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MINIREVIEWS

Reprogramming of germ cells into pluripotency

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Abstract

Primordial germ cells (PGCs) are precursors of all gametes, and represent the founder cells of the germline. Although developmental potency is restricted to germ-lineage cells, PGCs can be reprogrammed into a pluripotent state. Specifically, PGCs give rise to germ cell tumors, such as testicular teratomas, *in vivo*, and to pluripotent stem cells known as embryonic germ cells *in vitro*. In this review, we highlight the current knowledge on signaling pathways, transcriptional controls, and post-transcriptional controls that govern germ cell differentiation and de-differentiation. These regulatory processes are common in the reprogramming of germ cells and somatic cells, and play a role in the pathogenesis of human germ cell tumors.

Key words: Primordial germ cell; Embryonic germ cell; Germ cell tumor; Reprogramming; Induced pluripotent stem cell; Small molecule compound; Gene; Signal; Transcription factor

Core tip: Primordial germ cells can be reprogrammed into pluripotent stem cells called as embryonic germ cells *in vitro* and into pluripotent germ cell tumors *in vivo*. Germ cell reprogramming can be regulated by signaling pathways, including PI3K/Akt signaling, mitogen-activated protein kinase signaling, transforming growth factor-β signaling, RA signaling. These mechanisms are also involved in somatic cell reprogramming, indicating that there exist common regulatory networks regulating germ and somatic cell reprogramming. On the other hand, regulators for germ cell development prevent germ cell dedifferentiation in unique manners.

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INTRODUCTION

The germ lineage is a privileged cell lineage that transmits genetic and epigenetic information from generation to generation[1]. Primordial germ cells (PGCs) are embryonic germ cell (EGC) precursors that eventually differentiate into sperm or oocytes[2,3]. In mice, a population of proximal epiblast cells in egg cylinder-stage embryos is committed to PGC precursors at embryonic day 6.25 (E6.25). During gastrulation, PGC precursors migrate out of embryos into the extraembryonic region, where a small number of nascent PGCs emerge at E7.0. PGCs return to embryos at E7.75, migrate through the hindgut and dorsal mesentery, and finally colonize the genital ridges until E11.5. PGCs actively proliferate and increase in number from E7.0 to E13.5, being transiently arrested in the G2/M phase at E8.5. In the gonads, PGCs undergo sex-dependent differentiation under the influence of somatic cells. Male germ cells enter into mitotic arrest after E13.5 and retain mitotic quiescence during embryogenesis. After birth, male germline stem cells (GSCs) called spermatogonia resume proliferation and produce sperm *via* meiosis and sperm morphogenesis (spermiogenesis). In contrast, female germ cells enter into meiosis at E13.5, and oocytes mature and are ovulated after birth.

Although totipotency is restored after fertilization, germ-lineage cells differentiate into only sperm or oocytes, but never into somatic cell types, during normal development. However, PGCs can be reprogrammed into pluripotency or can de-differentiate under experimental and pathological conditions as described below. In this review, we present an overview of the molecular mechanisms underlying germ cell preprogramming and germ cell tumor pathology, and discuss the features shared by germ cell and somatic cell reprogramming.

DIFFERENTIATION AND DE-DIFFERENTIATION OF PGCS

PGC differentiation

A number of events take place during PGC specification[2,3]. These include transcriptional activation of germ cell-specific genes [Stella and Deadend-1 (*Dnd1*)], reactivation of pluripotency-related genes (*Sox2* and *Nanog*), and repression of the somatic cell differentiation program. Epigenetic reprogramming occurs concomitantly. DNA methylation is globally erased through two waves by passive and active demethylation mechanisms, and unique genome-wide histone modification patterns are established (acquisition of H3K27me3 and loss of H3K9me2).

Three transcription factors, Blimp1 (Prdm1), Prdm14, and Tfap2c (AP2γ), play central roles in the specification of PGCs from the epiblast. Blimp1 expression commences in PGC precursors, the most proximal layer of the epiblast, at E6.25[4]. Expression of *Prdm14* follows soon after the onset of *Blimp1* expression in the precursors[5]. *Tfap2c* may be a downstream target of Blimp1[6]. In mice lacking these transcription factors, PGC precursors and nascent PGCs have abnormal gene expression patterns and epigenetic status. Gene expression analysis has revealed that Blimp1 represses somatic cell gene expression and Prdm14 activates germline and pluripotency genes[5,7]. Additionally, forced expression of these three transcription factors sufficiently promotes the differentiation of PGC-like cells from embryonic stem cells (ESCs) in culture[8,9].

