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Regulation of spermatogonial stem cell self-renewal and proliferation in mammals

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Summary. The generation of functional sperm relies on spermatogonial stem cells (SSCs) as they can maintain a stem cell pool for continuous generation of functional spermatozoa. The maintenance of SSCs is regulated by several factors. In this paper, we summarize the niche and intrinsic factors in regulating SSC self-renewal and proliferation. GDNF regulates SSC self-renewal through Ras-ERK1/2, SFC, PI3K/Akt and MEK/ERK-mTOR signaling pathways. FGF activates MAPK2K1, ERK and Akt pathways and EGF activates ERK and Akt pathways to induce SSC proliferation. Wnt ligands regulate SSC self-renewal and proliferation through both β-catenin dependent and independent pathways. SCF1 and CXCL12 are also found to have roles in SSC maintenance. As for intrinsic factors in SSCs, ETV5, Bcl6b, Lhx1, ID4 and Nanos2 are regulated by niche factors. They act as the downstream factors of niche factors in regulating SSC self-renewal and proliferation. Transcriptional factors OCT4 and PLZF, as well as FOXO1 in SSCs can directly regulate SSC self-renewal and proliferation. Although we have identified the factors, the detailed mechanism of these factors in regulating SSC fate determination is largely unknown. Here, we summarize factors which have roles in SSC fate determination and hope it will be beneficial for further study and treatment of male infertility.

Key words: Spermatogenesis, Spermatogonial stem cell, Self-renewal, Proliferation, Niche

Introduction

Germ cells are regarded as the most important cells in most organisms, they are unique as they provide genetic information to the offspring. The formation of male germ cells depends on spermatogenesis, which can produce thousands of spermatozoa daily in the male testis. Infertility is a very big problem in the world affecting couples, with males accounting for 50% (Schlegel, 2009). In testis, there is a group of cells which are called spermatogonial stem cells (SSCs). As SSCs can be induced to generate the function haploid spermatids, SSCs are considered to be a suitable source for treating male infertility (Yang et al., 2014). As stem cells, SSCs also have the ability to differentiate to the primary germ layers (Kossack et al., 2009). In addition, SSCs are a good source for regenerative medicine as they can directly transdifferentiate to functional hepatocytes and dopaminergic neurons (Yang et al., 2015; Chen et al., 2016a,b, 2017). SSCs have the

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Abbreviations. SSC, spermatogonial stem cell; PGC, primordial germ cell; dpc, days post coitum; Sry, sex determining region Y; Sox9, Sry-box 9; Rspo1, R-spondin 1; Wnt4, wingless-type MMTV integration site family, member 4; Foxl2, forkhead box L2; dpp, days post partum; GDNF, glial cell line-derived neurotrophic factor; TGF-β, transforming growth factor-β; FSH, follicle-stimulating hormone; NGFI-B or Nur 77, nerve growth factor inducible gene B; NICD, NOTCH intracellular domain; TNF-α, tumor necrosis factors alpha; NF-κB, nuclear factor-κB; FGF, fibroblast growth factor; MAPK, mitogen-activated protein kinase; PI3K, phosphatidyl inositol 3-kinase; FGFR1, fibroblast growth factor receptor 1; CaN, calcineurin; NFAT, nuclear factor of activated T-cells; EGR-1, growth response protein 1; CARF, collaborator of ARF; GDNFR-α or GFRA, glial cell line-derived neurotrophic factor receptor alpha; RET, REarranged during Transfection; SFK, Src family kinase; ERK, extracellular signal-regulated kinases; Bcl6b, B cell CLL/lymphoma 6 member B; Erm or ETV5, Ets variant 5; Lhx1, LIM homeobox 1; Grb2, growth factor receptor-bound protein 2; CREB-1, cAMP responsive element-binding protein 1; ATF-1, activating transcription factor-1; CREM-1, cAMP response element modulation protein 1; CSF1, colony stimulating factor 1; Csf1r, colony stimulating factor 1 receptor; JNK, Jun N-terminal kinase; PAK1, P21-activated kinase 1; EGF, epidermal growth factor; IGF-I, insulin-like growth factor I; CXCL12, chemokine (C-X-C motif) ligand 12; CCL9, chemokine (C-C motif) ligand 9; Chdl1, chromodomain helicase/ATPase DNA binding protein 1-like gene, ID4, inhibitor of DNA binding 4; DND1, Dead end1; OCT4, octamer-binding transcription factor 4; PLZF, promyelocytic leukemia zinc-finger; MKK7, mitogen-activated protein kinase kinase 7; THY1, thymus cell antigen 1; Med1, mediator complex subunit 1; DMRT1, doublesex and Mab-3-related transcription factor 1; KLF, Kruppel-like factor; GHR, growth hormone receptor.

capacity of stem cells for both self-renewal and differentiation (de Rooij and Russell, 2000). SSC self-renewal provides a stem cell pool which allows for the continuous generation of functional sperm throughout the majority of a male's life span. SSC differentiation results in the formation of differentiated germ cells, which can be further transformed into mature sperm after spermiogenesis (Oatley and Brinster, 2008; Tseng et al., 2015). The two different fates of SSCs above are relative to two potentially different division pathways, symmetrical division or asymmetrical division (Oatley and Brinster, 2008, 2012). Two new SSCs or two progenitor spermatogonia are generated by symmetrical division of SSCs, while the asymmetrical division produces one new SSC and one progenitor spermatogonium. Regardless of symmetrical division or asymmetrical division, the new SSCs or progenitor spermatogonia generated by the division of SSCs represent two different fate determinations of SSCs. Both fates of SSC contribute to male fertility. Reduction in or loss of SSC function can have effects on spermatogenesis, which leads to male subfertility and infertility (de Rooij and Grootegoed, 1998). In recent years, there are studies indicating that the balance between the two fates of SSCs is regulated by several extrinsic and intrinsic factors (Oatley and Brinster, 2008; Mei et al., 2015; Tseng et al., 2015).

The biology of SSCs

The SSCs are derived from primordial germ cells (PGCs) (Clermont and Perey, 1957; Sapsford, 1962). It was very difficult to identify the PGCs in the embryos of mammals until the utilization of the alkaline phosphomonoesterase activity in PGCs, which could be used to identify the PGCs at 8.5 days post coitum (dpc) (Chiquoine, 1954). Significantly earlier than the research above, Ginsburg and colleagues (Ginsburg et al., 1990) found that PGCs in the mouse could be identified at least as early as 7 dpc. Then the following migration pathway of PGCs from the epiblast toward the genital ridges was identified by Mintz and Russell, which showed that the PGCs arrive at the genital ridges, the site of embryonic gonads at about 11.5 dpc (Mintz and Russell, 1957). After the PGCs reach the genital ridges, the PGCs transform into gonocytes (Clermont and Perey, 1957). At the genital ridges, the PGCs undergo an important fate determination, namely develop into testis or ovary. The sex determination is regulated by certain sex determining genes and signaling pathways, such as *Sex determining region Y* (*Sry*) and *Sry-box 9* (*Sox9*) in male, *R-spondin 1* (*Rspo1*), *wingless-type MMTV integration site family, member 4* (*Wnt4*) and *forkhead box L2* (*Foxl2*) in female (She and Yang, 2014, 2017). The gonocytes will sustain until birth, and then develop into a population of undifferentiated cells, which occurs at 5 days post partum (dpp) in mice (Bellve et al., 1977), but may take several months in human and primates (Oatley and Brinster, 2008). The differences between primates and rodents of the undifferentiated cells developing from the gonocytes were reviewed by Oatley and colleagues (Oatley and Brinster, 2008, 2012). The A_{single}, A_{paired}, and $A_{aligned}$ spermatogonia are the undifferentiated spermatogonia in rodents. The A_{paired}, $A_{aligned}$, A1, A2, A3, and A4 spermatogonia are derived from A_{single} spermatogonium, which are mostly considered as the true SSCs. The $A_{aligned}$ undifferentiated spermatogonia can develop to A1 differentiated spermatogonia, and will further develop into type B spermatogonia, which finally results in the formation of spermatozoa. Both type A1-A4 and type B spermatogonia belong to differentiated spermatogonia. They can be identified used different molecular makers. As differentiated spermatogonia, both of them have high levels of c-KIT, which cannot be found in undifferentiated spermatogonia (Schrans-Stassen et al., 1999; Gassei and Orwig, 2013). However, type A1-A4 spermatogonia still express several proteins in undifferentiated spermatogonia, such as SOHLH1, CDH1, Nanos3, SALL4, PLZF and LIN28, which can hardly be found in type B spermatogonia (Ballow et al., 2006; Tokuda et al., 2007; Suzuki et al., 2009; Gassei and Orwig, 2013; Chakraborty et al., 2014). In primates, the undifferentiated spermatogonia are two types of A spermatogonia, namely A_{dark} and A_{pale}, which are similar to the A_{single} in rodent testes (Clermont, 1966, 1969). The A_{pale} can give rise to B1 differentiated spermatogonia, followed by B2-4, and then preleptotene spermatocytes. It has been suggested that A_{dark} and A_{pale} are the functional SSCs in primates (Hermann et al., 2009). However, in recent years several studies have indicated that there may be a transiting spermatogonial population between A_{dark} and A_{pale} (Sharma et al., 2019). The exploration of the biology of SSCs and the identification of molecular makers of SSCs significantly increase our knowledge of how to characterize them. The characterization of germ stem cells of mouse and human will significantly promote the study of the identification of factors which have a function in SSC self-renewal and differentiation (Hofmann et al., 2005; Hou et al., 2015).

Niche of SSCs

In seminiferous tubules, SSCs are surrounded by a specific microenvironment called "niches", and a considerable number of studies found that the fate decisions of the SSCs can be regulated by the niche (de Rooij, 2009). In mammalians, the seminiferous tubule is the basic unit of the testis, and it is also the site of spermatogenesis. Germ cells and somatic Sertoli cells are the two main kinds of cells found in seminiferous tubules. Sertoli cells locate in the basement of seminiferous tubule, and can provide a framework for germ cells (Petersen and Soder, 2006). The tight junctions between Sertoli cells provide a safe environment for the SSCs or spermatogonia, and can also provide many critical factors for the development of the SSCs and spermatogonia (Griswold, 1998). In

addition, the peritubular myoid cell and Leydig cells can also contribute to the niche of the SSCs (Cao et al., 2017). Peritubular myoid cells surround the outside basement membrane of the seminiferous tubules, while Leydig cells are a kind of somatic cell between different seminiferous tubules (Skinner et al., 2009). Over the last decades, evidence is accumulating that the niche cells above contribute to spermatogenesis by providing the growth factors required for SSC fate determination (Skinner et al., 2009; Oatley and Brinster, 2012).

