Front. Agr. Sci. Eng. 2019, **6**(1): 28–32
https://doi.org/10.15302/J-FASE-2018222
Available online at http://journal.hep.com.cn/fase

REVIEW

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

Qijing LEI[1], Qin PAN[1], Shuai YU[1], Na LI[1], Shulin CHEN[1], Kuldip SIDHU (✉)[2], Jinlian HUA (✉)[1]

1 College of Veterinary Medicine/Shaanxi Centre of Stem Cells Engineering and Technology, Northwest A&F University, Yangling 712100, China
2 Centre for Healthy Brain Ageing, UNSW Medicine, Randwick NSW 2031, Australia

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 undertood 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 lager animals is still largely unachieved. In this review, we discuss the recent advances and procedures for obtaining PSCs from pig PGCs.

Received October 6, 2017; accepted November 11, 2017

Correspondences: Kuldip.Sidhu@WorldBrainMapping.org, jinlianhua@nwsuaf.edu.cn

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[21,22], 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 SB431542 (TGF-β receptor inhibitor) and replaced Klf4 with kempaullone (inhibitor of glycogen synthase kinase-3 and cyclin-dependent kinases). In addition, hypoxia induces reprogramming of PGCs by deregulating expression of Oct4[26]. In human PGCs, endogenous expression of Klf4 and cMyc is similar to EGCs, but the expression of Sox2 and Oct4 is lower than EGCs. Thus, the reprogramming of PGCs into iPSCs can be achieved with two transcription factors, Sox2 and Oct4[9].

However, the establishment of 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[32]. 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[38]. 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 transfected transcription factors Oct4, Sox2, Nanog, Klf4, Lin28 and cMyc, and form chimeric offspring. However, there have been no subsequent reports about porcine chimeras produced from porcine iPSCs. In particular, there are no reports that porcine PGCs can be reprogrammed into iPSCs.

The biggest obstacle for obtaining porcine iPSCs is that these cells cannot be induced into an authentic pluripotent state, which generates chimeric offspring[33,39,40]. Furthermore, the evidence suggests that the expression of exogenous reprogramming transcription factors must be strongly silenced when endogenous reprogramming transcription factors are already activated to generate

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]

