⌘ *Author's Choice*

A Germ Cell-specific Gene, *Prmt5*, Works in Somatic Cell Reprogramming*[S]

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Germ cells possess the unique ability to acquire totipotency during development *in vivo* as well as give rise to pluripotent stem cells under the appropriate conditions *in vitro*. Recent studies in which somatic cells were experimentally converted into pluripotent stem cells revealed that genes expressed in primordial germ cells (PGCs), such as *Oct3/4*, *Sox2*, and *Lin28*, are involved in this reprogramming. These findings suggest that PGCs may be useful for identifying factors that successfully and efficiently reprogram somatic cells into toti- and/or pluripotent stem cells. Here, we show that *Blimp-1*, *Prdm14*, and *Prmt5*, each of which is crucial for PGC development, have the potential to reprogram somatic cells into pluripotent stem cells. Among them, Prmt5 exhibited remarkable reprogramming of mouse embryonic fibroblasts into which *Prmt5*, *Klf4*, and *Oct3/4* were introduced. The resulting cells exhibited pluripotent gene expression, teratoma formation, and germline transmission in chimeric mice, all of which were indistinguishable from those induced with embryonic stem cells. These data indicate that some of the factors that play essential roles in germ cell development are also active in somatic cell reprogramming.

Yamanaka and colleagues (1) showed that somatic cells could be reprogrammed by specific factors. To achieve this, the authors induced somatic cells to adopt an embryonic stem (ES)[4] cell-like state by introducing *Oct3/4*, *Sox2*, *Klf4*, and c-*Myc* into mouse fibroblasts. This technique for producing induced pluripotent stem (iPS) cells has also been applied to human somatic cells (2, 3). Because the generation of iPS cells is not plagued by either ethical considerations or immune rejection, the potential of this technology is of great importance for the field of regenerative medicine. Apart from the potential clinical applications, iPS cell generation also provides an excellent model system for investigating the mechanisms underlying cellular reprogramming *in vitro* through the identification of alternative or additional factors involved in this process. So far, a number of reprogramming factors, including *Oct3/4*, *Sox2*, *Klf2/4/5*, c-*Myc*, and *Lin28*, as well as reprogramming-inhibitory factors such as *p53* and *let7*, have been identified in this way (3–5). Of note, each factor is not only active during reprogramming but it also has biological significance in development and homeostasis in the cells and tissues. Frequency of the conversion into iPS cells by transfection with these four reprogramming factors is still very low, implying that other, unidentified reprogramming factors may exist.

Before the discovery of iPS cells, pluripotent cells could only be established from germ cells in post-implantation embryos. Primordial germ cells (PGCs) and spermatogonia are known to de-differentiate into pluripotent stem cells, embryonic germ (EG) cells and multipotent germline stem cells, respectively (6, 7). Consistent with their ability to de-differentiate, cells from the germ cell lineage express key factors that maintain pluripotency, such as *Oct3/4* and *Sox2* (8). In addition, the efficiency of deriving of EG cells from PGCs is much higher than the efficiency of generating iPS cells from mouse embryonic fibroblasts (MEFs), suggesting that germ cells already possess critical reprogramming factors. Recent studies have identified several factors that are crucial for germ cell specification and development (9). Of particular interest, a pair of PR domain-containing factors, *Blimp-1*/*Prdm1* and *Prdm14*, is essential for early PGC development. *Blimp-1* is detectable at the earliest stage of PGC specification and is essential for repressing somatic programs such as *Hox* gene expression. *Prdm14* expression follows *Blimp-1* expression in PGCs and also plays a pivotal role in germ cell specification (10, 11). Both mutants of these genes fail to allocate the PGC population, which has been attributed to the impaired repression of somatic programs followed by genome-wide reprogramming. Although it is unclear whether PR domains are essential for reprogramming, *Blimp-1* is known to interact with many epigenetic factors, including the argin-

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1 and Fig. S1.
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[4] The abbreviations used are: ES, embryonic stem; iPS, induced pluripotent stem; PGC, primordial germ cell; PKO cells, Prmt5, Klf4, and Oct3/4-induced pluripotent cells; MEF, mouse embryonic fibroblast; EG, embryonic germ.

ine-specific histone methyltransferase, *Prmt5*. *Prmt5* is thought to play a role in epigenetic reprogramming in germ cells (12, 13). During germ cell development, epigenetic reprogramming occurs via massive DNA demethylation and altered histone modification that normally occurs in the embryonic gonad (14), suggesting that the combined expression of *Blimp-1*, *Prmd14*, and *Prmt5* evokes proper epigenetic reprogramming in PGCs. Likewise, during iPS cell induction, somatic cell gene expression is repressed and histone modification patterns are markedly altered (15). These findings suggest that there is a functional relationship between germ cell development and somatic cell reprogramming.

Here, we investigated whether *Blimp-1*, *Prdm14*, and *Prmt5*, which are important for reprogramming in PGCs, are also active in somatic cell reprogramming. We screened cells expressing combinations of these transcription factors and found that all of these factors had reprogramming activity. Somatic cell reprogramming of MEFs was especially effective when *Prmt5* was introduced in combination with *Klf4* and *Oct3/4* (PKO cells). PKO cells were indistinguishable from ES cells in terms of their gene expression profile, DNA methylation status, capacity to differentiate into the three germ layers, and germline transmission. Here we show that factors with essential roles in germ cell development are also active in somatic cell reprogramming.