The balance between two fates of SSCs relies on both extrinsic and intrinsic factors. The factors provided by the niche of the SSCs contribute to the extrinsic regulation, while the downstream factors of the extrinsic factors in the SSCs constitute intrinsic factors in SSC fate determination.

Extrinsic factors in the niche

Glial cell line-derived neurotrophic factor (GDNF)

In the mammal testis, the maintenance of the SSCs requires extrinsic factors from the niche. As the SSCs are surrounded by Sertoli cells, Sertoli cells contribute greatly to SSC self-renewal. GDNF was originally identified in midbrain dopaminergic neurons, which was important for dopaminergic neuron survival and differentiation (Lin et al., 1993). Subsequent studies indicated that the GDNF also had an important role in other nervous systems, which could rescue and prevent the atrophy of facial motoneurons and promote the survival and phenotype of the locus coeruleus neurons (Henderson et al., 1994; Arenas et al., 1995; Airaksinen and Saarma, 2002). GDNF also had a role in fetal development, as mice with the GDNF knockout died shortly after birth. It wasn't until 2000 that the GDNF was found to have a role in the fate determination of undifferentiated spermatogonia, the overexpression of GDNF increased undifferentiated spermatogonia, while $GDNF^{+/-}$ mice lost stem cells (Meng et al., 2000).

GDNF belongs to the transforming growth factor-β (TGF-β) superfamily, as GDNF has the same seven conserved Cys residues which are common to all of the TGF-β members (Lin et al., 1993). GDNF is widely expressed in most tissues such as testis, stomach and kidney (Trupp et al., 1995). GDNF is produced by Sertoli cells and peritubular myoid cells in testis (Meng et al., 2000; Johnston et al., 2011; Chen et al., 2014, 2016a,b). The regulation of GDNF is shown in Fig. 1. GDNF is regulated by the gonadotropic pituitary hormones and testosterone. Follicle-stimulating hormone (FSH) significantly stimulated GDNF secretion (Tadokoro et al., 2002). It was reported that the FSH could stimulate the mRNA level of both GDNF and nuclear receptor transcription factor nerve growth factor inducible gene B (NGFI-B, also known as Nur77) in Sertoli cells (Ding et al., 2011). Nur77 can directly bind to the promoter of *Gdnf*, and the changes of Nur77 expression significantly affected the mRNA level of *Gdnf* (Ding et al., 2011). FSH was also found to regulate the secretion of GDNF by inhibiting autophagy. FSH can inhibit biogenesis of lysosome, and then inhibited the fusion of autophagosome and lysosome, which extended the half-live of GDNF in Sertoli cell and increased the secretion of GDNF (Xi et al., 2022). In addition to FSH, testosterone was able to induce GDNF secretion in peritubular myoid cells (Chen et al., 2014). Also, spermatogenesis was restored in sterile mice after being transplanted with testosterone-treated peritubular myoid cells. And melatonin was also able to increase GDNF in Sertoli cells (Niu et al., 2016). Testicular undifferentiated cells might also regulate GDNF expression through negative feedback regulation (Garcia et al., 2017; Jabarpour and Tajik, 2018). The undifferentiated spermatogonia can express the JAG1 ligand to activate NOTCH signaling. Activated NOTCH signaling resultes in the translocation of NOTCH intracellular domain (NICD) to nucleus. NICD can interact with RBP-J to activate the expression of HES1 and HEY1. HES1 and HEY1 can directly repress GDNF by binding to its promoter (Garcia et al., 2014, 2017; Parekh et al., 2019). Tumor necrosis factors alpha (TNF-α) inhibited the GDNF by a nuclear factor-κB (NF-κB) dependent way through promoting the HES1 (Persio et al., 2020). The cytokine fibroblast growth factors (FGFs) also have a role in the regulation of mRNA for GDNF. It has been reported that in the testis FGF2 can be expressed by Sertoli cells, Leydig cells, and also differentiated germ cells (Mullaney and Skinner, 1992; Han et al., 1993), and the receptors of FGF2 can be expressed in germ line and somatic cells (Sakai et al., 2018). FGF2 produced by germ cells can signal to the receptors in Sertoli cells to regulate the *Gdnf* mRNA. And the increase of *Gdnf* mRNA induced by FGF2 can be reduced after inhibiting MAPK and PI3K pathways (Simon et al., 2007). In recent years, GDNF was also found to be produced by the testicular endothelial cells, and the production of GDNF can also be regulated by FGF2 (Bhang et al., 2018). FGF2 binds to the FGF receptor 1 (FGFR1) to activate calcineurin (CaN)-nuclear factor of activated T-cells (NFAT) pathway in the testicular endothelial cells. NFAT cannot directly regulate the GDNF but can regulate early growth response protein 1 (EGR-1), which can bind to the promoter of *Gdnf* (Bhang et al., 2018). The Wnt pathway also regulates the GDNF in Sertoli cells through the regulator collaborator of ARF (CARF). Deletion of CARF decreases GDNF, and the decrease can be rescued by the activation of the Wnt pathway (Cui et al., 2020).

The roles of GDNF in regulating SSC self-renewal are shown in Fig. 2. Jing et al. discovered that GDNF receptor alpha (GDNFR-α, also known as GFRA) was found to be a cell surface receptor for GDNF (Jing et al., 1996). GDNF specifically binds to GFRA, and then activates the RET (REarranged during Transfection) protein tyrosine kinase (Jing et al., 1996). RET has many phosphorylation sites and can activate multiple

pathways. In the mammalian testis, GFRA1 and RET were restrictively expressed in SSCs, the mutant of GFRA1 or RET inhibited SSC proliferation and resulted in the depletion of SSCs (Naughton et al., 2006). The silencing of *Gfra1* decreased the phosphorylation of RET, while the mutant of tyrosine 1062 in RET inhibited SSC self-renewal, which indicated that the phosphorylation of tyrosine 1062 in RET regulated SSC self-renewal by RET/GFRA (He et al., 2007; Jijiwa et al., 2008). RET has been shown to interact with the Src

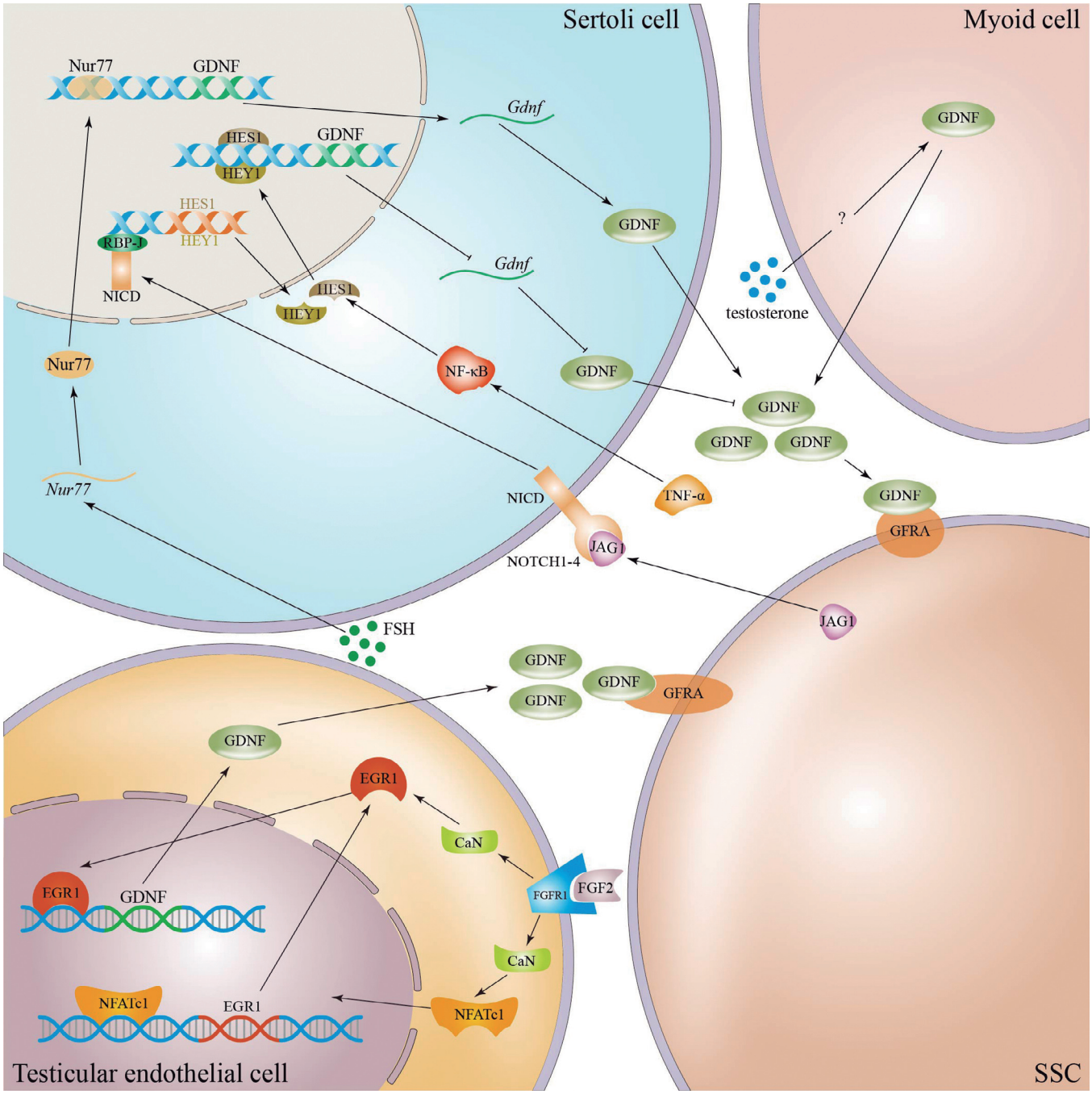

Fig. 1. Schematic diagram illustrating the regulation of GDNF in the niche of spermatogonial stem cells. (1) Two hormones regulate the expression of GDNF in the niche of SSCs, FSH regulates the expression of Nur77, and then promotes Gdnf in Sertoli cells, testosterone promotes the expression of GDNF in myoid cells. (2) JAG1 from SSCs regulates the transcriptional repressors HES1 and HEY1 through NOTCH signaling in Sertoli cells, HES1 and HEY1 directly repress the GDNF expression. (3) FGF2 binds with FGFR, and then activates CaN which regulates transcription factor EGR1, which regulates GDNF expression. (4) TNF-α promotes HES1 to inhibit GDNF by the NF-κB pathway.

family kinase (SFK) and activates Akt (Melillo et al., 1999; Encinas et al., 2001). The PI3K-Akt pathway, SFK signaling and extracellular signal-regulated kinases (ERK) pathway were found to function in the intracellular mechanism for SSC self-renewal and proliferation (Braydich-Stolle et al., 2007; Lee et al.,

