—Mini Review—

Generation of Functional Primordial Germ Cells from Pluripotent Stem Cells

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Abstract: *Primordial germ cells (PGCs), origin of the germ cell lineage, arise from epiblast cells in response to BMP4 secreted by adjacent extraembryonic ectoderm. We recently reconstituted the PGC specification in vitro using mouse embryonic stem cells (mESCs) as well as induced pluripotent stem cells (iPSCs). In the culture system, mESCs/iPSCs first differentiated into epiblast-like cells (EpiLCs) and then induced PGC-like cells from the EpiLCs. This manner of differentiation from mESCs to PGCs reproduces the manner of PGC specification in vivo. PGCs produced from mESCs, termed PGC-like cells (PGCLCs), were fully potent, since they differentiated into spermatozoa and in turn the fertilized eggs with the spermatozoa gave rise to healthy individuals. Although many attempts have been made to produce fully potent PGCs, this study was the first study demonstrating the successful production of healthy individuals from PGCLCs. This achievement was made possible by knowledge accumulated on the manner of PGC specification in vivo, the nature of self-renewing pluripotent stem cells, and growth factors endowing EpiLC formation and PGCLC induction in vitro. This article reviews the research advance that made it possible to reconstitute PGC specification in vitro from mESCs.*

Key words: *Primordial germ cells, Epiblast, Pluripotent stem cells*

Introduction

Pluripotent stem cells have the ability to proliferate indefinitely under certain culture conditions *in vitro* while maintaining the capability of differentiating into any type of cell in the body. Pluripotent stem cells are derived from a group of pluripotent cells in the embryo, mouse embryonic stem cells (mESCs) and epiblast stem cells (EpiSCs), which are derived from the inner cell mass (ICM) of the blastocyst and epiblast of the post-implantation embryo, respectively [1–4]. Recently, Yamanaka and colleagues succeeded in reprogramming terminally differentiated cells into pluripotent cells, named induced pluripotent stem cells (iPSCs), by introducing the expression of defined transcription factors [5, 6]. Their findings demonstrate that any type of cell can be a source of pluripotent stem cells. In addition to demonstrating the plasticity through which terminally differentiated cells become pluripotent stem cells, a significant impact of iPSC generation is that it makes possible not only the production of patient-specific pluripotent stem cells for transplantation, which do not induce immune rejection, but also the analysis of mechanisms causing diseases.

To apply ESCs and iPSCs to regenerative medicine, several key steps remain to be elucidated. Among them, one of the most primary steps is to establish an *in vitro* culture system in which pluripotent cells differentiate into specific types of cells that are as functionally potent as their counterparts *in vivo*. To achieve this primary step, it is first necessary to clarify the manner of differentiation *in vivo*, since this information may eventually lead to the identification of the key growth factor(s) and/or transcription factor(s) that regulate the differentiation of specific types of cells. Identification of key growth factor(s) and/or transcription factor(s) would assist in the development of a culture system that properly reproduces the manner of differentiation *in vivo*.

We recently developed a culture system in which mouse primordial germ cells (PGCs), the precursors of spermatozoa or oocytes, can be derived from pluripotent stem cells such as mESCs and iPSCs [7]. The PGCs produced *in vitro* from mESCs, termed PGC-like cells

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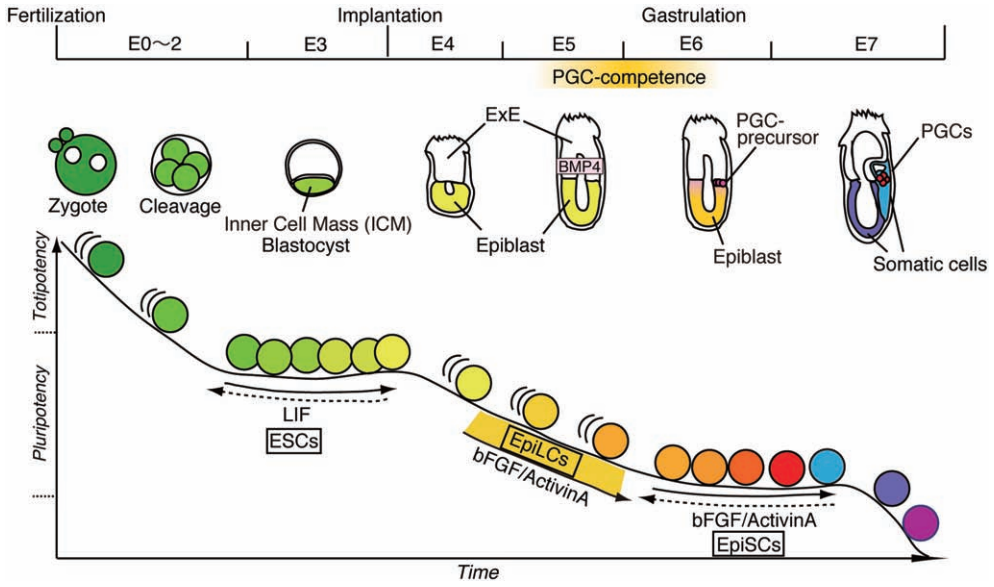

