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Human trophoblast stem cell-state acquisition from pluripotent stem cells and somatic cells

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For an extended period of time, research on human embryo implantation and early placentation was hindered by ethical limitation and lack of appropriate in vitro models. Recently, an explosion of new research has significantly expanded our knowledge of early human trophoblast development and facilitated the derivation and culture of self-renewing human trophoblast stem cells (hTSCs). Multiple approaches have been undertaken in efforts to derive and understand hTSCs, including from blastocysts, early trophoblast tissue, and, more recently, from human pluripotent stem cells (hPSCs) and somatic cells. In this concise review, we summarize recent advances in derivation of hTSCs, with a focus on derivation from naive and primed hPSCs, as well as via reprogramming of somatic cells into induced hTSCs. Each of these methods harbors distinct advantages and setbacks, which are discussed. Finally, we briefly explore the possibility of the existence of trophectoderm-like hTSCs corresponding to earlier, preimplantation trophoblast cells.

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Introduction

The placenta, deemed the least-understood human organ [1,2], is a highly complex entity that is formed as the developing embryo attaches to the wall of the uterus. The trophoblast cells, which comprise the functional epithelium of the placenta, are a product of the trophectoderm (TE) of the blastocyst, following the first cell-fate decision in mammalian embryogenesis [3]. Despite the prevalence and profound implications of placental dysfunction on fetal and maternal health, precise molecular mechanisms underlying early placental development have remained largely unknown due to the lack of appropriate modeling systems. While mouse trophoblast stem cells (mTSCs) were successfully derived in 1998 [4], until recently, establishment of long-term cultures of self-renewing human trophoblast cells was impossible due to lack of knowledge regarding suitable culture conditions, while continual access to fresh early placental tissue is technically and ethically challenging. In recent years, significant breakthroughs have been made in the ability to derive and culture human trophoblast stem cells (hTSCs), owing to continuing advances in knowledge of hTSC gene circuitry, expansion of pluripotent stem cell (PSC) differentiation potential, and derivation methods from somatic cells.

Initial derivation of human TSCs

For an extensive period of time, all attempts to isolate and propagate hTSCs in vitro had failed due to lack of knowledge of the culture conditions required for the maintenance of these cells, up to a point where the very existence of hTSCs was questioned [5]. In 2018, a transformative paper was published in which hTSCs were successfully derived from blastocysts and first-trimester placentas and propagated in vitro [6]. This was accomplished by adding agonists of the Wnt pathway and Epidermal growth factor (EGF), along with inhibitors of Transforming growth factor beta (TGFβ), histone deacetylase, and Rho kinase to the culture medium. This was the first widely accepted two-dimensional in vitro culture system for hTSCs, closely followed by two additional studies utilizing a comparable medium composition to produce the first three-dimensional trophoblast organoids from first-trimester placentas, which enabled a more complex model system of interactions among trophoblast stem cells and differentiated cell types [7,8]. A number of surprising discoveries pertaining to these hTSCs included a complete lack of expression of *CDX2* and a very minimal expression of E74 Like ETS Transcription Factor 5 (*ELF5*), along with lack of expression of other genes that are known to be crucial for mTSC maintenance, such as Sex determining region Y-Box 2 (*Sox2*), *Esrrb*, and *Eomes* [6,9]. Additionally, culture conditions of mouse Trophoblast stem cells (TSCs) (mTSCs) require TGFβ activation, as opposed to inhibition of this pathway required in hTSCs [10]. Though an important discovery, these cells may only be isolated during the first

trimester. As access to first-trimester trophoblast is limited, efforts were undertaken to obtain hTSCs through alternate methods.

Derivation of human trophoblast stem cells from pluripotent stem cells: does naive state really matter?

