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c-kit and its related genes in spermatogonial differentiation

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Abbreviations: A_s, A single; A_{al}, A aligned; A_{pr}, A paired; ADH, alcohol dehydrogenase; ALK3 (also called BMPR1A), bone morphogenetic protein receptor, type 1A; AKT, thymoma viral proto-oncogene 1; BAD, BCL2-associated agonist of cell death; BMP4, bone morphogenetic protein 4; BMP8b, bone morphogenetic protein 8b; CBP, sarcoplasmic calcium-binding protein; cdk2, cyclin-dependent kinase 2; c-Fos, FBJ osteosarcoma oncogene; c-Jun, Jun oncogene; CRABP, cellular retinoic acid binding protein; CYP26B1, cytochrome enzyme P450; Dmc1, DMC1 dosage suppressor of mck1 homolog; dpc, days post coitum; dpp, days post partum; E2F, E2F transcription factor; ERK, elk-related tyrosine kinase; FGF2, fibroblast growth factor 2; FSH, follicular stimulating hormone; FSHR, FSH receptor; GDNF, glial cell line-derived neurotrophic factor; GRB2, growth factor receptor-bound protein 2; Kit, Kit receptor; Kitl, Kit ligand; Kitm, membrane form of Kit; Kits, soluble Kit; Kitlm, membrane form of Kitl; Kitls, soluble kit ligand; LH, luteinizing hormone; MAPK, mitogen-activated protein kinases; NRE, nanos-responsive element; p70S6K, Rps6kb1, ribosomal protein S6 kinase; PGCs, primordial germ cells; PI3K, phosphoinositide-3-kinase; PLCG, phospholipase Cgamma; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; RAR, retinoic acid receptor; RARE, retinoic acid responding element; RAS, RAt Sarcoma; Rb, retinoblastoma protein; RBP, Retinoic acid binding protein; SCF, stem cell factor; SCP3, synaptonemal complex protein 3; SiRNA, small interfering RNA; Smad5, MAD homolog 5; Spg, spermatogonia; SRC, rous sarcoma oncogene; Sry, Sex-determining region Y; SSC, spermatogonial stem cell; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling; VAD, vitamin A deficient

Genes: *Aldh1a2*, also called Raldh 2, aldehyde dehydrogenase family 1, subfamily A2; *Bcl-2*, B-cell leukemia/lymphoma 2; *Bcl6b*, B-cell CLL/lymphoma 6, member B; *c-kit,* also called CD117, kit oncogene; *Dazl*, deleted in azoospermia-like; *Dmc1*, also called Dmc1 h, dosage suppressor of mck1 homolog; *Kitl*, also called SCF, kit ligand; *Mvh*, also called Ddx4, DEAD (Asp-Glu-Ala-Asp) box polypeptide 4; *Nanos2*, nanos homolog 2; *Nanos3*, nanos homolog 3; *Neurogenin3*, also called neurogenin3; *Oct*$^{3/4}$, also called Pou5f1, POU domain, class 5, transcription factor 1; *Sox3*, SRY-box containing gene 3; *Stra8*, stimulated by retinoic acid gene 8; *Sycp3*, also called Scp3 and Cor1 Synaptonemal complex protein

Spermatogenesis is the process of production of male gametes from SSCs. The SSCs are the stem cells that differentiate into male gametes in the testis. In the mean time, the Spg are remarkable for their potential multiple trans-differentiations, which make them greatly invaluable for clinical applications. However, the molecular mechanism controlling differentiation of the Spg is still not clear. Among the discovered spermatogenesis-related genes, *c-kit* seems to be expressed first by the Spgs thus may play a central role in switching on the differentiation process. Expression of Kit and the activation of the Kit/Kitl pathway coincide with the start of differentiation of Spgs. Several genes have been discovered to be related to the Kit/Kitl pathway. In this review, we have summarized the recent discoveries of *c-kit* and the Kit/Kitl pathway-related genes in the spermatogenic cells during different stages of spermatogenesis.

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Introduction

It has long been believed that "differentiating" Spg are irreversibly committed to differentiation. In other words, the *c-kit* positive differentiated Spg can't reverse to *c-kit* negative stem cells. But in male and female *Drosophila melanogaster*, it is shown that differentiating germ cells can revert to functional stem cells that can restore germinal lineage.[1,2] A study by Barroca et al. reported that in mouse, purified *c-kit* positive Spg, although committed to differentiate, can repopulate when transplanted into the γ-irradiated germ-cell-depleted adult mice testes. GDNF and FGF2 are found to be able to reprogram in vitro Spg for reverse differentiation.[3] As a marker of Spg differentiation, functions of *c-kit* include anti-apoptosis in PGCs, promoting cell replication in PGCs and Spg, and initiating the entry of Spg into meiosis.[4] As the fate of germ cell lineage in mammals is defined by a chain of inductions, we will discuss the role of *c-kit* and its signaling pathway (Kit/Kitl pathway) in Spg differentiation by structure, function and interacting factors.[5]