Fig. 1. Schematic view of a series of pluripotent cells *in vivo* and *in vitro*, and PGC specification
After fertilization, the zygote undergoes cleavage divisions. The zygote and to some extent early blastomeres are totipotent. A pluripotent cell population is established within the inner cell mass (ICM) of blastocysts, which is origin of ESCs. After implantation some cells in the ICM become post-implantation epiblast. *Blimp1*-expressing PGC precursors are derived from the epiblast around E6.25 in response to BMP4 secreted from the extraembryonic ectoderm (ExE), and in turn PGCs are specified by E7. Between E5.5 and E6.5, the epiblast harbors high PGC-competence, the capability of differentiation into PGCs. The lower drawing depicts states of pluripotent stem cells and EpiLCs. Under specific culture conditions, pluripotent stem cells are maintained in a metastable state that corresponds to their cells of origin. LIF and bFGF/ActivinA are required for self-renewal of ESCs and EpiSCs, respectively. EpiLCs are in a transient state between ESCs and EpiSCs, which is induced by culturing ESCs with bFGF/ActivinA and is PGC-competent.

(PGCLCs), were functional, as they gave rise to fertile spermatozoa and the fertilized eggs with the spermatozoa developed into healthy individuals. Successful development of this culture system was based on an accumulated understanding of the differentiation manner of PGCs *in vivo*, the nature of pluripotent stem cells, and the growth factors endowing EpiLC formation and PGCLC induction *in vitro*.

PGC Specification and Attempts at PGC Reconstitution *In Vitro*

During embryogenesis, PGCs segregate from the somatic cell lineage at a relatively early developmental stage. In mice, PGCs are derived from the post-implantation epiblast around embryonic day (E) 6.25 in response to BMP4 secreted by the extraembryonic ectoderm, a tissue adjacent to the epiblast (Fig. 1). BMP4 seems to be the essential and sufficient factor to initiate PGC specification from the epiblast, since BMP4-deficient embryos lack PGCs [8], and epiblast cultured *in vitro* with BMP4 differentiates into PGCs [9]. Near the time of PGC specification, about six cells of the posterior proximal epiblast at E6.25 start to express *Blimp1/Prdm1*, a zinc finger transcriptional repressor, and these *Prdm1*-positive cells are lineage-restricted to become PGCs [10]. One day later, about 40 *Stella*-expressing PGCs are located at the posterior end of the primitive streak (Fig. 1). Upon specification, PGCs start to express germ cell-specific genes, such as *Prdm14* and *Nanos3*, as well as pluripotency-associated genes, such as *Nanog* and *Sox2*. Following the cell-specific gene expression, PGCs undergo a unique program of germ cell development that includes epigenetic reprogramming, meiosis, and morphological change during gametogenesis [11, 12].

Since PGCs are specified around gastrulation, it seems that they are one of the earliest cell lineages segregated from the post-implantation epiblast, a group of