While PSCs are by definition able to differentiate into any fetal tissue, it has long been believed that they are not capable of faithfully assuming the identity of TSCs [11,12], due to the fact that PSCs are analogous to cells that exist after the first cell-fate decision in which the inner cell mass and TE fates diverge. Thus, the establishment of PSCs with expanded differentiation potential, from which extraembryonic lineages could be derived, was intriguing. This area has recently undergone revolution in both mouse and human, through multiple works demonstrating establishment of a totipotent-like stem cell state from PSCs with the ability to give rise to both embryonic and extraembryonic cells [13–17]. One of the first works that described derivation of TSCs from PSCs utilized porcine PSCs as a tool for illuminating culture conditions for expanded potential stem cells (EPSCs, [18]). Porcine TSCs were established through culturing EPSCs in the recently established hTSC medium [6], while primed PSCs were unable to produce TSCs in this manner. Several following studies were similarly unable to derive hTSCs from primed human pluripotent stem cells (hPSCs), but successfully utilized various naive or ground-state conditions for hTSC derivation, primarily through subjecting the cells to hTSC culture medium [19–21].

Guo et al. developed a protocol of hTSC derivation from naive hPSCs by inhibition of Extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) as well as TGFβ and Activin/Nodal signaling with small molecules PD0325901 and A83-01 [22], while Io et al. utilized a pretreatment step with BMP4 and JAK inhibitor I before seeding in hTSC medium [23]. These facilitated tracing of differentiation dynamics from naive hPSCs to trophoblast lineage cells. Moreover, Guo et al. demonstrated that epiblast cells derived from late human blastocysts transdifferentiate into TE with similar treatment as naive hPSCs [22]. This strengthened the notion that human naive-like PSCs as well as their in vivo counterparts retain the plasticity required to form TE, along with an apparent loss of this capability as PSCs undergo capacitation from naive to primed.

Importantly, and in contrast to the mouse [11], these hPSC-derived TSCs harbored appropriate methylation levels throughout several key gatekeeper genes, known to be aberrantly methylated in mouse models of PSC–TSC derivation [21]. However, some aberrations in methylation have been observed in hTSCs derived from naive-like PSCs [21], notably in imprinted genes, which may play a substantial role in trophoblast function. This may be linked to global hypomethylation observed in naive-like hPSCs [24,25].

Nevertheless, derivation of hTSCs from naive hPSCs has already yielded useful information in exploring early development dynamics and disease modeling [8]. Utilizing previously established conditions for three-dimensional culture of hTSCs, Io et al. and Karvas et al. were successful in establishing trophoblast organoid cultures from naive hPSCs, which inversely recapitulated the architecture of first-trimester trophoblast in placental villi [23,26] and displayed susceptibility to known placental viral pathogens [26]. Karvas et al. additionally exploited X-chromosome reactivation in naive hPSCs and subsequent X-chromosome deactivation (XCI) in naive-hPSC-to-hTSC transition to show that trophoblast organoids undergo clonal XCI, resulting in clusters of cells with maternal/paternal XCI, similar to previous reports of human placenta [27–29].

Though the aforementioned works depicted the inability of primed hPSCs to produce hTSCs, several recent papers present findings that support hTSC derivation from primed hPSCs, many of which utilize Bone morphogenetic protein 4 (BMP4)-based conversion protocols. Long-standing controversy exists surrounding the ability of BMP4 treatment to produce bona fide trophoblast lineage cells from hPSCs [12,30–33]. A similar debate has developed involving the capacity of BMP4 to facilitate derivation of hTSCs from hPSCs. While some works deny the ability of BMP4 to induce hTSCs from primed hPSCs [19,22,23], and present evidence that BMP4 treatment of primed hPSCs results in cells with characteristics of amnion [22,23], additional works argue for faithful derivation of hTSCs from primed hPSCs utilizing specialized BMP4 treatment protocols [34–37]. For example, as BMP4 mesendodermal differentiation was evidenced to be dependent on Wingless (WNT) signaling [38], Soncin et al. utilized a BMP4 treatment protocol with the addition of WNT inhibitor IWP2 to convert primed PSCs into hTSCs [36]. Jang et al. demonstrated that prolonged exposure to BMP4 resulted in a mixed cell population with upregulation of amnion genes, while short BMP4 exposure times of 48 h resulted in optimal hTSC derivation [35]. These proponents of BMP4-directed TSC derivation demonstrate that the resultant converted cells were true TSC and not amnion by examining gene expression markers [34–36]. Indeed, Seetharam et al. compared transcriptomic data from multiple BMP4 protocols and concluded that differing elements in the protocols resulted in significantly altered consequent cell populations [39]. As such, small alterations in protocols of BMP4 treatment may be the underlying cause of expression of amnion markers in some experiments, rather than an inherent inability of primed hPSCs to be converted into genuine hTSCs. Nevertheless, full characterization of