PGC specification is regulated by interactions with surrounding somatic-lineage cells. Bone morphogenetic protein 4 (BMP4) is secreted from extraembryonic ectoderm, and is critical for the induction of PGC precursors and mesodermal cells from the epiblast *in vivo*[10]. Furthermore, treatment of epiblast explants with BMP4 activates the expression of *Blimp1* and *Prdm14* and induces the formation of PGC-like cells in culture[11], which suggests that BMP4 is an upstream regulator of *Blimp1* and *Prdm14*. Other BMP family proteins, BMP8b and BMP2 (which are secreted from extraembryonic ectoderm and visceral endoderm, respectively), may support PGC specification along with BMP4[11-14]. Wnt3a is also essential for the specification of PGCs and mesodermal cells. Since epiblast explants isolated from *Wnt3a*-deficient mice do not generate PGC-like cells in response to BMP4[11], Wnt3 seems to enable epiblast to respond to BMP4. Finally, the suppression of mitogen-activated protein kinase (MAPK) signaling is critical for the induction of PGC-like cells in the lineage choice between germ and mesodermal cells[15].

Testicular teratomas

Germ cell tumors are classified into two groups: Germinomas (seminomas) and non-germinonatous tumors[16,17]. Testicular teratomas belong to the latter group, and contain a variety of differentiated cells and tissue structures, which belong to the ectoderm, endoderm, and mesoderm lineages. Undifferentiated cells called embryonal carcinoma cells (ECCs) are also found in testicular teratomas[18]. ECC lines can be established from teratomas and maintained indefinitely in culture. However, these cell lines are usually multipotent rather than pluripotent because the cells differentiate into a limited number of cell types *in vitro* and *in vivo*. Teratomas often occur outside of the testis. Non-germinonatous germ cell tumors include yolk sac tumors and choriocarcinomas.

The etiology of testicular teratomas has been extensively studied using the 129/Sv inbred mouse strain, which frequently develops juvenile testicular teratomas[18]. Early teratomatous foci can be detected in E15.5 testes. Seminiferous tubule structures are disorganized, and teratomatous cells are found outside of the tubules thereafter. The foci contain a number of mitotically active cells, suggesting that these cells have failed to enter into mitotic arrest.

Teratoma onset is considered to be at around E12.5

in 129/Sv mice based on two lines of evidence. First, investigation of the sizes of the spontaneous tumors at various embryonic ages has indicated that tumor onset occurs at E12.5[18]. Secondly, when E12.5 gonads of 129/Sv mice were transplanted into the testes of adult 129/Sv mice, about 80% of the grafts developed into teratomas; conversely, the incidence of experimental teratomas was dramatically lower when E13.5 gonads were transplanted[19]. It is noteworthy that testicular teratomas do not develop in other inbred mouse strains both spontaneously and experimentally, suggesting that the genetic background affects the occurrence of teratomas.

The homozygous steel (*Sl*) mutant mouse has been used to show that testicular teratomas originate from germ cells in the gonads[20]. The *Sl* locus encodes a growth factor Kit ligand (KITLG, also known as stem cell factor), which activates the receptor tyrosine kinase c-Kit. c-Kit is expressed in migratory and gonadal PGCs, and its signaling is required for their proliferation and survival *in vivo*. When E12.5 gonads of 129/Sv mice carrying the homozygous *Sl*/*Sl* mutation were transplanted, no grafts developed into experimental teratomas, clearly demonstrating that teratomas are derived from PGCs.

EGCs

Studies that searched for PGC growth factors uncovered methods for reprogramming PGCs into pluripotent EGCs *in vitro*[21,22]. Treatment of PGCs with individual growth factors, such as KITLG, leukemia inhibitory factor (LIF), or basic fibroblast growth factor (bFGF), can promote the proliferation and survival of PGCs in culture. PGCs are responsive to these growth factors for only a few days, and eventually die *via* apoptosis. However, when LIF, KITLG, and bFGF are simultaneously added in culture, PGCs actively proliferate to form ESC-like, dome-shaped colonies (EGC colonies) within 5-7 d. In contrast, PGCs cultured in the presence of KITLG and LIF generate scattered colonies of cells with elongated morphology and do not lead to EGC formation.