pluripotent cells. Due to the early segregation from the pluripotent cells, it seems relatively feasible to reconstitute the differentiation step(s) *in vitro* using pluripotent stem cells. It is known that early PGCs transplanted into the testis are capable of differentiating into spermatozoa [13]. Although the manner in which PGCs transplanted into the testis undergo spermatogenesis is obscure, it suggests that PGCs autonomously regulate a stepwise process of the development, such as reorganization of genomic imprinting. More practically, the transplantation analysis allows us to test whether PGCs produced from pluripotent cells *in vitro* are fully functional. There have been several attempts to produce PGCs, and gametes, from mESCs *in vitro* [14–17]. Despite these efforts, the generation of healthy individuals from PGCs derived from mESCs had not been achieved. This was partly due to the inappropriate differentiation of mESCs into cells that had an epiblast-like state. Differentiation of mESCs into epiblast had thus far been accomplished by either monolayer culture without LIF, a critical growth factor for self-renewal of mESCs, or formation of embryoid bodies that largely mimic early post-implantation development. However, it has been unclear how great the similarity is between differentiating mESCs under these conditions and the epiblast *in vivo*. Moreover, mESCs passively differentiate under such undefined conditions, thereby making cell cultivation uncontrollable. These findings and the fact that no healthy individual had been obtained by these methods led us to develop a culture system in which ESCs efficiently and properly differentiate into epiblast under defined conditions. To this end, at least two goals needed to be met: the clarification of the nature of self-renewing ESCs as a starting material and the identification of the set of growth factors that promote differentiation of ESCs into epiblast.

Pluripotent Stem Cells: Their State, Heterogeneity and Relation to Their Cells of Origin

In mouse development, the pluripotent state is first established in the ICM of the blastocyst (Fig. 1). After implantation, the ICM immediately differentiates into two types of cell lineages, the post-implantation epiblast and primitive endoderm. The former is pluripotent and the source of the embryo proper. The post-implantation epiblast then starts to differentiate into various somatic cells or PGCs. As seen in a series of differentiations from ICM to post-implantation epiblast and in turn to the various somatic cells, the pluripotent state *in vivo* appears transient (Fig. 1). In contrast, pluripotent stem cells self-renew indefinitely while maintaining the potency of their counterparts *in vivo*; mESCs and EpiSCs maintain some, though not all, of the properties of the ICM and post-implantation epiblast, respectively [18, 19].

Given that the definition of self-renewal of stem cells is that one cell produces two daughter cells identical to the original cell, self-renewing mESCs would be a group of homogenous cells. However, several research groups, including ours, have found that mESCs are not a unique population, but are composed of heterogeneous subpopulations [20–22]. Based on these researches, genes such as *Zfp46*, *Nanog*, and *Stella*, all of which are known as pluripotent cell-specific genes, are not uniformly expressed in mESCs, but rather expressed in a subpopulation of mESCs. Detailed analyses of the gene expression in each subpopulation, such as in the *Zfp46*-positive subpopulation and *Zfp46*-negative subpopulation, have revealed that the *Zfp46*-positive subpopulation exhibits a gene expression pattern similar to ICM, whereas the *Zfp46*-negative subpopulation is similar to the post-implantation epiblast. The *Zfp46*-negative subpopulation expresses post-implantation epiblast marker genes, such as *Fgf5*. Interestingly, both subpopulations are mutually interchangeable: *Zfp46*-positive cells give rise to *Zfp46*-negative cells, and *vice versa*. These results suggest that mESCs fluctuate between two states, the ICM-like and post-implantation epiblast-like states (Fig. 1). Consistent with this observation, *Nanog* and *Stella* are preferentially expressed in the ICM-like subpopulation of the mESCs. Although the ICM-like and post-implantation epiblast-like subpopulations are mutually interchangeable, the ICM-like subpopulation is prone to conversion into the epiblast state, whereas the post-implantation epiblast-like subpopulation is hard to convert into the ICM-like state [21]. This might be because conversion of the ICM-like state into the post-implantation epiblast-like state follows an inherent developmental program from the ICM toward the epiblast, whereas the reciprocal conversion is contrary to the normal developmental program *in vivo*. This may account for the fact that the latter conversion takes place less readily. Taken together, these results indicate that the pluripotency of mESCs is maintained in a metastable state that corresponds to a range of pre- and post-implantation stages *in vivo* (Fig. 1).

The meta-state is controlled by intrinsic and extrinsic cues. For example, mouse embryonic fibroblasts (MEFs) play a role in accelerating the conversion into the ICM state [21]. In contrast, Fgf-mediated signaling promotes differentiation into the post-implantation epiblast state. The Fgf-mediated signaling is activated by an autoinductive mechanism: Fgf4 generated by mESCs activates its pathway through Fgf-receptors expressed in the mESCs