EXPERIMENTAL PROCEDURES

Mice—A *Nanog-GFP-IRES-puro* transgenic mouse (RBRC02290) was provided by RIKEN BRC, which is participating in the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (16). C57BL/6 and Balb/c nude mice were purchased from Japan SLC (Shizuoka, Japan). Animal care was in accordance with the guidelines of Keio University for animal and recombinant DNA experiments. MEFs were prepared from E13.5 embryos obtained by crossing *Nanog-GFP* mice with C57BL/6.

Cell Culture—PKO cells were cultured on mitomycin C-treated STO cells with knock-out DMEM containing 15% FCS, LIF, penicillin/streptomycin, L-glutamine, β-mercaptoethanol, and non-essential amino acids. The PKO cells were depleted of feeder cells by being incubated twice on a 2% gelatin-coated dish. Collected cells were used for DNA and RNA analysis.

Feeder Cell Preparation—STO cells were treated with 12 μg/ml of mitomycin C for 2 h and plated at a density of 1×10^6 cells/55 cm^2.

Plasmids—Retroviral plasmids for iPS cell induction were provided by Addgene as follows: pMXs-*Sox2* (Addgene plasmid 13367), pMXs-*Oct3/4* (Addgene plasmid 13366), pMXs-*Klf4* (Addgene plasmid 13370), and pMXs-c-*Myc* (Addgene plasmid 13375). The *Blimp-1-IRES-GFP* vector was a gift from Dr. Kiyoshi Takatsu and the IRES-GFP region was excised (17). *Prdm14* and *Prmt5* were cloned by PCR, inserted into the pGEM-T-easy plasmid (Promega), and converted to pMXs via the BamHI and XhoI sites. The PCR primers used were: *Prdm14*, forward, 5′-CCGCGGTCCGCAAAACTCAGGCC-ACCATGG-3′, and *Prdm14*, reverse, 5′-CTCGAGAACCAT-

GCCCACGCGACACAGACA-3′; *Prmt5*, forward, 5′-GGAT-CCGGCCGCGCGAGGGCCACCATGGCG-3′, and *Prmt5*, reverse, 5′-CTCGAGAACCAGCAGATGTTCTACACC-TTC-3′.

Reprogramming of MEFs by Blimp-1, Prdm14, and Prmt5— PKO cells were generated by the iPS cell induction method as described previously (16), except for cell density at the time of re-seeding. MEFs transfected with *Prmt5*, *Klf4*, and *Oct3/4* were reseeded on mitomycin C-treated STO cells at 5 days after infection, at the cell densities indicated under Table 2. Primary ES cell-like colonies of MEFs transfected with *Blimp-1*, *Prdm14*, and c-*Myc* were reseeded onto a 0.1% gelatin-coated dish without STO feeder cells at a density of 580,000 cells/35-mm dish.

Gene Expression Analysis—Primers and probes for *Oct3/4*, *Sox2*, *Klf4*, and c-*Myc* were designed to distinguish between endogenous and viral transcripts (see supplemental Table S1). Transcript levels were determined using the 7500 Fast Real Time PCR system (Applied Biosystems).

Bisulfate Sequencing—Bisulfate reactions were performed with the EpiTecht Bisulfite Kit (Qiagen) according to the manufacturer's instructions. Primers used for the PCR have been described previously (7, 16). PCR products were cloned into the pGEM-T Easy plasmid (Promega) and sequenced by conventional methods.

Teratoma Formation—To produce teratomas, 1.0×10^6 cells were suspended in BD Matrigel (BD Biosciences) and injected into nude mice. Three to 4 weeks later, tumors were fixed with 4% paraformaldehyde in PBS, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Creation of Chimeric Mice—To analyze the germ cell contribution of PKO cells, PKO cells were injected into blastocysts from ICR mice. Gonads were isolated at E12.5 and examined for PKO cell-derived *Nanog-GFP* fluorescence. At the same time, the contribution of PKO cells to the eyes was also analyzed by the examination of pigment cells. To generate adult chimeric mice from PKO cells, blastocysts with a B6 background were used. Functional germ cell development was analyzed by the fluorescence of *Nanog-GFP* in the F1 morula.

Microarray Data Analysis—Expression profiles were analyzed using the three-dimensional Gene Mouse Oligo chip 24K (Toray Industries, Tokyo, Japan). The fluorescence intensities were detected using the Scan-Array Life Scanner (PerkinElmer Life Sciences). PMT levels were adjusted to achieve 0.1–0.5% pixel saturation. Each TIFF image was analyzed using GenePix Pro version 6.0 software (Molecular Devices, Sunnyvale, CA). The data were filtered to remove low confidence measurements and globally normalized per array, such that the median signal intensity was adjusted to 50 after normalization (accession number GSE18813).

Detection of Introduced Alleles—Genomic integration of introduced factors were analyzed with the following primers: *Virus Klf4*, forward, 5′-CATCCTCTAGACTGCCGGATCT-3′ and *Virus Klf4*, reverse, 5′-GATTCAATATAAACCGGCG-ATGTC-3′; *Virus Sox2*, forward, 5′-TTAAGGATCCCAGTG-TGGTGGTA-3′, and *Virus Sox2*, reverse, 5′-TTCAGCTCCG-TCTCCATCATG-3′; *Virus Oct3/4*, forward, 5′-CATCCTCT-AGACTGCCGGATCT-3′, and *Virus Oct3/4*, reverse,

5′-TGCTTCAGCAGCTTGGCAAACTGT-3′; *Virus* c-*Myc*, forward, 5′-CATCCTCTAGACTGCCGGATCT-3′, and *Virus* c-*Myc*, reverse, 5′-AGGTCATAGTTCCTGTTGGTGAAGT-3′; *Virus Prmt5*, forward, 5′-CATCCTCTAGACTGCCGGA-TCT-3′ and *Virus Prmt5*, reverse, 5′-AATTCAGGTCCCTC-CCGCTGGACA-3′; control (*IL-2*), forward, 5′-CTAGGCCA-CAGAATTGAAAGATCT-3′, and control (*IL-2*), reverse, 5′-GTAGGTGGAAATTCTAGCATCATCC-3′.