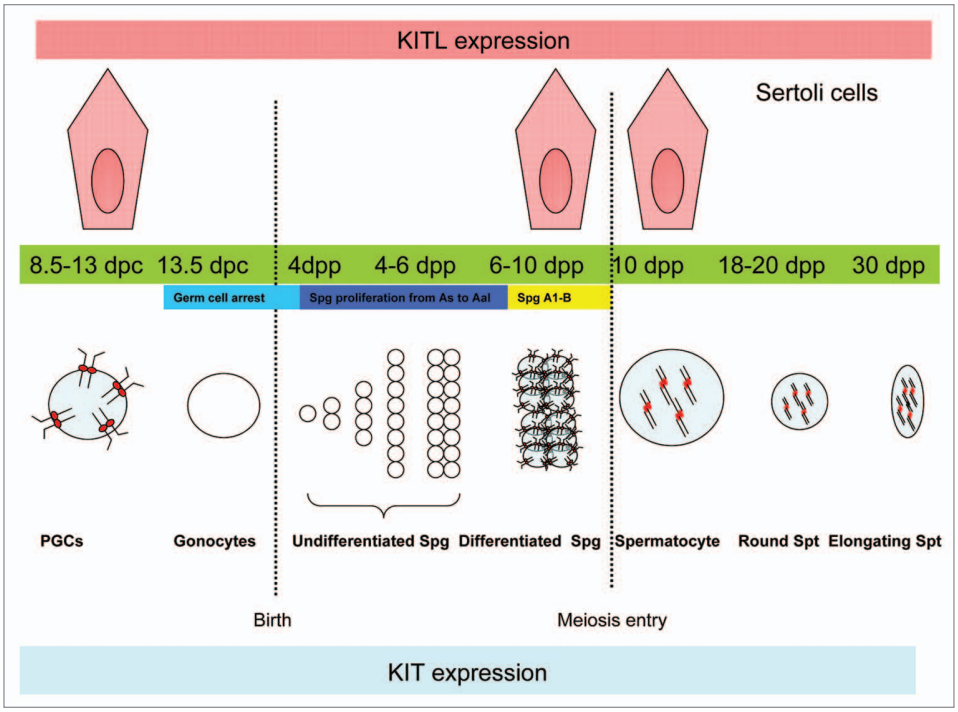

Figure 1. Schematic representation of expression of *c-kit* in mouse germ cells from fetus to 30 days postpartum. PGCs from 8.5–13 dpc express full-length Kit which is located on the membrane. From 13.5 dpc to 3 dpp, the PGCs lose Kit expression and turn into *c-kit*$^-$ gonocytes which are arrested. At 3 dpp, the gonocytes start to proliferate into A_s and A_{al} Spg, which are undifferentiated without expressing Kit. Expression of *c-kit* is re-started at around 7 dpp when the A_1-B stage Spg appear. A truncated isoform of *c-kit* starts to be expressed (Tr-Kit) in the cytocol of the spermatocytes and is kept expressed in later stage germ cells (spermatocytes, spermatids and spermatozoa). Sertoli cells express Kitl from 8.5 dpc to 13.5 dpc and 6–10 dpp only. Entry of germ cells into meiosis occurs around 10 dpp. Expression of Kit and Kitl in the spermatogenic cells and Sertoli cells respectively is represented by the red color in the membrane, cytosol and nucleus.

Spermatogenesis and *c-kit*

Spermatogenesis is a highly regulated process of differentiation and complex morphologic alterations that leads to the formation of sperm in the seminiferous epithelium. In the adult male mammals, it can be subdivided into three main phases: spermatogonial proliferation, meiosis of spermatocytes and spermiogenesis of haploid spermatids. In the adult testes, spermatogenesis starts from diploid SSCs. The SSCs, also called type A_s Spg, are located on the basal membrane of the seminiferous tubules. The A_s Spg can self-renew or produce the type A_p Spg. After successive divisions, A_p Spg differentiate and form chains of 4, 8 or 16 type A_{al} Spg and migrate along the basal membrane. According to morphological criteria, the SSCs, A_p and A_{al} Spg are classically called undifferentiated Spg. A_{al} Spg differentiate into more committed A1 Spg that will further divide and differentiate into A2, A3, A4, intermediate and B Spg, which will undergo meiosis after a final mitosis.[3] The "undifferentiated" (A_s, A_p and A_{al}) and the "differentiating" (A1, A2, A3, A4, intermediate and B) Spg differ in the expression of *c-kit*.[6] The transition of undifferentiated Spg into differentiating Spg coincides with the gain of Kit. The presence of Kit in Spg has been routinely used as a marker to identify differentiating Spg.[7,8] **Figure 1** summarizes *c-kit* expression during spermatogenesis. Kit continues to be expressed until meiosis and play essential roles in the survival of the Kit-expressing cells.[6,9] Kitl is expressed in the Sertoli cells which there have extensive contacts with the germ cells. Kit is activated only after binding with Kitl and the Kit/Kitl pathway is considered to be crucial for the proliferation, migration, survival and maturation of the germ cells both in the embryonic and the postnatal gonads.[9-18] Mice carrying a mutation rendering a constitutively active Kit kinase have an interrupted transition from the round into the elongating spermatids.[19]

c-kit plays a key role in maintaining the ratio between self-renewal and differentiation of SSCs.[20] In normal seminiferous epithelium, the ratio between self-renewal and differentiation of spermatogonial stem cells is maintained at 1.0. Alterations in this ratio entail greater Spg self-renewal leading to Kit$^+$ tumor cells in the seminiferous epithelium.[21] In the mean time, the alterations also entail stem cell depletion resulting in Sertoli cells only syndrome.[22]