After secondary cultures, EGCs can be propagated indefinitely in the presence of LIF, but without KITLG and bFGF[21]. When transplanted into blastocysts, EGCs can be incorporated into development and contribute to the three germ layers and germline in chimeric mice, indicating that EGCs have pluripotency equivalent to ESCs. However, when PGCs are transplanted into blastocysts immediately after isolation without culture, they never contribute to chimeric mice[23]. Thus, stimulation with KITLG, LIF, and bFGF can reprogram germline-committed PGCs into pluripotent EGCs. bFGF can be replaced by retinoic acid (RA) or forskolin[24,25], which increases the intracellular cyclic AMP (cAMP) concentration and leads to the activation of protein kinase A (PKA).

EGC derivation efficiency gradually decreases as germ cell differentiation proceeds. Efficiency is highest in E8.5 migratory PGCs, and sharply declines in E13.5 PGCs[21]. No EGCs can be derived from germ cells after E15.5[26]. In contrast to testicular teratomas, EGCs can be derived not only from 129/Sv mice but also from various other mouse strains. This indicates that PGCs intrinsically have the potential to be reprogrammed, regardless of genetic background, although genetic background has a strong influence on the pathogenesis of testicular teratomas *in vivo*.

PI3K/AKT SIGNALING

PI3K/Akt signaling in germ cell reprogramming

As stimulation with KITLG, LIF, and bFGF is required for the derivation of EGCs, signaling pathways downstream of these growth factors are likely critical for PGC reprogramming. Phosphoinositide-3 kinase (PI3K) is a lipid kinase activated by these growth factors. PI3K produces phosphatidylinositol 3,4,5-triphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2) and transmits signals *via* downstream effector proteins, such as the serine/threonine kinase Akt and the small GTPases Rac1 and Cdc42[27]. Akt promotes physiological and pathological processes, such as proliferation, survival, metabolism, and tumorigenesis, through the phosphorylation of various target proteins[28]. On the other hand, the tumor-suppressor gene product phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a lipid phosphatase that converts PIP3 to PIP2 and antagonizes PI3/Akt signaling.

PGC-specific *Pten*-deficient mice develop juvenile testicular teratomas with a high frequency despite their mixed genetic background[29]. In mutant mice, PGC differentiation appears normal until E13.5, because the expression of germ cell-specific genes such as mouse vasa homolog (Mvh) is activated in mutant PGCs as well as in control PGCs. However, mutant PGCs do not enter into mitotic arrest and a number of PGCs undergo apoptosis after E14.5. Teratomatous foci, which are weakly positive or negative for Mvh, are detected in the E15.5 testes of mutant mice. Additionally, EGC derivation efficiency is much higher in E11.5 PGCs isolated from *Pten* mutant mice than in those from control mice. These findings show that *Pten* is essential for the establishment of the male germ lineage, and suggest that hyperactivation of PI3K reprograms PGCs into pluripotent cells *in vivo* and *in vitro*.

The effects of downstream Akt signaling have been examined using transgenic mice expressing the Akt-Mer fusion protein, which is composed of the myristoylated active form of Akt and mutated ligand-binding domain of estrogen receptor (Mer)[26,30]. The kinase activity of Akt-Mer can be turned on or off by the addition or withdrawal, respectively, of the Mer ligand, 4-hydroxytamoxifen (4OHT). When E11.5 PGCs from transgenic mice are cultured in the presence of KITLG, LIF, and bFGF, EGC derivation efficiency is greatly enhanced by 4OHT treatment. Furthermore, whereas bFGF is essential for EGC derivation, EGCs can be efficiently derived from transgenic PGCs cultured with 4OHT, KITLG, and LIF but without bFGF, showing that Akt hyperactivation can replace bFGF. Thus, the PI3K/Akt signaling axis plays

pivotal roles in PGC reprogramming.