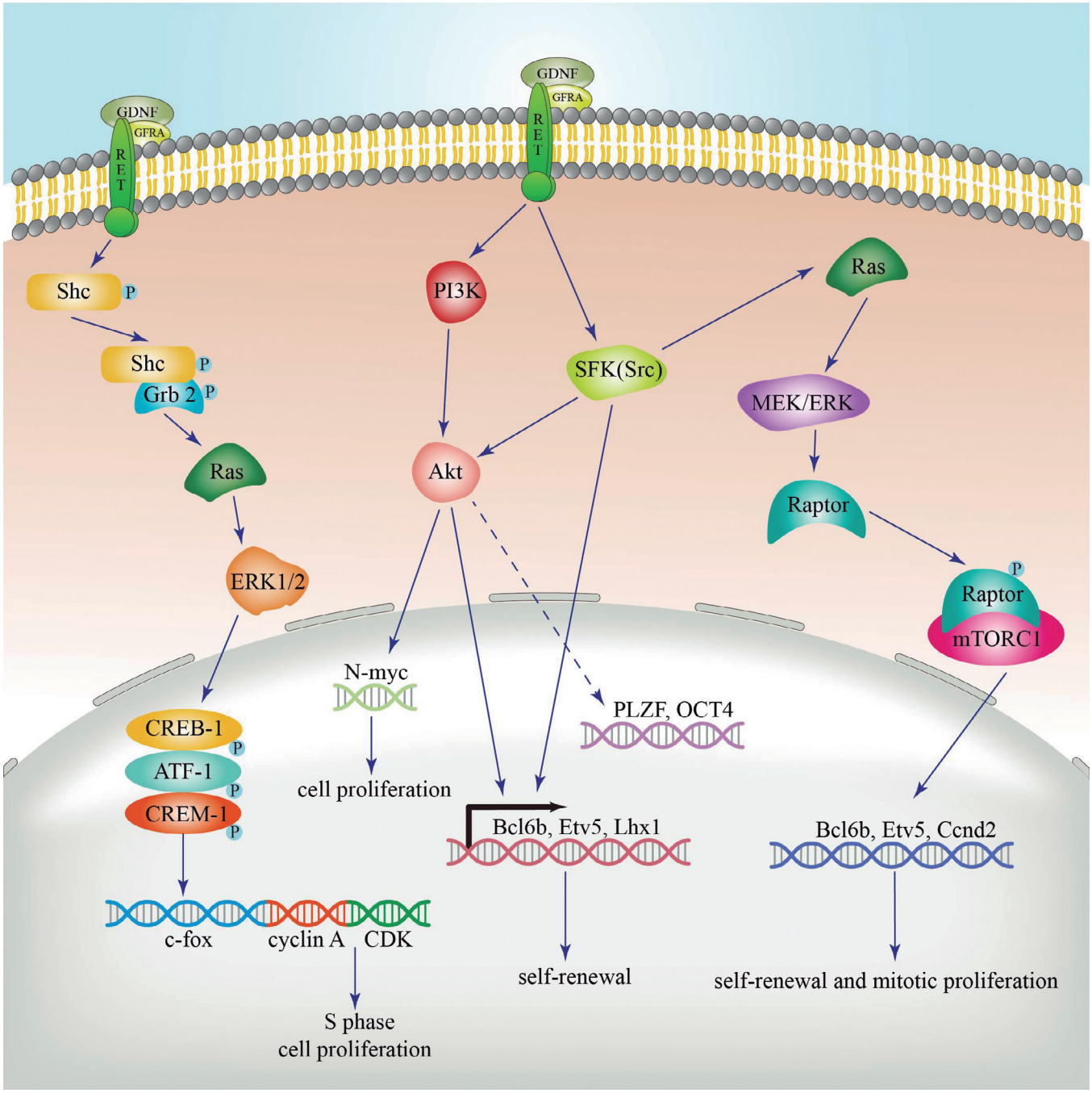

Fig. 2. Schematic diagram illustrating the role of GDNF in regulating cell proliferation and self-renewal of spermatogonial stem cells. (1) GDNF binds with GFRA to activate RET in the membrane. Activated RET results in the phosphorylation of Shc and Grb 2. Phosphorylated Shc and Grb 2 can further activate Ras. Activated Ras phosphorylates CREB-1, ATF-1, and CREM-1 through ERK1/2. And phosphorylated CREB-1, ATF-1, and CREM-1 finally upregulate c-fos, cyclin A and CDK2 to promote the cell proliferation. (2) GDNF also regulates SSC self-renewal and proliferation through the PI3K/Akt pathway, which can promote Bcl6b, Etv5, Lhx1 and N-myc. Although PLZF and OCT4 can be downregulated by Akt inhibition, they cannot be regulated by GDNF. (3) GDNF also promotes the phosphorylation of the mTORC1 component Raptor by MEK/ERK, and then upregulates the expression of the self-renewal associated genes ETV5, Bcl6b and Ccnd2 to increase the self-renewal and proliferation of SSCs.

2007; Oatley et al., 2007). GDNF induced the phosphorylation of Akt, and the inhibition of PI3K can significantly prevent SSC self-renewal (Lee et al., 2007). GDNF significantly stimulate the expression of B cell CLL/lymphoma 6 member B (*Bcl6b*), LIM homeobox 1 (*Lhx1*), and Ets variant 5 (*Erm* or *ETV5*) genes in the SSC. And the stimulation induced by GDNF can be completely blocked by Akt inhibitor (Oatley et al., 2007). Except for the three important genes above, two GDNF-independent genes, PLZF and OCT4, were also reduced by the Akt inhibitor (Oatley et al., 2007). Similarly with PI3K, the inhibitor of SFK also blocked the increase of phosphorylated Akt induced by GDNF stimulation. And the inhibitor of SFK also blocked the increase of *Bcl6b, Lhx1*, and *ETV5* stimulated by GDNF, but not of PLZF and OCT4. Compared with Akt, SFK inhibitor resulted in smaller cell clumps rather than single cells, which indicated that the SFK signaling might function in two different ways, first by regulating Akt, and second by directly targeting *Bcl6b, Lhx1*, and *ETV5* gene (Oatley et al., 2007). As a member of SFK, the inhibition of the Src kinase can abolish the effects induced by GDNF indicating that GDNF acted through the Src pathway, and the Src pathway can exert its action through the PI3K/Akt pathway to promote the proliferation of SSCs by upregulating *N-myc* (Braydich-Stolle et al., 2007). Another pathway regulated by GDNF signaling is the ERK pathway. GDNF binds to GFRA inducing Shc and RET phosphorylation, the phosphorylated Shc binds to the growth factor receptor-bound protein 2 (Grb2) and then activates Ras. The activated Ras results in the phosphorylation of activating transcription factor-1 (ATF-1), cAMP responsive element-binding protein 1 (CREB-1), and cAMP response element modulation protein 1 (CREM-1) through the ERK1/2 signaling pathway, which can further promote cell proliferation through upregulating cyclin A and CDK2 (He et al., 2008). MEK/ERK signaling can maintain SSCs by suppressing the expression of the differentiation-associated genes *STRA8* and *c-KIT* (Hasegawa et al., 2013). Wang and colleagues identified the phophorylated sites induced by GDNF, the most frequently phophorylated sites could be recognized by ERK1/2. The inhibitor of ERK1/2 significantly inhibited cell proliferation and the expression of *ETV5, Bcl6b* and *Ccnd2*. GDNF upregulated the phosphorylation of mTORC1 component Raptor at Ser863, and the upregulated phosphorylation could be reduced by ERK1/2 inhibitor. These results suggested that GDNF regulated the phosphorylation of mTORC1 component Raptor at Ser863 through MEK/ERK pathway to stimulate spermatogonial progenitor cell proliferation (Wang et al., 2017). As the complicated regulation of GDNF on SSCs, more details still need to be further studied.

Fibroblast growth factor (FGF)

FGF, another cytokine, was also found to function in SSC self-renewal (Kubota et al., 2004), as is shown in Fig. 3. In recent years, the mechanism of the function in the involvement of FGF has been demonstrated. It is reported that the FGF2 produced by Sertoli cells can stimulate the expression of *Etv5* and *Gdnf*, through MAPK and PI3K signaling cascades (Simon et al., 2007). In addition, FGF2 can also directly regulate the proliferation of SSCs through increasing the phosphorylation of AKT and ERK in SSCs (Choi et al., 2010; Zhang et al., 2012). The activated MEK/ERK pathway induced by FGF2 can further upregulate *Etv5* and *Bcl6b* in SSC (Ishii et al., 2012). However, in recent years, more and more controversial conclusions were found about the function of FGF2 in SSC. In vivo experiments indicated that the depletion of FGF2 in the testis increased the *Gdnf* and enriched SSCs (Takashima et al., 2015). Another study indicated that FGF2 suppressed GDNF production, and the spermatogonial clusters induced by FGF2 and GDNF in vivo were distinct as the FGF2-cultured spermatogonia showed strongly differentiated characteristics (Masaki et al., 2018). The different conclusions of FGF2 function in SSCs suggest the complexity of SSC biology, and more details should be further studied. Apart from FGF2, more and more FGF members were found to act as niche factors in regulating the fate of SSCs. FGF8 maintained the undifferentiated spermatogonia through FGFR1, FGF8 activated MEK/ERK signaling to inhibit the differentiation in SSCs and induce the expression of GFRA1 and RET (Hasegawa and Saga, 2014). FGF5 also promoted the proliferation of SSCs by enhancing Cyclin A2 and Cyclin E1 through activating ERK and AKT signaling (Tian et al., 2019). FGF9 expressed by somatic cells was also found to regulate SSC proliferation. FGF9 enhanced the number of SSCs, which could be negated by the inhibition of p38 MAPK phosphorylation (Yang et al., 2021).