epigenetic reprogramming in these cells is incomplete. Furthermore, some works found reduced expression levels of the trophoblast-associated C19 miRNA cluster [12]. Interestingly, a recent work by Kobayashi et al. demonstrated that upregulation of the C19 miRNA cluster in primed hPSCs before a BMP4-based conversion protocol to hTSCs restored a proliferative phenotype and rescued the ability of the resultant hTSCs to differentiate into extravillous trophoblast (EVT)-like cells, which was found in this work to be deficient [40]. However, other works demonstrated suitable differentiation capacity into EVT-like cells in hTSCs derived from primed hPSCs without the need for C19 miRNA cluster upregulation [34–36,41,42].

hPSC responsiveness to BMP4 appears to be specific to the primed state [20], though one study reported increased efficiency of derivation of hTSCs from naive-like hPSCs by adding BMP4 [23], as well as observations of increased responsiveness to BMP4 in the EPSC state [18]. A comprehensive overview of BMP4-dependant trophoblast derivation from PSCs has recently been published [43].

Further supporting the notion that primed hPSCs are capable of conversion to hTSC, Wei et al. demonstrated that hTSCs could be derived from primed PSCs independently of exogenous BMP4, albeit with low efficiency, simply through prolonged culture in hTSC medium with subsequent colony picking and expansion. Nonetheless, this process was greatly enhanced through BMP4 treatment [34]. BMP4-independent derivation of hTSCs was refined by Viukov et al., who described an induction step of primed PSCs using TGFβ and Activin/Nodal inhibitor A83-01, before seeding in hTSC medium [41]. Moreover, they showed that there was no difference in GATA Binding Protein 3 (GATA3) upregulation between primed hPSCs that underwent induction with A83-01, and cells that were subjected to a BMP4-dependent method of hPSC- to-trophoblast conversion, namely with BMP4, A83-01, and ERK/MAPK inhibitor PD0325901 (BAP). This was based on a previously established protocol for derivation of trophoblast from primed hPSCs, which included the FGF receptor inhibitor PD173074 rather than PD0325901 [32].

An interesting, albeit less widely utilized, alternative method of derivation of trophoblast cells from primed hPSCs exploits environmental cues by culturing hPSCs in mechanical conditions that encourage TE formation. Before establishment of hTSC culture conditions, cystic structures comprised of trophoblast lineage cells were reported to have been obtained from culturing hPSCs on micromesh culture systems [44,45]. hTSCs were later derived by culturing hPSCs in a similar environment and passaging the resultant trophoblastic cysts in hTSC medium [42].

A recent study examined expression dynamics during hPSC primed-to-naive-like induction and demonstrated emergence of a subpopulation of cells with PE- and TE-like signatures during this process. This TE-like subpopulation was able to give rise to hTSCs. Interestingly, this work provided evidence that this subpopulation is also able to ultimately undergo induction to naive-hPSC state through a prolonged induction process [46]. This provides further evidence for the plasticity present in early human embryonic lineages, and for the complex interplay between these cell states.

