Reprogramming of the pig primordial germ cells into pluripotent stem cells: a brief review

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Abstract Primordial germ cells (PGCs) are regarded as unipotent cells that can produce only either spermatogonia or oocytes. However, PGCs can be converted into the pluripotent state by first dedifferentiation to embryonic germ cells and then by reprogramming to induce them to become pluripotent stem cells (iPSCs). These two stages can be completely implemented with mouse cells. However, authentic porcine iPSCs have not been established. Here, we discuss recent advances in the stem cell field for obtaining iPSCs from PGCs. This knowledge will provide some clues which will contribute to the regulation of reprogramming to pluripotency in farm species.

Keywords pig, pluripotent stem cells, primordial germ cells, reprogramming

1 Introduction

Pluripotent stem cells (PSCs) are defined as the cells that can differentiate into multiple cell lineages. They include embryonic stem cells (ESCs) derived from the inner cell mass (ICM), embryonic germ cells (EGCs) derived from gonadal primordial germ cells (PGCs) and induced pluripotent stem cells (iPSCs). PGCs are unipotent cells which can only produce either spermatogonia or oocytes[1,2]. It is now understood how to convert these unipotent cells to the pluripotent state. One study[3] showed that somatic cells could be reprogrammed into iPSCs when the transcription factors, Oct4, Sox2, Klf4 and cMyc (also called OSKM), are introduced into target cells. This is a preliminary study that has received a great deal of attention and much public interest. Subsequently, many groups around the world independently replicated the protocol[4–7]. By contrast, the PGCs are quite different from somatic cells in their differentiation potential because they can give rise to EGCs under appropriate in vitro cell culture[2,8]. However, they can also be reprogrammed into iPSCs using the standard protocol of inducing somatic cells into iPSCs[9]. However, the reprogramming of PGCs into PSCs for larger animals is still largely unachieved. In this review, we discuss the recent advances and procedures for obtaining PSCs from pig PGCs.

2 Specification of primordial germ cells

Many studies have shown that PGCs are derived from the proximal epiblast cells. Then PGCs migrate through the dorsal mesentery to the genital ridges[10]. In the earlier studies, PGCs could be identified by alkaline phosphatase activity in mice[11]. With the advent of transgenic and molecular markers, the methodology for identification of PGCs has been updated. Evidence suggested that Blimp1 (also called Prdm1) is critical for the process of PGC formation at E6.5–7.5 embryos[12]. Moreover, Blimp1 is regarded as a pluripotency gatekeeper protein in PGCs because Blimp1 deletion promotes PGCs dedifferentiation into EGCs[13]. However, Blimp1 is not an exclusive marker of PGCs because it also controls expression of visceral endoderm[1,12]. In addition, studies have suggested that Prdm14, a PR-domain containing transcriptional regulator, is exclusively expressed in pluripotent cells and the germ cells[14]. In porcine PGCs, previous studies have shown that the Sda/GM2-glycan is a surface marker of porcine PGCs[15]. Basic fibroblast growth factor (bFGF) is not only important in the initiation of PGCs dedifferentiation by impeding Blimp1 nuclear expression, but also maintains the viability of PGCs[16]. Dolichos biflorus agglutinin (DBA) can bind to PGCs and gonocytes, and a small number of spermatogonia can also be marked by DBA[17]. After arriving in the genital ridges, porcine PGCs undergo...
extensive DNA methylation, which is similar to murine PGCs. The methylation levels of early migrating porcine PGCs are remarkably low, compared to somatic cells at E15\(^{[18]}\). PGCs from 26-day-old embryos can generate a cell line possessing DNA methylation, including imprints, regardless of their sex chromosomes\(^{[19]}\). With the exception of DNA methylation, porcine PGCs also undergo a major histone modification between E15 and E21, where the levels of histone H3 lysine9 mono- and dimethylation 1/2 (H3K9me1/2) decrease and levels of H3K27me3 increase\(^{[20]}\).

### 3 Pluripotent stem cells derived from primordial germ cells

Compared to other cell lines, PGCs are unique cells, because they can express specific genes of the germ-cell lineage, such as Dazla and Vasa\(^{[20,22]}\), and also pluripotency marker genes, such as Oct4 and Nanog\(^{[23,24]}\). Moreover, these cells can dedifferentiate into EGCs when cultured in vitro in the presence of bFGF, stem cell factor and leukemia inhibitory factor (LIF)\(^{[8,25]}\). EGCs are in the pluripotent state, and the developmental potency of EGCs are equivalent to PSCs. A study by Kimura et al.\(^{[2]}\) showed that PGCs can be reprogrammed into induced EGCs using small molecule compounds and transcription factors Oct4 and cMyc. They replaced Sox2 with kempaullone (inhibitor of glycogen synthase kinase-3 β) and replaced Klf4 with SB431542 (TGF-β receptor inhibitor) and replaced Klf4 with kempaullone (inhibitor of glycogen synthase kinase-3 β). In human PGCs, endogenous expression of Oct4, Nanog and Sox2 in the zona-enclosed blastocysts is different from those in mice and humans\(^{[18]}\). Thus, porcine iPSCs are probably different from those of mice. In the Fig. 1, we describe the differentiation fate of pig PSCs. The ESCs are derived from the ICM of blastocysts, and these cells cultured in vitro can form chimeric piglets when they are injected into blastocysts. PGCs can dedifferentiate into EGCs when they are cultured in vitro in the presence of bFGF and LIF, and EGCs can form chimeras. Porcine somatic cells can be reprogrammed into iPSCs by transfection with the six human reprogramming factors (Oct4, Sox2, Nanog, Klf4, Lin28 and cMyc) and the production of chimeric offspring\(^{[15,12]}\). However, there have been no subsequent reports on the production of porcine chimeras. Most iPSC lines fulfilled most pluripotency criteria, but their cells could not contribute to chimeras or generate cloned piglets\(^{[33-37]}\).