Other extrinsic factors

In addition to GDNF and FGF, many other factors were also found to function in SSC self-renewal, such as the colony stimulating factor 1 (CSF1) and Wnt ligands, as shown in Fig. 3. In mammalian testes, CSF1 was mainly expressed in myoid and Leydig cells, the self-renewal of SSCs can be significantly enhanced by CSF1 through colony stimulating factor 1 receptor (Csf1r) at the surface of cultured $THY1^+$ cells (Kokkinaki et al., 2009; Oatley et al., 2009). The deficiency of CSF1 induced by the loss of Leydig cells can significantly damage SSC self-renewal (Du et al., 2018). The Wnt signaling pathway is known as a critical pathway in stem cell self-renewal regulation (Reya and Clevers, 2005; Clevers et al., 2014). Wnt signaling is mediated by a series of Wnt ligands. WNT3A has a role in SSC stemness regulation through β-catenin pathway (Golestaneh et al., 2009). Also, Wnt6 from niche Sertoli cells contributes to the proliferation of SSCs by activating the β-catenin pathway (Takase and Nusse,

2016). Wnt5a from Sertoli cells promotes the survival of SSCs through non-canonical Wnt/β-catenin pathway, wnt5a phosphorylated JNK to maintain SSC self-renewal (Yeh et al., 2011). P21-activated kinase 1 (PAK1) was regulated by epidermal growth factor (EGF), the knockdown of PAK1 significantly decreased the phosphorylation of ERK1/2 and AKT, as well as the cyclin A level. The study reveals a novel network of EGF

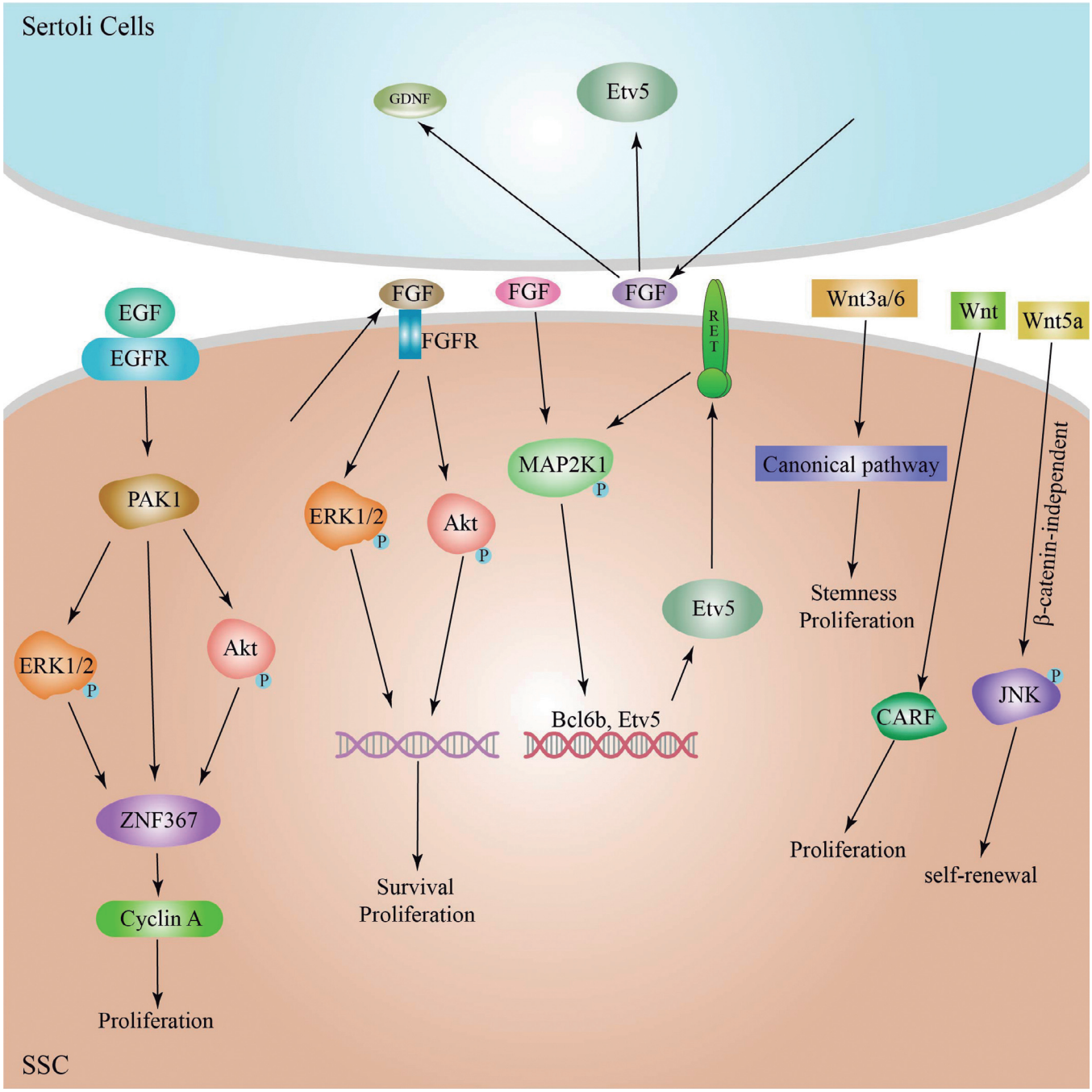

Fig. 3. Schematic diagram illustrating the role of EGF, FGF, Wnt ligands in regulating the cell proliferation and self-renewal of spermatogonial stem cells. (1) FGF regulates GDNF and Etv5 in Sertoli cells, and they can also directly regulate Bcl6b and Etv5 in SSCs through the MAPK pathway to promote the survival and proliferation of SSCs. (2) EGF binds with EGFR in SSCs to regulate PAK1 and promotes the proliferation of SSCs by upregulating cyclin A through ERK1/2 and Akt. (3) Wnt pathway participates in SSC self-renewal and proliferation of both β-catenin dependent and independent pathways. Wnt3a/6 promotes SSC proliferation and maintains the stemness through the β-catenin pathway, Wnt5a phosphorylates JNK to support the self-renewal of SSCs.

in regulating human SSCs via ERK1/2 and AKT pathways (Fu et al., 2018). Studies also indicate that the insulin-like growth factor I (IGF-I) may be another growth factor influencing SSC self-renewal, which can increase the stem cell activity of SSCs by transplant assay (Kubota et al., 2004). CXCL12-CXCR4 signaling also contributes to the maintenance of SSCs. The chemokine (C-X-C motif) ligand 12 (CXCL12) and its receptor CXCR4 were expressed by both undifferentiated spermatogonia and Sertoli cells (Yang et al., 2013). Although the addition of CXCL12 in the medium of undifferentiated spermatogonia did not have any effects on the numbers and morphology of SSC, while the inhibition of CXCL12 receptor CXCR4 significantly decreased the numbers of SSC, which demonstrates that CXCL12 is an extrinsic factors in SSC self-renewal via its receptor CXCR4 in SSCs (Yang et al., 2013).

Intrinsic factors

Growth factor-dependent factors

The intrinsic factors in the mammalian testes can be divided into two categories: growth factor-dependent and -independent. As we mentioned above, GDNF and FGF can regulate ETV5, Bcl6b and Lhx1 to affect SSC self-renewal. ETV5 is a transcription factor belonging to the ETS family, it has essential roles in SSC self-renewal. Etv5$^{-/-}$ male mice only had Sertoli cells, and the germ cells were lost with increasing age (Chen et al., 2005; Zhang et al., 2021). ETV5 can be expressed in both Sertoli cells and SSCs. In Sertoli cells, ETV5 was essential to the secretion of chemokine (C-C motif) ligand 9 (CCL9) (Simon et al., 2010). In the SSCs, the mutant of ETV5 can significantly decrease the RET

Table 1. Factors in regulating the self-renewal and proliferation of spermatogonial stem cells.

Name	Expression cells	Method	Induced changes	Reference
Extrinsic factors				
GNDF	Sertoli cell	One allele depletion (GDNF+/-)	Depletion of spermatogonia	Meng et al., 2000
GDNF	Peritubular myoid cell	Coculture THY1+ spermatogonia with testosterone	GFRA1+ undifferentiated spermatogonia increased	Chen et al., 2014
FGF2	Sertoli cell, Leydig cell	Culture cells with FGF2 and transplantation	Induce germ cell tumor	Ishii et al., 2012
FGF8	Spermatogenic Cells	Conditional knock-out, overexpression	Reduce NANOS3+ cells, increase GFRA1+ and NANOS3+ cells	Hasegawa and Saga, 2014
FGF5	Sertoli cell	Culture C18-4 cell lines with FGF5	Promote the proliferation of C18-4	Tian et al., 2019
FGF9	Niche somatic cell	Overexpression in vivo and transplantation	Accumulation of undifferentiated spermatogonia	Yang et al., 2021
CSF1	Leydig and myoid cells	Culture Thy1+ germ cells with CSF1	Increase SSCs numbers	Kokkinaki et al., 2009; Oatley et al., 2009
WNT3A	Spermatogonia	Culture C18-4 cells with WNT3A	Increase of C18-4 cells and primary spermatogonia	Golestaneh et al., 2009
Wnt5a	Sertoli cells	Culture Thy1+ cells with Wnt5a	Increase the SSC numbers and decrease the apoptosis	Yeh et al., 2011
EGF	-	Culture SSC cell lines with EGF	Promote SSC proliferation by upregulating PAK1	Fu et al., 2018
CXCL12	Sertoli cell	Inhibition of the CXCL12 receptor CXCR4	Loss of SSCs due to decreased proliferation	Yang et al., 2013
Intrinsic factors				
ETV5	Sertoli cell and Thy1+ cells	Knockout and transplantation assay	Decrease the SSC colony numbers	Chen et al., 2005; Oatley et al., 2007
Bcl6b	Germ cell	Knockdown in vitro and knockout in vivo	Decrease SSC clump and colonies, Sertoli cell-only phenotypes	Oatley et al., 2006
Lhx1	Germ cells	Knockdown in vitro and transplantation analysis	Decrease the germ cell clumps and SSC colony numbers	Oatley et al., 2007
ID4	A_{single} spermatogonia	Knockout in vivo and knockdown in vitro	Sertoli cell-only phenotype and reduced colony numbers	Oatley et al., 2011
Nanos2	A_{single} and A_{paired} spermatogonia	Conditional knockout	Decreased cell recovery rate and upregulate differentiation genes	Suzuki et al., 2009; Zhou et al., 2015
OCT4	Undifferentiated spermatogonia	Conditional knockout, knockdown, transplantation	Apoptosis of primordial germ cells. Reduced colonization	Kehler et al., 2004; Dann et al., 2008
PLZF	Undifferentiated spermatogonia	Knockout	Progressive loss of spermatogonia with age	Costoya et al., 2004
FOXO1	Gonocytes and SSCs	Conditional knockout	Reduced spermatogonia at P7 and no spermatozoa	Goertz et al., 2011
CARF	Undifferentiated spermatogonia	Knockout	Reduced proliferation of undifferentiated spermatogonia	Cui et al., 2020
MKK7	Undifferentiated spermatogonia	Knockdown	Inhibited SSC proliferation and increased apoptosis	Huang et al., 2021