The establishment of protocols for deriving hTSCs from hPSCs constitutes a critical breakthrough in the study of early human development and in creating models for human placental function and pathology. It facilitates the procurement of hTSCs from post-gestational tissue, which allows the study of hTSCs with a specific genetic load associated with a known pregnancy outcome [47]. It additionally enables the production and study of isogenic lines of hPSCs and hTSCs, facilitating deeper understanding of the roles of various cell types in the context of specific pathologies and the interactions between them. Nevertheless, there exist some unresolved issues pertaining to derivation of hTSCs from both primed and naive-like hPSCs, including faithful epigenetic identity and expression of certain trophoblastic markers, which may be inherently linked to the derivation of hTSCs from a pluripotent state. Owing to this, efforts were undertaken to derive hTSCs without the need for intermediary pluripotency.

Derivation of human TSCs through nuclear reprogramming: is there a link to pluripotency?

Direct lineage conversion of somatic cells is an alternative to hTSC derivation from hPSCs. Through this method, cells are directly converted into a target cell identity without passing through a pluripotent state [48,49]. One possible benefit of a direct conversion approach includes circumventing the global methylation erasure associated with pluripotent states. This may facilitate retainment of an epigenetic signature reflective of a specific intrauterine environment, which may be valuable in studying certain placental dysfunctions and exposures throughout gestation. Additionally, hTSCs directly converted from somatic cells may display a more accurate gene expression profile, as explored in a recent study by Naama et al., and which has been well established in the mouse model [11].

The first evidence supporting direct conversion of somatic cells into induced TSCs was published in 2015 by Benchetrit et al. and Kubaczka et al., who demonstrated that forced expression of Gata3, Eomes, Tfap2c and Myc (GETM), or Ets2 in mouse fibroblasts can produce fully

functional mouse induced TSCs (miTSCs) that are equivalent to blastocyst-derived mTSCs in all examined parameters [50,51]. Of the first works to present a possible evidence of direct reprogramming to hTSCs was Liu et al., who mapped single-cell transcriptomics during reprogramming of fibroblasts using forced expression of the classic Yamanaka pluripotency factors, OCT4, SOX2, Krüppel-like family Transcription Factor 4 (KLF4), and not an acronym (MYC) (OSKM, [52]). In this work, fibroblasts undergoing reprogramming with OSKM were transitioned into hTSC medium at day 8 of reprogramming, presumably before the formation of induced pluripotent stem cells (hiPSCs). Thus, the successful derivation of human induced TSCs (hiTSCs) directly from fibroblasts was implied. Similarly, Castel et al. transitioned fibroblasts in OSKM reprogramming into hTSC medium at day 7, though they reported that hiTSC derivation was more efficient following culture in hiPSC TeSR Essential 7 (E7) reprogramming medium during days 7–21, with transfer to hTSC medium afterward [19]. Protocols detailing these hiTSC derivation methods have recently been published [53,54]. In both works, it is not completely clear whether these cells underwent direct lineage conversion, or acquired an early, transient pluripotent state as was shown to occur during short expression of OSKM in the mouse system [55,56].

Proposing an alternate approach for converting post-gestational somatic cells into hTSCs, two works reported successful in derivation of hiTSCs from term villous cytotrophoblasts (vCTs) [57,58], which are considerably more accessible than early placental tissue [59]. This was achieved by Bai et al. following forced expression of TE-associated transcription factors TFAP2C, TEAD4, CDX2, and ELF5, as well as ETS2, in term cytotrophoblasts. Additionally, Wang et al. characterized an antagonistic relationship between Glial Cells Missing Transcription Factor 1 (GCM1) and the stemness-associated marker ΔNp63α in human trophoblast [58]. ΔNp63α upregulation was enhanced by culturing term cytotrophoblasts in hTSC medium under hypoxic conditions, thereby facilitating derivation of hTSCs [58]. Previous works have shown extended mutagenesis and epigenetic changes acquired in placental tissue during the course of pregnancy [60,61]. This arguably poses a significant limitation to derivation of hTSCs from term placentas. Nevertheless, it is possible to conceptualize that in certain contexts, these elements would prove to be an important advantage, as the derived hTSCs may retain epigenetic properties reflecting a specific intrauterine environment during the pregnancy. Thus, these cells may prove especially helpful in modeling certain pathologies affecting placental function.

