCs in vitro.
IGF	IGF-II	Yes	Yes	
	IGF1R	Yes	Yes	
	IGFBP2	Yes	Yes	
TGFβ/Activin/Nodal	TGFβ1	Yes	Yes	Dispensable in vitro for mESCs. However, Smad2/3 activation supports the ex vivo pluripotency of mouse ICM cells.
	Nodal	Yes	Variable[a]	Placental defects and death at gastrulation
	TDGF1	Yes	Yes	Mice homozygous for disruptions in this gene display abnormalities in rostral-caudal axis formation, embryonic development, and heart development.
	ACVR2A/B	Yes	Yes	Supportive in vitro for hESCs through Smad2/3 activation.
	SMAD2/3	Yes	Yes	Embryonic lethal
	ACVR1	Yes	Yes	Embryonic lethal
	TGFBR1	Yes	No	Lethal mid-gestation
	TGFBR2	Yes	No	Targeted null mutations die in midgestation with impaired yolk sac hematopoiesis and vasculogenesis
	Lefty1	Yes	Yes	Lethality/prenatal-perinatal
	SMAD4/7	Yes	Yes	Lethal prior to gastrulation/prenatal lethality

This table shows components of the main pathways used by mESCs and hESCs to maintain self-renewal and pluripotency. Most data are based on differences in gene expression between the two cell types.

[a], Inconsistent expression depending on the ESC line, culture conditions, or the different isoforms of the gene.

[b], Based on the phenotype of mouse homozygous null embryos for key genes in each pathway, with respect to their effects on the formation/maintenance of the ICM/epiblast or ability to derive ESC lines and early embryonic phenotypes compiled from the Mouse Genome Informatics database (http://www.informatics.jax.org/).

Abbreviations: ESCs, embryonic stem cells; FGF, fibroblast growth factor; hESCs, human embryonic stem cells; ICM, inner cell mass; mESCs, mouse embryonic stem cells; TGF, transforming growth factor.

germ cells) [10, 36, 37]. Loss of Oct4 is lethal for embryos at the blastocyst stage and its expression is required for mESC self-renewal [38], indicating that Oct4 is necessary for the establishment and maintenance of ESC properties, both in vivo and in vitro. Tight regulation of Oct4 is crucial since changes to Oct4 levels induce different lineages in mouse and human ESCs. Slight increases in Oct4 cause spontaneous differentiation of mESCs into a mesoderm/endoderm population [39], yet it solely promotes endoderm differentiation in hESCs [40]. Cell lines derived from Oct4 mutant blastocysts produce only trophoblast lineages in the mouse. In a similar fashion, knockdown of Oct4 expression causes differentiation into trophectoderm in both mouse and human ESCs, but also produces mixed mesoderm/endoderm in hESCs [39–44]. This differential lineage induction demonstrates that the targets and effectors governing cell fate decisions are distinct

Table 2. Mouse embryonic stem cell genes with promoter regions bivalently modified by H3K4 and H3K27 trimethylation that are not conserved in human embryonic stem cells

Homologene	Gene Symbol	Human Embryonic Stem Cells Histone Modification	Gene Ontology/KEGG Pathway	Human Expression	Mouse Expression
1548	*GAS1*	K4	HH	36.325	101.518295
4115	*SMO*	K4	HH	57.795	172.7919222
30997	*IGF1R*	K4	IGF	109.36	335.8302661
676	*IGF2R*	K4	IGF	351.525	154.4100593
21183	*GDF11*	K4	Mesoderm development	12.525	8.780931393
55948	*IKZF1*	K4	Mesoderm development	100.972	16.53815125
7755	*HES5*	K27	Notch	n/d	8.332498883
180	*JAG1*	K4	Notch	108.575	261.7592217
22475	*LFNG*	K4	Notch	nd	10.43208785
7324	*FST*	K4	TGF beta	108.5278	23.06754931
1633	*ID3*	K4	TGF beta	296.543	86.21146922
1186	*ID4*	K4	TGF beta	29.7695	393.2877649
4079	*SMAD6*	K4	TGF beta	165.65	85.48667548
21198	*SMAD9*	none	TGF beta	n/d	4.573483267
2432	*TGFB2*	K4	TGF beta	n/d	8.336018456
2435	*TGFBR2*	K4	TGF beta	n/d	16.51366538
31142	*THBS1*	K4	TGF beta	140.615	3911.474756
7230	*ARNT2*	K4	Transcription factor activity	n/d	21.54494477
1265	*ATF3*	none	Transcription factor activity	211.677	23.60002681
15639	*DMBX1*	none	Transcription factor activity	n/d	6.374510141
49239	*FOXD3*	K4	Transcription factor activity	n/d	8.146804412
69103	*IKZF4*	K4	Transcription factor activity	6.915	3.986881885
3168	*NFE2L3*	K4	Transcription factor activity	220.153	18.87667668
2220	*RXRA*	K4	Transcription factor activity	123.59	34.68518265
5143	*SOX21*	K4	Transcription factor activity	n/d	57.40186271
2338	*SOX4*	K4	Transcription factor activity	474.658	1107.698792
22631	*SOX6*	K27	Transcription factor activity	n/d	3.86324189
3420	*AXIN2*	K4	WNT	9.768757	27.37608111
11878	*CXXC4*	K4	WNT	n/d	6.375443061
8095	*FRAT2*	K4	WNT	358.783	159.0072666
7325	*FZD4*	K4	WNT	18.115	28.33728888
2617	*FZD6*	K4	WNT	63.0525	12.75970293
22876	*PLCB1*	none	WNT	64.31517	3.612781037
55679	*PRKCA*	K4	WNT	58.2625	9.181787496
68433	*RAC3*	K4	WNT	n/d	119.2522574
62175	*WNT16*	none	WNT	3.8075	4.839108693
22526	*WNT2B*	none	WNT	n/d	3.803357438
22531	*WNT7B*	none	WNT	n/a	11.20936815
7553	*WNT8A*	K27	WNT	6.6875	6.562692287

