Cell

Leading Edge
Review
A Decade of iPS Cells

Stem Cells: A Renaissance in Human Biology Research

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http://dx.doi.org/10.1016/j.cell.2016.05.043

The understanding of human biology and how it relates to that of other species represents an ancient quest. Limited access to human material, particularly during early development, has restricted researchers to only scratching the surface of this inherently challenging subject. Recent technological innovations, such as single cell "omics" and human stem cell derivation, have now greatly accelerated our ability to gain insights into uniquely human biology. The opportunities afforded to delve molecularly into scarce material and to model human embryogenesis and pathophysiological processes are leading to new insights of human development and are changing our understanding of disease and choice of therapy options.

Introduction

For centuries, biologists have been fascinated by the question of what is unique about humans compared to other species. More recently we have come to understand the limitations of modeling human disease and treatment options in other animals. To tackle these challenges, we need to understand human biology at molecular, cellular, tissue, and organismal levels during different developmental stages. Advances have been severely hampered by the lack of accessibility to early embryos and limited supply of primary tissues. Therefore, in vitro, ex vivo, and xeno models using human material that can reproduce many aspects of human development, physiology, and disease hold potential to provide novel insights into human biology.

The ability to isolate and propagate human stem cells including embryonic stem cells (ESCs), fetal progenitors, and adult stem cells (ASCs), as well as to generate induced pluripotent stem cells (iPSCs), provides powerful means for modeling human development in a dish. These stem cells can renew virtually limitlessly in culture and can, to different extents, differentiate into various lineages akin to in vivo development and/or injury repair. These properties of human stem cells not only lend themselves to learning about developmental and regenerative processes, but also to disease modeling, drug screening, and prospectively, cell-replacement therapies. Stem cells have also recently been coaxed to form organoids, three-dimensional cellular structures resembling tiny versions of the organ they are meant to model. The potential for organoids to provide more dynamic and physiological relevant models for developmental processes, drug toxicity screening, disease modeling, and personalized medicine has generated much excitement in the academic and pharmaceutical research communities.

Concomitant with progress in human stem cell research, researchers have been able to maximize molecular studies of scarce primary human tissue by applying single-cell technologies such as single-cell transcriptomics.

In this review, we will follow the human developmental timeline to provide a brief account of our current understanding of human development, how it has been enhanced by recent methodological breakthroughs, particularly in stem cell technology, and the impact that this knowledge is having on our understanding of human biology and disease.

Pre-implantation Human Development

Although eutherian mammals essentially follow a similar pre-implantation path, the developmental timing differs. Mammalian embryogenesis starts with a single cell, the zygote that contains genetic information from both parents. The zygote is transcriptionally quiescent, and initial embryo development solely relies on maternal factors, such as RNAs and proteins, contributed by the egg cytoplasm that initiates the first cell division and gives rise to two equal blastomeres of the 2-cell embryo. In mice, during the first cell division, developmental control is transferred from the mother to the embryo following a process called zygotic genome activation (ZGA) (Lee et al., 2014). In humans ZGA is deferred to between the 4- and 8-cell stages (Blakeley et al., 2015). The first lineage segregation in mice, the extraembryonic trophectoderm (TE) on the outside and the inner cell mass (ICM) on the inside, can be clearly recognized by the 32-cell stage. Recent work indicated that this process could be initiated as early as the 4-cell stage (Wu and Izpisua Belmonte, 2016). Further development leads to compaction and cavitation to form the blastocyst with a fluid-filled cavity (blastocoel) (Graham and Zernicka-Goetz, 2016). Subsequent cell divisions lead to the second cell fate decision within the ICM, with some cells fated toward early epiblast (EPI) and the others toward primitive endoderm (PE). Compared to mouse, human embryos are delayed in their timing of compaction and blastocyst formation and undergo at least an additional round of cell division before implantation (Niakan et al., 2012). At about embryonic day 7 in humans, the hatched blastocyst starts to implant into the uterine wall and

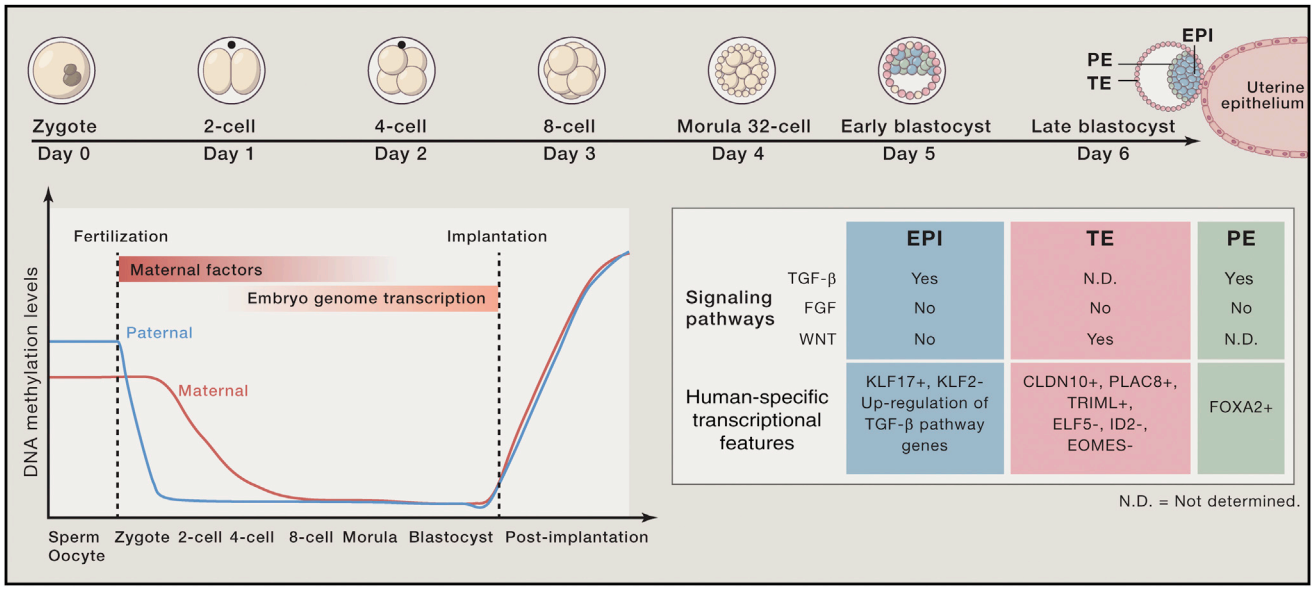

Figure 1. Human Pre-implantation Development

The first six days of human life starts from a single zygote, which develops into a late blastocyst containing three embryonic cell types: EPI, PE, and TE. In humans, zygotic genome activation occurs between 4-cell and 8-cell stages, and paternal genome is demethylated much faster than the maternal genome. Unlike other species, TGF-β signaling pathway affects the number of epiblast cells in human blastocyst. FGF signaling pathway, which is important for PE-EPI segregation in mice, has no apparent effect on human pre-implantation lineage formations. WNT signaling pathway seems to affect human TE development but has a negligible role in EPI. Single-cell transcriptomic studies reveal several human-specific transcriptional features.

continues on its path toward post-implantation embryonic and fetal development (Figure 1). Like apes, human embryos invade into the endometrium with the help of TE-derived cytotrophoblasts in a process called interstitial implantation. By contrast, in other primates, implantation is superficial, where the blastocysts remain in the uterine lumen (Carter et al., 2015), whereas rodent embryos attach to a cleft in the uterine wall and are subsequently encapsulated (secondary interstitial implantation) (De Paepe et al., 2014).

