Human Germline Development from Pluripotent Stem Cells *in vitro*

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Abstract

Mammalian primordial germ cells (PGCs) are specified in the early post-implantation embryo. Attempts have been made to establish *in vitro* PGC development since the derivation of embryonic stem cells (ESCs) from blastocysts. Despite the advances made with mouse models, similar studies in human germ cell development have not progressed because practical and ethical reasons prevent the use of early human embryos. Recently, we and others developed a robust *in vitro* system for producing human primordial germ cell-like cells (hPGCLCs) from ESCs and induced pluripotent stem cells (iPSCs) by inducing competency for germ cells. Strikingly, the molecular mechanism for germline differentiation is not fully conserved between mouse and human, probably because of the differences in their early embryogenesis and regulation of the pluripotent state. Here, we present a review of the current status in the field of *in vitro* germ cell production from pluripotent stem cells, and discuss how its usefulness could be extended to clinical applications.

Introduction

The fate of human germ cells is determined during weeks 2-3 of embryonic development [1, 2]. Since early human embryos are not accessible for practical and ethical reasons, most of the knowledge of mammalian germ cell development is based on studies of mice. While some animals, such as *Xenopus laevis, Caenorhabditis elegans* and *Drosophila melanogaster*, carry maternal pre-determinants of germ cell fate, the mouse, as well as the axolotl and cricket, show specification of PGCs from a subset of competent precursors induced by signaling factors that include bone morphogenetic protein (BMP) and Wnt which are secreted from surrounding tissues [3-5]. The nascent PGCs express key transcription factors, such as BLIMP1, TFAP2C and SOX17 (in human), establishing a molecular network for germ cell fate, which also initiates the germline epigenetic program [6]. Following gastrulation, PGCs migrate through the hindgut towards the embryonic testes/ovaries, called genital ridges or gonads. The specified PGCs then undergo epigenetic resetting which includes DNA de-methylation and changes in histone modifications [3, 7]. Finally the PGCs undergo sex-specific differentiation and enter meiosis to complete gametogenesis (Figure 1) [8].

The mouse has been the main model organism for studying mammalian early germ cell

development. Based on knowledge obtained from *in vivo* studies, defined and robust protocols for inducing PGC-like cells (PGCLCs) *in vitro* from mouse pluripotent stem cells (PSCs) have been established [9, 10]. PSCs are embryonic stem cells (ESCs) derived from the inner cell mass (ICM) of blastocysts, or induced pluripotent stem cells (iPSCs) derived from somatic cells [11-14]. When introduced back into blastocysts, PSCs have the potential to contribute to the germline *in vivo*. Furthermore, mouse PGCLCs (mPGCLCs) can be induced from naïve PSCs through the induction of competent "epiblast-like cells (EpiLCs)"[9]. After injecting mPGCLC into mouse testes or ovaries, they can further develop into functional sperm or eggs, respectively [9, 10]. Recently, Zhou et al. claimed that their culture protocol allows the produce of haploid male germ cells from *in vitro* mPGCLCs through meiosis [15].

Researchers have made various attempts to understand human PGC development. Although our current knowledge of PGC development originates from mouse studies, the morphology of early human embryos, especially the epiblasts where PGCs are induced, differs from the mouse. Extra-embryonic ectoderm (ExE) that develops over the epiblast tissue in mice, and is the source of BMP4 that induces PGC fate in mice, is not present in human postimplantation embryos [16, 17]. While mouse epiblasts develop as an egg-

cylinder, human epiblasts develop as a flat bi-laminar disc, a feature that is also observed in a number of non-rodent mammals [16].