mRNA and protein expression indicating that ETV5 in SSCs might regulate SSC self-renewal through GDNF/RET/GFRA1 signaling (Tyagi et al., 2009). In addition, ETV5 can also mediate Bcl6b, Lhx1, Brachyury and CXCR4 (Wu et al., 2011). ETV5 also contributes to tight junctions between Sertoli cells, which provide a specific microenvironment for the development of spermatogonia (Morrow et al., 2009). GDNF dependent ETV5 expression is regulated by many factors. RNA-binding protein Lin28a stimulated ETV5 by increasing GFRA1 (Ma et al., 2016). The knockout of *Ddx5* down-regulated the expression of ETV5 (Xia et al., 2021). Knockdown of chromodomain helicase/ATPase DNA binding protein 1-like gene (Chd1l) also significantly decreased *Etv5* transcripts (Liu et al., 2016). Bcl6b was the most responsive gene regulated by GDNF in SSC, the mutant of Bcl6b in the male resulted in the Sertoli cell-only phenotypes, which was similar to the *Etv5*$^{-/-}$ male mice (Oatley et al., 2006). Infertile mouse transplanted with Bcl6b-expressed germline stem cells were able to form germ cell tumor (Ishii et al., 2012). Although Bcl6b was regulated by ETV5, the mutants of these two genes shared few overlapping genes compared to the control group (Wu et al., 2011). Genes regulated by Bcl6b were largely enriched in cell interactions, while ETV5 can regulate the genes associated with cellular and molecular functions, which indicated the different mechanisms of Bcl6b and ETV5 in SSC self-renewal (Wu et al., 2011). As a transcription factor, the location of Bcl6b in the nucleus was found to be regulated by ETV5, and constitutively active *Etv5* significantly increased Bcl6b in the nucleus (Morimoto et al., 2019). As we mentioned above, Chd1l also regulated *Bcl6b* and *Lhx1* (Liu et al., 2016). Lhx1 can be induced by GDNF. Knockdown of Lhx1 by siRNA significantly decreased cell clumps, and functional transplantation analysis indicated that the interference significantly reduced SSC colonies (Oatley et al., 2007). Inhibitor of DNA binding 4 (ID4) was the only ID protein expressed in A_{single} spermatogonia (Sablitzky et al., 1998; Oatley et al., 2011). The expression of ID4 can be upregulated by the stimulation of GDNF. *ID4*-null mice showed lost undifferentiated spermatogonial population, and the interference of ID4 in the THY1^{+} cells did not have significant effects on the total germ cell, but abolished the colony numbers after transplantation (Oatley et al., 2011). Spermatogenesis in the mouse was disrupted by the overexpression of ID4, and ETV5, Bcl6b and Lhx1 have the corresponding expression with ID4 (Helsel et al., 2017). ID4 is a molecular marker of SSC in mouse (Chan et al., 2014). However, in bovine system, ID4 was found in Sertoli cells, which showed the differences of spermatogenesis between bovine and rodent (Park et al., 2021). Nanos2 was specifically expressed in the cells with stemness (Suzuki et al., 2009). The deficiency of *Nanos2* showed the loss of germ cells due to the depletion of SSCs (Sada et al., 2009). The knockout of Nanos2 in pig and goat showed complete germline ablation, and the spermatogenesis was able to regenerate after transplantation of wild-type SSC (Ciccarelli et al., 2020). Nanos2 was found to be regulated by GDNF, as the expression of *Nanos2* was lost upon the knockout of *Gfra1*, and the overexpression of *Nanos2* was able to rescue the loss of SSC phenotype induced by the knockout of *Gfra1* (Sada et al., 2012). In recent years, Nanos2 was found to mediate translational repression of differentiation-related transcripts, and sequestrate mTOR protein by promoting the condensation of cellular messenger ribonucleoprotein in the cytoplasm (Zhou et al., 2015). Nanos2 also interacted with CCR4-NOT complex and directly bound to the mRNAs important for SSC self-renewal to regulate their stability (Codino et al., 2021). Nanos2 regulated A_{single} and A_{paired} spermatogonia proliferation by interacting with DND1 (Dead end1), the deplete of DND1 reduced A_{single} and A_{paired} spermatogonia (Niimi et al., 2019).

Other factors

OCT4, PLZF and FOXO1 have been confirmed to contribute to SSC self-renewal without the stimulation of growth factors. Octamer-binding transcription factor 4 (OCT4, also known as OCT3 and POU5F1) was the first gene to have functions in the maintenance of embryonic stem cell multipotency (Niwa et al., 2000). In germ cell, OCT4 was down-regulated in the endoderm and mesoderm, but was mainly expressed in the germline after gastrulation and was able to persist in the gonocytes, the precursors of SSCs (Rosner et al., 1990; Pesce et al., 1998; Li et al., 2015). Loss function of OCT4 in mouse showed the embryonic lethal, the conditional knockout of OCT4 in the primordial germ cells led to germline cell death (Nichols et al., 1998; Kehler et al., 2004). Knockdown of OCT4 in the germline stem cell was performed using short hairpin RNA (shRNA), OCT4 shRNA treated cells showed less colonization after transplantation (Dann et al., 2008). Promyelocytic leukemia zinc-finger (PLZF) was initially found to be associated with acute promyelocytic leukemia (Costoya and Pandolfi, 2001). Azoospermia contained a nonsense mutation of PLZF demonstrating that PLZF might be responsible for the azoospermia phenotype (Buaas et al., 2004). PLZF-null mice underwent loss of spermatogonia (Costoya et al., 2004). As a transcriptional repressor, the first target gene of PLZF in regulating SSC self-renewal was the *c-Kit* gene, which can promote SSC differentiation (Filipponi et al., 2007). PLZF was also found to maintain the SSCs by upregulating Redd1, which was the inhibitor of mTORC1. Thus PLZF can inhibit mTORC1 to maintain SSCs (Hobbs et al., 2010). In recent years, a new mechanism of PLZF in regulating SSC proliferation was found in dairy goat. PLZF suppressed mir146a, the downregulated mir146a resulted in the expression of CXCR4, which promoted the proliferation of SSCs might through ERK1/2 pathway (Mu et al., 2016). FOXO belongs to the forkhead transcription factor

family, and functions in cellular proliferation and survival (Salih and Brunet, 2008). FOXO1 was expressed in germline stem cells (Goertz et al., 2011). Mice with germ cell conditional knockout of FOXO1 had normal numbers of gonocyte at P1 but had depleted spermatogonia at P7. And this regulation was mainly by targeting RET (Goertz et al., 2011). As we mentioned above, CARF can regulate GDNF in Sertoli cells to promote SSC self-renewal. CARF also acted as an intrinsic factor, as the loss of CARF in undifferentiated spermatogonia inhibited the proliferation of undifferentiated spermatogonia. However, the rescue of CARF in the $CARF^{-/-}$ Sertoli cell could not rescue the proliferation of undifferentiated spermatogonia, which suggested the important role of intrinsic CARF (Cui et al., 2020). Mitogen-activated protein kinase kinase 7 (MKK7) expressed by SSCs also participated in regulation of SSC proliferation. Lack of MKK7 impaired SSC proliferation, which was similar to the inhibition of phosphorylation of JNKs (Huang et al., 2021).

At present, infertility in humans is increasing. As SSCs can be induced to generate functional haploid spermatids with fertilization capacity, SSCs represent a valuable application to treat male infertility. The normal functions of SSCs rely on two different fate determinations, differentiation and self-renewal. SSC self-renewal lays the foundation of continuous production of mature sperm. SSC self-renewal can be regulated by many extrinsic and intrinsic factors. In this paper, we summarize the factors which are essential for SSC self-renewal. However, due to complexity of the regulation in SSC self-renewal, further studies focus on the mechanism of SSC self-renewal will advance our knowledge on SSC biology, which will be beneficial for the treatment of infertility.

Summary and perspectives

SSCs play important roles in the generation of functional sperm, which have two different fates, differentiation and self-renewal. SSC differentiation results in the formation of functional sperm. SSC self-renewal provides a stem cell pool which allows the continuous generation of functional spermatozoa throughout the mature part of a male's life span. Both fates are regulated by a series of factors. In the present review, we summarize the factors important for SSC self-renewal and proliferation. The extrinsic factors are mainly GDNF, FGF2, EGF, Wnt, CSF1, IGF-I and CXCL12. GDNF regulates SSC self-renewal through Ras-ERK1/2, SFC, PI3K/Akt, and MEK/ERK-mTOR signaling pathways. FGF activates the MAPK2K1, ERK and Akt pathways to regulate SSC proliferation and self-renewal. Similarly, EGF can also activate ERK and Akt to promote SSC proliferation. Both of the canonical and β-catenin independent Wnt pathway have roles in SSC proliferation and self-renewal. For the intrinsic factors, we divided them into two groups: growth factor dependent and independent. ETV5, Bcl6b, Lhx1, ID4 and Nanos2 regulate SSC self-renewal and proliferation in growth factor dependent ways. They can be regulated by GDNF, FGF and other factors, while the transcription factors OCT4 and PLZF, as well as FOXO1 and CARF are found to directly regulate SSC self-renewal.

Due to complexity of SSC biology, there are several mechanisms unknown in SSC self-renewal and proliferation as yet. Compared with GDNF, the details of other extrinsic factors, such as FGF, EGF and Wnt, in regulating SSC proliferation and self-renewal are largely unknown. While GDNF is known to be regulated by FSH, testosterone, TNF-α, and JAG1, little is known about EGF, FGF, and Wnt regulation. The transcription factors OCT4, PLZF and FOXO1 can regulate SSC proliferation and self-renewal. However, the genes regulated by these transcription factors still need to be investigated. As we can find in this paper, some opposite conclusions are found in SSC self-renewal regulation. We speculate that this is mainly due to the identification of SSCs. Most of the studies identified the SSCs using different markers. But during the development of germ cells, there might be a transition stage containing both undifferentiated and differentiated markers, which means that the cell stemness is lost (Liao et al., 2019). To further investigate the regulation of SSC self-renewal, we should pay more attention to the characterization of SSCs used in the studies and investigate the potency of these factors in the treatment of male infertility.

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References

Airaksinen M.S. and Saarma M. (2002). The GDNF family: signalling, biological functions and therapeutic value. Nat. Rev. Neurosci. 3, 383-394.

Arenas E., Trupp M., Åkerud P. and Ibáñez C.F. (1995). GDNF prevents degeneration and promotes the phenotype of brain noradrenergic neurons in vivo. Neuron 15, 1465-1473.

Ballow D., Meistrich M.L., Matzuk M. and Rajkovic A. (2006). Sohlh1 is essential for spermatogonial differentiation. Dev. Biol. 294, 161-167.

Bellve A.R., Cavicchia J.C., Millette C.F., O'Brien D.A., Bhatnagar Y.M. and Dym M. (1977). Spermatogenic cells of the prepuberal mouse. Isolation and morphological characterization. J. Cell Biol. 74, 68-85.

Bhang D.H., Kim B.J., Kim B.G., Schadler K., Baek K.H., Kim Y.H., Hsiao W., Ding B.S., Rafii S., Weiss M.J., Chou S.T., Kolon T.F., Ginsberg J.P., Ryu B.Y. and Ryeom S. (2018). Testicular endothelial cells are a critical population in the germline stem cell niche. Nat. Commun. 9, 4379.