This table shows transcription factors and core pluripotency pathway genes that possess a bivalent promoter status in mESCs but are differentially marked in hESCs. Global histone modifications were determined from published ChIP-chip and ChIP-seq data [96-98]. Human ESC expression data were obtained from our own Affymetrix expression profiles [114, 115] and normalized using Dchip (biosun1.harvard.edu/complab/dchip). Mouse ESC expression data were obtained from published expression profiles [96]. Homologous gene pairs between mouse and human ESCs were designated from genomewide ChIP-Chip and ChIP-Seq data using HomoloGene (release 63; http://www.ncbi.nlm.nih.gov/homologene).

Abbreviations: ESCs, embryonic stem cells; hESCs, human embryonic stem cells; KEGG, Kyoto Encyclopedia of Genes and Genomes; mESCs, mouse embryonic stem cells; n/a - not represented on HGU133A/B; n/d, not detected.

between mice and humans (supporting Table S1). Accordingly, capitalizing on Oct4 as a target to regulate differentiation toward therapeutic use will require focused study of the hESC system.

Sox2 is a member of the Sox (*S*RY-related HMG b*ox*) gene family [45]. Despite its expression in several differentiated lineages [46–48], Sox2 was identified as a marker for pluripotent cells in the ICM and in vitro counterparts of both mice and humans [49]. Sox2 is required to maintain the mouse epiblast in a cell autonomous manner [49]. Repression of Sox2 commonly results in trophectoderm differentiation in mESCs and hESCs [41, 50]. Upregulation of Sox2 promotes mESC differentiation into cell types other than endoderm [51]. Much less is known functionally about Sox2 in hESCs, with the exception of its partnership with Oct4 and Nanog, and knockdown-induced trophectoderm differentiation [50]. The necessity of Sox2 for hESCs is questionable as it has been shown to be absent in some lines [52] and possibly replaced by alternate isoforms or Sox factors (Sox4, Sox11, Sox15) [53] in contrast with the mouse. However, the conserved requirement of Sox2 in reprogramming both mouse and human somatic cells to a PSC-state suggests that Sox2 may have roles in conferring pluripotency in both species, likely via an Oct4-dependent mechanism [14, 54].

The third member of the pluripotency hub, Nanog, is a divergent homeobox transcription factor identified as a pivotal regulator of ESC properties [55, 56]. Like Oct4, Nanog is nearly exclusively expressed in the ICM, early germ cells, and ESCs of mice and humans [36, 55, 56], with a few exceptions in adult mouse tissues [57]. Downregulation of Nanog induces distinct differentiation programs in mouse and human ESCs [41, 44, 55, 58], whereas