Since early human embryos are not available for research purposes, it would be a significant breakthrough to have a robust *in vitro* human germ cell development model that allows the study of the mechanism regulating germ cell fate and epigenetic dynamics. Moreover, such as *in vitro* model could be used for investigating the inheritance of mutated epigenetic modifications and mitochondria, causes of infertility, germ cell tumors and other related disorders. However, it is not straightforward to directly adopt the mouse *in vitro* PGCLC induction model for similar studies for inducing human germ cells from PSCs, since the regulation of pluripotency, including cell morphology and required signaling, differs between mouse and human [16, 17]. Attempts to induce germ cells *in vitro* using conventional human PSCs are dependent on their spontaneous differentiation which occurs with low efficiency, and the resulting PGC/germ cell-like cells are not well characterized [18, 19].

Recently, we and others have reported a robust and defined protocol for inducing human PGCLCs (hPGCLCs) from competent hPSCs [20, 21]. The global gene expression profile of the hPGCLCs induced using our method is similar to those of *in vivo* human PGCs and

seminoma, a testicular cancer known to have features characteristic of hPGCs. Our hPGCLC induction system has allowed the identification of key hPGC specification factors and cell surface markers that are crucial for the isolation of hPGCLCs as well as hPGCs and seminoma [20]. Interestingly, hPGCLCs induced from both of the two protocols demonstrate the characteristics of pre-migratory PGCs that do not develop further under the same culture condition [20, 21]. Below, we discuss the current progress of *in vitro* germ cell development from pluripotent stem cells based on the *in vivo* differentiation, and potential applications of this system.

Germ cell development: human versus mouse

After fertilization, zygotes undergo cleavage divisions and form blastocysts that consist of trophectoderm and ICM after 3.5 days. ICM cells develop into primitive endoderm and epiblast, which give rise to all the cell lineages of the embryo proper. Importantly, ESCs can be derived from ICM cells *in vitro*, which can self-renew and maintain their pluripotency with the potential to differentiate into three germ layers and germ cells. Blastocysts develop into post-implantation epiblasts, where PGCs are specified, around week 2 in human, and embryonic day (E) 6.5 in mice. Notably, the morphology of the epiblast stage embryos of rodents and non-rodents, including those of humans and pigs, is clearly different [16, 22].

Rodents have cup-shaped epiblasts with ExE on top. ExE secrets BMP4, a critical factor for inducing PGCs in the subpopulation of epiblast cells in the proximal posterior region [3, 23]. Wnt signaling from the posterior primitive endoderm is also important for PGC specification [24, 25]. Human epiblasts on the other hand, are disc-like and consist of two layers, an epiblast with a primitive endoderm (hypoblast) beneath. Notably, the tissue equivalent to ExE in the mouse is not observed in human epiblasts. The bilaminar epiblast seems to be more common in mammals such as rabbits and pigs. In the rabbit, specified PGCs expressing BLIMP1 can be found in the epiblast during the postimplantation development stage when BMP2 and BMP4 are expressed in the surrounding tissue [26]. Expression of BMP2 and BMP4 in the equivalent stages of development has also been demonstrated in pig embryos [27]. These findings suggest that BMP-signaling seems to be important for PGC specification in non-rodent species as well. In mice, nascent PGCs establish the molecular network for germline development by expressing the key factors of BLIMP1, TFAP2C and PRDM14, while the expression of these factors has not been found in humans [28, 29]. The specified PGCs start to migrate in peri-gastrulation embryos around week 4-5 in humans and E9.5 in mice and go through the hindgut to reach the genital ridge [2, 3]. At this stage, PGCs undergo dynamic epigenetic resetting including global DNA demethylation and changes in histone modifications [1, 2, 3, 7, 8, 30]. PGCs in the gonads undergo sex-specific

differentiation around week 9-10 in human and E12.5 in mice [2, 8]. Germ cell sex determination is largely dependent on interactions with somatic cells in the gonads. Male sex determination is marked by the expression of the sex determining region Y (SRY) and the SRY-related HMG-box 9 (SOX9) in gonadal somatic cells. Male PGCs enter mitotic arrest and remain in G_1/G_0 until after birth, when the cell cycle, meiosis and spermatogenesis resume. On the other hand, female PGCs undergo meiosis through the leptotene, zygotene and pachytene stages in the fetal ovaries and get arrested at the diplotene stage of prophase I around the time of birth. Female germ cells undergo folliculogenesis, followed by the first meiotic division upon hormonal stimulation after birth and the completion of the second meiotic division upon fertilization [8]. Retinoic acid (RA) is one of the critical factors required for meiotic entry in both female and male germ cells. RA induces meiotic entry in the embryonic ovary; however, it is degraded by Cyp26b1 in the embryonic testis in order to prevent the mitosis/meiosis transition during development, since male germ cells undergo meiosis only after birth [31].