Male GSCs in the testes of postnatal mice also reportedly de-differentiate into pluripotent cells in culture, albeit much less frequently than do PGCs. For example, it has been shown that GSCs, which are established from neonatal mouse testis, spontaneously generate ESC-like colonies during long-term culture[31]. These cells are called multipotential GSCs (mGSCs), and show pluripotency equivalent to ESCs and EGCs. Although both PGCs and GSCs are germ-lineage cells, Akt activation does not enhance the emergence of mGSCs from GSCs[32].

Cellular processes and target molecules in the reprogramming of germ and somatic cells

Somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) by the introduction of the transcription factors Oct4, Sox2, Klf4, and c-Myc (OSKM)[33,34]. E-Ras is an ESC-specific small GTPase that activates PI3K. Overexpression of E-Ras and downstream active Akt enhance OSKM-induced iPSC derivation efficiency[35,36]. In this section, we discuss the cellular processes and target molecules downstream of PI3K/Akt signaling by comparing the germ cell and somatic cell reprogramming systems.

The tumor suppressor Trp53 is a gatekeeper that checks the balance between proliferation and apoptosis[37]. The amount and activity of Trp53 are regulated transcriptionally and post-transcriptionally by intrinsic and external stimuli that cause DNA damage and oncogenic activation. Mice lacking *Trp53* frequently develop testicular teratomas against the 129/Sv genetic background[38]. Akt activation in cultured PGCs inhibits nuclear accumulation of Trp53 and the phosphorylation required for maximal transcriptional activation of Trp53[26], suggesting that Akt inhibits Trp53 activity in PGCs during reprogramming. Furthermore, deletion of *Trp53* not only enhances the derivation efficiency of EGCs in the presence of KITLG, LIF, and bFGF, but also can replace bFGF[26]. This shows that Trp53 inhibition is a critical event downstream of Akt signaling.

Deletion or knockdown of *Trp53* also greatly enhances iPSC induction[39]. Whereas OSKM introduction and/or culture conditions induce cell cycle arrest in somatic cells during reprogramming, inhibition of Trp53 suppresses cell cycle arrest, promotes cell proliferation, and eventually leads to a high frequency of iPSC production. Moreover, the cell proliferation rate is well-correlated with reprogramming efficiency in iPSC production[40], suggesting the existence of proliferation-dependent reprogramming processes. Likewise, PGC reprogramming also seems to be proliferation-dependent as failure of mitotic arrest in both 129/sv mice and *Pten*-deficient mice *in vivo* leads frequent incidence of PGC dedifferentiation[19,29]. Akt activation enhances proliferation but suppresses apoptosis in cultured PGCs *in vitro*[26,30]. In addition to inhibiting Trp53, Akt is known to promote proliferation through many other target proteins, such as cyclin D and cyclin-dependent protein kinase inhibitors (CDKIs), p21Cip1, and p27Kip1[28,41]. In fact, mutation in *INK4* CDKI promotes incidence of spontaneous testicular teratomas in the absence of *Trp53*[42]. Cell cycle arrest represents a roadblock for reprogramming that can be overridden by higher proliferative activity both in somatic and germ cells.

Metabolic reprogramming, shifting from oxidative phosphorylation to glycolysis, is required for somatic cell reprogramming toward iPSCs[43]. Akt signaling promotes glycolysis by phosphorylation of the Foxo family transcription factors[28,41]. Foxo1 regulates the expression of genes involved in glycogenesis and gluconeogenesis, as well as in proliferation and apoptosis[44]. Akt inhibits the transcriptional activity of Foxo1 through its exclusion from the nuclei, leading to enhanced glycolysis. In fact, forced expression of the dominant-negative form of Foxo1 enhances the derivation efficiency of iPSCs[36]. The mechanistic target of rapamycin complex 1 (mTORC1) is another target of Akt that regulates metabolism[45]. As activation of mTORC1 by Akt inhibits mitophagy, Akt can promote oxidative phosphorylation in mitochondria and thereby antagonize metabolic reprogramming[46,47]. On the other hand, little is known about the metabolic status of PGCs or metabolic changes during germ cell reprogramming.