Transcription and Translation of *c-kit* and its Ligand in Spermatogenic Cells

c-kit is allelic to the W locus on mouse chromosome 5.[23] The 21-exon gene encodes a 5,150 bp transcript which is translated into a product of 145 kDa protein with 979 amino acid residues which is called Kit.[24] *c-kit* mRNA and protein synthesis are regulated separately possibly by circulating hormones as the undifferentiated Spg contains only *c-kit* mRNA but not

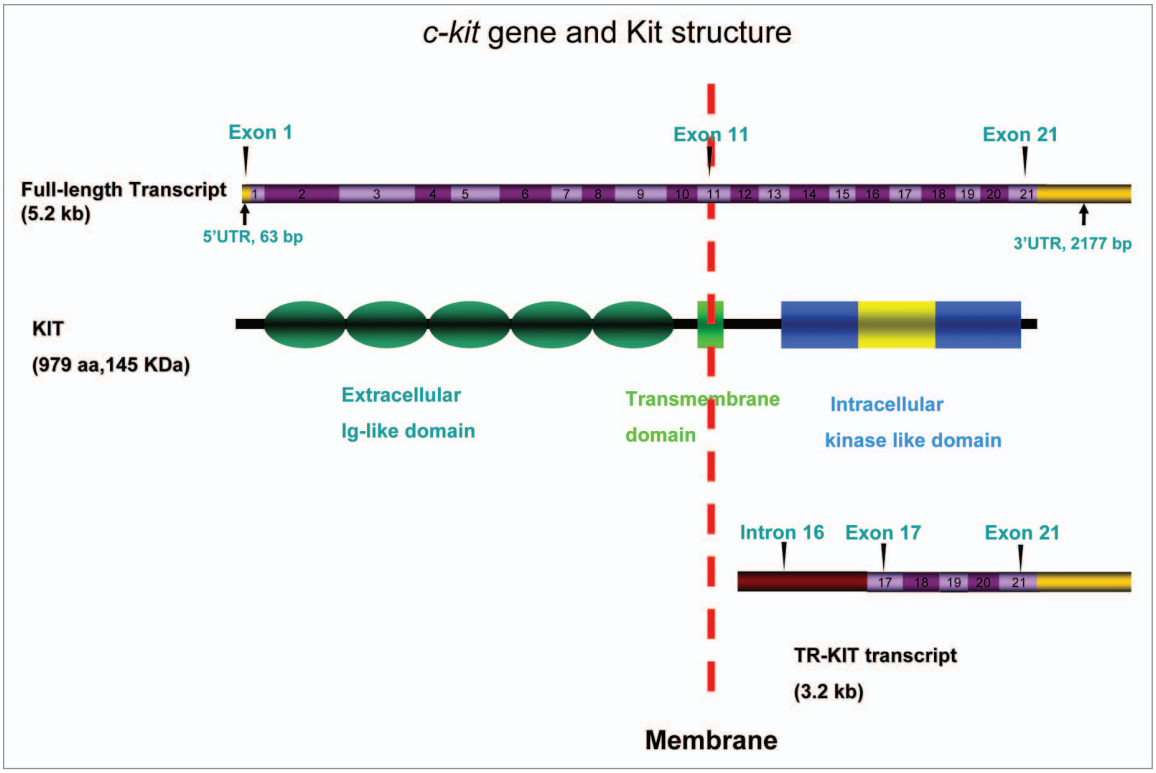

Figure 2. *c-kit* gene and Kit structure. The *c-kit* DNA is 5,150 bp in length, consists of 21 exons and encodes a 150 KDa full-length protein with 979 amino acids. Exons 1–10 encode the extracellular components with 5 Ig-like domains and the signal sequences. Exon 11 encodes the transmembrane segment. Exons 12–20 encodes the intercellular segments including the juxtamembrane segment, proximal kinase domain, kinase insert domain and distal kinase domain. Exon 21 encodes the C-terminal tail. Truncated mRNA from intron 16 to exon 21 encodes the TR-Kit.