Induction of human germ cell fate from pluripotent stem cells

Previous attempts to induce human germ cell fate were based on spontaneous differentiation using conventional hPSCs. They demonstrated expression of some germ cell

markers such as VASA and DAZL. However, global gene expression profiling and further characterization were not performed due to the low efficiency of the induction [32-34]. Recently, we and others established an efficient and defined protocol for *in vitro* human PGCLC induction from pluripotent stem cells [20, 21]. Taking into account the step-wise induction of mouse PGCLCs, it was important to first establish the “competent state” in human PSCs as a crucial step towards efficient hPGCLC induction [9, 20, 21]. In our system, when human PSCs are cultured with 4 inhibitors (4i) of GSK3β, MEK, p38 and JNK, they exhibit increased competency for germ cell fate [20]. The 4i state can be maintained and reversed to the conventional state in PSCs. Approximately 10-50% of 4i hPSCs can be specified as hPGCLCs in the presence of BMP2/4 and the other cytokines by forming small cell aggregates, called embryoids. The 3D structure seems to be important for the hPGCLC specification event. The cell number in the embryoids increases over 5 days with germ cell-specific NANOS3-positive cells appearing around day 3 after hPGCLC induction. The resulting PGCLCs have a global transcriptome similar to those of *in vivo* gonadal PGCs and seminoma cells. Interestingly, these PGCLCs show the initiation of epigenetic resetting which is indicated by increased levels of hydroxymethyl-cytosine together with down-regulated methyl-cytosine in a global and locus specific manner [1, 20]. Sasaki et al. cultured hPSCs in preformulated manufactured feeder-free medium and showed there was PGCLC

induction with an efficiency of around 20% after the addition of BMP4 and the other cytokines to the cell aggregates [21]. Incipient mesoderm-like cells (iMeLCs) are induced from hPSCs in the presence of Activin A and a Wnt-signaling agonist. The hPSCs obtain competency toward PGCLC differentiation with up to 60% efficiency between 42 and 48 hours after iMeLC induction. The resulting PGCLCs show a global gene expression profile similar to hPGCLCs induced from 4i hPSCs. Both iMeLCs and 4i hPSCs express mesoendodermal markers, which suggests that the competent states are a peri-gastrulation epiblast-like state [20, 21]. Importantly, PGCLCs induced using both protocols do not express later gonadal PGC markers such as DDX4 and DAZL, even after prolonged culture, suggesting that early human PGC specification and the ensuing development require distinct signaling pathways and environments as is also the case for the mouse (see below).

Molecular mechanism of human PGC specification

Global analysis of the commonly expressed genes of the three human germ cell related cells, hPGCLCs, gonadal PGCs and seminoma, has shown there is a unique gene expression pattern compared to mouse PGCs [1, 20]. One of the SOX family transcriptional factors, SOX17, rather than SOX2 in the case of the mouse, has been found to be expressed in human PGCs/PGCLCs/seminoma, while BLIMP1 is expressed as a conserved gene in both