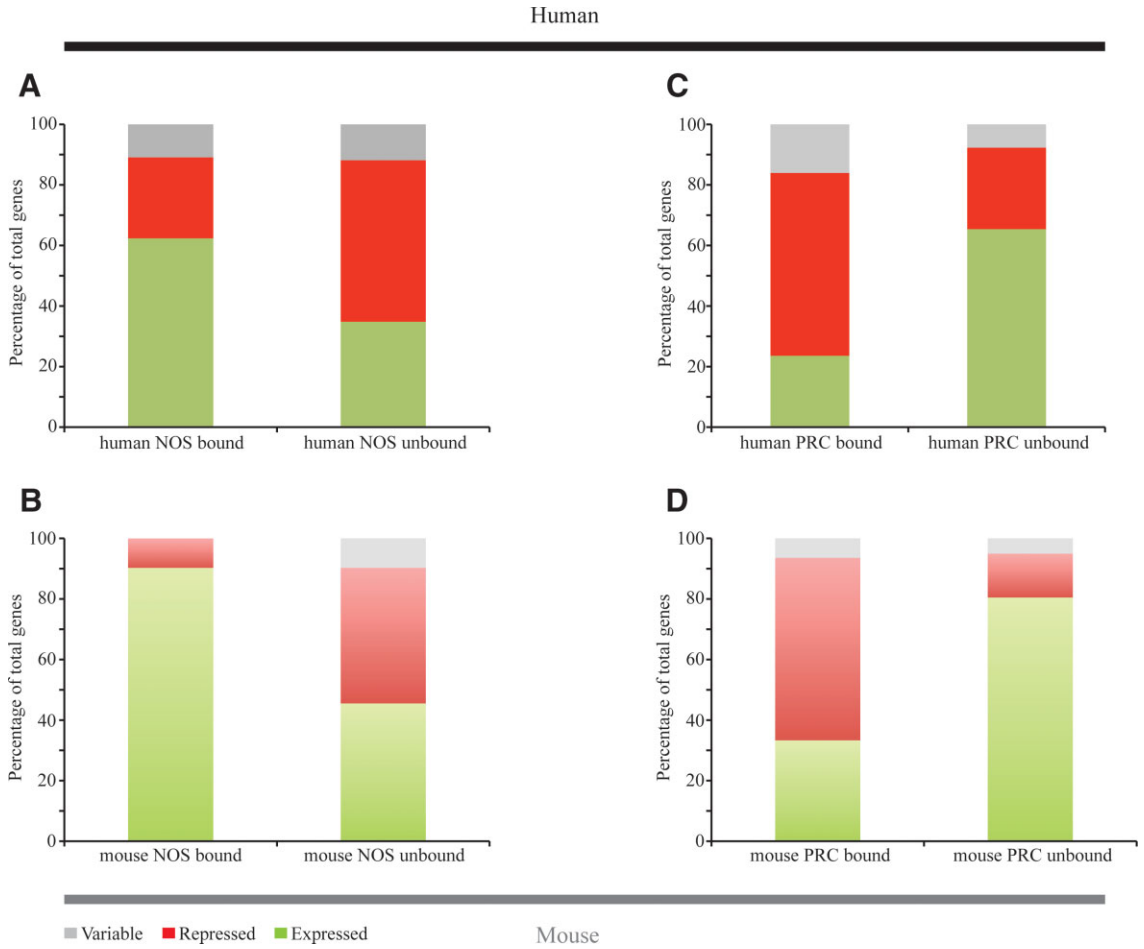

Figure 2. **(A–D):** Expression of Nanog/Oct4/Sox2 (NOS) and polycomb repressive complexes one and two (PRC) target genes in human and mouse embryonic stem cells (ESCs). We used our laboratory's human embryonic stem cell (hESC) expression data [114, 115] and mouse embryonic stem cell (mESC) public expression profiles (GSE9244) [126] to assign whether NOS and PRC targets were associated with gene expression, repression, or had variable expression across replicate samples. Genes bound by NOS in both **(A)** human and **(B)** mouse ESCs were correlated with gene expression, whereas genes bound by PRC were associated with gene repression in both species **(C,D)**. Abbreviations: NOS, Nanog/Oct4/Sox2; PRC, polycomb repressive complexes.

its overexpression maintains pluripotency in both species [56, 59, 60]. However, the role of Nanog in maintenance of the pluripotency is unclear. Nanog is required to maintain the ICM [55], yet its role in ESCs appears to be species specific. It was recently suggested that Nanog is dispensable for self-renewal in mESCs [61] and likewise for reprogramming mouse and human somatic cells to a pluripotent state [15, 18, 21, 62]. While Nanog has a role in the maintenance of both human and mouse ESCs pluripotency, we and other groups have observed that hESCs are dependent upon Nanog for self-renewal in contrast with mESCs (our unpublished data), [44, 61]. Thus the unique dependency on Nanog for both fundamental properties of human PSCs, pluripotency, and self-renewal is not recapitulated in mESCs.

Dissimilar Actions of the Self-Renewal Machinery in Mouse and Human PSCs

Nanog/Oct4/Sox2 (NOS) regulate global transcriptional networks in both mouse and human ESCs [63–65], although most known/putative targets are not conserved (supporting Table S1). In both mouse and human ESCs, Oct4 and Sox2 co-operatively regulate their own transcription and the expression of self-renewal genes such as Nanog, Utf1, Fgf4, and Fbxo15 [66–73]. However, Nanog acts independently of the Oct4-Sox2 complex in mouse and human ESCs [64, 74]. NOS/Oct4 target genes in human and mouse ESCs, respectively, are mainly correlated with gene expression as shown in Figure 2A and 2B. Only a proportion of repressed targets bound by NOS/Oct4 (hESC 66%; mESC 31%) share a similar mechanism of repression via the polycomb repressive complexes (PRC1 and/or PRC2) (supporting Table S1).

Functional validation of the targets and unique effects of the Oct4/Sox2/Nanog hub is required to determine whether the obvious species-specific differences are the most relevant toward directed differentiation and expansion (that is, Nanog) of human cells. This suggests that focused experiments using hPSCs are required for developing the clinical potential of differentiated progeny for cell replacement therapy.

MicroRNAs: Master Regulators of Multilineage Differentiation?