Despite morphological similarities, pre-implantation human and mouse embryos harbor key molecular differences, e.g., in expression of lineage-specific transcription factors: CDX2, a key transcription factor for mouse TE specification in morula stage, is not expressed until after blastocyst formation in humans; OCT4, a master regulator of pluripotency, is not restricted to the ICM until implantation and shows a protracted co-localization with CDX2 in the TE, a feature distinct from both rhesus monkey and mouse (Niakan and Eggan, 2013; Chen et al., 2009); and LAMININ is a basement membrane component thought to play an important role in cell sorting to separate murine ICM into distinct PE and EPI domains (Chazaud et al., 2006; Yamanaka et al., 2006). Interestingly, there is lack of appreciable LAMININ expression in the presumptive human PE, suggesting divergence in PE specification between the two species (Niakan and Eggan, 2013).

Signaling pathways involved in lineage segregation also differ between human and other species (Figure 1). FGF signaling is known to be the principle driver of PE-EPI segregation in rodents, and inhibition of FGF signaling pathway ablated PE formation and increased EPI population (Boroviak et al., 2015; Roode et al., 2012). By comparison, FGF signaling seems to play a reduced role in bovine and marmoset embryos. FGF pathway inhibition only leads to partial abrogation of PE lineage in bovine blastocysts and results in marmoset embryos containing cells co-expressing EPI/TE or EPI/PE markers (Kuijk et al., 2012; Boroviak et al., 2015). In contrast, treating human pre-implantation embryos with FGF pathway inhibitors does not block PE formation (Kuijk et al., 2012; Roode et al., 2012; Van der Jeught et al., 2013). Moreover, unlike mouse, human TE does not respond well to FGF, probably due to the low expression levels of FGF receptors (Kunath et al., 2014; Rossant, 2015). Species differences have also been noted in TGF-β signaling. In one study, TGF-β inhibitor SB431542-treated human embryos showed abrogated NANOG and SOX17 and reduced OCT4 expression, demonstrating an indispensible role of TGF-β signaling in maintaining key pluripotency and PE marker expressions in human ICM (Blakeley et al., 2015). In another study, however, SB431542 treatment of human blastocysts resulted in increased epiblast proliferation with a significant higher number of NANOG-positive ICM cells, but no difference in GATA6 positive PE cells (Van der Jeught et al., 2014). The discrepancy is likely due to the different dosages of SB431542 used, potentially resulting in a complete versus a partial inhibition of the TGF-β signaling pathway. Regardless, there was no effect on NANOG and OCT4 expression in both mouse and bovine embryos treated with TGF-β pathway inhibitors (Blakeley et al., 2015; Kuijk et al., 2012). Likewise, treating marmoset blastocysts with the type I TGF-β/activin/Nodal receptor inhibitor A83-01 also did not disrupt lineage segregation, and the numbers of NANOG- and GATA-6-positive cells were comparable to control embryos (Boroviak et al., 2015). These results suggest that dependency on TGF-β signaling is potentially a human-specific trait. Finally, mouse

studies showed that WNT/β-catenin signaling is dispensable for blastocyst lineage formation (Biechele et al., 2011). And study with bovine embryos revealed a minor role of WNT in the segregation of PE and EPI (Kuijk et al., 2012). In contrast, inhibition of WNT/β-catenin signaling significantly increased NANOG expression in marmoset embryos and, in conjunction with ERK inhibition, also led to a profound reduction of PE cells (Boroviak et al., 2015), suggesting a WNT-dependent ICM lineage segregation in a non-human primate (NHP) species. Intriguingly, a recent study found canonical WNT signaling was involved in TE development but has a negligible effect on the ICM in human blastocyst (Krivega et al., 2015). Likewise, treatment of a canonical WNT activator CHIR99021 didn't reduce NANOG-positive EPI compartment in human embryos (Roode et al., 2012).

The rapidly evolving field of single-cell "omics" (Wang and Bodovitz, 2010) has expanded our knowledge of the cellular and molecular intricacies underlying pre-implantation human development at an unprecedented pace. Recent advances in single-cell RNA sequencing (RNA-seq) are greatly facilitating quantification of global gene expression using limited human pre-implantation embryo samples and enriching our understanding of transcriptional programs during early human embryogenesis (Figure 1). Cross-species comparison indicated that, although human and mouse share core transcriptional programs, they differ in their developmental stage specificity and timing, reflecting species-specific difference in length of development during this period (Xue et al., 2013). Single-cell RNA-seq also uncovered novel long non-coding RNAs (lncRNAs) during human pre-implantation development (Yan et al., 2013). With a focus on lineage specification, a recent single-cell transcriptomic comparison of human and mouse pre-implantation embryos revealed several genes exclusively expressed in the human EPI, including *KLF17*, suggesting its species-specific function in human pluripotency. The expression levels of several key genes of the TGF-β signaling pathway were also found elevated in human EPI, in agreement with its indispensable role in maintaining pluripotency in human ICM. In contrast, known mouse ICM genes were either absent (*KLF2*) or restricted to PE and/or TE cells (*ESRRB* and *BMP4*) in human embryos (Blakeley et al., 2015). Interestingly, similar expression patterns of pluripotency-related genes were also observed in marmoset embryos, indicating embryonic pluripotency programs are more conserved among primates (Boroviak et al., 2015). Regarding the TE lineage, key mouse factors such as *Elf5* and *Eomes* were absent in humans, while CLDN10, PLAC8 and TRIML showed human-specific expression (Blakeley et al., 2015). A most recent report by Lanner and colleagues generated a comprehensive single-cell RNA-seq dataset of 1,529 individual cells from 88 pre-implantation human embryos (Petropoulos et al., 2016). Unlike mice, in which TE/ICM segregation occurs before EPI/PE, Petropoulos et al. found that separation of all three lineages (TE, EPI, and PE) occurs simultaneously in humans, coinciding with the formation of the blastocyst at E5, in agreement with the delayed CDX2 expression observed by Niakan and Eggan (2013). Also, co-expression of lineage-specific genes was observed before human blastocyst formation. Moreover, in contrast to mice, *XIST* and other X-linked genes showed biallelic expression in human E7 blastocysts (Petropoulos et al., 2016).

Mammalian pre-implantation development is also marked by genome-wide reprograming of parental methylomes. Recently, reduced representation bisulphite sequencing (RRBS) and whole-genome bisulphite sequencing (WGBS) have been applied to human pre-implantation embryos from the zygote stage through to the peri-implantation stage (Guo et al., 2014; Okae et al., 2014; Smith et al., 2014) and provided the first genome-scale DNA methylation maps of human pre-implantation development. As revealed by RRBS, methylome dynamics are overall similar but do show distinct features between human and mouse pre-implantation embryos (Smith et al., 2014). In contrast to mouse, the major wave of genome-wide demethylation is complete at 2-cell stage in humans (Guo et al., 2014). Compared to maternal genome, the demethylation of the paternal genome is much faster in human embryos (Figure 1) (Guo et al., 2014). RRBS only covers 5%–10% of all CpGs in the genome. A higher-resolution methylome map provided by WGBS covering >70% of genomic CpGs led to the discovery of more species differences: human maternal genome was found less demethylated than mouse, several regions of the human paternal genome such as SINE-VNTR-Alu were protected from being demethylated, and unlike mice, human oocytes contain a hypermethylated centromeric satellite repeats (Okae et al., 2014). Future work using NHP embryos will help clarify whether these features of epigenetic regulation found in humans are conserved among primates. Other intriguing questions include whether these species-specific epigenetic signatures are of any functional importance and whether there is epigenetic variability among human embryos with same and/or different genetic background. Answers to these questions may be informative for understanding epigenetic flexibility and stability during pre-implantation period and its impact on post-implantation embryo development.