protein.[15] Kit belongs to a family of growth factor receptors with intrinsic tyrosine kinase activity that transduces growth regulatory signals across the plasma membrane. Kit has three main functional regions: the extracellular domain, the transmembrane region and the intracellular domain. The extracellular domain consists of five immunoglobin-like repeats with about 520 amino acids which are required for ligand binding and dimerization.[25] The transmembrane region is a 23 amino acid hydrophobic domain, which anchors the receptor to the cell membrane. The 433 amino acid intracellular domain consists of three domains, with a proximal kinase region for ATP binding, a 70–100 amino acid non-conserved insert and a distal phosphotransferase kinase region.[26] Binding to the Kitl induces a rapid and complete receptor dimerization that involves activation by autophosphorylation of the tyrosine kinase residues (**Fig. 2**).[27] The phosphoTyrosine (pTyr) residues in the intracellular juxtamembrane domain subsequently serve as docking sites for signal transduction molecules.[28]

Kit has function not only during spermatogenesis, but also throughout all stages of male germ cell development before and after birth. Northern blot analysis of germ cells at different developmental stages has shown the presence of two alternative mRNA of *c-kit*, 3.2 and 2.3 kb in length respectively, in the haploid cells of the mouse testis.[29] With an Open Reading Frame (ORF) that starts in the intron 16th mouse *c-kit*, the alternative spermatid-specific *c-kit* transcripts contain all the downstream exons, and encode for a truncated form of the Kit protein (~30 kDa), called Tr-Kit, including 12 hydrophobic amino acids followed by the last 190 carboxy terminal residues of the Kit.[30,31] Tr-Kit derived from alternative promoter in the intron 16 of *c-kit* and encodes part of the non-conserved insert from the C-terminal tail region and the distal phosphotransferase kinase region, and it lacks the entire extracellular and the transmembrane domain.[32] This intronic promoter of the *c-kit* is only active in the late stages of spermatogenesis, suggesting a role for this truncated protein either during spermatid differentiation or for the function of mature sperm.[33] Tr-Kit is found in the residual sperm cytoplasm and there is evidence for its serving a function in the activation of oocyte at fertilization in mice.[31]

Kitl and Spermatogenesis

Kitl, also called SCF, is produced in the Sertoli cells and is a cytokine essential for haematopoiesis, melanogenesis and development of germ cells. Kitl has been identified as an analogue of the murine steel (*Sl*) locus and is located on chromosome 12 in humans. Two isoforms of Kitl are generated from the same gene by alternative splicing—a soluble ($Kitl_s$) and a transmembrane ($Kitl_m$) form.[34,35] The soluble form arises after proteolytic cleavage of a membrane-bound precursor.[36] In the Spg proliferating stage, the membrane isoform is a predominant