Based on studies in the mouse, it has been suggested that miRNAs could play major roles in regulating pluripotency. MiRNAs

are endogenous noncoding RNAs that are cleaved by the RNases Drosha and Dicer into ~22 nucleotide sequences that bind to multiple complementary target mRNAs, mainly leading to post-transcriptional silencing, most commonly by RNA interference (RNAi) [75]. These small molecules are intriguing candidates as global regulators of multilineage differentiation based on their affinity for a constellation of targets, usually within the same pathways [76], and specific miRNA promoters can be occupied in a conserved manner by Oct4, Sox2, and Nanog in both mESCs and hESCs [64, 65, 77]. In addition, the observation that RNAi machinery and polycomb group (PcG) proteins (discussed below) colocalize at human target promoters [78] suggests a link between these regulatory systems.

Since miRNA transfer is highly efficient, miRNA targeting will likely be an avenue for future hPSC-based regenerative therapies, similar to using ligand/receptor-based approaches to direct differentiation of hESCs. Initial comparison of mouse and human ESCs has revealed, not surprisingly, different profiles and chromosomal distributions of miRNAs between the species [79–86] (supporting Table S2). Thus, exploitation of small RNAs for directed differentiation of human PSCs may not be informed by the mouse expression profiles. Regardless, further investigation into interactions between the self-renewal machinery of PSCs and miRNAs is warranted for several reasons. Loss of both Drosha and Dicer blocks differentiation in mESCs [87–89], and miRNAs (miR-1 and miR-133) have been shown to promote specific lineage differentiation in both mESCs and hESCs [90]. Thus, while the mechanisms regulating the effect of miRNAs on multilineage differentiation are likely to be conserved between mouse and human ESCs, the species-specific differences in miRNA profiles may provide significant control over protein-coding genes regulating the balance between pluripotency and differentiation. We propose that inhibition of the species-conserved pluripotency machinery coupled with human specific investigations of miRNAs associated with differentiation may prove to be an effective strategy toward refining human PSC-lineage specification. In terms of technical feasibility, the discovery of small molecules that can inhibit the pluripotency machinery or mimic cell-specific miRNAs will be critical in making the leap to clinical applications.

Epigenetic Landscapes Influence Lineage Differentiation

In addition to transcriptional and post-transcriptional regulation, genome accessibility determined by epigenetic histone/DNA modifications represents the upper echelon of gene expression regulation promoting the pluripotent state versus lineage differentiation, and vice versa. Epigenetic modifications regulating gene expression include direct DNA methylation or covalent modifications of histone residues (that is, acetylation and methylation). For example, trimethylation of histone H3 lysine residue four (H3K4me3) is strongly associated with transcriptional activation, whereas H3K27me3 is indicative of repression. Epigenetic regulators such as the PcG proteins mediate repression, while activation can be controlled via Trithorax group proteins (TrxGs) (reviewed in [91]).

In the past few years, several landmark studies have suggested the importance of specific epigenetic signatures on pluripotency in mouse and human cells. To globally define active and inactive promoters/genes in mammalian ESCs, several groups have focused on whole-genome interrogation of specific post-translational histone modifications using chromatin immunoprecipitation (ChIP) coupled with sequencing or hybridization technologies [92–98]. The term "bivalent domains" was introduced to describe promoter regions of genes containing both active (H3K4me3) and repressive (H3K27me3) marks [93], which were first identified in undifferentiated ESCs. This was later suggested to be a general feature of repressed genes that may require rapid and dynamic regulation in hESC-differentiated progeny [96–98] but may be overestimated in hESC cultures as a consequence of cellular heterogeneity (unpublished observations). Bivalency was thus proposed as a default state underlying pluripotency because of "repression" of lineage specific genes. This hypothesis was supported by the conservation of comodified genes (56-70%) between mammalian ESCs [99], though not all bivalent genes were conserved between mouse and human ESCs, for example, *Tgfbr2*, while bivalent in mESCs is exclusively marked by H3K4me3 in hESCs (Table 2) consistent with its expression in hESCs and absence in mESCs (Table 1). Conversely, *Tcl1a*, is bivalent in hESCs while this gene only possessed an active chromatin mark in mESCs, in hESCs was bivalent although this gene possessed only an active chromatin mark in mESCs, in keeping with its recently discovered role in maintaining mESC identity [41] (Table 3). The epigenetic profiles were more similar between mouse EpiSCs and hESCs than compared with mESCs, in support of their similar expression profiles and developmental potential [12, 13]. It is possible however, since these studies were performed on bulk ESC cultures, that bivalency is in part an artifact of developmentally heterogeneous cultures in which some cells have begun to transition away from a pluripotent state, thus relieving repression of differentiation programs (unpublished observations). Dissection of ESC cultures based on expression of lineage-specific markers will be required to address this ambiguity. Overall, these studies provide insight into a mechanism by which the cells' epigenetic status regulates developmental potentials in mammalian PSCs.