human and mouse PGCs. In addition to SOX17, SOX15 is expressed in human PGCLCs and PGCs [21, 30]. Moreover, some other genes such as KLF4, TEAD4 and GATA4 are uniquely expressed in human PGCs, but not in the mouse counterpart. Conversely, the pluripotency gene ESRRB seems to be more dominantly expressed in mouse than in human PGCs [1, 20]. During mouse PGC development, the mesodermal gene T is expressed in PGC competent epiblast cells and is important for the induction of BLIMP1 and PRDM14 in mouse PGCs [25]. Expression of T is observed at a low level in competent human 4iPSCs and it increases in the whole cell aggregates after 24 hours of PGCLC induction. Interestingly, T expression is lower in PGCLCs than in the surrounding cells [20]. This suggests that human PGCLCs are specified in mesodermal-type cells, which are subsequently repressed after hPGCLC specification [20]. SOX17 is found to be one of the earliest genes expressed in a subset of the cells scattered in the cell aggregates. This marks the initiation of PGCLCs, and is followed by BLIMP1 expression in most of these cells that also become NANOS3 positive PGCLCs. Notably, while loss of SOX17 abolishes hPGCLC differentiation, loss of BLIMP1 induces mutant nascent PGCLCs that show up-regulation of somatic genes including endodermal markers, which might be induced by SOX17 [20]. This suggests that although SOX17 is important for the endodermal lineage, the combination of SOX17 and BLIMP1 is the key intrinsic driver for human PGC specification. The temporal

and spatial regulation of cell fate determination by SOX17 and BLIMP1 would be of great interest to investigate. BLIMP1, TFAP2C and PRDM14 are known to be the key PGC specifiers in the mouse [28, 29]. While BLIMP1 and TFAP2C expression are present during human PGCLC differentiation, PRDM14 expression is not detected in day 1 and day 2 hPGCLCs [20], suggesting that PRDM14 is not involved in early human PGC specification unlike mouse but seems important for later events such as epigenetic resetting. Sugawa et al. reported that the knock down of PRDM14 doesn't affect their hPGCLC induction [35]. The other pluripotency genes OCT4 and NANOG are expressed in ～75% and ～35% of nascent PGCLCs, respectively [20]. Thereafter, almost all the specified PGCLCs become positive for OCT4 and NANOG. The pluripotency genes, which are expressed in the competent precursors, diminish in expression in the nascent PGCs but their expression is restored in specified PGCs, similar to in the mouse [3]. Since PGCs are one of the unique cell types that express pluripotency genes, it is interesting to investigate how pluripotency genes are involved in epigenetic programing in the PGCs for the next generation. Interestingly, while the naïve markers TFCP2L1 and KLF4 are expressed in hPGCs/PGCLCs in addition to human ICM and reset hPSCs, KLF17 seems to be specific to ICM and reset cells [1, 36-38]. The *in vitro* human PGCLC differentiation system demonstrates that BMP2/4 signaling is critical for PGC specification, similar to in the mouse. It most likely activates the downstream

SMAD1/5/8 signaling pathway. This suggests that the same signaling pathways establish different molecular networks in human and mouse germ cell lineage. It might be related to fundamental differences in early embryogenesis and/or the epigenetic setup in the competent germ cell precursors of human and mouse PGCs.

Isolation of human PGCs

It is very important to be able to purify and separate the PGC/PGCLC population from the tissue or heterogeneous cell culture population for downstream applications. We have established hESC lines carrying mCherry with a 2A peptide linker tethered to the PGC specific marker NANOS3 immediately upstream of the stop codon [20]. Sasaki et al. have established hiPSC lines with BLIMP1-2A-tdTomato and TFAP2C-2A-EGFP [21]. These allow the isolation of the hPGCLC population from the aggregates after PGCLC induction, based on the gene expression. However, since we cannot perform gene editing on the cells in *in vivo* tissue, instead specific cell surface markers can be used for isolating and detecting the PGC/PGCLC population from *in vivo* cells and other *in vitro* cell lines. We recently identified a combination of two cell-surface proteins, tissue-nonspecific alkaline phosphatase (TNAP) and CD38, as markers specific to human PGC [20]. TNAP is a classical and conserved