Knockdown of Prmt5—Previously reported sequences were used for the knockdown of *Prmt5* (18). The sequences were introduced with a retroviral expression vector (19). Virus production and infection during the reprogramming process were performed as described as above (16). The viruses for knockdown were added at a 3 times higher concentration than the reprogramming factors.

Purification of MEFs after Three- or Four-factor Infection—MEFs infected with three or four factors were harvested at days 3 and 12. The cells were incubated with anti-*FcγR* antibody (2.4G2) (eBioscience) at 4 °C for 30 min. Then, cells were continuously incubated with allophycocyanin-conjugated anti-*Thy-1* monoclonal antibody (53-2.1) (BD Biosciences) and PE-conjugated anti-*SSEA-1* monoclonal antibody (MC-480) (BD Biosciences) for 30 min at 4 °C. After washing, samples were sorted by FACS AriaII (BD Biosciences).

RESULTS

Screen for the Reprogramming Activity of Blimp-1, Prdm14, and Prmt5—The unique relationship between PGCs and pluripotency led to an investigation of the reprogramming activity of factors involved in PGC development, EG cell derivation, or both. Factors that met the following criteria were selected: (i) preferential expression in early PGCs during reprogramming, (ii) a role in early PGC development, and (iii) involvement in pluripotent EG cell derivation. This screen yielded three candidate genes: *Blimp-1* (also known as *Prdm1*), *Prdm14*, and *Prmt5*.

Subsequently, the reprogramming activity of *Blimp-1*, *Prdm14*, and *Prmt5* was examined in somatic cells. Various combinations of the three factors were introduced, along with *Oct3/4*, *Sox2*, *Klf4*, or c-*Myc*, into MEFs carrying the *Nanog-GFP* reporter gene (Table 1). Although no *Nanog*-positive colonies grew after the expression of each of the three factors alone, *Nanog-GFP*-positive colonies were detected in two conditions when the cells were co-infected with the three known factors. In the first instance, MEFs co-infected with *Blimp-1*, *Prdm14*, and c-*Myc* (Table 1, case 4) produced primary colonies with an ES cell-like morphology (Fig. 1). A few colonies occasionally expressed *Nanog-GFP* but most did not (Fig. 1), and the *Nanog*-positive colonies exhibited severely inhibited growth. Moreover, cell growth was arrested when the *Nanog-GFP*-positive colonies were isolated and re-plated. Thus, despite the emergence of primary *Nanog-GFP*-positive colonies after infection with *Blimp-1*, *Prdm14*, and c-*Myc*, the MEFs did not become completely reprogrammed pluripotent stem cells. Because *Blimp-1* is not expressed in ES cells (Fig. 1), it is possible that this factor acts only during the induction phase. Indeed, overexpression of Blimp-1 in ES cells resulted in cell growth defects (Fig. 1). This may explain why the appearance of *Nanog*-

GFP colonies was very rare and why a cell line could not be established by infection with *Blimp-1*, *Prdm14*, and c-*Myc*. Complete silencing of *Blimp-1* may be necessary to establish a cell line during reprogramming.

Successful Generation of a Stable Line of Nanog-GFP-positive Cells by the Introduction of Prmt5, Klf4, and Oct3/4—The second instance in which *Nanog-GFP*-positive primary colonies were detected occurred following the co-infection of cells with *Prmt5*, *Klf4*, and *Oct3/4* (Table 1, case 18). Under these conditions, we observed ES cell-like primary colonies, many of which were positive for *Nanog-GFP* (Fig. 1). In contrast to the primary colonies derived from *Blimp-1/Prdm14*/c-*Myc* co-infection, *Nanog-GFP*-positive colonies from *Prmt5/Klf4/Oct3/4* co-infection grew and were successfully maintained as stable lines (Fig. 1). These cells were subsequently designated PKO cells (*Prmt5*, *Klf4*, and *Oct3/4*). Genomic PCR analysis showed that PKO cells actually integrated exogenous *Prmt5*, *Klf4*, and *Oct3/4*, but not *Sox2* or c-*Myc* (Fig. 1). PKO cells were successfully re-plated and their growth rate was comparable with the growth rate of ES cells derived from the inner cell mass of the blastocyst. Even with continuous passage, the level of *Nanog-GFP* expression in PKO cells remained as high as during the early passage. Upon induction of differentiation, *Nanog-GFP* expression was down-regulated, supporting that PKO cells are reprogrammed pluripotent stem cells with ES cell characteristics.

Gene Expression and DNA Methylation Patterns in PKO Cells—The gene expression patterns in PKO cells were analyzed. PKO cells exhibited up-regulated endogenous expression of many known reprogramming factors, including *Oct3/4*, *Sox2*, *Klf4*, and c-*Myc* (Fig. 2). In addition, PKO cells expressed other ES cell-specific genes such as *Nanog*, *ECAT-1*, and *Eras*, whereas parental MEFs did not (Fig. 2) (20, 21). Although PKO cells were induced by *Prmt5*, which is critical for germ cell specificity, the gene expression patterns of PKO cells were distinct from those of germ cells, as shown by the expression of *MVH* (mouse vasa homolog) and *Blimp-1* (Fig. 2) (10, 22). Furthermore, microarray analysis of PKO cells showed that the gene expression patterns of PKO cells became ES cell-like (Fig. 2).