These comparative genomic/epigenomic studies and functional interrogations have provided us with an initial map charting the first few days of human development. With further technical advancements, we should have a clearer picture of this developmental window. Despite the information accumulated on pre-implantation human development, the reduced total number of cells that can be obtained from limited human IVF embryos have made certain analyses difficult, e.g., chromatic dynamics, metabolomics, and proteomics studies. An alternative source of pre-implantation embryos can be achieved using nuclear reprograming somatic nuclei by an enucleated egg known as somatic nuclear transfer (SCNT) (Tachibana et al., 2013). However, SCNT faces a similar problem of limited supply of human oocytes. In addition to limited material, lack of genetic models in human embryos preclude dissecting gain or loss of function of gene(s) involved in the regulation of cell potency and early cell fate commitment. In this regard, in vitro expandable cell lines are ideally suited for obtaining large quantities of cells. Although method(s) to expand early embryonic cells in vitro is under intensive investigation, to date, we still cannot stably propagate cells in culture with blastomere and/or early ICM properties in any species.

Dynamic Pluripotent Stem Cell States

All the cell types within the adult are derived from the ICM of a blastocyst. This cell population is normally short lived during embryogenesis and undergoes sequential differentiation to form the embryonic precursors of different lineages. It is these cells, cultured in conditions that allow indefinite self-renewal and maintenance of the pluripotent state, that we know as "embryonic stem cells" (ESCs) (Evans and Kaufman, 1981; Martin, 1981; Thomson et al., 1998). Human ESCs (hESCs) have been conducive to understanding both functional and dysfunctional aspects of early human development and potentially offer an inexhaustible supply of cells, tissues, and organs for replacement therapies. Remarkably, nine years after the first derivation of hESCs, an ESC-like pluripotent cell type was successfully generated from human somatic cells through a cellular reprograming approach with co-expression of four pluripotency-related transcription factors (Aasen et al., 2008; Park et al., 2008; Takahashi et al., 2007; Wernig et al., 2007; Yu et al., 2007). These cells, coined as induced pluripotent stem cells or iPSCs, can be generated using different somatic cell types and share many defining features of embryo-derived hESCs, including the expression of core pluripotency factors, amenability for long-term culture while maintaining genomic stability, and the generative capability to derive tissues of all three germ lineages. Importantly, iPSCs can also be generated from patient cells with known mutations and phenotypes, thereby offering unprecedented opportunities to model human disease and perform gene corrections as well as personalized drug screenings.

Although mouse ESCs (mESCs) and hESCs are both sourced from pre-implantation ICMs, they exhibit distinct features: (1) mESCs colonies assume a dome-shaped morphology with cells tightly clustered together, while hESCs colonies appear more flattened, (2) the LIF/STAT3 signaling pathway promotes self-renewal of mESCs, reminiscent of the requirement of LIF/STAT3 signaling for prolonged maintenance of in vivo epiblasts during embryonic diapause, a temporary arrest of embryonic development as a protective response to unfavorable environments (Nichols et al., 2001), and in contrast, LIF/STAT3 signaling is dispensable for maintenance of self-renewal in hESCs (Daheron et al., 2004), (3) inhibition of FGF signaling pathway in mESCs suppresses differentiation, and in contrast, bFGF is indispensible for self-renewal of hESCs, (4) TGF-β signaling is dispensable for mESCs but required for maintaining pluripotency of hESCs, (5) hESCs survive poorly after single-cell dissociation and can only be effectively passaged as small clumps, unlike mESCs, which show high single-cell cloning efficiency, and (6) female mESCs harbor two activated X chromosomes while one of the two X chromosomes becomes inactivated in female hESCs. Historically, these and other differences have been attributed to species-specific pluripotency features. In 2007, two papers reported the derivation of another pluripotent stem cell line from a developing mouse embryo, the epiblast stem cells (EpiSCs) (Brons et al., 2007; Tesar et al., 2007). EpiSCs are derived from pluripotent post-implantation epiblasts and, surprisingly, share many features characteristic of hESCs, lending support for a post-implantation identity of established hESCs.

mESCs and EpiSCs have been proposed to represent two major phases of pluripotency: naive versus primed (Nichols and Smith, 2009). Naive pluripotency refers to the unbiased developmental potential of a cell to give rise to derivatives of all three germ layers, including the germline. In vivo, naive pluripotency arises during epiblast specification and accumulating evidence has indicated that naive pluripotency is also captured in mESCs (Boroviak et al., 2014). Although mESCs are commonly sourced from the blastocyst, they can also be derived from various earlier pre-implantation stages, even from single blastomeres (Chung et al., 2006; Tesar, 2005). Notwithstanding the source, mESCs exhibit similar characteristics, suggesting that during derivation, early embryonic cells are corralled into a common self-renewal state by mESC cultures. Clonal derivation and single-cell transcriptional profiling showed that mESCs paralleled the E4.5 epiblast (Boroviak et al., 2014). Molecularly, naive pluripotency is characterized by expression of a set of naive-related markers, such as *Klf2*, *Esrrb*, *Rex1*, a hypomethylated genome, bivalent metabolic features, among others. Functionally, naive mESCs are able to re-enter embryogenesis in pre-implantation embryos and generate germline competent blastocyst chimeras or complement a tetrapoid blastocyst and give rise to an entire fertile adult (Tam and Rossant, 2003). On the other hand, primed pluripotency captured in EpiSCs corresponds to the peri-gastrulation stage epiblast. Primed pluripotency is marked by molecular features such as expression of primed specific genes *Fgf5*, *Otx2*, and *T*, a hypermethylated genome, and an exclusive glycolytic metabolic pathway (Hackett and Surani, 2014; Wu and Izpisua Belmonte, 2015a). The developmental potency of EpiSCs was revealed by grafting them to the late epiblast of a gastrulating mouse embryo for the generation of ex vivo embryonic chimeras. Once inside the post-implantation epiblast, EpiSCs can proliferate and differentiate into the three embryonic germ lineages (Huang et al., 2012). Interestingly, mESCs are unable to engraft into post-implantation epiblasts, highlighting the importance of matching temporal properties of cultured PSCs to in vivo developmental stages for their chimeric contributions.

Like EpiSCs, primed human pluripotent stem cells (hPSCs) are positioned to provide novel insights into the developmental window during which somatic and germ-cell lineages are first specified, around gastrulation (Chenoweth et al., 2010). In contrast to mouse post-implantation development, we know very little about this period of human development beyond morphological descriptions. Human early post-implantation development is one of biology's impenetrable black boxes, largely due to ethical barriers in obtaining primary tissue. Robust culturing protocols based on principles extrapolated from mouse studies have been successfully used to guide hPSCs toward the three embryonic germ lineages: endoderm, mesoderm, and ectoderm, as well as the germline during in vitro differentiation. More developmentally advanced fetal cell types have also been generated that mimic some of the functions of their in vivo counterparts. In addition to differentiation in vitro, primed hPSCs grown in several different culture conditions have also shown the capacity to engraft into mouse post-implantation epiblasts and generate lineage precursors ex vivo (Mascetti and Pedersen, 2016; Wu et al., 2015). Interestingly, different culture conditions seem to generate different grafting outcomes. Wu et al. (2015) demonstrated that human H9 ESCs grown in mTeSR medium (containing a high concentration of bFGF) showed limited engraftment to

post-implantation mouse epiblasts, regardless of grafting location (anterior, posterior, or distal). In contrast, Mascetti and Pedersen (2016) showed a broader engraftment of hESCs grown in different cultures (containing much lower concentrations of bFGF), although only grafting to posterior or distal epiblasts was examined. This discrepancy suggests that subtle differences in culture parameters can affect the outcome of xeno-engraftment of hPSCs to a developing mouse embryo, and the role of bFGF in this context warrants future investigations. In agreement with this, a modified mTeSR medium containing IWR1, a Wnt/β-catenin signaling pathway inhibitor, nudged human H9 ESCs into a primed sub-state where they selectively engraft to the posterior part of the post-implantation mouse epiblast (region-selective PSCs [rsPSCs]) (Wu et al., 2015). Although much work is needed to formally demonstrate the usefulness of the epiblast-grafting platform, it holds the potential to become a tractable experimental model for studying human gastrulation.