It has been suggested that only a fraction of cells are randomly selected for reprogramming because of the stochastic nature of the epigenetic reprogramming processes[40]. A number of repressive epigenetic modifications, such as DNA methylation, H3K9me3, and H3K79me2, and their regulators, have been identified as barriers to somatic cell reprogramming[48]. In addition, inhibition of histone deacetylase complex enhances iPSC induction[49,50]. Mbd3 is a component of the nucleosome remodeling deacetylase (NuRD) complex, which is involved in heterochromatin formation. It has been reported that the majority of cells are reprogrammed into iPSCs by knockdown of *Mbd3* in the secondary iPSC induction system[51], showing that the NuRD complex is one of the most important epigenetic roadblocks. In addition, the deletion of *Mbd3* also enhances the efficiency of EGC derivation from PGCs[51]. Gene expression analysis during PGC reprogramming shows that a great number of Mbd3 target genes are affected by Akt activation[52]. Additionally, Akt activation decreases expression of Mbd3 during somatic cell reprogramming. Collectively, the evidence suggests that PI3K/Akt signaling may promote germ and somatic cell reprogramming through multiple pathways, including proliferation, survival, metabolic change, and epigenetic regulation.

PI3K/Akt signaling in human germ cell tumors

Mutants and variants of *KIT* and *KITLG* have been identified as risk factors for human germ cell tumors[17]. A strong association between a variant of *KITLG* and the occurrence of testicular teratomas has been reported. *KIT* mutations, which activate kinase activity in a ligand-independent manner, are found frequently in testicular seminomas but not in testicular teratomas or yolk sac tumors[53,54]. *CBL* mutations have been found in

teratomas, yolk sac tumors, and mixed-type tumors composed of germinomas and non-germinomatous tumors, all of which occur intracranially[54]. Because *CBL* encodes ubiquitin ligase for receptor tyrosine kinases, including KIT, mutations may lead to KIT overexpression.

The PI3K/Akt and MAPK signaling pathways are associated with the occurrence of germ cell tumors. *KRAS* and *NRAS* mutations, which activate both PI3K/Akt and MAPK signaling, are frequently detected in seminomas and teratomas[54]. Single nucleotide polymorphisms (SNPs) of *PTEN* have been identified as risk factors for testicular teratomas[55]. In addition, mutations in *MTOR* and *TRP53* and copy number gains in *AKT1* are frequently observed in intracranial teratomas and yolk sac tumors[54,56]. On the other hand, variants of sprouty-4, encoding a negative regulator for MAPK signaling, are associated with testicular teratomas[53]. Thus, the KIT, PI3K/AKT, and MAPK signaling pathways could be promising therapeutic targets for human germ cell cancers, including testicular teratomas.

REPROGRAMMING BY SMALL MOLECULE COMPOUNDS

In somatic cell reprogramming, reprogramming-inducing transcription factors can be replaced by chemical compounds. For example, the effects of Sox2 and Klf4 can be reproduced by transforming growth factor-β receptor inhibitor (TGFβRi, SB431542 and A83-01)[57-59] or Kempaullone[60], respectively. Kempaullone is an inhibitor of kinases, including glycogen synthase kinase-3 (GSK3) and cyclin-dependent protein kinases. Oct4 can be substituted by forskolin, 2-methyl-5-hydroxytryptamine, and D4476[61]. As forskolin substitutes for bFGF in PGC reprogramming[24], the cAMP/PKA axis mediates cellular reprogramming in both somatic and germ cells.

PGCs are never converted to EGCs when cultured on mouse embryonic fibroblast (MEF) feeder layers with LIF, which is a standard culture condition for ESCs. When post-migratory PGCs at E11.5 are treated with TGFβRi under ESC culture conditions, EGCs can be derived without KITLG and bFGF, showing that TGFβRi can reproduce the effects of KITLG and bFGF[62]. Although Kempaullone alone does not induce EGCs from E11.5 PGCs, simultaneous treatment with TGFβRi and Kempaullone synergistically enhances EGC induction efficiency. In contrast, when E13.5 PGCs are cultured under ESC culture conditions, Kempaullone efficiently induces EGCs, while TGFβRi merely promotes EGC derivation. In addition, the effects of Kempaullone are inhibited completely by TGFβRi in E13.5 PGCs. It remains to be elucidated how PGCs respond differentially to these compounds in a differentiation stage-dependent manner.