Next, the DNA methylation states of the *Oct3/4-* and *Nanog*-regulatory elements were examined (16). In contrast to the promoter regions of these two genes in MEFs, the promoter regions of these two genes were demethylated in PKO cells (Fig. 3). Compared with ES cells, the *Oct3/4* locus of PKO cells was partially methylated, indicating that the PKO was composed of heterogeneous cells, some of which could have been incompletely reprogrammed. To distinguish whether PKO cells were derived from the direct reprogramming of MEFs or by mediating MEF-derived PGCs from *Prmt5/Klf4/Oct3/4* co-infection, the DNA methylation pattern of the imprinted loci, *Igf2r*, was examined. Because germ cells lose the parental methylation pattern, even the early stage PGCs, the methylation status would be altered if the PKO cells were derived from PGCs. However, the *Igf2r* locus was not altered after reprogramming (Fig. 3). Thus, PKO cells did not progress through the germ cell phase during

TABLE 1
Screened combination of introduced genes

Case	Name	Induced factors							
		GFP[a]	Klf4	Sox2	Oct3/4	c-Myc	Blimp-1	Prdm14	Prmt5
1	K,1,14		+				+	+	
2	S,1,14			+			+	+	
3	O,1,14				+		+	+	
4	M,1,14	+				+	+	+	
5	K,1,5		+				+		+
6	S,1,5			+			+		+
7	O,1,5				+		+		+
8	M,1,5					+	+		+
9	K,14,5		+					+	+
10	S,14,5			+				+	+
11	O,14,5				+			+	+
12	M,14,5					+		+	+
13	K,S,1		+	+			+		
14	K,S,14		+	+				+	
15	K,S,5		+	+					+
16	K,O,1		+		+		+		
17	K,O,14		+		+			+	
18	K,O,5	+	+		+				+
19	S,O,1			+	+		+		
20	S,O,14			+	+			+	
21	S,O,5			+	+				+
22	K,S,1,14		+	+			+	+	
23	K,O,1,14		+		+		+	+	
24	S,O,1,14			+	+		+	+	
25	K,S,1,5		+	+			+		+
26	K,O,1,5		+		+		+		+
27	S,O,1,5			+	+		+		+
28	K,S,14,5		+	+				+	+
29	K,O,14,5		+		+			+	+
30	S,O,14,5		+	+			+	+	
31	1,14						+	+	
32	1,5						+		+
33	14,5							+	+
34	1						+		
35	14							+	
36	5								+
37	K,1		+				+		
38	S,1			+			+		
39	O,1				+		+		
40	M,1					+	+		
41	K,14		+					+	
42	S,14			+				+	
43	O,14				+			+	
44	M,14					+		+	
45	K,5		+						+
46	S,5			+					+
47	O,5				+				+
48	M,5					+			+
49	1,14,5						+	+	+
50	K,S,O	+	+	+	+				
51	K,S,O,M	+	+	+	+	+			

[a] *Nanog-GFP* positive colonies.

reprogramming, indicating that they are similar to ES cells but not EG cells.

In Vivo Differentiation Capacity of PKO Cells—Finally, we tested the pluripotency of PKO cells. When PKO cells were injected into nude mice, PKO cells produced teratomas that contained tissues derived from all three germ layers (Fig. 4). Although some PKO cells contained a methylated *Oct3/4*-regulatory element, PKO cells were able to differentiate into germ cells through the formation of a chimeric embryo (Fig. 4). PKO cells were also detected in the eyes of chimeric embryos (Fig. 4). In addition to their presence in the embryo, adult chimeras were also produced from PKO cells and some of these demonstrated the incorporation of PKO cells by their coat color (Fig. 4). Furthermore, chimeric mice produced PKO cell-derived F1 progeny, confirmed by *Nanog-GFP* fluorescence of the morula (Fig. 4). These results demonstrated that somatic cells were reprogrammed to a pluripotent status by expression of reprogramming factors *Prmt5*, *Klf4*, and *Oct3/4*.