marker of PGCs [39, 40]. Although TNAP is expressed in hPSCs, it becomes hPGCLC-specific in differentiated aggregates on day 4. CD38 expression, on the other hand, can be detected in hPGCs and NANOS3-positive PGCLCs, but not at earlier stages. In contrast to embryonal carcinoma (EC) and hPSCs, seminomas express CD38 rather than CD30 [41]. It would be of great interest to investigate the respective relationships of CD30/SOX2 and CD38/SOX17 in EC/ESC and PGC/seminoma [42]. KIT is also expressed in hPGCs and can be used as a specific marker of PGCs in combination with TNAP [1, 32]. While KIT is expressed in PGCLCs, it is undetectable at the cell surface. This is most likely due to the presence of stem cell factor (SCF) in the PGCLC induction medium, which induces the internalization of the ligand, KIT [43, 44]. Finally, other cell surface markers, such as EpCAM, INTEGRINα6, and INTEGRINβ3, can also be used to isolate PGCLCs [1, 20, 21, 32].

Maturation of in vitro-induced PGCs

The currently defined protocols for PGCLC induction from pluripotent stem cells provoke early PGC specification and the resulting cells halt at a developmental stage before the onset of meiosis and gametogenesis under these culture conditions. While human PGCLCs display characteristic features of pre-migratory PGCs, mouse PGCLCs induced with BMPs are equivalent to the mouse PGCs at E9.5-12.5 which are migratory-gonadal PGCs (Figure

1) [9, 10, 15, 20, 21]. Importantly, these mouse PGCLCs are able to develop further and differentiate into functional gametes following injection into mouse tissues [9, 10]. Purified male PGCLCs undergo spermatogenesis 10 weeks after injection into seminiferous tubules of neonatal mice. The spermatozoa developed from PGCLCs can be used for fertilization by intracytoplasmic sperm injection (ICSI) and give rise to healthy offspring by transfer of the resulting embryos into a foster mother. The efficiency of mouse PGCLC maturation and function seems to be comparable to the same procedure using *in vivo* PGCs [9]. For females, PGCLCs are isolated and reconstituted with female gonadal somatic cells from E12.5 to form aggregates [10, 45]. PGCLCs in the aggregates start to express late PGC markers DDX4 and DAZL after 3-6 days, as well as the meiotic markers SYCP3 and Stra8 after day 9. Day 3-4 aggregates show X chromosome reactivation and imprinting erasure. The aggregates can be transplanted into the ovarian bursa of adult immunodeficient mice where they undergo oogenesis to form follicles. However, the PGCLC-reconstituted ovaries show instability in cumulus cell-oocyte complex formation, and the germinal vesicle stage oocytes from PGCLCs exhibit increased frequency of cytoskeletal immaturity and/or fragility [10]. PGCLC-derived oocytes can undergo *in vitro* maturation followed by *in vitro* fertilization, and develop into two-cell embryos with an efficiency comparable to that of *in vivo* oocytes. Around 40% of the two-cell embryos from PGCLCs further develop into blastocysts *in vitro*.

Half of the PGCLC-derived zygotes formed by *in vitro* fertilization (IVF) produce three pronuclei at the pronuclear stage, which is abnormal. Embryos transferred to foster mothers gave newborn pups from the PGCLC-derived two-cell embryos at an efficiency of around 4%, and the offspring displayed a normal imprinting pattern and was fully fertile [10].

Recently, Zhou et al. demonstrated a method to induce meiosis *in vitro* using mouse male PGCLCs [15]. They optimized the mouse PGCLC induction protocol published by Hayashi et al., and obtained PGCLCs differentiated to a stage equivalent to mouse E12.5. Male PGCLCs were mixed with early postnatal (day 2 to 8) testicular somatic cells that support further male germ cell development. The mixed cells were then cultured in the presence of retinoic acid, BMPs and ActivinA for 6 days and started to express later germ cell markers such as *Ddx4*, *Stra8* and *Dmc1*, whose expression is normally found in spermatocytes. On the other hand, the earlier markers Blimp1 and Stella were down-regulated at this stage. On day 7, the protocol dictates a switch from cytokines to sex hormones, follicle-stimulating hormone, testosterone and bovine pituitary extract. The meiotic marker, Prm1 started to be expressed after 10 days of culture. In addition to Prm1, expression of haploid spermatid markers such as Tp1, acrosin and haprin were observed from day 14. In agreement with this, around 14-20% of the cells showed haploid (1C) DNA content. The spermatid-like cells