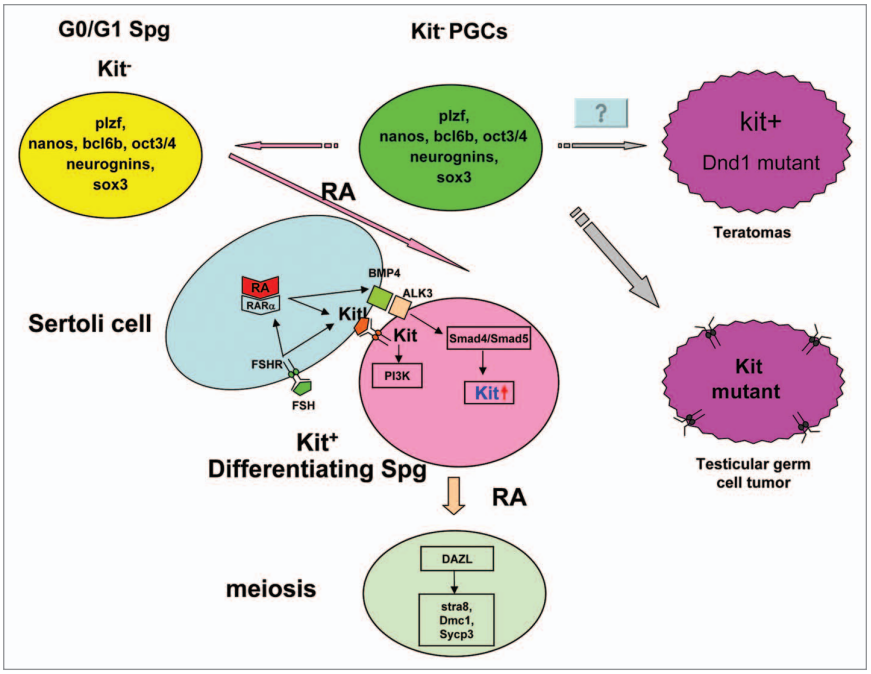

Figure 3. Summary of Kit/Kitl signaling during spermatogenesis. The *c-kit*$^{+}$ PGCs lose *c-kit* expression and turn into the undifferentiated Spg which characteristically express *Plzf, Nanos, Bcl6b, Oct*$^{3/4}$, *neurogenin3* and *Sox3*. Most of those undifferentiated Spg will go into *c-kit*$^{-}$ mitotic arrest (G_0/G_1 stage) under control of meiosis-inhibiting factor Cyp26b1. Undifferentiated Spg arrested during mitosis can return to normal spermatogenesis triggered by various differentiation signals. The lack of *c-kit* negative mitotic arrest disorders will lead to teratomas. RA, FSH and BMP4 may facilitate Spg differentiation as well as the activation of the Kit/Kitl signal pathway. Active RA acts on Sertoli cells through the RARα to stimulate the synthesis of growth factors Kitl, which bind to Kit to activate kit/Kitl/PI3K pathway. ALK3, receptor of BMP4, is specifically expressed in the mitotic Spg during the first week after birth. BMP4 action is mediated by a rapid nuclear translocation of Smad4 and Smad5 which make the Spg start to express *c-kit*. Mutations of *c-kit* (resulting in self-activation of Kit) at post-migration stage of Spg will cause bilateral testicular germ cell tumors. Differentiated Spg will go into meiosis after the activation of Kit/Kitl signaling. *Dazl* is a competence factor for meiosis initiation and germ cells start to express *Stra8*, *Dmc1* and *Sycp3* once get into meiosis.

one; whereas in the Spg quiescent stage, the soluble form dominates. Actions of Kit/Kitl system include stimulation of PGC migration, enhancement of proliferation of PGCs/Spg and anti-apoptosis of PGCs.[4] However, the down-stream signaling pathways involved on Spg proliferation and anti-apoptosis seem to be different. In vitro addition of Kitl to A1-A4 Spgs results in a transient activation of Erk1/2 and PI3K which lead to Spg proliferation.[37] Kitl existed in the seminal plasma in human significantly correlate with the sperm count.[38] Mutation in Kitl in human has significant association of idiopathic male infertility.[39] Together with the soluble growth factors including GDNF and FGF2, Kitl might contribute to construct the potential niche which stimulates stem cell divisions.[40] Kitl is also found to be able to increase the percentage of sperm undergoing acrosome reaction when cultured in vitro.[41]

Kit/Kitl-Dependent Mechanisms during Spermatogenesis

Four pathways are known to be activated in response to Kit/Kitl activation in the Spg. The PI3K pathway results in cell survival (via AKT and BAD regulation), adhesion (via c-JUN and c-FOS activation) and proliferation (via AKT and p70S6K). The PI3K/AKT pathway appears to be critical exclusively in postnatal stage spermatogenesis. Mice with a mutant form of Kit are incapable of PI3K recruiting and are sterile caused by reduced proliferation and increased apoptosis in the Spg.[42] Through PI3K pathway, Kit/Kitl facilitate the upregulation and nuclear accumulation of cyclin D3 as well as Spg proliferation suggesting that cyclin might be one of the targets of Kit/Kitl pathway within the testis.[37,43] Secondly, the SRC pathway involves the association of SRC family proteins with the intracellular juxtamembrane domain of Kit and affects cell migration and AKT phosphorylation in mice PGCs.[44] Thirdly, Tr-Kit activated PLCG through the PLCG pathway, mediates the resumption of meiosis of the fertilized eggs.[45] Lastly, the MAPK cascade is activated by RAS with the binding of Kit and GRB2. MAPK directly mediates gene transcription in PGCs and proliferation in Spg.[37,44,46]

Roles of *c-kit* in Embryonic and Neonatal Spermatogenic Cells

***c-kit* and spermatogenic cell proliferation and restoration.** In mouse, at around 7.2 dpc, somatic signals earmark a small cohort of proximal epiblast cells as potential germ cell precursors.[47] This group of cells moves into the extraembryonic tissue at the base of the allantois, where a second round of selection occurs which results in a group of about 45 cells specified to be germ cell precursors or PGCs. After specification, the germ cells become transcriptionally silent at 9.5 dpc and are subject to an extensive reprogramming of their genomes by histone modifications and alterations in the state of DNA methylation.[48] *C-kit* mRNA is first detected in the PGCs at 6.5–7 dpc and persists during their subsequent proliferation and migration to the genital ridge (7.5–13.5 dpc).[49,50] In the mean time, the somatic cells along the migratory pathway and genital ridges synthesize Kitl.[16,17,51,52] In the absence of either Kit or Kitl, mice are sterile and with a reduced number of PGCs.[17] Kitl secreted by the somatic cells seems to be an attractant for germ cells migration and are required for their adhesion, proliferation, migration and survival prior to 10 dpc after which downregulation of Kitl is associated with switching on the intrinsic apoptotic pathway in ectopic germ cells.[16,44,53] We wonder if Kit/Kitl

pathway may facilitate SSCs survival by suppressing apoptosis as that in the ES cells.[54]

Unlike in the female where kit expression in oogonia continues into meiosis, the male PGCs arrest in G_0/G_1 of the mitotic cycle around 13.5 dpc and resume mitosis around 3 days post partum (dpp) during which Kit expression is markedly reduced in mice (**Fig. 3**).[49] At around 3 dpp, expression of Kit is still low when the male PGCs actively proliferate again. The transition from *c-kit* independent type to *c-kit* dependent type occurs at about 5 dpp when the competence to enter meiosis is reached.[12,55,56] Expression of Kit (3 dpp) is before the expression of Kitl (6–8 dpp) and their expression is closely coordinated.[57-59]