Despite the usefulness of primed hPSCs, whether mESC-like naive hPSCs can be captured and stabilized in culture has been a major focus of human stem cell biology in recent years (Weinberger et al., 2016). Several notable advantages associated with naive pluripotency, such as high single cell cloning efficiency, facile genome editing capability and higher developmental potential, can facilitate gene targeting, relieve researchers from tedious clump passaging, and generate more mature cells for transplantation. To date, several studies reported de novo derivation of naive hPSCs from blastocysts (Gafni et al., 2013; Guo et al., 2016; Theunissen et al., 2014; Ware et al., 2014). It is important to note that diverse media formulations have been utilized to derive and cultivate naive hPSCs, and consequently, cultured cells display distinct molecular and cellular characteristics. A systems biology approach employed to assess the conservation of gene networks revealed that transcriptomes vary considerably among established naive human lines despite a consensus gene network in RNA processing, ribosome biogenesis, and mitochondrial metabolism (Huang et al., 2014). Regardless, when compared with primed hPSCs, all established naive hPSCs examined showed a clear resemblance to human late pre-implantation embryos. Also, cross-species comparison indicated that gene networks between naive human and mouse PSCs were not well conserved and better resembled their respective blastocysts (Huang et al., 2014).

Blastocyst chimeras are used for functional evaluation of mESCs, but due to ethical considerations, naive hPSCs cannot be tested in this context. Instead, interspecies chimeric embryo formation using animal hosts can potentially help validate human naivety or generate functional human organs in animal hosts (Wu and Belmonte, 2016; Wu and Izpisua Belmonte, 2015a). However, it remains controversial whether naive hPSCs can cross species boundaries at the blastocyst stage and generate chimeric fetuses. In one report, Gafni et al. (2013) successfully generated E10.5 chimeric mouse embryos using NHSM-cultured naive hPSCs. In contrast, Theunissen et al. (2014) reported the opposite: both NHSM- and 5iL/A-cultured naive hPSCs could not generate interspecies human-mouse chimeric embryos. It is interesting to note that reproducible ICM incorporation of naive hPSCs into mouse blastocysts has been reported (Gafni et al., 2013; Takashima et al., 2014). Thus, it seems that naive and primed hPSCs can robustly integrate into developing mouse embryos at blastocyst and gastrula stages, respectively, and likely, divergent early post-implantation (pre-gastrulation) development between human and mouse precludes ICM-integrated naive hPSCs from proper patterning, and they are thus out-competed by resident mouse embryonic cells. To this end, co-transplanting hPSC-derived hypoblast cells and/or trophoblast cells in which the signals for early human epiblast patterning originated may help to circumvent this barrier. Alternatively, choosing a species physiologically more similar and/or evolutionarily closer to humans likely can help human naive cells cross species boundaries.

The ability of hPSCs to contribute to early development of another species raises several intriguing questions: (1) how conserved early developmental programs, in particular the early epiblast and gastrulation, are shared between human and other mammals, (2) whether it is possible for hPSCs to generate functional cells in a xeno-environment, and (3) whether one can isolate or engineer novel types of hPSCs (Wu and Izpisua Belmonte, 2014) that can more efficiently cross xeno-boundaries and contribute to early development of another species. To answer these questions, continued efforts to understand pre-, peri-, and post-implantation embryonic pluripotency of humans and other closely related species, such as NHPs, would be needed (Boroviak et al., 2015). As for primed hPSCs, culture conditions that support embryo development beyond the stage afforded by current methods will extend the utility of ex vivo interspecies chimeras.

Derivation of naive hPSCs analogous to mESCs has another advantage. Compared to primed cells, naive hPSCs likely resemble human epiblasts from an earlier developmental stage, and thus, state transitions between naive and primed provide an in vitro model system to examine the very early steps of human epiblast development and helps enrich our knowledge of the signaling, genetic, epigenetic, and metabolic pathways during this process. For instance, a recent study by Rouhola-Baker and colleagues uncovered a novel metabolic switch for the naive-to-primed state transition in humans, highlighting an intricate interplay between the metabolome and epigenome conferred by nicotinamide N-methylatransferase (NNMT) (Sperber et al., 2015; Wu and Izpisua Belmonte, 2015b).

Through genome editing, hPSCs can provide invaluable gain- or loss-of-function genetic models that facilitate our understanding of the functions of genes and regulatory elements in human pluripotency. When combined with in vitro differentiation, transgenic hPSC lines also offer genetic insights into lineage formation. Alternatively, human DNA can be directly inserted into animal genomes such as the mouse or zebrafish and create transgenic animals for interrogation of human-specific traits in vivo. Transgenic animal models have been instructive for understanding the functions of several coding and non-coding human DNA sequences (reviewed by Franchini and Pollard [2015]). Another application of the transgenic approach is to generate improved "humanized" mouse models by introducing human specific factors, such as human leukocyte antigen (HLA) molecules and cytokines, into immunodeficient mice for better and broader engraftment of multiple types of human cells and

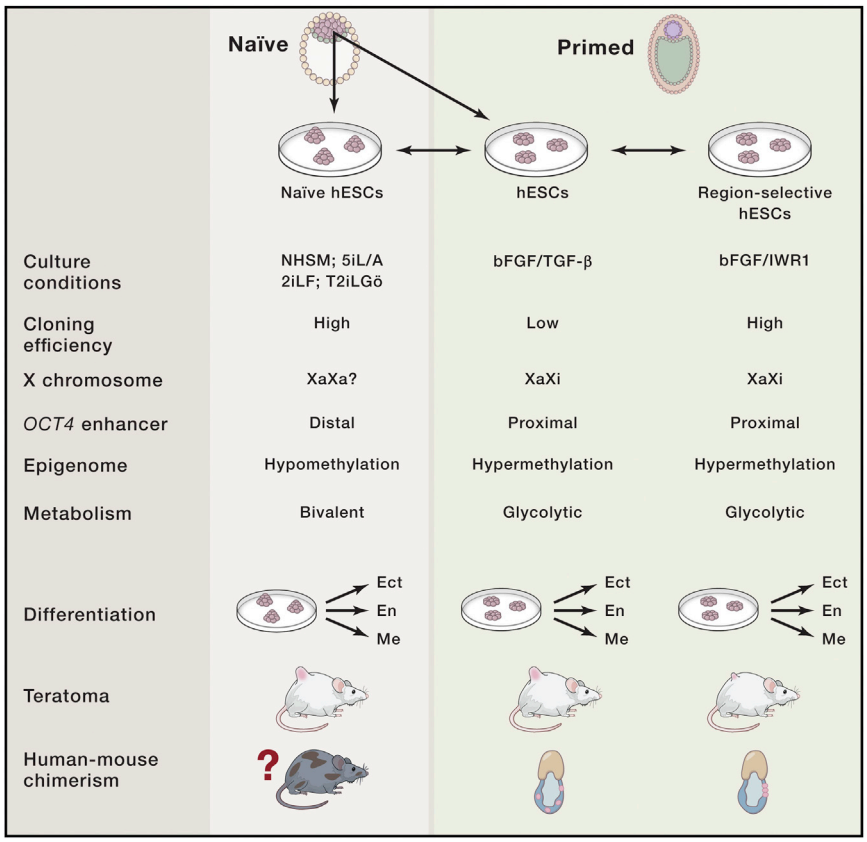

Figure 2. Dynamic Human Pluripotent Stem Cell States

Conventional hESCs derived under bFGF/TGF-β are believed to be at the primed pluripotent state. Modulation of hESC culture condition by including a WNT signaling pathway inhibitor IWR1 nudges the hESCs into a distinct region-selective primed state. Recently naive hESCs reminiscent of mESCs have also been derived from human blastocysts, which exhibit distinct molecular and functional features from both primed hESCs and region-selective hESCs.

tissues (Brehm et al., 2014). Humanized mouse models are instrumental for in vivo functional interrogation of hPSC derivatives, direct research of various human diseases, mechanistic understanding of human immune disorders, and development of vaccines against human-specific infectious agents (Ito et al., 2012). In addition, recent advances in genetic engineering have made possible knockin of human DNA in large animals, such as the pig, and will not only provide a more physiological model for human disease but one day may allow xenotransplantation of organs from humanized pig direct into humans to solve the severe shortage of organ donations worldwide (Prather et al., 2013).