ESCs are derived from the epiblast in blastocysts before implantation, whereas epiblast stem cells (EpiSCs) are established from the epiblast in post-implantation stage embryos[63,64]. While mouse ESCs can be propagated in the presence of LIF and form multi-layered colonies, mouse EpiSCs can be expanded and form mono-layered colonies in the presence of bFGF and TGF-β family member activin. These differences may reflect the distinct developmental stages of epiblast. On the other hand, primate ESCs resemble mouse EpiSCs in terms of colony morphology and growth factor requirements. While the pluripotent states of mouse ESCs are called naïve pluripotency, those of mouse EpiSCs and primate ESCs are called primed pluripotency.

Mouse ESCs can be maintained in a more undifferentiated state, so-called "ground-state" pluripotency, when cultured with LIF and two inhibitors (2i), namely inhibitors of MAPK/ERK kinase and GSK3 (PD0325901 and CHIR99021, respectively)[14]. The efficiency of iPSC production is enhanced by treatment with 2i[65,66]. Furthermore, EGCs are derived from migratory PGCs at E8.5 by 2i without KITLG and bFGF[67]. Treatment with 2i also increases EGC derivation efficiency in post-migratory PGCs at E11.5, and the effect is further enhanced by TGFβRi treatment[68].

It has recently been reported that iPSCs can be derived from MEFs by sequential treatment with chemical compounds alone[61,69]. These compounds include TGFβRi (616452), GSK3i (CHIR99021), a cAMP/PKA agonist (forskolin), an RA agonist (AM580), a histone deacetylase complex inhibitor [valproic acid (VPA)], an inhibitor of H3K4 demethylase LSD1 (tranylcypromine), inhibitors of H3K79 methyltransferase DOT1L (EPZ004777 and SGC0946), and a DNA methyltransferase (Dnmt) inhibitor (5-aza-dC). Despite their positive effects on somatic cell reprogramming, VPA and 5-aza-dC inhibit EGC derivation from E11.5 PGCs, indicating differences in epigenetic status between somatic and germ cells[68].

REGULATORS OF GERM CELL DEVELOPMENT

A homozygous Teratoma (*Ter*) mutation dramatically increases the occurrence of testicular teratomas against the 129/Sv genetic background[70,71]. Although germ cells in *Ter*/*Ter* mutant mice appear normal until E13.5, the cells do not enter into mitotic arrest after E14.5, undergo massive apoptosis, and generate early teratomatous foci after E15.5, which are essentially the same phenotype as those of *Pten*-deficient mice. However, the *Ter*/*Ter* mutant mice, against other genetic backgrounds such as C57/BL6, do not develop testicular teratomas but exhibit germ cell deficiency. A homozygous *Ter* mutation causes germ cell death during embryonic development regardless of the genetic background. There exist genetic and epigenetic modifiers required for teratoma formation in the 129/sv genome.

Dnd1 is a gene responsible for *Ter* mutation phenotype[72]. Dnd1 is an evolutionarily conserved RNA-binding protein that counteracts micro RNA (miRNA)-mediated translational inhibition of target mRNAs in zebrafish and mammals[73-75]. The miRNA targets include mRNAs for negative cell cycle regulators (*p27*, *Lats*,

Trp53), pluripotency and germ cell-related genes (*Oct4*, *Sox2*, *Nanos1*) and anti-apoptotic factors (*Bax*, *Bclx*). As translation of these target mRNAs is de-repressed by Dnd1, *Ter* mutation brings about decreased levels of these proteins, which can lead to germ cell deficiency and uncontrolled cell proliferation and survival. Dnd1 is a binding partner of the RNA-binding protein Nanos2, which interacts with the CCR4-NOT deadenylase complex and regulates the stability of mRNAs for germline genes such as *Sycp3*, *Dazl*, *Nanog*, and *Stra8*[76]. Deregulation of RNA metabolism may also be implicated in tumorigenesis in *Ter* mutant germ cells.