Even though its activation represents the start of differentiation in Spg, *c-kit* is an important stem cell marker for many types of stem cells such as hematopoietic stem cells.[60] Expression of Kit in the SSCs is contradictory. In the early studies, Kit expression in the adult testis is detected by immunohistochemical analysis and in situ hybridization in the differentiating type A (A1–A4), intermediate, and type B spermatogonia, as well as preleptotene spermatocytes and interstitial Leydig cells, but not in the undifferentiated spermatogonia and Sertoli cells.[6,9] Hence, activation of the Kit/Kitl signaling pathway is not required for SSCs self-renewal.[20,61] More recent studies demonstrate that both Kit^- and Kit^+ cells showed comparable levels of stem cell activity after germ cell transplantation.[3,20,62] As SSCs can change their phenotype according to their microenvironment, Kit^+ cells might be an intermediate state during SSCs self-renewal.[20] Izadyar et al. further characterized the Kit^+ SSCs and find that the $POU5F1^+/Kit^+$ subset of mouse SSCs generates cell lines that express pluripotent ES markers and can differentiate into multiple lineages. But in vivo testes regeneration assay shows that only the $POU5F1^+/Kit^-$ SSCs will regenerate the spermatogenesis of the recipient tests.[63] Inactivation of c-kit by Imatinib caused an impairment of Spg self-renewal.[64] Therefore, Kit seems not to affect SSCs self-renewal directly, instead, it might affect the size of SSCs pool by playing a role during the phenotypic transitions of SSCs (**Fig. 4**). Intriguingly, Kit is expressed by SSCs such as A_s, A_{pr} and A_{al} even though activation of the Kit/Kitl signaling pathway is not required for their self-renewal which demonstrates the comlex dynamics of spermatogonial differentiation.[20,61,65-67]

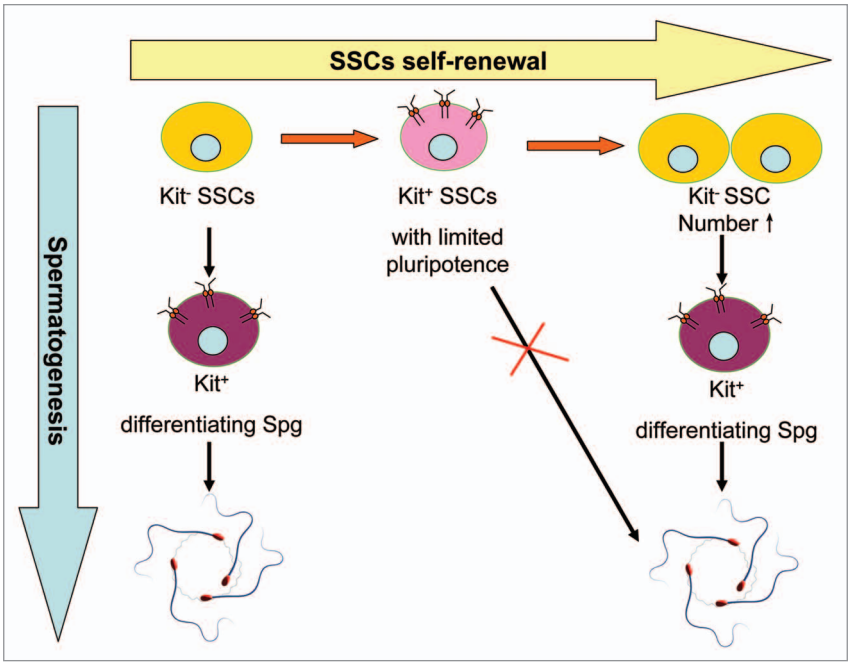

Figure 4. *c-kit* and phenotypic transition of SSCs during spermatogenesis. There are two kinds of SSCs including the Kit^+ and Kit^- SSCs in the early stage of postnatal spermatogenesis in mice. The Kit^- SSCs is the performer of normal spermatogenesis. In order to complete self-renewal, Kit^- SSCs change their phenotype to Kit^+ SSCs according to their microenvironment. Unlike the Kit^- SSCs, Kit^+ SSCs have limited pluripotence and cannot re-enter spermatogenesis after in vivo transplantation to the recipient mice.