The ability to stabilize different states of hPSCs in artificial culture milieus highlights the plastic nature and dynamic developmental processes of human epiblast cells in vivo (Figure 2). Through the lens of expandable pluripotent human cell lines we have obtained snapshots of a myriad of molecular and cellular landscapes during early post-implantation epiblast development. We have come to know the signaling pathways regulating self-renewal and differentiation of gastrula-stage epiblast cells, epigenetic signatures, metabolic requirements, and transcriptional regulations. We also have tasted the different flavors of hPSC naivety, a rapidly evolving research topic. Despite these and other advances, a burning question remains: to what extent is the information derived from the studies of hPSCs reflective of true in vivo processes? To address this, examining primary tissue from NHPs, generating interspecies early post-implantation chimeras, and developing methods to extend cultured human blastocyst to gastrula stage of development may be useful.

A Germ-Cell View of Human Post-implantation Development

Morphogenic differences in early post-implantation development between mice and humans affect the source, duration, and nature of signaling molecules that confer competence for specific cell fates. Recent technical advances in culturing human IVF embryos beyond the early post-implantation period will help enrich our understanding of the significance of these differences (Deglincerti et al., 2016; Rossant, 2016; Shahbazi et al., 2016). Regardless, the lack of primary tissues as well as technical and ethical barriers for culturing human embryos beyond day 14 makes hPSC differentiation the primary choice for studying early lineage commitments in humans. Here, by focusing on the germ cells, we will highlight the efficacy of modeling human development using hPSC differentiation. For other cell types, please refer to a recent review for more information (Tabar and Studer, 2014).

The germ-cell lineage is separated from the somatic lineage early in development. In mice, primordial germ cells (PGCs), the common precursor of oocytes and spermatozoa, are specified from posterior-proximal epiblast between E5.5 and E6.5 stages by BMP signals from the extraembryonic ectoderm (Ohinata et al., 2009). Thereafter, PGCs increase in numbers while actively migrating through the hindgut to colonize the developing gonads, where they initiate differentiation into either oocytes or spermatozoa (Ohinata et al., 2009). Due to their limited numbers and the technical difficulties for isolation at early developmental stages, there is a growing need to model germ-cell development in vitro to gain novel insights into this dynamic and complex process. By treating mESCs with bFGF, ActivinA (ACTA), and 1% KSR for two days, Hayashi et al. (2011) obtained transient epiblast-like cells (EpiLCs) resembling E5.75 pre-gastrulating epiblast, which is within PGC fate competency window. Indeed, stimulating EpiLCs grown as 3D aggregates with a combination of growth factors led to the generation of PGC-like cells (PGCLCs), which could contribute to normal gametogenesis

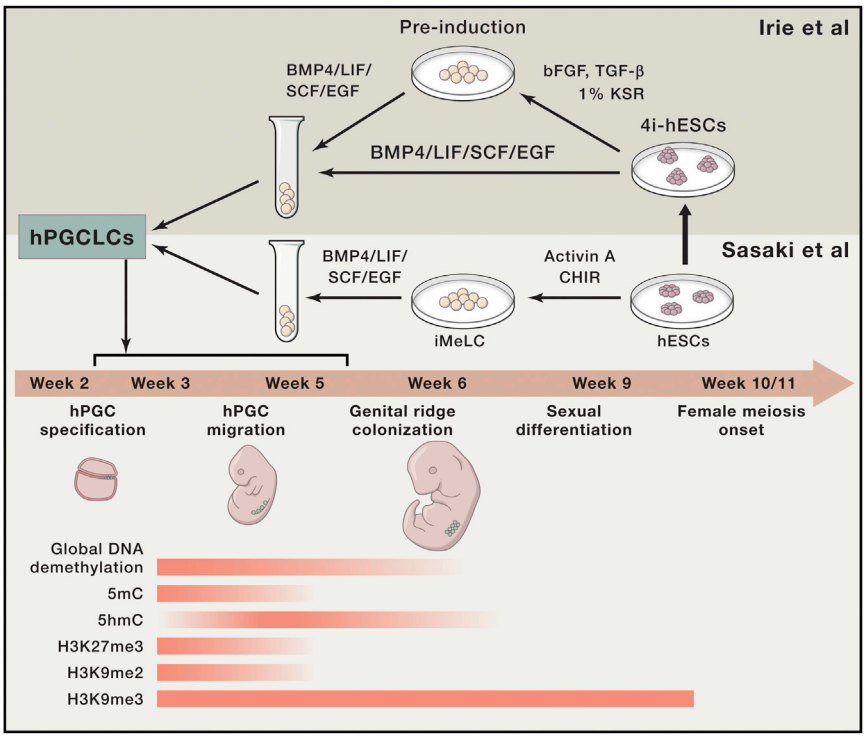

Figure 3. Human Primordial Germ Cell Development

Top, two in vitro methods have been developed to generate human primordial germ-cell-like cells (hPGCLCs) from hESCs. hPGCLCs resemble pre-gonadal hPGCs. Bottom, epigenetic reprograming during week 4 to week 9 of hPGC development.

after in vivo transplantation (Hayashi et al., 2011, 2012; Nakaki et al., 2013).

In humans, PGC specification occurs approximately between E12–E16 in the posterior epiblast, a timing that precludes direct investigation in early human embryos (Surani, 2015). Between week 3 and 5 of gestation, roughly corresponding to E8–E10.5 in mice, specified human PGCs migrate from the yolk sac wall through the hindgut and enter into the fetal genital ridge, where they proliferate and commit to sex-specific development (Tang et al., 2015). The success in obtaining functional PGCLCs from mESCs raises the intriguing possibility of using hPSCs to study human germ-cell development in vitro (Figure 3). Irie et al. (2015) used a naive hESC line cultured with four kinase inhibitors (thus named 4i medium) and found 4i-hESCs could be efficiently induced to hPGCLCs via transient pre-induction with bFGF, TGF-β, and 1% KSR. Interestingly, unlike mouse EpiSCs, primed hPSCs could also be efficiently differentiated into hPGCLCs via intermediate primitive streak-like cells (iMeLCs) induced by exposure to ACTA and a WNT signaling agonist (Sasaki et al., 2015). Global gene expression analyses indicate that the hPGCLCs generated by Sasaki et al. bear similar transcription profiles to primary hPGCs and hPGCLCs obtained by Irie et al. Interestingly, Irie et al. also demonstrated that, instead of pre-treatment with bFGF/TGF-β/1% KSR, direct induction using 4i-hESCs could also give rise to hPGCLCs. This suggests that 4i-hESCs are likely not in the postulated naive state, but rather, similar to iMeLCs, represent a peri-gastrulating epiblast-like pluripotent state (Sasaki et al., 2015). These results further highlight the complexity underlying human pluripotency.