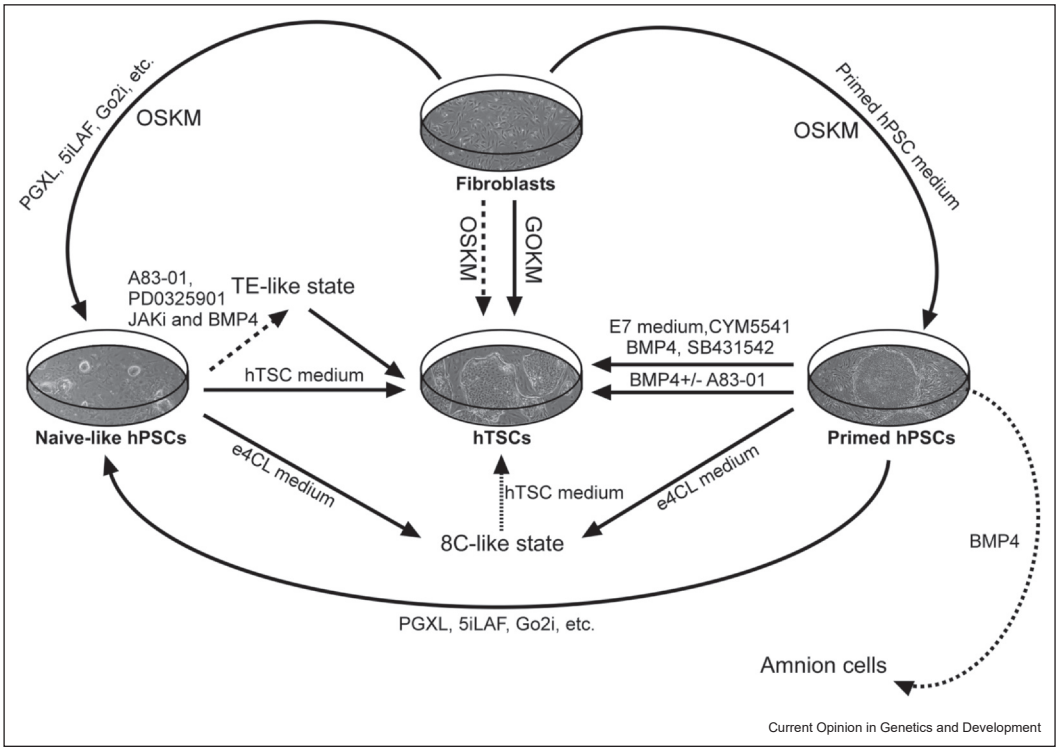
A recent study describes a method of direct lineage conversion of dermal fibroblasts into hiTSCs and three-dimensional trophoblast organoids through forced expression of GATA3, OCT4, KLF4, and MYC (GOKM) [62]. Bypassing a pluripotent state was confirmed in this work through a number of approaches, including monitoring gene expression patterns throughout the reprogramming process and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-mediated knockout of essential pluripotency genes before reprogramming. Additionally, in-depth chromatin accessibility and activity analysis revealed that while GOKM activate hTSC-specific genes and *loci* to acquire the hTSC state, OSKM primarily do so by activating genes and *loci* that are shared between hPSCs and hTSCs [62]. This was recently shown to be the case also in mouse, as OSKM and the TSC reprogramming combination GETM were shown to activate a different set of genes and *loci* from the onset of the reprogramming process, thus establishing distinct reprogramming trajectories [62]. Although assessment of the epigenetic landscape of GOKM–hiTSCs through reduced-representation bisulfite sequencing (RRBS) displayed minor differences in methylation levels from blastocyst-derived hTSCs in certain hypomethylated *loci*, gene expression profiles of GOKM–hiTSCs demonstrated a more similar transcriptome to blastocyst-derived hTSCs than OSKM–-hiTSCs [62]. This highlights the need for refinement of derivation protocols both from hPSCs and through direct lineage conversion.