[23]. Interestingly, ablation of Fgf-signaling from mESCs, either by genetic modification or addition of an inhibitor to the culture, prevents mESCs switching into the epiblast state, thereby resulting in a relatively homogenous ES cell population [24]. mESCs with a disrupted *Fgf4* gene are refractory to differentiation into the ectoderm and mesoderm. Consistently, disruption of the Fgf transducer protein prevents mESCs from differentiating [23]. Based on these observations, Smith and colleagues developed a serum-free culture medium in which mESCs maintain their pluripotency [25]. The medium contains small molecules that inhibit GSK3*β*, Fgf receptor, or MEK, a transducer of Fgf signaling. Under this condition, mESCs exhibit a homogeneous pattern of gene expression in which, for example, Nanog is uniformly expressed in almost all the mESCs. It is of note that the culture condition allows mESCs to maintain pluripotency even without LIF, suggesting that mESCs have an intrinsic mechanism for maintaining pluripotency without an extrinsic signal. This possible state, in which mESCs are maintained without extrinsic cues, has been tentatively termed the "ground state".

Recently, a different type of pluripotent stem cell, named EpiSC, has been established from post-implantation epiblast [3, 4]. The gene expression and epigenetic states in EpiSCs are distinct from those of mESCs. Many of the genes expressed in ICM-like mESCs, such as *Stella* and *Zfp46*, are downregulated, whereas expression of *Fgf5* becomes prominent. Furthermore, one of the X chromosomes in female EpiSCs is transcriptionally inactivated, which is an epigenetic feature of the post-implantation epiblast, but this is not the case in ICM and ES cells. Apart from the distinct genetic and epigenetic status of EpiSCs [3, 4], the signaling pathways important for self-renewal of EpiSCs are also different from those for mESCs: Activin/Nodal- and Fgf-mediated pathways play crucial roles, whereas LIF does not have a significant impact on the self-renewal of EpiSCs. It is of note that, like mESCs, EpiSCs are also composed of heterogeneous subpopulations (see below), each of which represents a developmental stage of the post-implantation epiblast (Fig. 1).

Reconstitution of Germ Cell Competence in Pluripotent Stem Cells

In PGC specification *in vivo*, there is a time window during which the post-implantation epiblast is capable of differentiating into PGC precursors in response to BMP4. An *ex vivo* culture experiment in which the post-implantation epiblast was exposed to BMP4 revealed that PGC precursors are efficiently induced from E5.5 to E6.5 epiblast [9] (Fig. 1). Although the molecular mechanisms underlying the time window remain unclear, this observation is of practical importance for reconstituting the PGC competence in pluripotent stem cells. Do mESCs or EpiSCs harbor the PGC competence? The responsiveness of each cell-type to BMP4 provides important clues leading to the answer to this question, as the growth factor is essential and sufficient for PGC induction from "PGC-competent" epiblast cells. It seems, however, that neither mESCs nor EpiSCs are highly PGC-competent. In response to BMP4, ESCs do not produce PGCs but rather promote self-renewal [26]. Although cells of the PGC-like population are slightly induced in EpiSCs by BMP4, most of the EpiSCs are stubborn [27]. Interestingly, self-renewing EpiSCs already include a subpopulation representing features of PGC precursors [27] (Fig. 1). The PGC precursor-like subpopulation in EpiSCs expresses *Blimp1/Prdm1* and other PGC-specific genes, such as *Prdm14*, *Nanos3* and *Dnd1*. The PGC precursor population of EpiSCs follows at least two kinds of cell fate: one differentiates further into PGCs, and the other reverts to the pluripotent population. The former forms oocyte-like cells, when cultured with fetal gonadal somatic cells [27]. These findings suggest that the meta-state of EpiSCs includes a PGC precursor state (Fig. 1), and some of the cells in the PGC precursor-like population lose their plasticity and differentiate into PGCs. It is likely that self-renewing EpiSCs contain not only a PGC precursor population but also an anterior epiblast-like population, which is a population placed in the opposite region of PGC precursors in the fate map of the epiblast. The heterogeneity of EpiSCs, including an anterior epiblast-like population, might be a reason why EpiSCs are barely able to induce PGCs in response to BMP4.

To reconstitute the PGC competence in pluripotent stem cells, it should be taken into consideration that mESCs introduced into the blastocyst acquire PGC competence by the chimeric embryo age of E5.5 at the earliest, which is only 2 days after the introduction. During those 2 days, mESCs would be exposed to various signals from the surrounding tissues and in turn differentiate into epiblast cells with PGC competence. Together with this observation, the fact that mESCs differentiate into EpiSCs when cultured under bFGF and ActivinA, a condition for EpiSC culture, led to the idea that there might be a time window in which cells acquire PGC competence during the transition from mESCs to EpiSCs (Fig. 1). To examine this possibility, we cultured mESCs under an EpiSC condition and tested whether PGC precursors were induced at each time point in response to BMP4.