exhibited male-specific imprinting patterns on H19 and Snrpn loci. Spermatid-like cells were fertilized by ICSI and about 90% of the injected zygotes developed to the two-cell stage after activation. Despite the low number of examples, 2-5% of the embryos that were fertilized with spermatid-like cells were born and developed normally to adulthood, while the birth rate following ICSI with round spermatids isolated from normal testes is 9.5%. The resulting mice, which developed from *in vitro* spermatid-like cells, were capable of producing offspring [15]. While the report of Zhou et al. is interesting, it needs further statistical support to establish the validity of its results.

Perspectives and applications of in vitro human germ cell development

Although efficient *in vitro* human PGC specification methods have been established, currently there is no available protocol to induce further development of nascent hPGCLCs, especially in a defined and robust manner. While mouse studies have proposed some methods for advanced *in vitro* PGCLC development, the fundamental differences in molecular mechanisms between mouse and human PGC development and the lack of access to human early embryos make this research rather challenging. Furthermore, *in vitro*-derived human PGCLCs exhibit features slightly earlier than in *in vitro*-induced mouse PGCLCs. This might be due to differences in the germline competent states of the

pluripotent stem cells of mice and humans, each of which depend on different signals [9, 10, 20, 21]. It is also possible that the timing of each PGC gene activation, epigenetic programming and signaling, such as BMPs and Wnts, during early PGC development may be fundamentally different between mice and humans, possibly due to differences in their epiblast morphologies and the timing of the initiation of gastrulation [16, 17]. Interestingly, early human embryogenesis is more similar to non-rodent mammals such as pigs and rabbits, than rodents. It would be of great interest to investigate non-human primate, rabbit or pig PGC development as potential models. Currently, one of the most advanced *in vitro* PGC development mouse models is still largely dependent on spontaneous differentiation induced by unknown factors from supporting cells, such as gonadal somatic cells, and the induction efficiency seems to be both low and variable. It would be worth examining the co-culturing of human PGCLCs with appropriate gonadal somatic cells, such as primate, pig and rabbit cells, to induce their further differentiation. It is also important to identify the key factors expressed by gonadal somatic cells that might support further development of human PGCs. In order to establish a human germline *in vitro* model, understanding of the stepwise and sexual dimorphic differentiation of the *in vivo* germline is crucial, and further detailed study of the development of human gonadal somatic cells and their dynamic interaction with germ cells is required.

Development of hPGC derived from hPSCs would be a powerful model for addressing molecular mechanisms involved in this cell fate decision and epigenetic dynamics, and the disorders that might cause their misregulation. For instance, patient specific iPSCs could be used to investigate mutations that might underlie specific diseases. In addition, new gene editing techniques, such as the CRISPR-Cas9 system, could facilitate gene manipulation of hPSCs to generate specific mutations. The germline is responsible for the transmission of genetic and epigenetic information to subsequent generations. Aberrant epigenetic modifications including imprinting and mutant mitochondria, and their transmission through the germline can cause human diseases such as metabolic disorders, obesity, neuronal disorders and cancers [46, 47, 48]. Aberrant information could be induced by environmental factors, which may have an enduring impact over many generations, although the mechanistic basis for such claims remains unclear. Recently, we found that some single copy loci evade germline epigenetic programming, and their predominant expression in the brain is apparently associated with neuronal disorders and other diseases such as obesity [1]. *In vitro* models may provide opportunities to elucidate the mechanisms behind their escape from reprogramming, which may also be relevant for the inheritance of environmentally induced epigenetic transgenerational modifications.