Direct lineage conversion offers a helpful alternative method for deriving hTSCs, though it is important to note that the optimal source of hTSCs may vary for specific applications and must be considered carefully. While first-trimester trophoblasts and human blastocysts remain the gold standard for hTSC derivation to date, the technical and ethical difficulties involved in their acquisition along with the inability to derive these cells after pregnancy outcome limit their widespread use. Derivation of hTSCs from hPSCs and somatic cells (see Figure 1 and Table 1 for all approaches and protocols) offers invaluable options for addressing these challenges, though questions remain whether they fall short of providing perfect interchangeability with early trophoblast- and blastocyst-derived hTSCs in gene expression, epigenetic landscape, and differentiation potential.

In search of a preimplantation human trophoblast stem cell state: beyond today's gold standard

To date, the vast majority of methods for derivation of hTSCs produce cells that are similar in transcriptome to those derived in the original paper by the Arima lab [6]. Careful examination of these cells, which are currently widely accepted as the gold standard for hTSCs, reveals that they are analogous to proliferative, bipotent cytotrophoblasts of the postimplantation embryo. By

Figure 1



Graphic representation of methods for deriving hTSCs from naive-like hPSCs, primed hPSCs, and fibroblasts according to the works presented in this review. Multiple works have demonstrated that it is possible to derive hTSCs from naive-like hPSCs, primarily by seeding the cells in hTSC culture conditions. A more specific technique of hTSC derivation from naive-like hPSCs can be achieved through inhibition of ERK/MAPK and TGFβ Activin/Nodal signaling with small molecules PD0325901 and A83-01. Primed hPSCs were shown to give rise to hTSCs following treatment with BMP4, though induction with BMP4 has also shown to produce amnion-like cells. A BMP4-independent method of hTSC derivation from primed hPSCs includes an induction step with A83-01. The possibility of transient acquisition of a TE-like state during derivation of hTSCs from both primed and naive-like hPSCs has been proposed, which has been further explored in two papers denoted in the review. In addition, both primed and naive-like cells have recently been converted into 8C-like cells, which are likewise capable of giving rise to hTSCs. Derivation of hTSCs from fibroblasts has been suggested in two works that isolated hiTSCs following reprogramming of fibroblasts with OSKM. Another method, which has been verified to be pluripotency-independent, is reprogramming of fibroblasts with alternate transcription factors GOKM. Dashed lines represent possible state of cells.

comparing gene expression of these cells to early human embryos, Castel et al. discovered that hTSCs are most similar to postimplantation day-8–10 cytotrophoblasts [19], while Dong et al. found that they share the strongest transcriptional correlation with human cytotrophoblasts at day 12 post fertilization [20].

As mentioned above, one striking difference between these cells and human TE is a lack of expression of the human and mouse TE marker, Caudal type homeobox 2 (CDX2) [69]. Interestingly, multiple works reported a transient upregulation of CDX2 during hTSC derivation from hPSCs [22,23,37,41]. Some studies additionally present evidence of a TE-like expression signature in early stages of various derivation protocols, through transcriptome comparison with early embryonic lineages [22,52]. Specifically, Guo et al. and Io et al. performed single-cell transcriptome analysis tracing differentiation trajectories during derivation of trophoblast from naive hPSCs and compared with data of early embryos. They uncovered transcriptional similarity to TE during early stages of differentiation, with progression to post-implantation cytotrophoblast in the following days [22,23]. These findings support the notion that cells transiently pass through an earlier TE-like state during the derivation process, which may not be supported under current hTSC culture conditions, and provide a valuable tool in studying the dynamics of cell-fate acquisition during early human embryonic development.