Role of *c-kit* in onset of meiosis. Activation of Kit signaling before meiosis and inactivation of Kit after meiosis are important for proper spermatogenesis. Synthesis of *c-kit* mRNA and protein is concordant with the first appearance of differentiating Spg, and persists at relatively lower levels in meiotic pachytene spermatocytes.[15] Followed by c-kit activation, early meiotic markers such as *Dmc1* and *Scp3* are activated.[68] It has been demonstrated that the timing of meiosis entry is indirectly controlled by the Sertoli cells through the activation of Kit/Kitl system when they are induced by RA.[11,13,69-71] In vitro experiment has proved that the addition of RA to the Kit expressing Spg will induce the onset of spermatogenesis but not the Kit negative Spg.[70] Kit/Kitl activation causes a transient activation of ERK1/2 and PI3K-dependent AKT kinase. These events are followed by a rapid nuclear redistribution of cyclin D3 and accumulation of cyclin E that promotes cell cycle progression via the PI3K/p70 S6 kinase pathway.[37,43] Hyperphosphorylation of retinoblastoma protein Rb by cyclin E/cdk2 is followed by the release of Rb-associated transcription factor E2F, which elicits a timely induction of other genes required for S-phase progression.[72] Silencing *c-kit* expression by siRNA in the Spg induces cell cycle arrest thus confirming the role of Kit on meiosis entrance.[73] Transcriptome analysis of the Spg treated with Kitl indicates that Kitl stimulates their entrance of meiotic program by upregulating the G_1/S transition inhibitors and G_2/M promoters and by downregulating the G_1/S promoters.[74]

Kit and mature sperm. *c-kit* gene translates to two kinds of proteins, Kit and Tr-Kit, during spermatogenesis. Tr-Kit protein is expressed during human spermiogenesis and maintained in human spermatozoa. Tr-Kit begins to be expressed in the post-meiotic haploid germ cells.[30,31] Cytometric analysis of several human sperm samples from volunteer donors, showed variable degrees of the Tr-Kit-specific immunolabeling, and a significant inverse correlation of the Tr-Kit positivity with markers of sperm damage, i.e., DNA fragmentation, as revealed by TUNEL

analysis and the intense clusterin positivity. So, the maintaining of Tr-Kit in the haploid spermatogenic cells appears to correlate with the next stage spermatozoa DNA integrity.[75] Whether Kit is present in these haploid germ cells is under debate. Muciaccia et al. found that Kit and its coding mRNA was not detected in the spermatozoa.[75] Feng et al. show that the mature human spermatozoa express Kit and its presence appears to be correlated with sperm capacitation and the acrosomal reaction. The percentage of sperm underwent acrosomal reaction declined and the percentage of head-to-head agglutination increased following blocking with Kit antibodies.[76]

Mechanisms Controlling the Activation of Kit/Kitl Pathway

The mechanisms controlling *c-kit* expression during spermatogenesis are not very clear. However, several upstream regulating factors have been discovered in the studies of PGCs, oogenesis and other organisms. Here we will discuss 3 factors which directly or indirectly relate to Kit/Kitl pathway activation in the Spg.

Vitamin A and its derivatives. Retinoic acid (RA), an active metabolite of vitamin A, is a vital signaling molecule for normal fetal development, pattern formation, cell proliferation and differentiation, and apoptosis.[77] RA is synthesized by the mesonephroi to which the gonads are attached.[78] ES cells will differentiate to PGCs when culture with 2 μM RA for 5 days.[79,80] RA may regulate proliferation and differentiation of Spg mainly through RARα mediated signal pathway. During post-natal development, RARα and RXRβ are confined to Sertoli cells, whereas RARγ is expressed in Spg followed by a colocalization of RARβ, RXRα and RXRγ to the step 7–8 spermatids.[81] RA acts to initiate meiosis both in male and in female. In male, exogenous RA can induce XY A_{al} staged germ cells in a cultured mouse fetal testis to enter meiotic prophase.[82] It is not yet known whether the action of RA in inducing differentiation and *c-kit* expression is directly, or indirectly via Kitl in the Sertoli cell. It is accepted that RA regulates the timing of meiosis indirectly via juxtacrine signaling by Sertoli cells.[70] Some studies show that RA directly act on spermatogenic cells by stimulating *Stra8* and *c-kit* gene expression, whereas others show exogenous RA could not stimulate *c-kit* expression in spermatogenic cells but cause apoptosis of the A_{al} Spg.[83-85] Besides, RA also upregulates Kitl levels in the Sertoli cells, resulting in increased levels of the early meiotic cell markers. This activation is independent of germ cell viability and occurs through the phosphatidylinositol-3-kinases (PI3K) and MAP kinase (MAPK) pathways.[70]

BMP4/ALK3/SMAD5 signaling pathway. BMP4, one of the TGFβ-BMP superfamily growth factor, is produced by Sertoli cells very early in the postnatal life and is downregulated during peri-pubertal phase. BMP4 treatment of the PGCs, SSCs and Spg increases Kit levels and causes a mitogenic response to Kitl.[86-88] BMP4 expression is significantly upregulated in the testes of VAD mice and is downregulated in freshly isolated germ cells treated by retinol. This reflects a direct requirement for retinoid by germ cells for the resumption of spermatogenesis in VAD animals via mechanisms that involve the suppression of BMP4 expression.[89] Receptors of BMP4 (ALK3 and BMPIIR) are specifically expressed in mitotic Spg during the first week after birth. BMP4 action is mediated by a rapid nuclear translocation of Smad4 and Smad5, where the Smad4/Smad5 complexes are able to recruit the transactivating factor CBP and to bind Smad-responsive DNA sequences.