Knockdown of Prmt5 Decreases the Formation of Nanog-GFP Colonies in 4- and 3-Factor-mediated Reprogramming—To investigate whether endogenous *Prmt5* also contributed to reprogramming by Yamanaka factors, we knocked down the expression of Prmt5 in combination with the exogenous expression of four or three known reprogramming factors (*Oct3/4*, *Klf4*, and *Sox2* with or without c-*Myc*). The knockdown efficiency was estimated in ES cells by RT-PCR (Fig. 5). The number of *Nanog-GFP* positive colonies was counted at day 14 for four factors and at day 18 for three factors, respectively (Fig. 5). In both cases, *Nanog-GFP* colonies were decreased by *Prmt5* knockdown, especially in the case of four factors. These data indicate that Prmt5 also participates in the reprogramming process mediated by Yamanaka factors.
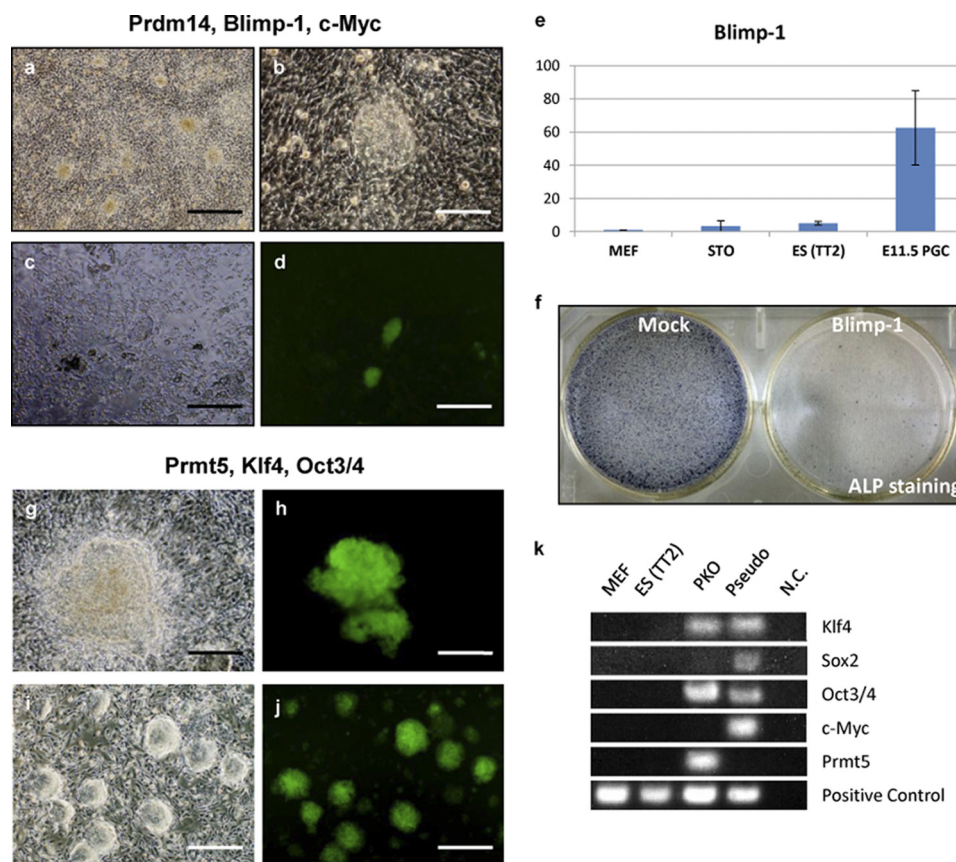
FIGURE 1. **Reprogramming of MEFs by *Blimp-1*, *Prdm14*, and *Prmt5*.** *a–d*, the morphology of MEFs transfected with *Prdm14*, *Blimp-1*, and c-*Myc*. ES cell-like colonies are shown at 16 days after induction (*a* and *b*). *Panel b* shows a higher magnification of *a*. *Nanog-GFP*-positive colonies are shown at 14 days after induction (*c* and *d*). Representative phase-contrast (*c*) and *Nanog-GFP* fluorescence (*d*) images are shown. *e* and *f*, endogenous expression and exogenous overexpression of *Blimp-1* in ES cells. The relative expression profiles of endogenous *Blimp-1* in ES cells compared with those in MEFs, STO cells, and E11.5 PGCs (*e*). ES cells transfected with a *CAG-Blimp-1* vector contained the *Blastsidin* resistance gene. Two days after transfection, cells were re-plated and 10 μg/ml of *Blastsidin* was added. After 4 days (6 days post-transfection), cells were fixed and stained for alkaline phosphatase (*ALP*) activity (*f*). *g-j*, morphologies of MEFs transfected with *Prmt5*, *Klf4*, and *Oct3/4*. *Nanog-GFP*-positive colonies at 28 days after induction (*g* and *h*) and subsequently established PKO cells (*i* and *j*) are depicted. *Scale bars* = 500 μm (*a*, *c*, *d*, *i*, and *j*), 100 (*b*), and 200 μm (*g* and *h*). *k*, integration of retroviral genes. Genomic DNA was purified from the indicated cells, and PCR with primers specific for *Klf4*, *Sox2*, *Oct3/4*, c-*Myc*, *Prmt5*, and the *IL-2* locus (control) was performed. Pseudo-colony cells were generated by infection with the four Yamanaka factors, but these cells were morphologically dissimilar to ES-like cells and negative for *Nanog-GFP*.

DISCUSSION

Because germ cells are closely related to pluripotent stem cells, certain endogenous factors in germ cells are predicted to have reprogramming activity. In this study, we demonstrated that the induction of *Blimp-1*, *Prdm14*, and c-*Myc* resulted in the partial reprogramming of somatic cells, whereas the induction of *Prmt5*, *Klf4*, and *Oct3/4* conferred pluripotency to somatic cells, making them comparable with ES cells with respect to gene expression, epigenetic modification of the promoter regions of *Nanog* and *Oct3/4*, and the potential to differentiate into all three germ layers. *Blimp-1* is an essential factor in germ cell specification, demonstrated by the failure of *Blimp-1*-deficient PGCs to suppress *Hox* gene expression (10). *Prdm14* is also essential for establishing PGCs, and *Prdm14* deficiency leads to the failure of *Sox2* reactivation in PGCs. More importantly, *Prdm14*-deficient PGCs fail to give rise to pluripotent EG cells (11). In addition, in human ES cells, *PRDM14* is important for maintaining pluripotency (23). The third factor, *Prmt5*, forms a complex with *Blimp-1* in PGCs and is thought to be important for PGC development (13). Furthermore, *Prmt5* is important for PGC conversion into EG cells