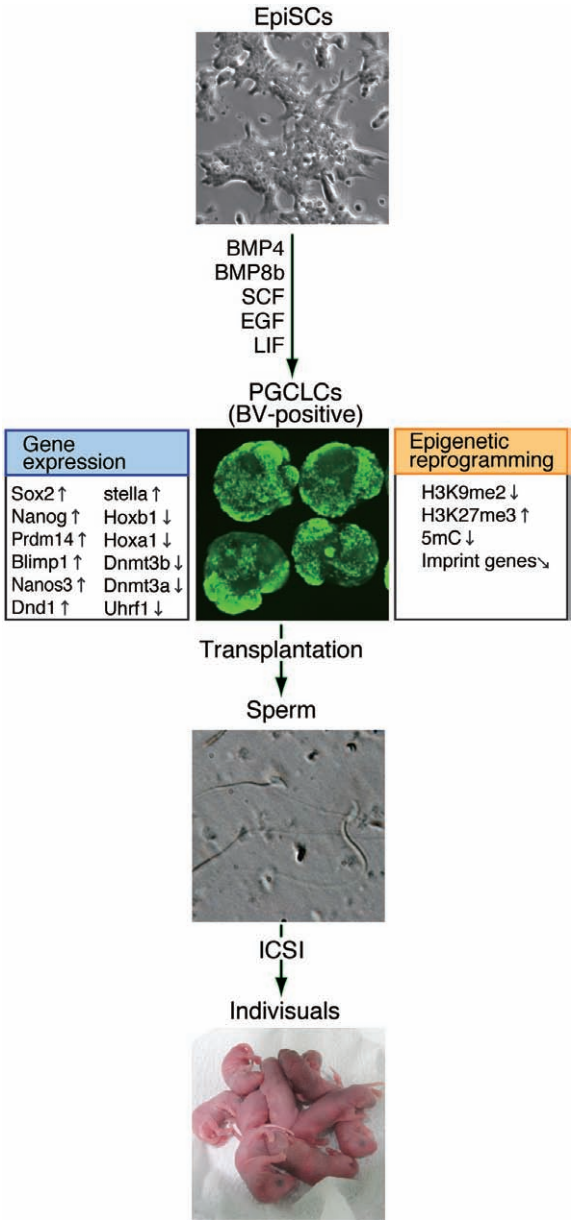

Fig. 2. Derivation of PGCLCs from EpiLCs
PGCLCs are induced from EpiLCs cultured with BMP4, BMP8b, SCF, EGF and LIF. In this culture system, PGCLCs are monitored by *Blimp1* promoter-driven *Venus* (BV) expression exhibiting green fluorescence. PGCLCs show patterns of gene expression and epigenetic reprogramming that reproduce those of PGCs *in vivo*. PGCLCs transplanted into the testis are capable of differentiating into spermatozoa. The spermatozoa are introduced to oocytes by intracytoplasmic sperm injection (ICSI). The fertilized eggs with the spermatozoa give rise to healthy individuals. Few spermatozoa were observed in the cauda epididymis possibly due to a small number of spermatogenic colonies in the testis. At present, it is not possible to perform conventional *in vitro* fertilization (IVF).

To realize homogeneous differentiation from mESCs to EpiSCs, mESCs were cultured in medium containing the FGF inhibitor PD0325901, GSK3*β* inhibitor CHIR99021, and LIF [25]. We used a reporter ES line with the membrane-bound *Venus* gene driven by a *Blimp1*-promoter (BV) that facilitates the detection of PGC differentiation [28]. The results clearly demonstrated that mESCs cultured for 2 days under an EpiSC condition acquired PGC competence, and properly differentiated into PGC precursors expressing the BV reporter gene [7] (Fig. 2). We named the PGC-competent cells epiblast-like cells (EpiLCs) to distinguish them from epiblasts *in vivo*. Microarray and quantitative PCR (qPCR) analysis clearly showed that the gene expression pattern of EpiLCs resembled that of epiblast *in vivo*. EpiLCs are in a transient state, as mESCs cultured for longer than 3 days lose the PGC competence. Thus, using ES cells, this culture system reconstitutes the differentiation processes from the ICM of the blastocyst to the post-implantation epiblast with PGC-competence.