Indeed, a study by Mischler et al. highlighted the formation of a CDX2-expressing cell population in conjunction with a hTSC derivation protocol from hPSCs [37]. These cells were maintained under distinct culture conditions that included sphingosine-1 phosphate receptor agonists CYM5541, CHIR99021, A83-01, and FGF10. However, these cells displayed an altered differentiation capacity, including an inability to form EVT-like cells, and failed to show transcriptional similarity with TE cells.

Table 1

Summary of the works describing derivation of hTSCs, which are presented in this review.

Study	Cells of origin	Target cells	Strategy
Mazid et al. [17]	Primed and naive hPSCs	8C-like cells	Primed and naive PSCs were cultured in e4CL medium (consisting of PD0325901, IWR-1, human LIF, Activin A, L-ascorbic acid, and high concentrations of DZNep and TSA) to produce 8C-like cells. These were able to be converted into TSCs by seeding in a modified hTSC medium.
Gao et al. [18]	Primed hPSCs	EPESCs	Inhibition of GSK3, SRC, and Tankyrases with small molecules CHIR99021, A419259, and XAV939, respectively, as well as supplementation with vitamin C, Activin A, IWR-1 and LIF. TSCs were established through culturing EPSCs in TSC conditions (Okae et al., 2018)
Castel et al. [19]	Fibroblasts, primed and naive PSCs	hTSCs	Overexpression of OSKM transgenes in fibroblasts and transferring to TSC medium produced hTSCs. hNPSCs [63] and extended hPSCs [64], but not primed hPSCs, converted into hTSCs following transition into hTSC medium.
Dong et al. [20]	Primed and naive PSCs	hTSCs	Naive PSCs cultured in 5i/L/A and PXGL conditions [65,66], but not primed hPSCs, produced hTSCs following transition into hTSC medium.
Cinkornpumin et al. [21]	Primed and naive PSCs	hTSCs	Naive PSCs cultured in PXGL and 5iLAF conditions [65,67], produced hTSCs following transition into hTSC medium. Primed cells produced a population that expressed markers of both hTSCs and amnion cells.
Guo et al. [22]	Naive PSCs and epiblast obtained from late human blastocysts	TE-like cells and hTSCs	Naive PSCs cultured in PXGL [67], as well as E6–E7 human epiblast were transdifferentiated through inhibition of ERK/MAPK and Nodal signaling with small molecules PD0325901 and A83-01.
Io et al. [23]	Primed and naive PSCs	TE-like cells and hTSCs	Naive PSCs were transdifferentiated into TE-like cells and hTSCs with A83-01, PD0325901, JAK inhibitor I, and BMP4; while primed PSCs treated with BMP4, A83-01, and PD173074 (BAP) exhibited characteristics of amnion.
Wei et al. [34]	Primed PSCs	hTSCs	Prolonged culture of primed PSCs in TSC medium and subsequent colony picking resulted in TSCs, which was enhanced with BMP4 treatment
Jang et al. [35]	Primed PSCs	hTSCs	Culture of primed PSCs in TSC medium with BMP4 for 48 h resulted in optimal hTSC derivation, prolonged exposure to BMP4 resulted in a mixed population with upregulation of amnion markers
Soncin et al. [36]	Primed PSCs	hTSCs	Primed PSCs were converted into hTSCs through treatment with BMP4 and a WNT inhibitor, IWP2 [68], and subsequent culturing in a modified TSC medium for 5 passages [57].
Kobayashi et al. [40]	Primed and naive PSCs	hTSCs	Naive hES cells were converted into hTSCs through culturing in hTSC medium following transient exposure to N2B27 medium without 5i/L/A. Primed hES cells were treated with BMP4 for three days and then cultured in hTSC medium. The resultant hTSCs exhibited deficient proliferation and differentiation capacities, which were restored upon upregulation of the previously underexpressed C19 miRNA cluster.
Viukov et al. [41]	Primed PSCs	hTSCs	Primed PSCs underwent an induction step with medium that included N2B27 and TGF-beta inhibitor A83-01 for 5 days, and then were seeded into hTSC medium. Homogeneous TSC populations emerged after 1 or 2 passages in TSC medium.
Li et al. [42]	Primed PSCs	hTSCs	hTSCs were derived by culturing primed PSCs on a micromesh culture system with formation of trophoblastic cystic structures, and subsequent seeding and passaging in hTSC medium
Liu et al. [52]	Fibroblasts	hiTSCs	Reprogramming of fibroblasts with OSKM factors and transfer to hTSC medium at day 8 produced hiTSCs
Bai et al. [57]	Term vCT	hiTSCs	hiTSCs were derived from term vCT through forced expression of TFAP2C, TEAD4, CDX2, ELF5, and ETS2
Wang et al. [58]	Term vCT	hiTSCs	After establishing an antagonistic relationship between GCM1 and stemness regulator ΔNp63α, hiTSCs were derived from term vCT through culture in TSC medium under hypoxic conditions, thereby causing downregulation of GCM1 and upregulation ΔNp63α.
Naama et al. [62]	Fibroblasts	hiTSCs	Fibroblasts were converted into hiTSCs through forced expression of GOKM in a pluripotency-independent manner