(24). Therefore, each of the three factors is essential factors for germ cell specificities.

iPS cell generation was first characterized by exogenous expression of transcription factors *Oct4*, *Sox2*, *Klf4*, and c-*Myc* (9). Thereafter, it became evident that the control of epigenetic regulators, such as histone deacetylase and *G9a* (histone H3K9-specific methyltransferase), could improve the efficiency of somatic cell reprogramming (25, 26). It is noteworthy that both histone deacetylase and *G9a* play inhibitory roles in reprogramming. Until now, no epigenetic factor was known to act as a positive regulator of somatic cell reprogramming. The work described in this study demonstrates that *Prmt5* could be one such factor, as it promoted somatic cell reprogramming when it was overexpressed in MEFs together with *Klf4* and *Oct3/4*. Furthermore, the knockdown of *Prmt5* showed that it also works in the reprogramming process mediated by Yamanaka factors. Taken together, it is important to consider the role of *Prmt5* from both genetic and epigenetic aspects of the reprogramming process. The reprogramming activity of Prmt5 could depend on its ability to symmetrically methylate arginine R3 of histone H2/4 and arginine R8 of H3 (27), a process that has been linked
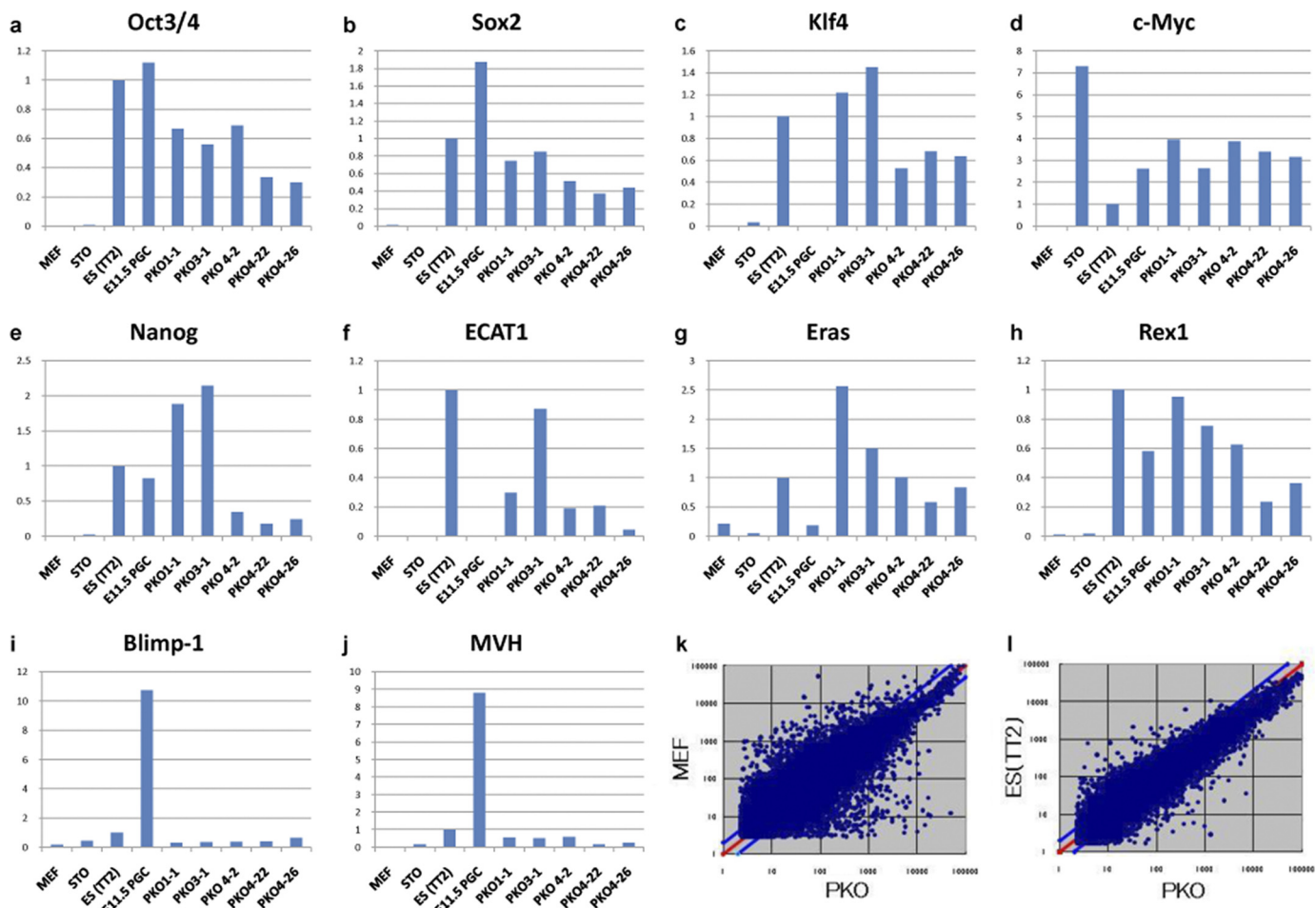
FIGURE 2. **Gene expression profiles of PKO cells.** *a–j,* the relative gene expression profiles of PKO cells were compared with those of ES cells, MEFs, STOs, and PGCs. The endogenous expression profiles of the four iPS cell-inducible factors (*a–d*), ES cell-specific markers (*e–h*), and germ cell markers (*i* and *j*) for each cell type are shown. *k* and *l,* microarray analysis of PKO cells. PKO cells were compared with MEFs (*k*) or ES cells (*l*) by microarray.
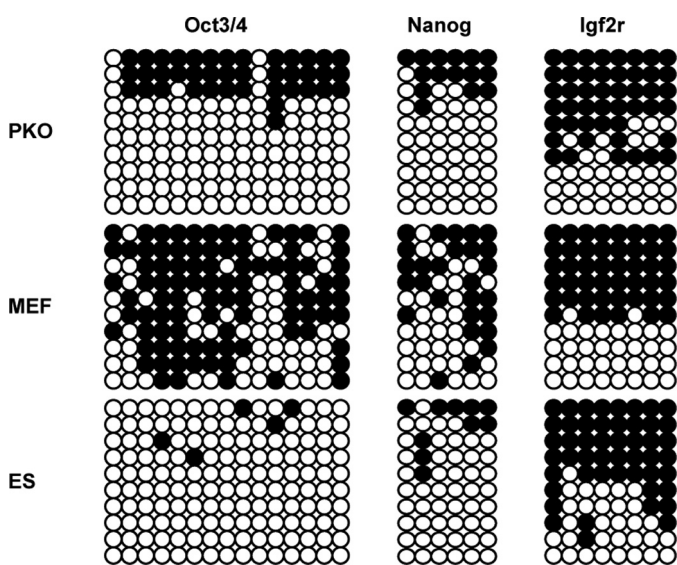