While it is clear that epigenetic modification of the genome is involved in initiation or repression of specific differentiation programs, it remains to be determined whether specific epigenetic marks are the consequence or cause of lineage-specific differentiation. The importance of epigenetic marks to successful directed differentiation and proper epiblast development is supported by studies in both mESCs and mouse blastocysts in which disruption of core PcG genes (Eed-/- and Suz12-/-) results in loss of H3K27me3 and subsequent activation of developmental genes [100, 101]. The role of epigenetic modification in regulation of cell fate is further supported by the report that knockdown of REST (required for the recruitment of histone deacetylases (HDACs) to repress neuronal target genes) in mESCs led to abrogated self-renewal and differentiation [102]. This was mediated, at least in part, via unchecked miR-21 expression and its negative consequence on the self-renewal machinery [102], which strongly suggests that REST may be an integral member of the self-renewal machinery in mouse and potentially human PSCs, supported by the conservation of the active chromatin mark in both mESCs and hESCs (supporting Table S3).

Based on our analysis of public ChIP data generated from mouse and human ESCs, we have identified conserved and species-specific PcG developmental targets catalogued in supporting Table S3 [100, 101, 103]. In general, 60% of PcG targets were not expressed in mouse or human ESCs (Fig. 2C, 2D). A large number of genes important to embryonic development, including members of the HOX, *PAX*, and WNT families, were conserved targets of PcG proteins in both mouse and human ESCs (supporting Table S3). In parallel to the pluripotency hub, there were significant differences in PcG repressed genes between mammalian PSCs which are likely to be important for lineage specification upon induction

Table 3. Human embryonic stem cells genes with promoter regions bivalently modified by H3K4 and H3K27 trimethylation that are not conserved in mouse embryonic stem cells

Homologene	Gene Symbol	Mouse Embryonic Stem Cell Histone Modification	Gene Ontology/KEGG Pathway	Human Expression	Mouse Expression
160	*TBX5*	K27	Embryonic development/ Transcription factor activity	n/d	4.194056759
498	*IGFBP1*	none	Insulin-like growth factor	51.7775	4.309527276
40711	*EYA2*	none	Mesoderm development	n/d	4.749169825
20322	*BMPR1B*	none	TGF beta	n/d	9.371719451
20906	*ACVR1B*	K4	TGF beta	n/d	889.4391503
26724	*ACVR1C*	none	TGF beta	n/a	9.855068646
55859	*CDKN2B*	K4	TGF beta	n/d	80.64371999
916	*BACH1*	K4	Transcription factor activity	140.738	1178.157748
3276	*ETV5*	K4	Transcription factor activity	294.34	5461.764274
3921	*MYCL1*	K4	Transcription factor activity	11.1925	31.97065629
4088	*NFIC*	K4	Transcription factor activity	n/d	160.3445821
4785	*KLF1*	K4	Transcription factor activity	n/d	3.975394968
7369	*HOXD12*	K27	Transcription factor activity	n/d	5.368295731
7773	*HOXD4*	K27	Transcription factor activity	n/d	4.244387837
10473	*HOXD8*	K27	Transcription factor activity	9.685	10.64486504
21428	*STAT1*	K4	Transcription factor activity	372.728	28.34876546
31110	*HHEX*	K4	Transcription factor activity	45.7975	88.83817333
48264	*E2F2*	K4	Transcription factor activity	n/d	47.10471848
68371	*PHOX2B*	K27	Transcription factor activity	n/d	4.872078416
1850	*MYF6*	none	Transcription factor activity	n/d	4.196193411
8140	*FOXI1*	none	Transcription factor activity	7.095	4.226372782
9666	*TBX22*	none	Transcription factor activity	n/d	7.38238431
3983	*CER1*	none	WNT	89.2375	5.149504706
4299	*APC2*	none	WNT	n/d	5.143304587
7565	*TCL1A/Tcl1*	K4	Protein binding	26.8875	1495.69899

This table shows transcription factors and core pluripotency pathway genes that possess a bivalent promoter status in hESCs but are differentially marked in mESCs. Genomewide histone modifications were determined from published ChIP-chip and ChIP-seq data [96-98]. Human ESC expression data were obtained from our own Affymetrix expression profiles [114, 115] and normalized using Dchip (biosun1.harvard.edu/complab/dchip/). Mouse ESC expression data were obtained from published expression profiles [96]. Homologous gene pairs between mouse and human ESCs were designated from genomewide ChIP-Chip and ChIP-Seq data using HomoloGene (release 63; http://www.ncbi.nlm.nih.gov/homologene).