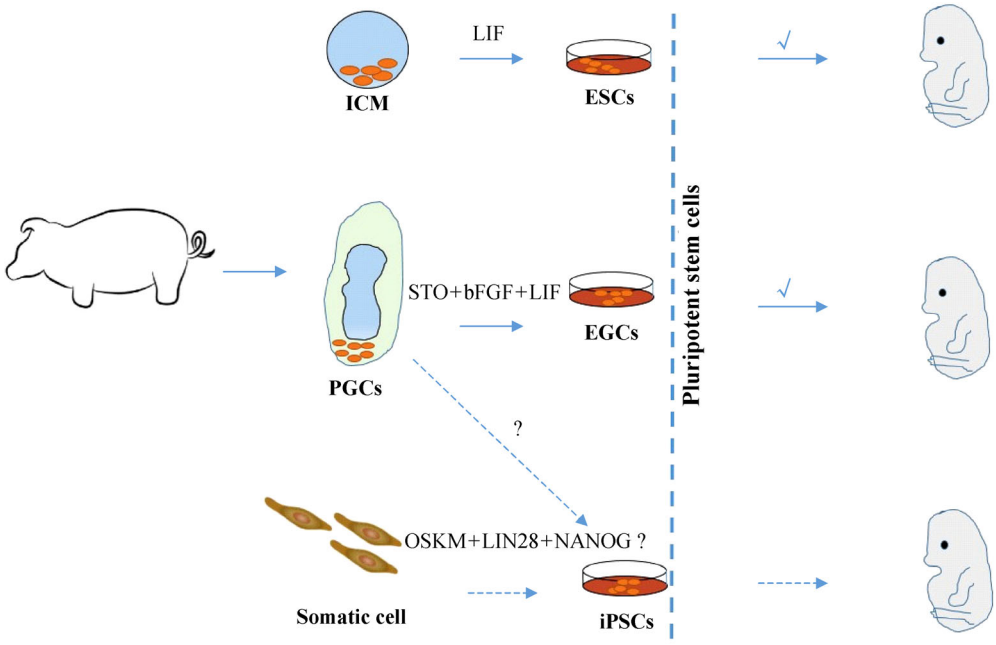

Fig. 1 Differentiation fate of pig pluripotent stem cells

chimeras[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[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[43]. As PSCs, expression profiles of genome-wide DNA demethylation in EGCs shows a high similarity to PGCs[44]. Human PGCs can only be directly reprogrammed into iPSCs by using two transcription factors, Sox2 and Oct4[9]. As an important reprogramming factor, Oct4 is continuously expressed in porcine PGCs[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[45]. This is because these differences lead to different consequences for the differentiation, dedifferentiation or self-renewal of PGCs and ESCs[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[47], but, notably, it can be continuously expressed in porcine PGCs[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.

Acknowledgements This work was supported by the National Basic Research Program of China (2016YFA0100203) and the National Natural Science Foundation of China (31572399, 31272518).

Compliance with ethics guidelines Qijing Lei, Qin Pan, Shuai Yu, Na Li, Shulin Chen, Kuldip Sidhu, and Jinlian Hua declare that they have no conflicts of interest or financial conflicts to disclose.