FIGURE 3. **DNA methylation of ES cell-specific genes and imprinted genes in PKO cells.** The bisulfite sequence at the promoter regions of *Oct3/4* and *Nanog* in PKO cells and the differentially methylated region of *Igf2r* are shown. *White circles* indicate unmethylated CpG dinucleotides, whereas *black circles* indicate methylated CpG dinucleotides.

to gene silencing (18). Alternatively, arginine methylation of proteins other than histones could be crucial for somatic cell reprogramming. Indeed, it has been reported that Prmt5-mediated arginine methylation of *p53* weakens its target specificity and function (28). Because p53 inhibits the generation of iPS cells (5), it is also feasible that functional inhibition of p53 by Prmt5 contributes to the generation of PKO cells. To evaluate these possibilities, it will be important to identify the target of Prmt5 methylation and the effect of p53 activity during PKO cell generation. Furthermore, to address the function of *Prmt5* during PKO cell generation, the subcellular localization of Prmt5 must also be evaluated because Prmt5 localization changes during the conversion of unipotent PGCs to pluripotent EG cells (24). Although the significance of the location and activation of Prmt5 is not yet clear, it is likely to be important for future identification of the molecular targets of Prmt5. Dign *et al.* (29) demonstrated that a combination with *Oct3/4* introduction, inhibitor for Prmt, and TGF-βR could induce iPS cells. A Prmt inhibitor, AMI-5, which they used inhibits the activities of Prmt1/3/4/6. It suggests unique reprogramming activity of Prmt5.

Although *Prmt5*, *Klf4*, and *Oct3/4* induced pluripotency in somatic cells, the process was largely inefficient and the timing of reprogramming was relatively late compared with the estab-
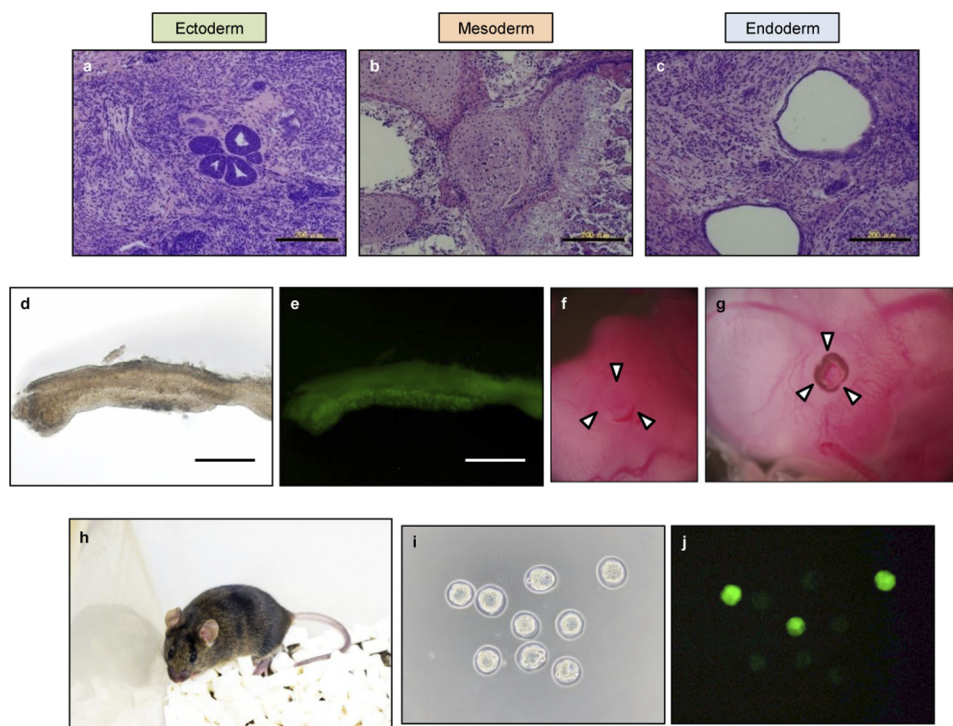
FIGURE 4. ***In vivo* differentiation capacity of PKO cells.** *a–c,* teratomas formed from PKO cells contained ectoderm (*a*), mesoderm (*b*), and endoderm (*c*). *Scale bars* = 200 μM. *d* and *e,* the genital ridge from an E12.5 PKO chimeric embryo. Phase-contrast image (*d*) and PKO cell-derived *Nanog-GFP* fluorescence (*e*). *Scale bars* = 500 μM. *f* and *g,* contribution of PKO cells in a chimeric embryo. PKO cell contribution to the eyes of a chimera (*g*) compared with a non-chimeric embryo (*f*). *h,* adult chimera of PKO cells. Chimerism was roughly estimated by aguti coat color. *i* and *j,* morula of F1 progeny. Phase-contrast image (*i*) and PKO cell-derived *Nanog-GFP* fluorescence (*j*) are shown.
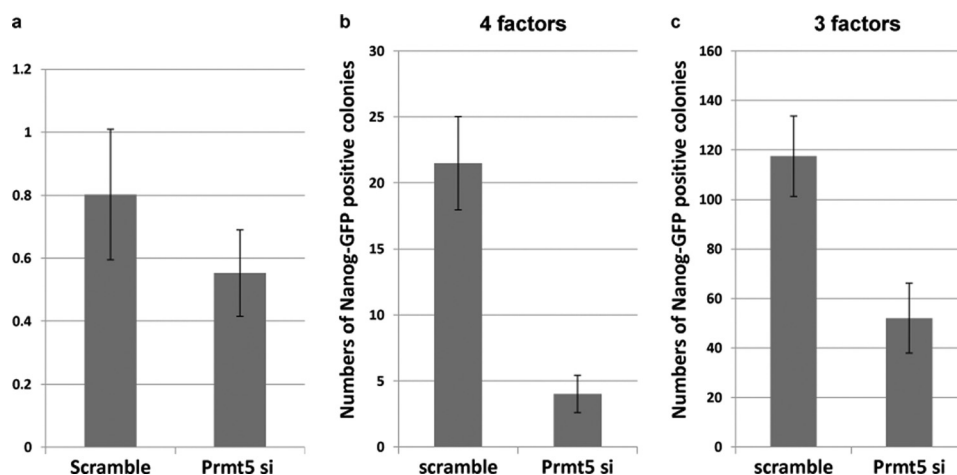