Abbreviations: ChIP-Chip, Chromatin immunoprecipitation on microarray; ChIP-Seq, Whole genome chromatin immunoprecipitation sequencing; ESCs, embryonic stem cells; hESCs, human embryonic stem cells; KEGG, Kyoto Encyclopedia of Genes and Genomes; mESCs, mouse embryonic stem cells; n/a, not represented on HGU133A/B; n/d, not detected; TGF, transforming growth factor.

of differentiation. Notably, a significant subset of PcG target genes (a third of the developmental transcription factors in hESCs) is co-occupied by Oct4/Sox2/Nanog [103], alluding to a role of these regulators in the recruitment of PcG proteins. Further investigation involving modulation of epigenetic regulators in both mouse and human ESCs will reveal whether changes to the epigenetic status of a gene results in species-specific developmental effects. Conditional knockouts of epigenetic regulators in committed progenitor cells have been shown to specify cell fate [104–107]; however, alteration of epigenetic regulators at an earlier developmental stage results in cell death, as seen in Eed-/- and Suz12-/- mouse embryos. These studies demonstrate that the chromatin landscape is important for differentiation and may provide an approach to induce or monitor specification of functional lineages from human PSCs. A major limitation that remains is whether a suitable level of control over the modulation of epigenetic regulators can be achieved to instruct PSC differentiation.

Induced Pluripotent Stem Cells: Artificial Modulation of Transcriptional Programs

Cellular reprogramming of mouse and human somatic cells to a pluripotent state has been achieved via ectopic expression of the same four transcription factors: Oct4, Sox2, Klf4, and c-Myc [14, 17, 20]. Although the same factors similarly resulted in human and mouse induced pluripotent cells (iPSCs), the cell lines demonstrate the developmental potential of their ESC counterparts [108]. Both mouse and human reprogramming absolutely require Oct4 [109], which represents the apex of the factor hierarchy in reinitiating the pluripotent stem cell state in somatic cells. The remaining factors improve the efficiency of reprogramming, however, the recent use of chemical inhibitors that target chromatin modifications or signaling pathways obviate their use [44, 110].

Comparison of the publicly available expression data generated from human and mouse iPSCs [20, 21, 62] further reinforces the differences between the molecular programs initiated in both species. The Venn diagram depicted in Figure 3 represents the degree of overlap between expressed genes detected in mouse and human iPSCs. Published gene expression profiles used were generated from mouse (GSM189665 and GSM189668— G4122F Agilent Mouse whole-genome arrays) or human (GSM241846—G4112F Agilent Human whole-genome array; GSM248203, GSM248205, GSM248206, GSM248207, GSM248208, GSM248211, GSM248212, GSM248215—Affymetrix HG–U133 Plus 2.0 Arrays) iPS of disparate origins. There are inherent caveats in comparing published gene expression profiles related to cellular variables used to generate these cells. We evaluated the expression profile of fibroblasts reprogrammed using the same four factors (Oct4, Sox2, Klf4, and c-Myc) to minimize variation caused by the

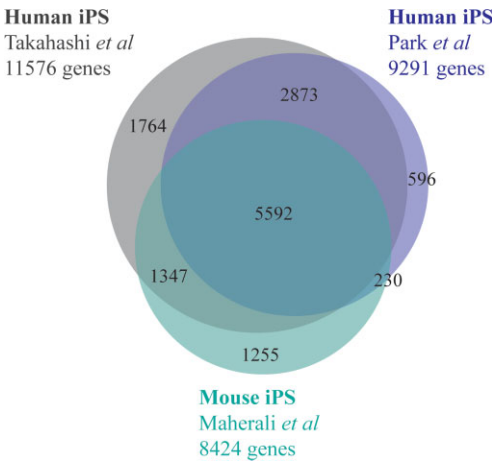

Figure 3. Comparison of global molecular profiles generated from reprogrammed mouse and human somatic cells to pluripotent cells. Published gene expression profiles were generated from mouse or human fibroblasts reprogrammed using the same four factors (Oct4, Sox2, Klf4, and c-Myc) to minimize variation caused by the mode of induction of reprogramming. Abbreviations: iPS, induced pluripotent stem cells.

mode of induction of reprogramming. The use of multiple samples of human iPS cross-validated by two different array types provides a robust dataset to determine a common gene signature; however, the mouse iPS expression analysis is limited to a single study and it remains to be determined if the species comparison is broadly applicable to all mouse iPS lines. The analysis in our review stringently considers genes that are differentially expressed by more than fivefold to provide as robust a comparison as possible. Comparison between mouse and human iPSCs transcriptome profiles resulted in 1,216 genes that demonstrated at least a fivefold change in relative gene expression (data shown in supporting Table S3 and Table 4). We provide Table 4 as a list of the top 100 up- and downregulated genes between mouse and human iPSCs that were shared between two microarray platforms. These genes were subcategorized according to the following gene ontologies: transcriptional regulators, cell-signaling, and differentiation-associated genes between mouse and human iPSCs. Differentially expressed genes ($\geq$ fivefold change) involved in transcriptional regulation, cell-cell interactions, differentiation, and proliferation are provided in supporting Table S3. We observed differential expression of core developmentally regulated epigenetic factors such as MLL4 (transcriptional activation; more abundant in miPSCs) and HDAC2 (transcriptional repression; more abundant in hiPSCs) (Table 4 and supporting Table S3). Several family members of Kruppel-like factors (KLF3, KLF4, KLF5, KLF6, KLF10) were upregulated in miPSCs, notably KLF4 and KLF5 which are among the most highly differentially expressed genes between the species (Table 4) and have both induced reprogramming of fibroblasts to a pluripotent state [18, 111]. The KLF family members have also been shown to interchangeably regulate self-renewal in mouse ESCs [112]. The redundant upregulation of KLF genes in miPSCs may be a major difference in the regulation of self-renewal between mouse and human pluripotent cells. We observed species-specific expression of genes associated with embryonic development/cell differentiation, the study of these gene sets should provide insight into what lineages are being primed in mouse or human pluripotent cells. For example, the expression of early mesoderm/hematopoietic differentiation genes KDR and KITLG [113–115] are more highly expressed in hiPSCs than its mouse counterpart (supporting Table S3). These initial comparisons support fundamental differences in epigenetic and transcription factors suggested to be important for maintaining an undifferentiated state and raises red flags in the applicability of using mouse iPS cells as a model for human PSC differentiation.