Braydich-Stolle L., Kostereva N., Dym M. and Hofmann M.C. (2007). Role of Src family kinases and N-Myc in spermatogonial stem cell proliferation. Dev. Biol. 304, 34-45.

Buaas F.W., Kirsh A.L., Sharma M., McLean D.J., Morris J.L., Griswold M.D., de Rooij D.G. and Braun R.E. (2004). Plzf is required in adult male germ cells for stem cell self-renewal. Nat. Genet. 36, 647-652.

Cao L.H., Zhang Q.L. and Lin X.H. (2017). Spermatogonial stem cell self-renewal and differentiation. Reprod. Dev. Med. 1, 171-178.

Chakraborty P., Buaas F.W., Sharma M., Snyder E., de Rooij D.G. and Braun R.E. (2014). LIN28A marks the spermatogonial progenitor population and regulates its cyclic expansion. Stem Cells 32, 860-873.

Chan F., Oatley M.J., Kaucher A.V., Yang Q.E., Bieberich C.J., Shashikant C.S. and Oatley J.M. (2014). Functional and molecular features of the Id4+ germline stem cell population in mouse testes. Genes Dev. 28, 1351-1362.

Chen C., Ouyang W.J., Grigura V., Zhou Q., Carnes K., Lim H., Zhao G.Q., Arber S., Kurpios N., Murphy T.L., Cheng A.M., Hassell J.A., Chandrashekar V., Hofmann M.C., Hess R.A. and Murphy K.M. (2005). ERM is required for transcriptional control of the spermatogonial stem cell niche. Nature 436, 1030-1034.

Chen L.Y., Brown P.R., Willis W.B. and Eddy E.M. (2014). Peritubular myoid cells participate in male mouse spermatogonial stem cell maintenance. Endocrinology 155, 4964-4974.

Chen L.Y., Willis W.D. and Eddy E.M. (2016a). Targeting the *Gdnf* Gene in peritubular myoid cells disrupts undifferentiated spermatogonial cell development. Proc. Natl. Acad. Sci. USA 113, 1829-1834.

Chen Z., Min. S., Yuan Q.Q., Niu M.H., Yao C.C., Hou J.M., Wang H., Wen L.P., Liu Y., Li Z. and He Z.P. (2016b). Generation of functional hepatocytes from human spermatogonial stem cells. Oncotarget 7, 8879-8895.

Chen Z., Niu M.H., Sun M., Yuan Q.Q., Yao C.C., Hou J.M., Wang H., Wen L.P., Fu H.Y., Zhou F., Li Z. and He Z.P. (2017). Transdifferentiation of human male germline stem cells to hepatocytes in vivo via the transplantation under renal capsules. Oncotarget 8, 14576-14592.

Chiquoine A.D. (1954). The identification, origin, and migration of the primordial germ cells in the mouse embryo. Anat. Rec. 118, 135-146.

Choi J.W., Kim S., Kim T.M., Kim Y.M., Seo H.W., Park T.S., Jeong J.W., Song G. and Han J.Y. (2010). Basic fibroblast growth factor activates MEK/ERK cell signaling pathway and stimulates the proliferation of chicken primordial germ cells. PLoS One 5, e12968.

Ciccarelli M., Giassetti M.I., Miao D.Q., Oatley M.J., Robbins C., Lopez-Biladeau B., Waqas M.S., Tibary A., Whitelaw B., Lillico S., Park C.H., Park K.E., Telugu B., Fan Z.Q., Liu Y., Regouski M., Polejaeva I.A. and Oatley J.M. (2020). Donor-derived spermatogenesis following stem cell transplantation in sterile NANOS2 knockout males. Proc. Natl. Acad. Sci. USA 117, 24195-24204.

Clermont Y. (1966). Renewal of spermatogonia in man. Am. J. Anat. 118, 509-524.

Clermont Y. (1969). Two classes of spermatogonial stem cells in the monkey (*Cercopithecus aethiops*). Am. J. Anat. 126, 57-71.

Clermont Y. and Perey B. (1957). Quantitative study of the cell population of the seminiferous tubules in immature rats. Am. J. Anat. 100, 241-267.

Clevers H., Loh K.M. and Nusse R. (2014). An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 346, 1248012.

Codino A., Turowski T., van de Lagemaat L.N., Ivanova I., Tavosanis A., Much C., Auchynnikava T., Vasiliauskaite L., Morgan M., Rappsilber J., Allshire R.C., Kranc K.R., Tollervey D. and O'Carroll D. (2021). NANOS2 is a sequence-specific mRNA-binding protein that promotes transcript degradation in spermatogonial stem cells. iScience 24, 102762.

Costoya J.A. and Pandolfi P.P. (2001). The role of promyelocytic leukemia zinc finger and promyelocytic leukemia in leukemogenesis and development. Curr. Opin. Hematol. 8, 212-217.

Costoya J.A., Hobbs R.M., Barna M., Cattoretti G., Manova K., Sukhwani M., Orwig K.E., Wolgemuth D.J. and Pandolfi P.P. (2004). Essential role of Plzf in maintenance of spermatogonial stem cells. Nat. Genet. 36, 653-659.

Cui W.H., He X.L., Zhai X.H., Zhang H., Zhang Y.W., Jin F., Song X.M., Wu D.Q., Shi Q.H. and Li L. (2020). CARF promotes spermatogonial self-renewal and proliferation through Wnt signaling pathway. Cell Discov. 6, 85.

Dann C.T., Alvarado A.L., Molyneux L.A., Denard B.S., Garbers D.L. and Porteus M.H. (2008). Spermatogonial stem cell self-renewal requires OCT4, a factor downregulated during retinoic acid-induced differentiation. Stem Cells 26, 2928-2937.

de Rooij D.G. (2009). The spermatogonial stem cell niche. Microsc. Res. Tech. 72, 580-585.

de Rooij D.G. and Grootegoed J.A. (1998). Spermatogonial stem cells. Curr. Opin. Cell Biol. 10, 694-701.

de Rooij D.G. and Russell L.D. (2000). All you wanted to know about spermatogonia but were afraid to ask. J. Androl. 21, 776-798.

Ding L.J., Yan G.J., Ge Q.Y., Yu F., Zhao X., Diao Z.Y., Wang Z.Q., Yang Z.Z., Sun H.X. and Hu Y.L. (2011). FSH acts on the proliferation of type A spermatogonia via Nur77 that increases GDNF expression in the Sertoli cells. FEBS Lett. 585, 2437-2444.

Du Z.Y., Xu S.S., Hu S.X., Yang H., Zhou Z., Sidhu K., Miao Y.L., Liu Z.H., Shen W., Reiter R.J., Hua J.L. and Peng S. (2018). Melatonin attenuates detrimental effects of diabetes on the niche of mouse spermatogonial stem cells by maintaining Leydig cells. Cell Death Dis. 9, 968.

Encinas M., Tansey M.G., Tsui-Pierchala B.A., Comella J.X., Milbrandt J. and Johnson Jr E.M. (2001). c-Src is required for glial cell line-derived neurotrophic factor (GDNF) family ligand-mediated neuronal survival via a phosphatidylinositol-3 kinase (PI-3K)-dependent pathway. J. Neurosci. 21, 1464-1472.

Filipponi D., Hobbs R.M., Ottolenghi S., Rossi P., Jannini E.A., Pandolfi P.P. and Dolci S. (2007). Repression of kit expression by Plzf in germ cells. Mol. Cell. Biol. 27, 6770-6781.

Fu H.Y., Zhang W.H., Yuan Q.Q., Niu M.H., Zhou F., Qiu Q.Q., Mao G.P., Wang H., Wen L., Sun M., Li Z. and He Z.P. (2018). PAK1 Promotes the proliferation and inhibits apoptosis of human spermatogonial stem cells via PDK1/KDR/ZNF367 and ERK1/2 and AKT pathways. Mol. Ther. Nucleic Acids 12, 769-786.

Garcia T.X., Farmaha J.K., Kow S. and Hofmann M.C. (2014). RBPJ in mouse Sertoli cells is required for proper regulation of the testis stem cell niche. Development 141, 4468-4478.

Garcia T.X., Parekh P., Gandhi P., Sinha K. and Hofmann M.C. (2017). The NOTCH ligand JAG1 regulates GDNF expression in Sertoli cells. Stem Cells Dev. 26, 585-598.

Gassei K. and Orwig K.E. (2013). SALL4 expression in gonocytes and spermatogonial clones of postnatal mouse testes. PLoS One 8, e53976.

Ginsburg M., Snow M.H.L. and McLaren A. (1990). Primordial germ

cells in the mouse embryo during gastrulation. Development 110, 521-528.

Goertz M.J., Wu Z.R., Gallardo T.D., Hamra F.K. and Castrillon D.H. (2011). Foxo1 is required in mouse spermatogonial stem cells for their maintenance and the initiation of spermatogenesis. J. Clin. Invest. 121, 3456-3466.

Golestaneh N., Beauchamp E., Fallen S., Kokkinaki M., Uren A. and Dym M. (2009). Wnt signaling promotes proliferation and stemness regulation of spermatogonial stem/progenitor cells. Reproduction 138, 151-162.

Griswold M.D. (1998). The central role of Sertoli cells in spermatogenesis. Semin. Cell Dev. Biol. 9, 411-416.

Han I.S., Sylvester S.R., Kim K.H., Schelling M.E., Venkateswaran S., Blanckaert V.D., McGuinness M.P. and Griswold M.D. (1993). Basic fibroblast growth factor is a testicular germ cell product which may regulate Sertoli cell function. Mol. Endocrinol. 7, 889-897.

Hasegawa K., Namekawa S.H. and Saga Y. (2013). MEK/ERK signaling directly and indirectly contributes to the cyclical self-renewal of spermatogonial stem cells. Stem Cells 31, 2517-2527.

Hasegawa K. and Saga Y. (2014). FGF8-FGFR1 signaling acts as a niche factor for maintaining undifferentiated spermatogonia in the mouse. Biol. Reprod. 91, 145.

He Z.P., Jiang J.J., Hofmann M.C. and Dym M. (2007). Gfra1 silencing in mouse spermatogonial stem cells results in their differentiation via the inactivation of RET tyrosine kinase. Biol. Reprod. 77, 723-733.

He Z.P., Jiang J.J., Kokkinaki M., Golestaneh N., Hofmann M.C. and Dym M. (2008). Gdnf upregulates c-Fos transcription via the Ras/Erk1/2 pathway to promote mouse spermatogonial stem cell proliferation. Stem Cells 26, 266-278.

Helsel A.R., Yang Q.E., Oatley M.J., Lord T., Sablitzky F. and Oatley J.M. (2017). ID4 levels dictate the stem cell state in mouse spermatogonia. Development 144, 624-634.