FIGURE 5. **Knockdown of Prmt5 in the reprogramming process.** *a,* vectors that contained the sequence for Prmt5 knockdown or a scrambled sequence were electroporated into ES cells. Two days after electroporation, bulk cells were collected and analyzed for expression of *Prmt5*. Relative expression compared with non-electroporated ES cells is shown. *b* and *c,* MEFs that contain the *Nanog-GFP-IRES-puro* allele were infected with a retrovirus containing the *Prmt5* knockdown sequence or a scrambled sequence and a combination of three or four factors (*Oct3/4*, *Klf4*, and *Sox2* with or without c-*Myc*). The numbers of puromycin-resistant colonies were counted at day 14 for four factors (*b*) or day 18 for three factors (*c*) post-infection, respectively.

lished method (Table 2). This indicates that reprogramming in these cells occurred in a novel manner. Supporting this idea, the expression of *Prmt5* was not up-regulated after infection of three or four factors (supplemental Fig. S1). The repression of somatic cell genes is an early event in reprogramming (30), and c-*Myc* overexpression has a unique effect on repression compared with the other three reprogramming factors (*Oct3/4*, *Klf4*, and *Sox2*) (31). Interestingly, our preliminary data showed that the activity of *Prmt5* in repressing the somatic cell gene, *Thy-1*, is similar to the activity of c-*Myc*. Because germ cell

development is accompanied by the repression of somatic lineage differentiation, it is likely that *Prmt5* may repress *Thy-1* expression.

PGCs arise directly from the epiblast (9). During the process of cell commitment, PGCs have a unique gene expression profile that includes *Sox2* reactivation and *Hox* gene repression (9). *Blimp-1* and *Prdm14* play central roles in establishing these gene expression patterns. Thereafter, PGCs change their genome-wide chromatin structure during development. As shown here, factors that are important in early PGCs also have

TABLE 2
PKO cell colony numbers

Well No.	Number of cells at reseeding	Puromycin selection	Numbers of *Nanog-GFP* positive colonies			
			Day 23	Day 26	Day 29	Total
	$\times 10^5/35$ mm	days after infection				
1	8.75	16	2	0	0	2
2	5.8	16	3	0	0	3
3	0.58	16	1	0	0	1
4	8.75	23	3	5	2	10
5	5.8	23	0	9	3	12
6	0.58	23	0	2	0	2
Yamanaka's method	0.58	14				

reprogramming activity, we show that several genes expressed in PGCs could be involved in the reprogramming of somatic cells. During development, genome-wide reprogramming that occurs in PGCs is the sole process by which differentiating epiblast cells are efficiently reprogrammed (32). As such, there are a number of similarities between the reprogramming in PGCs and generation of iPS cells, such as the expression of *Sox2* and *Nanog*, a functional requirement for *Lin28*, and repression of *G9a* activity (3, 26, 32, 33). These data, together with the results of the present study, strongly indicate that other reprogramming factors exist that are involved in both PGC development and iPS cell derivation. A more thorough understanding of how germ cells undergo genome-wide cellular reprogramming should provide useful clues about the identities of these factors and increase our knowledge of somatic cell reprogramming.

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