Foundation Toward Prospective Use of Human PSCs

The fact that sources of human somatic stem cells are limited and heterogeneous has drastically hampered their use in large-scale clinical applications. The isolation of hESCs, and more recently iPS cells, has introduced new possibilities for regenerative medicine. Both human ESCs and iPS cells are expected to provide a significant control over the limitations of somatic cell- or animal-based models, however, a number of accomplishments must be achieved prior to clinical application of human PSC-based therapies. In particular, the search to implement strategies that rely on ligands or small molecules of developmental pathways to robustly expand and coax differentiation of hESCs toward a specific lineage is ongoing. Design of new assays to functionally test human PSC-derived cells may be required to extend beyond current single cell assays or animal-based models. Increasing our knowledge of the molecular basis of "stemness" in hESCs, which has considerably fuelled iPS cell derivation, will remain critical for a better understanding of human cell fate specification from both cell types. Although it is too premature to envision which reprogramming method will be most appropriate for human personalized applications, the clearest advantages of iPS technology include 1) circumventing the use of human embryos, 2) the provision of an experimental system for modeling normal and pathologic phenotypes as well as for diagnostic, drug, and toxicology screenings, and 3) the generation of clinically relevant cell types that are genetically compatible for patients. The major challenge that the PSC field still faces is the efficient differentiation to functional cells; the answers to which will likely be found through understanding how regulatory pathways and mechanisms interact to control the balance between self-renewal and differentiation.

Core genetic and epigenetic mechanisms regulating stem cell maintenance and differentiation are conserved cross-species, however, it is clear from the above comparison of published molecular profiling data that the specific targets of both Oct4/Sox2/Nanog and epigenetic regulators differ significantly between species. These differences likely induce the fundamental differences observed in developmental potentials, culture requirements, transcriptome profiles, and epigenetic landscapes between mouse and human PSCs. Based on these studies, the justification for continued use of mouse models could be questioned, however, the accessibility and genetic manipulation of the mouse prevents it from becoming obsolete. Many procedures and "proof of principle" studies have been achieved with the mouse and proved to translate to the human, including derivation of both ESCs and iPS cells [11, 17, 20, 54]. However, the approaches for directed differentiation of mESCs has not been as successful in hESCs [115], therefore assuming a flawless translation between species will hamper progress in the development of human-specific protocols and ultimately delay the transition into the clinic.

Conclusion

Mouse pluripotent stem cell studies have led to the discovery of the basic machinery regulating PSC properties that

Table 4. Most highly differentially expressed genes between mouse and human induced pluripotent stem cells