Henderson C.E., Phillips H.S., Pollock R.A., Davies A.M., Lemeulle C., Armanini M., Simpson L.C., Moffet B., Vandlen R.A., Koliatsos V.E. and Rosenthal A. (1994). GDNF: a potent survival factor for motoneurons present in peripheral nerve and muscle. Science 266, 1062-1064.

Hermann B.P., Sukhwani M., Simorangkir D.R., Chu T.J., Plant T.M. and Orwig K.E. (2009). Molecular dissection of the male germ cell lineage identifies putative spermatogonial stem cells in rhesus macaques. Hum. Reprod. 24, 1704-1716.

Hobbs R.M., Seandel M., Falciatori I., Rafii S. and Pandolfi P.P. (2010). Plzf regulates germline progenitor self-renewal by opposing mTORC1. Cell 142, 468-479.

Hofmann M.C., Braydich-Stolle L., Dettin L., Johnson E. and Dym M. (2005). Immortalization of mouse germ line stem cells. Stem Cells 23, 200-210.

Hou J.H., Niu M.H., Liu L.H., Zhu Z.J., Wang X.B., Sun M., Yuan Q.Q., Yang S., Zeng W.X., Liu Y., Li Z. and He Z.P. (2015). Establishment and characterization of human germline stem cell line with unlimited proliferation potentials and no tumor formation. Sci. Rep. 5, 16922.

Huang Z.H., Huang C., Ji X.R., Zhou W.J., Luo X.F., Liu Q., Tang Y.L., Gong F. and Zhu W.B. (2021). MKK7-mediated phosphorylation of JNKs regulates the proliferation and apoptosis of human spermatogonial stem cells. World J. Stem Cells 13, 1797-1812.

Ishii K., Kanatsu-Shinohara M., Toyokuni S. and Shinohara T. (2012). FGF2 mediates mouse spermatogonial stem cell self-renewal via upregulation of Etv5 and Bcl6b through MAP2K1 activation. Development 139, 1734-1743.

Jabarpour M. and Tajik P. (2018). Evaluating the behavior of cultured sertoli cells in the presence and absence of spermatogonial stem cell. Stem Cell Investig. 5, 1.

Jijiwa M., Kawai K., Fukihara J., Nakamura A., Hasegawa M., Suzuki C., Sato T., Enomoto A., Asai N., Murakumo Y. and Takahashi M. (2008). GDNF-mediated signaling via RET tyrosine 1062 is essential for maintenance of spermatogonial stem cells. Genes Cells 13, 365-374.

Jing S.Q., Wen D.Z., Yu Y.B., Holst P.L., Luo Y., Fang M., Tamir R., Antonio L., Hu Z., Cupples R., Louis J.C., Hu S., Altrock B.W. and Fox G.M. (1996). GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-a, a novel receptor for GDNF. Cell 85, 1113-1124.

Johnston D.S., Olivas E., DiCandeloro P. and Wright W.W. (2011). Stage-specific changes in GDNF expression by rat Sertoli cells: a possible regulator of the replication and differentiation of stem spermatogonia. Biol. Reprod. 85, 763-769.

Kehler J., Tolkunova E., Koschorz B., Pesce M., Gentile L., Boiani M., Lomeli H., Nagy A., McLaughlin K.J., Scholer H.R. and Tomilin A. (2004). Oct4 is required for primordial germ cell survival. EMBO Rep. 5, 1078-1083.

Kokkinaki M., Lee T.L., He Z.P., Jiang J.J., Golestaneh N., Hofmann M.C., Chan W.Y. and Dym M. (2009). The molecular signature of spermatogonial stem/progenitor cells in the 6-day-old mouse testis. Biol. Reprod. 80, 707-717.

Kossack N., Meneses J., Shefi S., Nguyen H.N., Chavez S., Nicholas C., Gromoll J., Turek P.J. and Reijo-Pera R.A. (2009). Isolation and characterization of pluripotent human spermatogonial stem cell-derived cells. Stem Cells 27, 138-149.

Kubota H., Avarbock M.R. and Brinster R.L. (2004). Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells. Proc. Natl. Acad. Sci. USA 101, 16489-16494.

Lee J., Kanatsu-Shinohara M., Inoue K., Ogonuki N., Miki H., Toyokuni S., Kimura T., Nakano T., Ogura A. and Shinohara T. (2007). Akt mediates self-renewal division of mouse spermatogonial stem cells. Development 134, 1853-1859.

Li R.L., Vannitamby A., Zhang J.G., Fehmel E.L., Southwell B.R. and Hutson J.M. (2015). Oct4-GFP expression during transformation of gonocytes into spermatogonial stem cells in the perinatal mouse testis. J. Pediatr. Surg. 50, 2084-2089.

Liao J.Y., Ng S.H., Luk A.C., Suen H.C., Qian Y., Lee A.W.T., Tu J.J., Fung J.C.L., Tang N.L.S., Feng B., Chan W.Y., Fouchet P., Hobbs R.M. and Lee T.L. (2019). Revealing cellular and molecular transitions in neonatal germ cell differentiation using single cell RNA sequencing. Development 146, dev174953.

Lin L.F.H., Doherty D.H., Lile J.D., Bektesh S. and Collins F. (1993). GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260, 1130-1132.

Liu S.S., Bai Y.S., Feng L., Dong W.W., Li Y., Xu L.P. and Ma N.F. (2016). Identification of CHD1L as an important regulator for spermatogonial stem cell survival and self-renewal. Stem Cells Int. 2016, 4069543.

Ma F.L., Zhou Z., Li N., Zheng L.M., Wu C.Y., Niu B.W., Tang F.R., He X., Li G.P. and Hua J.L. (2016). Lin28a promotes self-renewal and proliferation of dairy goat spermatogonial stem cells (SSCs) through regulation of mTOR and PI3K/AKT. Sci. Rep. 6, 38805.

Masaki K., Sakai M., Kuroki S., Jo J.I., Hoshina K., Fujimori Y., Oka K., Amano T., Yamanaka T., Tachibana M., Tabata Y., Shiozawa T., Ishizuka O., Hochi S. and Takashima S. (2018). FGF2 has distinct

molecular functions from GDNF in the mouse germline niche. Stem Cell Rep. 10, 1782-1792.

Mei X.X., Wang J. and Wu J. (2015). Extrinsic and intrinsic factors controlling spermatogonial stem cell self-renewal and differentiation. Asian J. Androl. 17, 347-354.

Melillo R.M., Barone M.V., Lupoli G., Cirafici A.M., Carlomagno F., Visconti R., Matoskova B., Di Fiore P.P., Vecchio G., Fusco A. and Santoro M. (1999). Ret-mediated mitogenesis requires Src kinase activity. Cancer Res. 59, 1120-1126.

Meng X.J., Lindahl M., Hyvonen M.E., Parvinen M., de Rooij D.G., Hess M.W., Raatikainen-Ahokas A., Sainio K., Rauvala H., Lakso M., Pichel J.G., Westphal H., Saarma M. and Sariola H.A. (2000). Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Science 287, 1489-1493.

Mintz B. and Russell E.S. (1957). Gene-induced embryological modification of primordial germ cells in mouse. J. Exp. Zool. 134, 207-237.

Morimoto H., Kanastu-Shinohara M., Ogonuki N., Kamimura S., Ogura A., Yabe-Nishimura C., Mori Y., Morimoto T., Watanabe S., Otsu K., Yamamoto T. and Shinohara T. (2019). ROS amplification drives mouse spermatogonial stem cell self-renewal. Life Sci. Alliance 2, e201900374.

Morrow C.M.K., Tyagi G., Simon L., Carnes K., Murphy K.M., Cooke P.S., Hofmann M.-C.C. and Hess R.A. (2009). Claudin 5 expression in mouse seminiferous epithelium is dependent upon the transcription factor ets variant 5 and contributes to blood-testis barrier function. Biol. Reprod. 81, 871-879.

Mu H.L., Li N., Wu J., Zheng L.M., Zhai Y.X., Li B., Song W.C., Wang J.L., Zhu H.J., Li G.P. and Hua J.L. (2016). PLZF-induced upregulation of CXCR4 promotes dairy goat male germline stem cell proliferation by targeting Mir146a. J. Cell. Biochem. 117, 844-852.

Mullaney B.P. and Skinner M.K. (1992). Basic fibroblast growth factor (bFGF) gene expression and protein production during pubertal development of the seminiferous tubule: follicle-stimulating hormone-induced Sertoli cell bFGF expression. Endocrinology 131, 2928-2934.

Naughton C.K., Jain S., Strickland A.M., Gupta A. and Milbrandt J. (2006). Glial cell-line derived neurotrophic factor-mediated RET signaling regulates spermatogonial stem cell fate. Biol. Reprod. 74, 314-321.

Nichols J., Zevnik B., Anastassiadis K., Niwa H., Klewe-Nebenius D., Chambers I., Scholer H. and Smith A. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 95, 379-391.

Niimi Y., Imai A., Nishimura H., Yui K., Kikuchi A., Koike H., Saga Y. and Suzuki A. (2019). Essential role of mouse Dead end1 in the maintenance of spermatogonia. Dev. Biol. 445, 103-112.

Niu B.W., Wu C.Y., Wu J., Yan Y., Shang R., Bai C.L., Li G.P. and Hua J.H. (2016). Melatonin promotes goat spermatogonia stem cells (SSCs) proliferation by stimulating glial cell line-derived neurotrophic factor (GDNF) production in Sertoli cells. Oncotarget 7, 77532-77542.

Niwa H., Miyazaki J.-i. and Smith A.G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat. Genet. 24, 372-376.

Oatley J.M. and Brinster R.L. (2008). Regulation of spermatogonial stem cell self-renewal in mammals. Annu. Rev. Cell Dev. Biol. 24, 263-286.

Oatley J.M. and Brinster R.L. (2012). The germline stem cell niche unit in mammalian testes. Physiol. Rev. 92, 577-595.

Oatley J.M., Avarbock M.R., Telaranta A.I., Fearon D.T. and Brinster R.L. (2006). Identifying genes important for spermatogonial stem cell self-renewal and survival. Proc. Natl. Acad. Sci. USA 103, 9524-9529.

Oatley J.M., Avarbock M.R. and Brinster R.L. (2007). Glial cell line-derived neurotrophic factor regulation of genes essential for self-renewal of mouse spermatogonial stem cells is dependent on Src family kinase signaling. J. Biol. Chem. 282, 25842-25851.

Oatley J.M., Oatley M.J., Avarbock M.R., Tobias J.W. and Brinster R.L. (2009). Colony stimulating factor 1 is an extrinsic stimulator of mouse spermatogonial stem cell self-renewal. Development 136, 1191-1199.

Oatley M.J., Kaucher A.V., Racicot K.E. and Oatley J.M. (2011). Inhibitor of DNA binding 4 is expressed selectively by single spermatogonia in the male germline and regulates the self-renewal of spermatogonial stem cells in mice. Biol. Reprod. 85, 347-356.