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

References

1. Bertocchini F, Chuva de Sousa Lopes S M. Germline development in amniotes: a paradigm shift in primordial germ cell specification. *BioEssays*, 2016, **38**(8): 791–800
2. Kimura T, Kaga Y, Sekita Y, Fujikawa K, Nakatani T, Odamoto M, Funaki S, Ikawa M, Abe K, Nakano T. Pluripotent stem cells derived from mouse primordial germ cells by small molecule compounds. *Stem Cells*, 2015, **33**(1): 45–55
3. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 2007, **131**(5): 861–872
4. Park I H, Zhao R, West J A, Yabuuchi A, Huo H, Ince T A, Lerou P H, Lensch M W, Daley G Q. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*, 2008, **451**(7175): 141–146
5. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science*, 2008, **322**(5903): 945–949
6. Li Y, Zhang Q, Yin X, Yang W, Du Y, Hou P, Ge J, Liu C, Zhang W, Zhang X, Wu Y, Li H, Liu K, Wu C, Song Z, Zhao Y, Shi Y, Deng H. Generation of iPSCs from mouse fibroblasts with a single gene, Oct4, and small molecules. *Cell Research*, 2011, **21**(1): 196–204
7. Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, Abujarour R, Lin X, Hahm H S, Hao E, Hayek A, Ding S. A chemical platform for improved induction of human iPSCs. *Nature Methods*, 2009, **6**(11): 805–808
8. Matsui Y, Zsebo K, Hogan B L. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell*, 1992, **70**(5): 841–847
9. Bazley F A, Liu C F, Yuan X, Hao H, All A H, De Los Angeles A, Zambidis E T, Gearhart J D, Kerr C L. Direct reprogramming of human primordial germ cells into induced pluripotent stem cells: efficient generation of genetically engineered germ cells. *Stem Cells and Development*, 2015, **24**(22): 2634–2648
10. Kobayashi T, Zhang H, Tang W W C, Irie N, Withey S, Klisch D, Sybirna A, Dietmann S, Contreras D A, Webb R, Allegrucci C, Alberio R, Surani M A. Principles of early human development and germ cell program from conserved model systems. *Nature*, 2017, **546**(7658): 416–420
11. McLaren A. Primordial germ cells in the mouse. *Developmental Biology*, 2003, **262**(1): 1–15
12. Ohinata Y, Payer B, O'Carroll D, Ancelin K, Ono Y, Sano M, Barton S C, Obukhanych T, Nussenzweig M, Tarakhovsky A, Saitou M, Surani M A. Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature*, 2005, **436**(7048): 207–213
13. Nagamatsu G, Saito S, Takubo K, Suda T. Integrative analysis of the acquisition of pluripotency in PGCs reveals the mutually exclusive roles of Blimp-1 and AKT signaling. *Stem Cell Reports*, 2015, **5**(1): 111–124
14. Yamaji M, Seki Y, Kurimoto K, Yabuta Y, Yuasa M, Shigeta M, Yamanaka K, Ohinata Y, Saitou M. Critical function of Prdm14 for the establishment of the germ cell lineage in mice. *Nature Genetics*, 2008, **40**(8): 1016–1022
15. Klisch K, Contreras D A, Sun X, Brehm R, Bergmann M, Alberio R. The Sda/GM2-glycan is a carbohydrate marker of porcine primordial germ cells and of a subpopulation of spermatogonia in cattle, pigs, horses and llama. *Reproduction*, 2011, **142**(5): 667–674
16. Zhang Y, Ma J, Li H, Lv J, Wei R, Cong Y, Liu Z. bFGF signaling-mediated reprogramming of porcine primordial germ cells. *Cell and Tissue Research*, 2016, **364**(2): 429–441
17. Goel S, Sugimoto M, Minami N, Yamada M, Kume S, Imai H. Identification, isolation, and *in vitro* culture of porcine gonocytes. *Biology of Reproduction*, 2007, **77**(1): 127–137
18. Hyldig S M W, Ostrup O, Vejlsted M, Thomsen P D. Changes of DNA methylation level and spatial arrangement of primordial germ cells in embryonic day 15 to embryonic day 28 pig embryos. *Biology of Reproduction*, 2011, **84**(6): 1087–1093
19. Petkov S G, Reh W A, Anderson G B. Methylation changes in porcine primordial germ cells. *Molecular Reproduction & Development*, 2009, **76**(1): 22
20. Hyldig S M, Croxall N, Contreras D A, Thomsen P D and Alberio R. Epigenetic reprogramming in the porcine germ line. *BMC Developmental Biology*, 2011, **11**(1): 1–11
21. Ruggiu M, Speed R, Taggart M, McKay S J, Kilanowski F, Saunders P, Dorin J, Cooke H J. The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. *Nature*, 1997, **389**(6646): 73–77
22. Tanaka S S, Toyooka Y, Akasu R, Katoh-Fukui Y, Nakahara Y, Suzuki R, Yokoyama M, Noce T. The mouse homolog of *Drosophila* Vasa is required for the development of male germ cells. *Genes & Development*, 2000, **14**(7): 841–853
23. Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, Okochi H, Okuda A, Matoba R, Sharov A A, Ko M S, Niwa H. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nature Cell Biology*, 2007, **9**(6): 625–635
24. Silva J, Nichols J, Theunissen T W, Guo G, van Oosten A L, Barrandon O, Wray J, Yamanaka S, Chambers I, Smith A. Nanog is the gateway to the pluripotent ground state. *Cell*, 2009, **138**(4): 722–737
25. Resnick J L, Bixler L S, Cheng L, Donovan P J. Long-term proliferation of mouse primordial germ cells in culture. *Nature*, 1992, **359**(6395): 550–551
26. Lopeziglesias P, Alcaina Y, Tapia N, Sabour D, Arauzobravo M J, Sainz de la Maza D, Berra E, O'Mara A N, Nistal M, Ortega S, Donovan P J, Schöler H R, De Miguel M P, Sainz d l M D, Berra E, Nunezomara A, Nistal M and Ortega S. Hypoxia induces pluripotency in primordial germ cells by HIF1α stabilization and Oct4 deregulation. *Antioxidants & Redox Signalling*, 2015, **22**(3): 205–223
27. Chen L R, Shiue Y L, Bertolini L, Medrano J F, BonDurant R H, Anderson G B. Establishment of pluripotent cell lines from porcine