Successful PGCLC induction from both human and mouse PSCs enables comparison of early PGC development. Although signaling requirements for PGCLC induction are similar, time course transcriptome analysis revealed distinct transcriptional dynamics during PGCLC induction between human and mouse and a lack of prominent transient activation and subsequent repression of the somatic mesodermal program in hPGCLC specification (Sasaki et al., 2015). BLIMP1, which had been identified as the first and key regulator of murine PGC fate (Ohinata et al., 2005) and as a potent transcriptional repressor of somatic genes in nascent murine PGCs (Kurimoto et al., 2008), appears required for the specification and maintenance of hPGCLCs (Irie et al., 2015; Sasaki et al., 2015). However, the precise role of BLIMP1 seems to have diverged between humans and mice: human *BLIMP1*$^{-/-}$ cells showed upregulation of endodermal genes while this was not observed in *Blimp1*-deficient mouse PGCs (Kurimoto et al., 2008; Vincent et al., 2005). Also, in humans, BLIMP1 exerts a less eminent effect on the repression of the somatic mesodermal program. Instead, it has been found to be critical in repressing genes involved in neuron differentiation (Sasaki et al., 2015). A surprising role of SOX17—known primarily for its role in endoderm (Wang et al., 2011) and hematopoietic differentiation (Clarke et al., 2013)—as a key regulator of hPGCLCs was also discovered (Irie et al., 2015). Importantly, *Sox17* does not play an analogous role in the specification of mouse PGCs (Hara et al., 2009). SOX17 was found acting upstream of BLIMP1, and SOX17-null 4i-hESCs were defective for hPGCLC specification. Strikingly, activation of SOX17 alone in the absence of inducing signals was sufficient to induce hPGCLCs from 4i-hESCs. Of note is that T/BRACHYURY, a key mesodermal transcription factor, was found critical in mPGC specification. Overexpression of T/BRACHYURY alone could bypass the requirement of inductive BMP4 signaling, likely analogous to SOX17's role in hPGCLCs (Aramaki et al., 2013). In this context, it will be interesting to investigate whether SOX17 and T/BRACHYURY play similar roles in mPGC and hPGC inductions respectively. These comparative analyses indicate a conserved upstream signaling requirement and divergent downstream transcriptional programs underpinning PGCLC inductions in both human and mouse. Whether similar observations can be extended into the specification of somatic lineages remain to be explored.

Recently, Zhou et al. (2016) have pushed in vitro germ-cell differentiation even further, achieving complete meiosis from mESCs in vitro by subsequent co-culture of mPGCLCs with neonatal testicular somatic cells and sequential exposure to morphogens and sex hormones. After intracytoplasmic injection into oocytes, mPGCLC-derived haploid spermatid-like cells could successfully produce viable and fertile offspring. It remains to be seen whether a similar approach can also be applied for humans. If so, this stepwise strategy will provide an invaluable tool and constitute a robust in vitro platform for investigating later human germ-cell development.

Mammalian germ-cell development is marked by a major wave of epigenetic reprogramming, a key process to restore full germline potency and transmission of genetic and epigenetic information across generations (Figure 3) (Hajkova et al., 2002). Most recently, three studies using primary hPGCs isolated from different gestation stages covering specification, migration, genital ridges colonization, and sex differentiation charted the transcriptional and epigenetic landscapes and provided novel insights into the dynamics of PGC development in humans (Gkountela et al., 2015; Guo et al., 2015; Tang et al., 2015). Informed by these studies, a general observation is that transcriptome dynamics and epigenetic reprograming in hPGCs are similar to mPGCs at comparable stages. However, hPGCs also show species-specific features that distinguish them from mPGCs. RNA-seq analysis revealed unique transcriptional programs in the human germline (Tang et al., 2015). hPGCs strongly expressed *KLF4* and *TFCP2L1*, genes associated with naive human pluripotency, while lacking expression of *ESRRB, SOX2, SOX3*, and *ZIC3*. Also, hPGCs express lineage specifiers such as *GATA4* and *TEAD4*. These transcriptional differences can be attributed to the distinct PGC gene regulatory networks between mouse (BLIMP1, PRDM14, and TFAP2C) and human (SOX17 and BLIMP1). Interestingly, Guo et al. (2015) reported a more homogenous and higher level of *SOX15* in early hPGCs, suggesting its possible role in hPGC development. DNA demethylation dynamics in hPGCs between week 4 and 19 aligned well overall with the demethylation events in mPGCs between E10.5 and 13.5, highlighting a conserved epigenetic reprograming of the germline in mammals (von Meyenn and Reik, 2015). With regard to chromatin reorganization, however, there were some notable differences (Figure 3). For example, while mPGCs showed a global loss of H3K9me2 and persistent enrichment of H3K27me3, hPGCs exhibited lower levels of H3K9me2 and H3K27me3 than soma (Tang et al., 2015). The hallmark of epigenetic resetting in the germline (Hackett et al., 2013; Kobayashi et al., 2013; Seisenberger et al., 2012), imprint erasure, was observed before genital ridge colonization in humans, at an earlier time than that observed with mPGCs. In addition, X chromosome reactivation in female PGCs also occurred earlier in humans (prior to 4 weeks) than mice (E8.5 to E12.5). Notably, Gkoutela et al. detected the expression of *XIST* noncoding RNA, a major effector of the X-inactivation process, in all stages of both female and male hPGCs examined, suggesting a novel X-inactivation independent role of *XIST* in the human germline. More interestingly, despite the comprehensive erasure of epigenetic marks, similar to the mouse, there were loci that retained significant levels of DNA methylation in the human germline, potentially representing hotspots of trans-generational epigenetic inheritance (Heard and Martienssen, 2014; Tang et al., 2015).

These germ-cell studies reinforce the notion that divergent mechanisms have evolved to govern early lineage specification since human and mouse branched off evolutionarily about 90 million years ago. They also demonstrate the value of hPSCs as a model system to study human-specific early fate commitment and, in conjunction with timed primary tissues, will help elucidate the dynamic processes involved in pre-natal human development. Another important message that can be gleaned from PGCLC induction is that a more physiological 3D culture environment is important for lineage specification and differentiation (Haycock, 2010).

Modeling Human Development and Disease with Organoids

Until recently, monolayer culture has been the main platform for directed differentiation of hPSCs. While these have been extremely informative on a number of fronts, a growing body of evidence indicates that 3D aggregate culture can lead to the generation of more complex tissue structures known as organoids that closely mimic in vivo primary tissues in both composition and architecture and that may be more physiologically relevant than monolayer culture (Fatehullah et al., 2016). By definition, the term organoid refers to an in vitro aggregate of several cell types derived from PSCs, tissue progenitors, or tissue-resident ASCs, which are capable of self-renewal, self-organization, and execution of key functions characteristic of in vivo tissue (Lancaster and Knoblich, 2014). The self-organizing ability of stem cells or progenitors allows in vitro recapitulation of organogenic processes in the form of organoid formation. Self-organization is mainly marked by three separate, but not necessarily independent processes: self-assembly, self-patterning, and self-morphogenesis (Sasai, 2013a, 2013b).