Evaluation of PGCs Derived from EpiLCs

Whether PGCs derived from pluripotent stem cells are functional is of interest, because, if so, such functionality could contribute to new technologies in reproductive engineering, and could confirm that the EpiLCs reconstituted *in vitro* are equivalent to epiblast cells. We therefore characterized PGCLCs by testing the gene expression, epigenetic reprogramming and capacity for spermatogenesis. Microarray analyses showed that the gene expression pattern of PGCLCs almost mirrors that of E9.5 PGCs *in vivo*. qPCR analyses revealed that during derivation of PGCLCs from EpiLCs, the gene expression program in EpiLCs exactly followed that observed during PGC specification *in vivo*. In addition to the similar pattern of the transcriptome, genome-wide conversion of histone modification, which is a feature of epigenetic reprogramming observed in nascent and migrating PGCs *in vivo*, took place in PGCLCs. Similar to PGCs *in vivo*, PGCLCs exhibit a decrease in histone 3 lysine 9 dimethylation (H3K9me2) and an increase in histone 3 lysine 27 trimethylation (H3K27me3) [7, 29, 30] (Fig. 2). In addition to the histone modification, the genome-wide level of DNA methylation also decreases in PGCLCs, which is also the case in PGCs *in vivo*(Fig. 2). The parental states of genomic imprinting gene loci tested were erased, even though they had not been completed. These results demonstrate that PGCLCs have the ability to undergo epigenetic reprogramming. The gold standard for evaluating the function of germ cells is whether they give rise to fertile

gametes and in turn an individual. It has been known that PGCs *in vivo* give rise to fertile spermatozoa, when transferred into the testes of germ cell-less *W/Wv* males [13]. Therefore, PGCLCs were transferred into *W/Wv* testes to test whether they are capable of differentiating into fertile spermatozoa. Transplantation of the PGCLCs rsulted in fertile spermatozoa, and fertilized eggs with the spermatozoa gave rise to healthy individuals with normally sized placentas (Fig. 2). The individuals, both male and female, grew normally and had the ability to bear the next generation. These findings clearly demonstrate that PGCLCs are properly derived from EpiLCs under these culture conditions.

EpiLCs were derived from iPSCs. The iPS-derived EpiLCs were also capable of differentiating into PGCLCs, and in turn, spermatozoa after transplantation into *W/Wv* testes [7]. The spermatozoa had the ability to fertilize oocytes, and the fertilized eggs gave rise to healthy individuals. These results demonstrate that the EpiLC culture system is a practical method for deriving fertile PGCLCs from both mESCs and iPSCs. However, only one of the three iPSC lines tested successfully differentiated into PGCLCs and then spermatozoa. Although it remains unclear why the other two iPSC lines failed, one possible reason would be clonal variation between the iPSC lines, which is known to contribute to differences in the efficiency of differentiation [31]. It is noteworthy that the three iPSC lines used differed with respect to how they were reprogrammed: the one, which gave rise to individuals, was made by retroviral transduction of Oct4/Sox2/Klf4/c-Myc, the other two were made by either retroviral transduction of Oct4/Sox2/Klf4 or transient expression of Oct4/Sox2/Klf4/c-Myc [6, 32, 33]. It might be that the distinct potential of PGCLC derivation is attributable to a different set of reprogramming factors. Nevertheless, this report is, to our knowledge, the first report that demonstrates the generation of healthy individuals from PGCs derived from mESCs and iPSC *in vitro.*

Perspectives

The EpiLC culture system allows us not only to address the mechanisms of PGC specification that have been remained elusive due to the limited number of nascent PGCs in the embryo, but also to uncover clues for clinical application.

It is apparent that the number of nascent PGCs is insufficient for several types of experiments, for example biochemical analysis. The EpiLC culture system allows the production of a huge number of PGCLCs almost equivalent to nascent PGCs, making it possible to perform such experiments. A remaining unanswered question is how epiblast cells acquire PGC competence. To address this issue, it is of particular importance to uncover how BMP4-mediated signaling evokes a PGC-specific gene cascade in EpiLCs. This will be done by, for example, chromatin immunoprecipitation (ChIP) analysis to identify target genes of Smad1, a BMP-specific transducer, which is known to be pivotal for PGC-specification [34, 35]. Drawing on a network of Smad1-oriented gene regulation would be informative for understanding the molecular mechanisms underlying PGC-competence. In contrast to EpiLCs, it is known that the Smad1-mediated pathway promotes self-renewal of ESCs by preventing differentiation into neuroectoderm [26]. Analysis of such cell context-dependent functions of Smad1 may reveal differences between ESCs and EpiLCs.