An *in vitro* hPGC developmental model could be used to advance research in many areas, for example for elucidating the role of non-coding RNAs, including piRNAs, on regulating transposable elements, and for further development towards gametogenesis. The regulation of the 'mitochondrial bottleneck' and mitochondrial mutations could also be examined in *in vitro* human PGCs, which are implicated in the pathogenesis of late onset disorders. Cancers of germ cell origin, such as seminomas and embryonal carcinomas, as well as paediatric brain tumours called germinomas, also merit further investigations, which could be investigated using *in vitro* models. Advances the production od in *in vitro* derived germ cells could also potentially lead to the development of gametes from somatic cells via iPSCs. This would be a valuable tool for understanding the molecular basis of treatments in germ cell diseases and infertility. However, there are ethical issues, which need to be considered carefully when considering research on germ cells.

Conclusion

Human gem cell development *in vitro* induced from hPSCs would advance many fields of research, such as germ cell biology, epigenetic research, reproductive medicine, and cancer research, since they would facilitate studies of molecular mechanisms, genetics and epigenetics, and potential drug screening using patient specific iPSCs. Numerous attempts

have been made to induce mouse and human germ cell development using *in vitro* pluripotent stem cells, ESCs and iPSCs, representing early embryos [18, 19]. In this review, we have mainly focused on the most defined and robust *in vitro* PGCLC production protocols for human and mouse. Thus, we have focused on early PGC specification and differentiation events with well-defined isolation protocols for PGCLCs. Surprisingly, the protocols and molecular mechanisms involved in PGC specification differ between humans and mice. This may be because humans and mice already display differences in early embryogenesis, especially in the epiblasts where PGCs are specified. The current protocols for human PGCLC induction produce germ cells that develop up to the pre-migratory stage. However, mouse PGCLCs can also differentiate into the peri-migratory or gonadal stages, and are able to become functional gametes that produce offspring with the support of appropriate cells or tissues from the mouse testis/ovary (Figure 1). Currently, mouse PGCLC maturation seems to require some factors from primary somatic cells inhabiting the testis or ovary. Thus, given that the embryology and the molecular mechanisms of PGC development are different between human and mouse, applying mouse protocols to humans and other mammals requires careful consideration. Although the development *in vitro* human germ cells would lead to broad scientific advances in basic science and clinical research, the ethical issues will need careful consideration.

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Figure legend

Figure 1. Germ cell development: *in vivo* versus *in vitro*

Comparison of "*in vivo*" and "*in vitro*" germ cell development in human and mouse. *In vitro* germ cells are induced from pluripotent stem cells. Mouse *in vitro* derived-PGCs can undergo meiosis *in vitro* or in mouse tissue, which can produce offspring through intracytoplasmic sperm injection (ICSI). On the other hand, human *in vitro* PGC development currently arrests at the stage, presumably equivalent to the pre-migratory PGCs in week 3-4 embryos.

Key words (5)

Human primordial germ cells, primordial germ cell-like cells, pluripotent stem cells, human development, SOX17

Japanese Abstract

哺乳類の始原生殖細胞は発生初期の着床後の胚において最初期に運命決定される細胞の一つである。ヒトでは倫理的な観点から着床直後の初期胚が扱えないため、着床前初期胚である胚盤胞から樹立される胚性幹細胞（ES 細胞）などの多能性幹細胞を用いて、始原生殖細胞の発生を試験管内で再構築する試みが進められてきた。最近、我々と他の研究グループはヒト始原生殖細胞様細胞を多能性幹細胞から効率的に誘導する系を確立した。その分化系では、多能性細胞から生殖細胞分化への反応性を持つ状態を誘導することが重要であった。興味深いことに、マウスとヒトでは始原生殖細胞発生の分子機構が異なっていた。これは、マウスとヒトにおける初期発生機構や多能性制御の違いにより説明できる可能性がある。本稿では、多能性幹細胞からの生殖細胞分化誘導培養系の現状とその臨床応用などの可能性について述べる。