Parekh P.A., Garcia T.X. and Hofmann M.C. (2019). Regulation of GDNF expression in Sertoli cells. Reproduction 157, 95-107.

Park J.K., Song Y., Kim D.W., Cho K., Yeo J.M., Lee R., Lim Y.S., Lee W.Y. and Park H.J. (2021). Helix-loop-helix protein ID4 expressed in bovine Sertoli cells. Acta Histochem. 123, 151800.

Persio S.D., Starace D., Capponi C., Saracino R., Fera S., Filippini A. and Vicini E. (2020). TNF-α inhibits GDNF levels in Sertoli cells, through a NF-κB-dependent, HES1-dependent mechanism. Andrology 9, 956-964

Pesce M., Wang X.Y., Wolgemuth D.J. and Scholer H. (1998). Differential expression of the Oct-4 transcription factor during mouse germ cell differentiation. Mech. Dev. 71, 89-98.

Petersen C. and Soder O. (2006). The Sertoli cell-a hormonal target and 'super' nurse for germ cells that determines testicular size. Horm. Res. 66, 153-161.

Reya T. and Clevers H. (2005). Wnt signalling in stem cells and cancer. Nature 434, 843-850.

Rosner M.H., Alessandra Vigano M., Ozato K., Timmons P.M., Poirier F., Rigby P.W.J. and Staudt L.M. (1990). A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature 345, 686-692.

Sablitzky F., Moore A., Bromley M., Deed R.W., Newton J.S. and Norton J.D. (1998). Stage- and subcellular-specific expression of Id proteins in male germ and Sertoli cells implicates distinctive regulatory roles for Id proteins during meiosis, spermatogenesis, and Sertoli cell function. Cell Growth Deffer. 9, 1015-1024.

Sada A., Suzuki A., Suzuki H. and Saga Y. (2009). The RNA-binding protein NANOS2 is required to maintain murine spermatogonial stem cells. Science 325, 1394-1398.

Sada A., Hasegawa K., Pin P.H. and Saga Y. (2012). NANOS2 acts downstream of glial cell line-derived neurotrophic factor signaling to suppress differentiation of spermatogonial stem cells. Stem Cells 30, 280-291.

Sakai M., Masaki K., Aiba S., Tone M. and Takashima S. (2018). Expression dynamics of self-renewal factors for spermatogonial stem cells in the mouse testis. J. Reprod. Dev. 64, 267-275.

Salih D.A. and Brunet A. (2008). FoxO transcription factors in the maintenance of cellular homeostasis during aging. Curr. Opin. Cell Biol. 20, 126-136.

Sapsford C.S. (1962). Changes in the cells of the sex cords and seminiferous tubules during the development of the testis of the rat and mouse. Aust. J. Zool. 10, 178-192.

Schlegel P.N. (2009). Evaluation of male infertility. Minerva Ginecol. 61, 261-283.

Schrans-Stassen B.H.G.J., Van De Kant H.J.G., De Rooij D.G. and Van Pelt A.M.M. (1999). Differential expression of c-kit in mouse undifferentiated and differentiating type A spermatogonia. Endocrinology 140, 5894-5900.

Sharma S., Wistuba J., Pock T., Schlatt S. and Neuhaus N. (2019). Spermatogonial stem cells: updates from specification to clinical relevance. Hum. Reprod. Update 25, 275-297.

She Z.Y. and Yang W.X. (2014). Molecular mechanisms involved in mammalian primary sex determination. J. Mol. Endocrinol. 53, 21-37.

She Z.Y. and Yang W.X. (2017). Sry and SoxE genes: How they participate in mammalian sex determination and gonadal development? Semin. Cell Dev. Biol. 63, 13-22.

Simon L., Ekman G.C., Tyagi G., Hess R.A., Murphy K.M. and Cooke P.S. (2007). Common and distinct factors regulate expression of mRNA for ETV5 and GDNF, Sertoli cell proteins essential for spermatogonial stem cell maintenance. Exp. Cell Res. 313, 3090-3099.

Simon L., Ekman G.C., Garcia T., Carnes K., Zhang Z., Murphy T., Murphy K.M., Hess R.A., Cooke P.S. and Hofmann M.C. (2010). ETV5 regulates sertoli cell chemokines involved in mouse stem/progenitor spermatogonia maintenance. Stem Cells 28, 1882-1892.

Skinner M.K., Nilsson E.E. and Bhandari R.K. (2009). Cell-cell signaling in the testis and ovary. 2nd ed. Bradshaw R.A. and Dennis E.A. (eds). Academic Press. London. pp 2663-2678.

Suzuki H., Sada A., Yoshida S. and Saga Y. (2009). The heterogeneity of spermatogonia is revealed by their topology and expression of marker proteins including the germ cell-specific proteins Nanos2 and Nanos3. Dev. Biol. 336, 222-231.

Tadokoro Y., Yomogida K., Ohta H., Tohda A. and Nishimune Y. (2002). Homeostatic regulation of germinal stem cell proliferation by the GDNF/FSH pathway. Mech. Dev. 113, 29-39.

Takase H.M. and Nusse R. (2016). Paracrine Wnt/β-catenin signaling mediates proliferation of undifferentiated spermatogonia in the adult mouse testis. Proc. Natl. Acad. Sci. USA 113, E1489-1497.

Takashima S., Kanatsu-Shinohara M., Tanaka T., Morimoto H., Inoue K., Ogonuki N., Jijiwa M., Takahashi M., Ogura A. and Shinohara T. (2015). Functional differences between GDNF-dependent and FGF2-dependent mouse spermatogonial stem cell self-renewal. Stem Cell Rep. 4, 489-502.

Tian R.H., Yao C.C., Yang C., Zhu Z.J., Li C., Zhi E.L., Wang J.L., Li P., Chen H.X., Yuan Q.Q., He Z.P. and Li Z. (2019). Fibroblast growth factor-5 promotes spermatogonial stem cell proliferation via ERK and AKT activation. Stem Cell Res. Ther. 10, 40.

Tokuda M., Kadokawa Y., Kurahashi H. and Marunouchi T. (2007). CDH1 is a specific marker for undifferentiated spermatogonia in mouse testes. Biol. Reprod. 76, 130-141.

Trupp M., Ryden M., Jornvall H., Funakoshi H., Timmusk T., Arenas E. and Ibanez C.F. (1995). Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. J. Cell Biol. 130, 137-148.

Tseng Y.T., Liao H.F., Yu C.Y., Mo C.F. and Lin S.P. (2015). Epigenetic factors in the regulation of prospermatogonia and spermatogonial stem cells. Reproduction 150, R77-91.

Tyagi G., Carnes K., Morrow C., Kostereva N.V., Ekman G.C., Meling D.D., Hostetler C., Griswold M., Murphy K.M., Hess R.A., Hofmann M.C. and Cooke P.S. (2009). Loss of Etv5 decreases proliferation and RET levels in neonatal mouse testicular germ cells and causes an abnormal first wave of spermatogenesis. Biol. Reprod. 81, 258-266.

Wang M., Guo Y.S., Wang M., Zhou T., Xue Y.Y., Du G.H., Wei X., Wang J., Qi L., Zhang H., Li L.F., Ye L., Guo X.J. and Wu X. (2017). The glial cell-derived neurotrophic factor (GDNF)-responsive phosphoprotein landscape identifies raptor phosphorylation required for spermatogonial progenitor cell proliferation. Mol. Cell. Proteomics 16, 982-997.

Wu X., Goodyear S.M., Tobias J.W., Avarbock M.R. and Brinster R.L. (2011). Spermatogonial stem cell self-renewal requires ETV5-mediated downstream activation of Brachyury in mice. Biol. Reprod. 85, 1114-1123.

Xia Q., Cui G.Z., Fan Y., Wang X.Q., Hu G.C., Wang L.S., Luo X., Yang L.L., Cai Q.Q., Xu K.B., Guo W.J., Gao M.H., Li Y.Y., Wu J., Li W., Chen J.Y., Qi H.Y., Peng G.D. and Yao H.J. (2021). RNA helicase DDX5 acts as a critical regulator for survival of neonatal mouse gonocytes. Cell Prolif. 54, e13000.

Xi H.M., Ren F., Li Y., Xian M., Wang L.Q. and Hu J.H. (2022). FSH inhibits autophagy and lysosomal biogenesis to regulate protein degradation in cultured goat Sertoli cells. Mol. Cell. Endocrinol. 540, 111505.

Yang Q.E., Kim D., Kaucher A., Oatley M.J. and Oatley J.M. (2013). CXCL12-CXCR4 signaling is required for the maintenance of mouse spermatogonial stem cells. J. Cell Sci. 126, 1009-1020.

Yang S., Ping P., Ma M., Li P., Tian R.H., Yang H., Liu Y., Gong Y.H., Zhang Z.Z., Li Z. and He Z.P. (2014). Generation of haploid spermatids with fertilization and development capacity from human spermatogonial stem cells of cryptorchid patients. Stem Cell Rep. 3, 663-675.

Yang H., Liu Y., Hai Y.N., Guo Y., Yang S., Li Z., Gao W.Q. and He Z.P. (2015). Efficient conversion of spermatogonial stem cells to phenotypic and functional dopaminergic neurons via the PI3K/Akt and P21/Smurf2/Nolz1 Pathway. Mol. Neurobiol. 52, 1654-1669.

Yang F., Whelan E.C., Guan X.B., Deng B.Q., Wang S., Sun J.C., Avarbock M.R., Wu X. and Brinster R.L. (2021). FGF9 promotes mouse spermatogonial stem cell proliferation mediated by p38 MAPK signalling. Cell Prolif. 54, e12933.

Yeh J.R., Zhang X.F. and Nagano M.C. (2011). Wnt5a is a cell-extrinsic factor that supports self-renewal of mouse spermatogonial stem cells. J. Cell Sci. 124, 2357-2366.

Zhang Y., Wang S., Wang X.X., Liao S.Y., Wu Y.J. and Han C.S. (2012). Endogenously produced FGF2 is essential for the survival and proliferation of cultured mouse spermatogonial stem cells. Cell Res. 22, 773-776.

Zhang X.Y., Zhao X., Li G.L., Zhang M., Xing P.P., Li Z.C., Chen B., Yang H.Q. and Wu Z.F. (2021). Establishment of Etv5 gene knockout mice as a recipient model for spermatogonial stem cell transplantation. Biol. Open 10, bio056804.

Zhou Z., Shirakawa T., Ohbo K., Sada A., Wu Q., Hasegawa K., Saba R. and Saga Y. (2015). RNA binding protein Nanos2 organizes post-transcriptional buffering system to retain primitive state of mouse spermatogonial stem cells. Dev. Cell 34, 96-107.

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