In the EpiLC culture system, bFGF and ActivinA are used for differentiation from ESCs into EpiLCs. ActivinA-mediated signaling shares many similarities with Nodal, which plays a central role in patterning of the epiblast and mesoderm formation. It is likely that ActivinA-mediated singling endows EpiLCs with PGC-competence, based on the following evidence. (1) Nodal is expressed in the epiblast where PGC precursors form. It is initially expressed in the proximal region of the epiblast and finally in the posterior region of the epiblast according to anterior-posterior axis formation [36–39]. (2) Nodal is essential for mesoderm formation, which is presumably the first step of PGC precursor formation [40]. (3) Smad2-mutant embryos in which Nodal is expressed throughout the epiblast show increased numbers of PGCs in the ectopic region of the epiblast [41]. It is possible that various cell lineages other than PGCs are induced from EpiLCs cultured with various sets of growth factors and inhibitors. For example, inhibition of ActivinA signaling may result in the formation of an anterior epiblast with a high potential for differentiation into neuroectoderm.

In the culture system reported, PGCLCs arrest at a stage corresponding to the stage between E8.5 and E9.5 *in vivo*. Consistent with PGCs at this stage, most of the PGCLCs showed cell-cycle arrest in the G2-phase, and there were few PGCLCs expressing Mvh, a marker gene of later PGCs [42]. To overcome the developmental arrest, PGCLCs needed to be exposed to another set of growth factors, or need culturing with cells supporting PGC proliferation and/or differentiation, for example gonadal somatic cells. If PGCLCs develop further, for example until sex determination, the culture system would be more valuable. This period contains important germ cell events, such as erasure of epigenetic marks in imprinting gene loci and meiosis in the case of females. Re-

constitution of such events *in vitro* would allow us to address the molecular mechanisms of PGC development. An ultimate goal of the culture system is to reconstitute the entire process of germ cell development by which fertile oocytes or spermatozoa are produced *in vitro* from mESCs. To this end, it is prerequisite to develop a reliable culture system that allows PGCs to differentiate into oogonia or gonocytes.

Successful production of PGCLCs from mESCs naturally leads to the idea that human ESCs and iPSCs can differentiate into PGCs *in vitro* by adaptation in the EpiLC culture system. However, it would probably be difficult to directly adapt human ESCs/iPSCs to the EpiLC system, for the reasons described below. First, we need to consider the difference between human and mouse ESCs. These cells are different in morphology, clonogenicity and growth factor requirements for self-renewal as well as for induction of differentiation. Most importantly, ActivinA/Nodal-mediated signaling promotes self-renewal of human ESCs [43, 44], whereas the same signal presumably plays a positive role in the differentiation of ESCs into EpiLCs. Second, mESCs can stably propagate under a serum- and feeder-free condition, whereas this condition has not been firmly established in human ESC culture; the growth factor requirement is slightly different between each human ESC clone. In the case of mESCs, the inhibitors of Fgf-signaling and the agonist of Wnt-signaling promote self-renewal of mESCs regardless of differences in genetic background [25, 45]. Moreover, mESCs under such culture conditions remain in the ground state. mESCs placed in the ground state might be important, as they allow homogenous differentiation in response to a set of growth factors. In contrast, a culture condition placing human ESCs/iPSCs in the ground state has not yet been identified. Finding such a culture condition may be a necessary condition for producing a large number of PGCs from human ESCs/iPSCs. Third, it is not clear which gene is optimal as a reporter gene that easily identifies nascent human PGCs from human ESCs/iPSCs. To identify such reporter genes, we need to know how PGCs are specified in human embryo. For ethical reasons, it is difficult to address this issue fully using human embryos, though it might be possible to use primates as a model of PGC specification in humans. Finally, there is no tool to evaluate whether or not human PGCs from human ESCs/iPSCs are functional. Otherwise, it would remain essentially unclear whether human PGCs are properly induced from human ESCs/iPSCs. This issue may be overcome in part by using primate ESCs. Accumulating evidence from primates and careful comparisons between primate and human ESCs will at least provide clues to the development of a culture system for producing human PGCs from human ESCs/iPSCs.

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