Organoids derived from hPSCs have been established for all the three germ lineages (Figure 4) (Spence et al., 2011; McCracken et al., 2014; Nakano et al., 2012; Lancaster et al., 2013; Muguruma et al., 2015; Takasato et al., 2015; Morizane et al., 2015). hPSC-derived organoids can bypass the limited availability of high-quality human fetal tissues and are valuable in vitro models for studying early human development. As organoids can faithfully retain the features of primary tissues, one can obtain detailed snapshots and gain novel mechanistic insights into the developmental processes leading to lineage specification and maturation in a dish. The self-renewing capacity of hPSCs facilitates the generation of large quantities of organoids needed for global "omic" studies to better understand the tissue- and organ-specific molecular dynamics observed during embryogenesis. Organoid formation is also useful to help elucidate the roles of signaling pathways and their crosstalk during embryonic tissue patterning. For example, Spence et al. (2011) have found that the combined activity of WNT3A and FGF4 is required for specifying human hindgut from definitive endoderm. A concerted interplay between WNT, FGF, and BMP signaling pathways that promotes a mid-hindgut fate has also been identified (McCracken et al., 2014). It is important to note that species differences in the development and architectural organization of several tissues, e.g., the stomach (McCracken et al., 2014), may

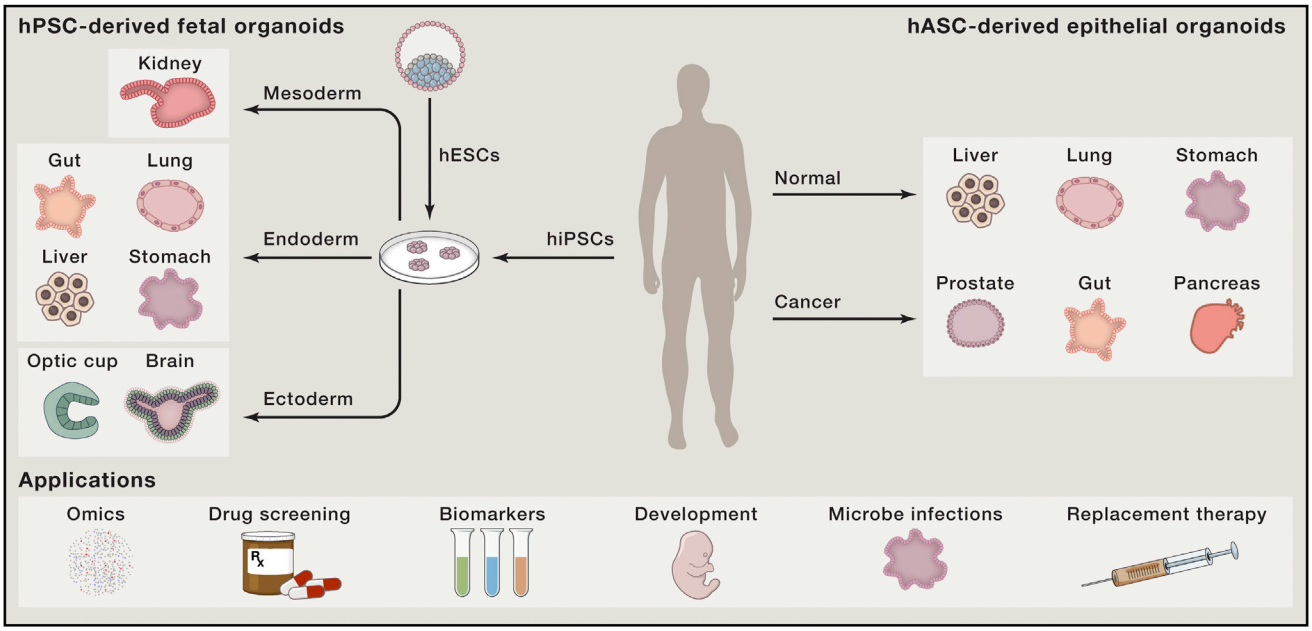

Figure 4. Organoids and Their Applications

Three-dimensional organoids that faithfully recapitulate in vivo tissue architectures can be generated from different embryonic germ lineages through differentiation of hPSCs. Also, organoids can be generated from tissue resident adult stem cells (ASCs). Organoids provide near-physiological model systems to study human development and diseases. Also, patient-specific organoids can be used for drug screening, diagnostic biomarkers, and cell and tissue replacement therapy.

make the use of animal models unsuitable for studying human organogenesis. In this regard, hPSC-derived organoids provide much-needed experimental models for human-specific developmental processes. Comparison of the same type of tissue organoids generated from both hPSCs and mPSCs revealed species differences and highlighted the importance of choosing human organoid models. For instance, when comparing retinal organoids generated from both mouse and human PSCs, several features unique to human were observed: (1) the size of the hPSC-derived optic cup was much larger than the mouse counterpart; (2) the hPSC-derived neural retina was thick and spontaneously curved in an apically convex manner, which was not observed in mouse; (3) hPSC-derived neural retinas contained both rods and cones, whereas cone differentiation was rare in mouse culture; and (4) in contrast to mouse, human photoreceptor differentiation was much slower but could be accelerated by a Notch inhibitor (Nakano et al., 2012). Similarly, species differences have also been observed between mouse and human PSC-derived gastric organoids. While mouse stomach organoids generated by Noguchi et al. were capable of secreting pepsinogen c and gastric acid and display rudimentary peristaltic contractions, gastric organoids generated from hPSCs only contained cell types from the gland, pit, and neck regions of the antral stomach, but lacked corpus cell lineages, suggesting a more complex gastric tissue patterning in humans (Fatehullah et al., 2016; McCracken et al., 2014; Noguchi et al., 2015).

Perhaps the organ that displays the most distinct features between human and other species is the brain, in which human-specific cognitive ability is generated. hPSC-derived brain organoids successfully recapitulated features specific to human cortical development, such as discrete cortical layers and the proliferation of progenitor zone organization with abundant outer radial glial cells in patterns reminiscent of the human fetal brain development (Lancaster et al., 2013; Muguruma et al., 2015). The ability to model human brain development using cerebral organoids offers an intriguing possibility toward the understanding of brain ontogenesis underlying the acquisition of higher cognitive functions in humans.

When combined with state-of-the-art genome editing such as the CRIPSR-Cas9 system (Cong et al., 2013; Mali et al., 2013) and/or patient-specific iPSCs, organoids can also deliver invaluable information regarding the onset and manifestation of human developmental and genetic disorders. This is particularly useful for certain human diseases that are difficult or impossible to model using animals, such as neurodevelopmental disorders. A good example is the human microcephaly that arises as a consequence of inactivating mutations in the *CDK5RAP2* gene. Cerebral organoid formation using patient-specific iPSCs revealed a previously unrealized premature neural differentiation in the progenitor zones, supporting a model in which the founder radial glial progenitors fail to properly expand, resulting in brain hypoplasia (Lancaster et al., 2013). Another example is using patient-specific iPSC-derived telencephalic organoids to understand pathophysiology associated with autism spectrum disorder (ASD) (Mariani et al., 2015). ASD-derived organoids exhibited an accelerated cell cycle and overproduction of GABAergic inhibitory neurons caused by increased *FOXG1* gene expression, lending support to the hypothesis that altered expression of FOXG1 drives a shift toward GABAergic neuron fate in ASD patients.

Despite recent exciting developments, it is only the beginning for complex tissue formation and disease modeling using hPSCs (Passier et al., 2016). There are still several challenges to be solved for hPSC-derived organoids: (1) the majority, if not all, of organoids differentiated from hPSC contain a mixture of other unwanted cell types that can potentially interfere with the interpretation of downstream assays; (2) hPSC differentiation mostly generates fetal cell types (Hrvatin et al., 2014; van den Berg et al., 2015), which also seems to be the case for hPSC-derived organoids (Dye et al., 2015; Finkbeiner et al., 2015; Takasato et al., 2015); and (3) the key to success of generation of organoids from hPSCs is the understanding of the successive signaling pathways controlling the whole differentiation process, the knowledge of which is primarily derived from animal studies and, in some cases, are not directly applicable for hPSC differentiation. Notwithstanding the success with some lineages, the lack of knowledge of many other tissues precludes the development of effective organoid generation protocols from hPSCs. Studies using primary human fetal tissues at different stages as well as using 3D organotypic cultures (Shamir and Ewald, 2014) may afford information that will facilitate broadening the utility of organoid technologies to a wider range of tissue types.