Table 1 (*continued*)

Study	Cells of origin	Target cells	Strategy
Mischler et al. [37]	Primed PSCs	hTSCs and TE-like cells	hESCs were transferred to E7 medium supplemented S1PR3 agonist CYM5541, BMP4, and activin inhibitor SB431542, which produced hTSCs and a CDX2+ population, which was cultured in medium containing CYM5541, CHIR99021, A83-01, and FGF10.

EPESCs, Extended potential embryonic stem cells; PD032590, XAV939, Gö6983, LIF (PXGL)

The creation of a stable in vitro cell system analogous to an earlier, preimplantation TE state would be highly useful in the study of human implantation dynamics and early placental development. Further study is needed in order to achieve this goal.

Successful derivation and culture of TE-like cells, as well as utilizing the full potential of current hTSCs, hinges on the ability to clarify mechanistically the establishment of the hTSC state from hPSCs, somatic cells, and in the early human embryo. Multiple recent works have provided insights into the molecular mechanisms of hTSC-state acquisition. As mentioned above, Kobayashi et al. suggested the importance of the C19 miRNA cluster in establishing self-renewing hTSCs with full differentiation potential [40], while other works highlighted ERK/MAPK, Janus kinase (JAK) I and TGFβ, and Activin/Nodal signaling inhibition in hTSC derivation from primed and naive hPSCs [22,23,41]. The role of BMP4 in trophoblast derivation remains somewhat controversial. Naama et al. demonstrated the specific importance of appropriate demethylation in obtaining fully functional hTSCs [62]. A recent work by Zijlmans et al. implicates polycomb-repressive complex 2 (PRC2) as a safeguard against trophoblast differentiation from hPSCs and demonstrated that inhibition of PRC2 activity results in accelerated hTSC derivation from hPSCs [70]. The utilization of recently developed methods for culturing human blastoids, which mimic properties of human blastocysts, will no doubt provide additional understanding of the dynamics in establishment of a TE-like state [71,72]. Further work in elucidating the mechanisms of hTSC derivation and TE-like state acquisition will be highly useful in advancing knowledge in this field of study.

Concluding remarks

In the past few years, astonishing advances have been made in the ability to culture and characterize human TSCs. These breakthroughs are expected to support further study of early human embryonic development, as well as study of placental development and dysfunction and its significant impact on human health, an area of knowledge that hitherto has been lacking. Multiple approaches have been taken in the endeavor to derive bona fide hTSCs from various sources, as described above. Nevertheless, work remains in fully characterizing and optimizing methods of hTSC derivation, and it is clear that future mechanistic work is vital to advance the field. We anticipate that this field will persist in its explosion of productivity and will continue to facilitate much-needed research in critical areas of study.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

There is no conflict of interest. Prof. Buganim and Dr. Naama hold an issued patent for the generation of hiTSCs from somatic cells.

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- •• of outstanding interest.

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