Homologene	Gene Symbol	Fold Change (mm vs. hs)[a]	Fold Change (mm vs. hs)[b]	Gene Ontology
2590	FZD5	0.0150613	0.0103036	Signal transduction, morphogenesis, cell differentiation
12358	ZNF398	0.0152395	0.0225073	Transcription regulator
2338	SOX4	0.0166485	0.0156823	Transcription regulator
40994	ABLIM1	0.0200437	0.0306834	System development, morphogenesis
4283	NET1	0.0229625	0.0309112	Signal transduction, morphogenesis
37912	CASP3	0.0260849	0.0657961	System development, signal transduction, cell differentiation, cell cycle
9269	SALL2	0.0283317	0.0436168	Transcription regulator
105405	ACTA1	0.0288135	0.0549023	System development, cell differentiation
3273	ERBB2	0.0316369	0.0734967	System development, signal transduction, morphogenesis, cell-cell signaling
23047	SEMA4C	0.0316837	0.081642	System development, cell differentiation
3236	DBN1	0.0318729	0.0863559	System development, morphogenesis, cell-cell signaling, cell differentiation
7561	TAF6	0.0320464	0.0591067	Transcription regulator, cell differentiation
68187	HDAC2	0.0340584	0.0337242	Transcription regulator
5184	SF3A2	0.0390227	0.0815408	Cell differentiation
7628	NR2F2	0.0430441	0.0485498	Transcription regulator, system development, signal transduction, morphogenesis, cell differentiation
55698	RAB13	0.043604	0.00986432	Signal transduction
2117	PTN	0.045191	0.0429547	System development, signal transduction, cell cycle
69220	RAB8B	0.0532706	0.0241414	Signal transduction
56386	CD59/CD59B	0.0590709	0.0255564	Signal transduction
84402	RP6-213H19.1	0.0597634	0.0460131	Cell differentiation
37856	RIPK2	0.0608177	0.0578024	Signal transduction, cell differentiation
9467	GULP1	0.06356	0.0182061	Cell differentiation
136	GJA1	0.065451	0.0300811	System development, signal transduction, cell-cell signaling, cell differentiation
3802	CBL	0.0676839	0.0794171	Transcription regulator, signal transduction
3168	NFE2L3	0.0837615	0.0713186	Transcription regulator
23391	ACD	0.0842073	0.055829	System development, morphogenesis, embryonic development
24376	CAPZA1	0.089406	0.0533562	Cell differentiation
41259	PLCXD1	0.0942358	0.0638663	Signal transduction
74392	DPYSL2	0.0950567	0.0612362	System development, signal transduction, cell differentiation
269	PXMP3	0.0977678	0.0831723	System development, cell differentiation
32094	HEPH	0.100206	0.0388935	System development, cell differentiation
4208	GPR64	0.105954	0.0561338	Signal transduction
32576	GNPTAB	0.106628	0.0639291	Cell differentiation
32142	ATF5	58.417	28.525	Transcription regulator, cell differentiation, cell cycle
1255	ARL4D	61.5696	27.1705	Signal transduction
31124	SKI	62.8228	25.9135	Morphogenesis, embryonic development, cell differentiation
88701	SLC7A7	71.3599	42.0237	Cell differentiation
7920	RNF12	72.1257	54.0971	Transcription regulator
38834	TADA2L	73.3064	35.458	Transcription regulator, cell cycle
48120	PLAUR	74.3813	23.5848	Signal transduction
55671	PLCG2	74.5178	36.7096	Signal transduction
9624	INCENP	75.3147	65.0775	Cell cycle
1793	MDM2	80.6792	47.6129	Cell differentiation, cell cycle
1223	ADRBK1	84.393	21.3655	System development, signal transduction
32055	PDGFA	90.5384	29.0538	System development, signal transduction, morphogenesis, cell-cell signaling, cell cycle
12873	SESN2	102.757	58.1884	Cell cycle
4397	H1FX	103.295	23.8797	Cell differentiation
1814	MKI67	115.769	27.9395	Cell cycle
20929	EPHA2	129.218	37.8984	Signal transduction
37520	KLF5	182.406	83.5024	Transcription regulator, system development, morphogenesis
68516	ERF	186	31.8239	Transcription regulator, cell cycle
1658	IRF1	188.769	36.089	Transcription regulator, cell cycle
3123	KLF4	217.416	38.7516	Transcription regulator, system development, morphogenesis, embryonic development, cell differentiation
20626	PTPRS	234.354	21.6862	Signal transduction
45872	ARID5B	248.986	70.0245	Transcription regulator
13196	AXUD1	250.536	45.2516	Cell differentiation

Genes were filtered based on gene ontologies associated with transcriptional regulation, differentiation, proliferation, and cell-cell interactions.

This table shows most highly differentially expressed genes between mouse and human iPS. Genes were filtered based on gene ontologies associated with transcriptional regulation, differentiation, proliferation, and cell-cell interactions. Publicly available microarray data from human and mouse iPS cells reprogrammed by Oct4, Sox2, Klf4 and c-Myc were obtained from the National Center for Biotechnology Information Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) and are accessible through GEO Series accession number GSE7815 (miPS; two libraries), GSE9561 (hiPS; one library), and GSE9832 (hiPS; six libraries) [20, 21, 62]. Functional assignments were determined using Fatigo (http://babelomics.bioinfo.cipf.es); miPS, mouse induced pluripotent stem cells; hiPS, human induced pluripotent stem cells.

[a], Fold change mouse (GSE7815) versus human induced pluripotent stem cells (GSE9561).

[b], Fold change mouse (GSE7815) versus human induced pluripotent stem cells (GSE9832).

absolutely requires focus on the human PSC epigenome and transcriptome to develop strategies for directed differentiation. Accordingly, what we currently understand of the genetic and epigenetic pathways underlying pluripotent stem cell biology has come from the incredibly valuable information in the mouse system. However, genetically engineered mESCs and animals, as detailed in this review, have yielded questionable relevance to hESCs. Conservation of classical pluripotency factors Oct4/Sox2 has an analogous role in both human and mouse PSCs, but downstream regulators are seemingly not as well conserved. This could be consistent with the notion that mouse and human ESCs represent distinct developmental stages evidenced by differential capabilities for self-renewal and subtle differences in the functional effect of pluripotency hub expression and their downstream targets [116], or be simply caused by the fact that human cells are not mouse cells. Consequently, using mouse PSCs to model differentiation in the human system may not be as advantageous as predicted.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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