The lack of mature phenotypes in hPSC derivatives is limiting their utility in modeling postnatal/adult human development and diseases. In this regard, organoids generated from tissue-resident ASCs are more relevant. Organoid generation from primary adult tissue relies on the self-organizing capability of actively cycling, quiescent, or facultative stem cells present in several organs. ASC-derived organoids have been established for both high-turnover organs (e.g., the stomach and intestine) and slow-turnover organs (e.g., the liver and the prostate; Figure 4). Actively dividing tissue stem cells compensating for high cellular turnovers have been identified in different regions of the gastrointestinal tract. In mice, based on 3D culture and knowledge of stem cell niche signals, a series of gastrointestinal organoids has been successfully propagated in vitro, covering tissues from the tongue through to the colon (Fatehullah et al., 2016). In humans, with modification to the mouse culture, organoids from esophageal, intestinal, colonic, and gastric tissues have also been achieved (Bartfeld et al., 2015; Dekkers et al., 2013; Sato et al., 2011; Schlaermann et al., 2016). In human liver, biliary epithelial cells residing in the Canals of Hering have been postulated to function as bi-potent facultative stem cells in response to toxin injury (Yanger and Stanger, 2011). This bi-potent ductal population turned out to be amenable for in vitro propagation as liver organoids (Huch et al., 2015). In addition to liver, pancreatic and prostate organoids have also been generated from primary samples (Boj et al., 2015; Karthaus et al., 2014). Interestingly, unlike mouse, TGF-β signaling inhibition is critical for long-term expansion of human liver, pancreatic, colon, stomach, and prostate organoids, highlighting a human-specific signaling requirement for ASC-organoid culture (Huch and Koo, 2015).

Remarkably, ASC-derived human organoids can be clonally expanded from single cells without compromising their genomic stability and their capacity to faithfully recapitulate in vivo tissue architecture. These properties, together with CRISPR-Cas9 based genome and epigenome editing, provide a robust in vitro model for dissecting genetic and epigenetic components of tissue generation, disease onset and progression, and gene correction of genetic disorders (Dekkers et al., 2013; Matano et al., 2015; Schwank et al., 2013). Patient-specific ASC organoids are useful for disease diagnosis, drug screening, and potential tissue replacement therapy (Dekkers et al., 2013; Huch et al., 2015; Sato et al., 2011). In particular, organoid formation from tumor biopsies constitutes a tractable system for probing molecular and cellular mechanisms underlying neoplastic progression in many forms of human cancers (Figure 4) (Boj et al., 2015; Gao et al., 2014). Another important application of ASC organoids, which has just been realized recently, is to study human-specific host-pathogen interactions. For example, human gastric organoids were successfully used to model pathology caused by *Helicobacter pylori* infection (Bartfeld et al., 2015; Schlaermann et al., 2016).

Another potential application of ASC-derived organoids is the study of human aging. Aging represents the major risk factor for most human diseases and can be defined as the progressive decline in the ability of a cell or an organism to resist stress, damage, and diseases (Kirkwood, 2005). Aging is characterized by a series of interconnected molecular and cellular hallmarks common to different organisms that include, among others, genomic instability, telomere attrition, mitochondrial dysfunction, epigenetic alterations, and stem cell exhaustion (López-Otín et al., 2013). The progressive decline in the regenerative capacity of tissue-resident ASCs during aging represents one of the most distinctive phenotypes of old organisms (López-Otín et al., 2013). ASC exhaustion is hypothesized to be the consequence of multiple types of cell-intrinsic as well as cell-extrinsic aging-associated damage (Oh et al., 2014; Pollina and Brunet, 2011). Examples of the progressive decline in the number and quality of ASCs during age have been described in the hematopoietic and musculoskeletal systems (Conboy et al., 2003; Morrison et al., 1996). The production of adaptive immune cells diminishes with age as a consequence of a myeloid versus lymphoid bias differentiation of old hematopoietic stem cells (HSCs) (Shaw et al., 2010). Moreover, old HSCs are characterized by decreased proliferation and engraftment capacity compared to young HSCs as a consequence of accumulation of DNA damage and expression of cell-cycle-inhibitory proteins such as $p16^{INK4a}$ (Janzen et al., 2006; Rossi et al., 2007). The establishment of aging models using ASC-derived organoids has not yet been reported. It will be interesting to compare organoids derived from young versus old ASCs in terms of their molecular characteristics and differentiation capability/bias as well as their tissue organization, among others. Aged ASC-derived organoids may also help distinguish extrinsic versus intrinsic factors involved in the aging process.

It should be noted that most, if not all, organoids generated to date lack stromal, endothelial, and immune cells and thus are of limited value in modeling certain tissue functions, for instance inflammatory responses to infections (Fatehullah et al., 2016). Co-culture with stromal and endothelial components may help to generate more complex and physiologically advanced organoids in the near future. Alternatively, the multilineage differentiation capability of hPSCs can be harnessed for co-differentiation of stromal cells, together with parenchymal cells for the generation of more complex tissue organoids (Guye et al., 2016). Although organoids recapitulate certain aspects of human

cell/tissue pathophysiology, they may not allow us to study complex systemic physiology and diseases in a dish. In this regard, generation of in vivo models using hPSCs and in the form of interspecies chimeras, although a distant and not straightforward possibility today (Wu and Izpisua Belmonte, 2015a), may help extract additional layers of information for understanding human biology and disease.

Conclusion

Strategies for reproducing physiological events in vitro with stem cells and highly sensitive tools for capturing molecular complexity at the single cell level are enabling a renaissance of new insights into human biology. We have obtained a global view of the dynamic transcriptional and epigenetic programs of pre-implantation embryos; broadened the concept of human pluripotency; gained in-depth understanding of early lineage specification; and are building better in vitro models for studying human development and disease. Moreover, equipped with comparative genomics and stem cell technologies, we are digging deeper, little by little, into the molecular traits that make us unique, a long and challenging journey to reach the core of understanding about human evolution and human origins.

Looking ahead, and in parallel to biological tools, engineering principles have also been incorporated into the study of mammals, including humans, for the design, analysis, and manipulation of biological processes. For example, synthetic biology approaches have started to see fruitful applications by modifying human cells for customized sensing and response behaviors (Morsut et al., 2016; Roybal et al., 2016). Also, the unprecedented multiplexing capability of the CRISPR-Cas9 system has been recently harnessed for building synthetic multi-gene transcriptional programs for rewiring cell fates (Zalatan et al., 2015). By combining these engineering approaches with those described above on human stem cells, one is tempted to paraphrase the visionary clinician and scientist Jonas Salk in that with our imagination and courage we may not be too far from bringing into reality our dream of translating basic knowledge of human biology into actual clinical therapies.

ACKNOWLEDGMENTS

We would like to thank Dr. Jose Maria Campistol for his encouragement and discussions on the importance of thinking on the patients while working with Petri dishes, Dr. Pedro Guillen for his continuous encouragement to bring basic biology discoveries to the patient's bedside, and all members from the J.C.I.B laboratory for their experimental and conceptual contributions, which led to some of the ideas presented in this review. We would like to thank May Schwarz and Peter Schwarz for their critical reading of the manuscript. Work in the laboratory of J.C.I.B. was supported by the G. Harold and Leila Y. Mathers Charitable Foundation, the Leona M. and Harry B. Helmsley Charitable Trust (2012-PG-MED002), the Moxie Foundation, the McKnight Foundation, the Universidad Católica San Antonio de Murcia (UCAM), and Fundacion Dr. Pedro Guillen.

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