### 4 Difficulties for acquisition of porcine induced pluripotent stem cells

Pigs, as an ideal animal model for human diseases and organ donation, are drawing more attention than before. However, porcine ESCs are quite different from those of mice in that porcine preimplantation development is distinctly different from mice and humans, and expression levels of Oct4, Nanog and Sox2 in the zona-enclosed porcine blastocyst are different from those in mice and humans\(^{[18]}\). Thus, porcine iPSCs are probably different from those of mice. In the Table 1, we summarize the comparison of pig PSCs. Briefly, the establishment of pig ESC or iPSC lines for large animals is more difficult than for mouse ESCs or iPSCs, especially for pig. In Table 1, we summarize the differentiation potential of pig PSCs. Briefly, Shim et al.\(^{[29]}\) isolated EGCs from cultured porcine PGSCs and these cells could differentiate and contribute to tissues of a chimeric piglet. Piedrahita et al.\(^{[30]}\) isolated a porcine EGC line and made transgenic chimeras. The scientists have tried to gain authentic iPSC lines using reprogramming factors. Remarkably, however, there is only one study reporting that porcine iPSC lines were established by transfection with the six human reprogramming factors (Oct4, Sox2, Nanog, Klf4, Lin28 and cMyc) and the production of chimeric offspring\(^{[12]}\). However, there have been no subsequent reports on the production of porcine chimeras. Most iPSC lines fulfilled most pluripotency criteria, but their cells could not contribute to chimeras or generate cloned piglets\(^{[33-37]}\).

### Table 1  Differentiation potential of pig pluripotent stem cells

| Cell type               | Cell source       | Induction/culture system                                                                 | Differentiation potential | Reference |
|-------------------------|-------------------|-----------------------------------------------------------------------------------------|---------------------------|-----------|
| ESCs                    | Blastocysts days 6–8 (in vivo) | STO feeder layer + FCS                                                                    | Chimera                  | [27]      |
|                         | Blastocysts day 7 (in vitro)    | MEF + bFGF + EGF + LIF + activin + 10% KSR                                              | Chimera                  | [28]      |
| EGCs                    | PGC-derived cells       | STO feeder layer + LIF + 15% FBS                                                        | Chimera                  | [29]      |
|                         | PGC-derived cells       | STO feeder layer + 15% FBS + LIF + bFGF                                                 | Chimera                  | [30]      |
|                         | PGC-derived cells       | STO feeder layer + 10% FCS + bFGF + SCF + LIF                                          | Chimera                  | [31]      |
| iPSCs                   | Porcine mesenchymal stem cells | Transduction with Oct4, Sox2, Nanog, Klf4, Lin28 and cMyc                              | Chimera                  | [32]      |
chimeras\textsuperscript{[41,42]}. One study showed that the reprogramming genes in porcine iPSCs could not be silenced or removed, which lead to failure in formation of chimeric offspring. The researchers suggested these genes probably are necessary to maintain the self-renewal of porcine iPSCs\textsuperscript{[33]}.

5 Induced pluripotent stem cells derived from pig primordial germ cells

As we have discussed, the generation of porcine iPSCs derived from somatic cells faces many obstacles. To our knowledge, currently there are no reports that pig PGCs can be induced into iPSCs. However, iPSCs derived from PGCs probably have more advantages over somatic cells because they can express many key transcription factors that facilitate pluripotency. In addition, PGCs have similar epigenetic characters to stem cells compared to somatic cells\textsuperscript{[43]}. As PSCs, expression profiles of genome-wide DNA demethylation in EGCs shows a high similarity to PGCs\textsuperscript{[44]}. Human PGCs can only be directly reprogrammed into iPSCs by using two transcription factors, Sox2 and Oct4\textsuperscript{[45]}. As an important reprogramming factor, Oct4 is continuously expressed in porcine PGCs\textsuperscript{[10]}. Compared to ESCs, the transcriptional profiles of Oct4 and its target genes in mouse PGCs show considerable differences because of the reduction of Klf4\textsuperscript{[45]}. This is because these differences lead to different consequences for the differentiation, dedifferentiation or self-renewal of PGCs and ESCs\textsuperscript{[45,46]}. Nanog, as another key pluripotency marker gene, determines the entry to pluripotency. Nanog is transiently repressed during mouse PGC 6.5–7.5 day\textsuperscript{[47]}, but, notably, it can be continuously expressed in porcine PGCs\textsuperscript{[10]}. These studies imply that the endogenous reprogramming genes of porcine iPSCs derived from PGCs may be more easily activated, and the exogenous reprogramming genes might be more likely to be removed because the endogenous reprogramming genes can probably maintain the self-renewal of porcine iPSCs. As for the problem that exogenous reprogramming genes in porcine iPSCs cannot be silenced or removed, we think that exogenous genes induced by small molecule compounds might be easily silenced compared with integrative lentiviral genes.

6 Conclusions

Together, these studies suggest that, unlike mouse iPSCs, Yamanaka 4 transcription factors (OSKM) may not be directly applied to induce porcine somatic cells into iPSCs with authentic pluripotency. The in vivo data for porcine iPSCs needs to be confirmed for pluripotency and complemented with further analyses. Here, with respect to porcine iPSCs, we hypothesize that porcine PGCs can probably be reprogrammed directly into iPSCs to reach an authentic pluripotent state.

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This article is a review and does not contain any studies with human or animal subjects performed by any of the authors.

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