preimplantation embryos. *Theriogenology*, 1999, **52**(2): 195–212

28. Vassiliev I, Vassilieva S, Beebe L F S, Harrison S J, McIlfatrick S M, Nottle M B. *In vitro* and *in vivo* characterization of putative porcine embryonic stem cells. *Cellular Reprogramming*, 2010, **12**(2): 223–230
29. Shim H, Gutiérrez-Adán A, Chen L R, BonDurant R H, Behboodi E, Anderson G B. Isolation of pluripotent stem cells from cultured porcine primordial germ cells. *Theriogenology*, 1997, **57**(5): 1089–1095
30. Piedrahita J A, Moore K, Oetama B, Lee C K, Scales N, Ramsoondar J, Bazer F W, Ott T. Generation of transgenic porcine chimeras using primordial germ cell-derived colonies. *Biology of Reproduction*, 1998, **58**(5): 1321–1329
31. Dong X, Tsung H, Mu Y, Liu L, Chen H, Zhang L, Wang H, Feng S. Generation of chimeric piglets by injection of embryonic germ cells from inbred Wuzhishan miniature pigs into blastocysts. *Xenotransplantation*, 2014, **21**(2): 140–148
32. West F D, Terlouw S L, Kwon D J, Mumaw J L, Dhara S K, Hasneen K, Dobrinsky J R, Stice S L. Porcine induced pluripotent stem cells produce chimeric offspring. *Stem Cells and Development*, 2010, **19**(8): 1211–1220
33. Du X, Feng T, Yu D, Wu Y, Zou H, Ma S, Feng C, Huang Y, Ouyang H, Hu X, Pan D, Li N, Wu S. Barriers for deriving transgenefree pig iPS cells with episomal vectors. *Stem Cells*, 2015, **33**(11): 3228–3238
34. Chakritbudsabong W, Sariya L, Pamonsupornvichit S, Pronarkngver R, Chaiwattanarungruengpaisan S, Ferreira J N, Setthawong P, Phakdeedindan P, Techakumphu M, Tharasanit T, Rungarunlert S. Generation of a pig induced pluripotent stem cell (piPSC) line from embryonic fibroblasts by incorporating LIN28 to the four transcriptional factor-mediated reprogramming: VSMUi001-D. *Stem Cell Research*, 2017, **24**: 21–24
35. Montserrat N, Bahima E G, Batlle L, Häfner S, Rodrigues A M, González F, Izpisúa Belmonte J C. Generation of pig iPS cells: a model for cell therapy. *Journal of Cardiovascular Translational Research*, 2011, **4**(2): 121–130
36. Ezashi T, Telugu B P V L, Alexenko A P, Sachdev S, Sinha S, Roberts R M. Derivation of induced pluripotent stem cells from pig somatic cells. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, **106**(27): 10993–10998
37. Wu Y, Li O, He C, Li Y, Li M, Liu X L, Wang Y, He Y. Generation and characterization of induced pluripotent stem cells from guinea pig fetal fibroblasts. *Molecular Medicine Reports*, 2017, **15**(6): 3690–3698
38. Hall V. Porcine embryonic stem cells: a possible source for cell replacement therapy. *Stem Cell Reviews*, 2008, **4**(4): 275–282
39. Onishi A, Iwamoto M, Akita T, Mikawa S, Takeda K, Awata T, Hanada H, Perry A C. Pig cloning by microinjection of fetal fibroblast nuclei. *Science*, 2000, **289**(5482): 1188–1190
40. Polejaeva I A, Chen S H, Vaught T D, Page R L, Mullins J, Ball S, Dai Y, Boone J, Walker S, Ayares D L, Colman A, Campbell K H. Cloned pigs produced by nuclear transfer from adult somatic cells. *Nature*, 2000, **407**(6800): 86–90
41. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, Plath K, Hochedlinger K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell*, 2007, **1**(1): 55–70
42. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein B E, Jaenisch R. *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*, 2007, **448**(7151): 318–324
43. Pashai N, Hao H, All A, Gupta S, Chaerkady R, De Los Angeles A, Gearhart J D, Kerr C L. Genome-wide profiling of pluripotent cells reveals a unique molecular signature of human embryonic germ cells. *PLoS One*, 2012, **7**(6): e39088
44. Saitou M, Kagiwada S, Kurimoto K. Epigenetic reprogramming in mouse pre-implantation development and primordial germ cells. *Development*, 2012, **139**(1): 15–31
45. Mise N, Fuchikami T, Sugimoto M, Kobayakawa S, Ike F, Ogawa T, Tada T, Kanaya S, Noce T, Abe K. Differences and similarities in the developmental status of embryo-derived stem cells and primordial germ cells revealed by global expression profiling. *Genes to Cells*, 2008, **13**(8): 863–877
46. Niwa H, Miyazaki J, Smith A G. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nature Genetics*, 2000, **24**(4): 372–376
47. Yamaguchi S, Kimura H, Tada M, Nakatsuji N, Tada T. Nanog expression in mouse germ cell development. *Gene Expression Patterns*, 2005, **5**(5): 639–646