Doublesex-related transcription factor (Dmrt1) promotes male differentiation in germ and somatic cells in fetal and neonatal testes. In the absence of *Dmrt1*, testicular germ cells prematurely enter into meiosis and Sertoli cells transdifferentiate into female somatic cells[77-79]. Like *Ter*/*Ter* mutant mice, over 90% of *Dmrt1*-deficient mice develop testicular teratomas against the 129/Sv genetic background, but not other genetic backgrounds. Conditional knockout mice demonstrate that the loss of *Dmrt1* in PGCs, but not in Sertoli cells, leads to teratoma formation[80]. Pluripotency-related genes and Nodal pathway genes are upregulated, whereas the glia-cell derived neurotrophic factor (GDNF) receptor genes including *Ret* and *Gfra1* are downregulated in mutant fetal testes[81]. As deletion of *Gfra1* in 129/Sv mice modestly increases the incidence of testicular teratomas[81], the effects of *Dmrt1* deletion are at least partly mediated by downregulation of GDNF signal. Alternatively, enhanced RA signaling in germ cells lacking *Dmrt1* may drive dedifferentiation, as RA treatment induces PGC reprogramming *in vitro*[25,77,79]. In addition to these effects on fetal germ cells, depletion of *Dmrt1*, together with *Trp53* depletion, increases the efficiency of mGSC derivation from GSCs[82]. It has been reported that SNPs near *DMRT1* are associated with testicular germ cell cancer in humans[83].

The transcription factors Blimp1, Prdm14, and Tfap2c are critical for the specification and differentiation of PGCs. While forced expression of *Blimp1* in ESCs reduces the expression of pluripotency genes, deletion of *Blimp1* in PGCs promotes the derivation of EGCs even in the absence of bFGF[52]. In addition, heterozygous *Tfap2c* mutant mice develop testicular teratomas against the 129/Sv background[84]. *In vitro*, PGC-like cells induced from homozygous *Tfap2c* mutant ESCs show upregulation of cell cycle regulators (*Cdk6*) and pluripotency genes (*Eras*, *Klf4*), but downregulation of germline genes (*Dmrt1*, *Nanos3*)[84]. Furthermore, the susceptibility locus for human testicular germ cell cancer has been found near *PRDM14*[85]. Collectively, these germline genes also function as gatekeepers of PGC dedifferentiation.

CONCLUSION AND PERSPECTIVES

Reprogramming of germ cells and somatic cells is controlled by common signaling pathways, which are activated by PI3K/Akt, MAPK, GSK3, TGFβ, RA, and cAMP/PKA. Therefore, it is critical to understand which downstream effectors are important for reprogramming, and which cellular processes are modulated by these signaling pathways during reprogramming. In contrast, the roles of epigenetic regulators on reprogramming seem to differ to some extent between germ and somatic cells. Furthermore, certain regulators of germ cell differentiation, which are essential for the establishment of the male germline, play critical roles in the prevention of germ cell dedifferentiation.

129/Sv mice frequently develop testicular teratomas. Additionally, mutations in *Dnd1*, *Dmrt1*, and *Tfap2c* lead to testicular teratomas in only the 129/Sv mouse strain. Therefore, it has been suggested that 10-15 susceptibility genes are present in the 129/Sv genome[86,87]. These modifiers include *Ter*, *Trp53,* testicular germ cell tumor 1, and primordial germ cell tumor 1[86-88]. *Ter* mutation increases the incidence of teratomas along with mutations in the genes encoding Trp53, KITLG, the translational regulator Eif2s2 (A^y mutation), and the cytidine deaminase Apobec1[89,90]. Furthermore, the introduction of chromosome 19 from MOLF mice into the 129/Sv background greatly increases the tumor incidence[86]. Investigating the genetic network among susceptibility genes will be necessary to understand the development of germ cell tumors.

Genome-wide association studies have revealed a number of candidate genes for human germ cell tumors. Variants have been found near genes involved in male germ cell development (*DAZL*, *HPGDS*, *SMARCAD1*, *SEPT4*, *TEX14*, *RAD51C*, *PPM1E*, and *TRIM37*), chromosomal segregation (*MAD1L1*, *TEX14*, and *SKA2*), the DNA damage response (*SMARCAD1*, *RFWD3*, and *RAD51C*), and epigenetic regulation (*JMJD1C/KDM3A* and *KDM2A*)[83,85,91]. Mouse models would help to evaluate the roles of these genes in the tumorigenesis of germ cells.

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