Another member of the TGFβ-BMP superfamily growth factor is BMP8b which stimulate both PGCs and Spg to proliferate. $BMP8b^{-/-}$ mice show impairment of PGC commitment, defects of Spg proliferation and spermatocyte apoptosis.[90,91]

FSH. FSH, LH and the testis androgen are involved in the process of orchestrated control of spermatogenesis. FSH is not essential for spermatogenesis but is required for quantitatively normal sperm production in both mice and human.[92-96] FSH works directly on Sertoli cells via FSHR. Kitl is expressed by Sertoli cells under FSH stimulation. So, the Sertoli cells of the genetic mutant mice lacking FSH receptor will produce less Kitl. These mutant mice exhibit reduced testis, epididymis, and seminal vesicle as well as low levels of testosterone. A significant increase in the percentage of *c-kit* positive Spg and non-germ cells and a significant decrease in the percentage of elongated spermatids are observed in these mice. The increase in the percentage of *c-kit*-positive cells and decrease in the testosterone values of FSH receptor mutant mice may be due to the reduced levels of Kitl available for intercellular communication in the absence of FSH receptor signaling.[97] In the Sertoli cells, FSH can regulate transcriptional function of the RARα, thus controlling cell proliferation and differentiation.[98] Therefore, it appears that it is FSH that determines the expression of *c-kit* in the Spg via Sertoli cell factors including Kitl and RARα.

Roles of Kit/Kitl Pathway and Related Genes in Spg Maintenance

Genes involved in Spg maintenance. *Plzf, Nanos, Bcl6b*, *Oct*$^{3/4}$, *neurogenin3* and *Sox3* are markers of the undifferentiated Spg. The DNA sequence-specific transcriptional repressor, *Plzf*, is considered to be involved in stem cell maintenance. Loss of *Plzf* function shifts the balance between spermatogonial stem cell self-renewal and differentiation toward differentiation at the cost of self-renewal and leads to an increase of post-meiosis apoptotic cells.[99,100] It is shown that *Plzf* directly represses the transcription of Kit.[101] *Nanos* encodes for a zinc-finger RNA-binding protein and shows a translational repression activity requiring the interaction with the ubiquitously expressed protein Pumilio. The Nanos-Pumilio protein complex binds to NRE in the 3' UTR of target mRNAs and represses their translation.[102] It has been indicated that *Nanos3* is required to prevent PGCs from undergoing apoptosis during migratio.[103] Overexpression of *Nanos3* causes an increase of G_1 stage undifferentiated Spg. RA significantly decreases the expression of *Nanos3* in the undifferentiated Spg.[104,105] So, *Nanos3* is important for maintaining the undifferentiated stage of Spg. *Nanos2* suppresses meiosis by preventing *stra8* expression. *Nanos2*$^{-/-}$ male PGCs go into apoptosis at 16.5 dpp and are completely lost before birth.[106] *Oct*$^{3/4}$ is the stem cell

and germ line specific marker encoding DNA binding domain POU[107] and it is also called *Pou5f1*. When RA binds to RARs, expression of Oct$^{3/4}$ is inhibited. In the germ cell-specific nulls of Pou5f1, XX and XY germ cells undergo apoptosis between 9.5–10.5 dpc, before colonization of the gonad.[108]

At around 13.5 dpc, germ cells in the testis enter mitotic arrest in G_0 until near birth.[109] At this stage, low level expression of meiosis-associated genes, such as *Sycp3* and *Dmc1*, indicates that all these germ cells are capable of entering meiosis.[68] It is hyphthesized that the mitotic arrest is induced by a testis-derieved meiosis-inhibiting factor.[110-112] Cyp26b1 seems to be one of the meiosis-inhibiting factors which regulate the amount of RA in the prenatal gonads.[113] Germ cells that fail to enter into mitotic arrest forms teratomas in the *Dnd1* mutant male mice and a finely tuned temporal expression of *c-kit* appears critical for normal spermatogenesis and surrpession of testicular tumors.[114-116]

***Dazl* is a competence factor for meiosis initiation.** The *Dazl* gene family encodes potential RNA binding proteins that are expressed in prenatal and postnatal germ cells of males and females. In the testis, this protein is localized to the nucleus of Spg but relocates to the cytoplasm during meiosis where it persists in spermatids and spermatozoa. *Dazl* is expressed from 11.5–12.5 dpc and is important for successful completion of meiotic prophase. Expression of *Dazl* is required for *stra8* responses to RA stimulation as RA could not stimulate *stra8* in *Dazl*$^{-/-}$ testis.[78,117,118] In male germ cells, *Dazl* is expressed in the A_{al} to A1 Spg.[119,120] The prime spermatogenic defect in the *Dazl*$^{-/-}$ mice is a failure of the great majority of the A_{al} Spg to differentiate into A1 Spg though most of the A Spg of *Dazl*$^{-/-}$ mice were positively stained for the *c-kit* protein. Most seminiferous tubules of *Dazl*$^{-/-}$ mice only contain actively proliferating A_s, A_{pr} and A_{al} Spg, with no further successful differentiation due to apoptosis of subsequent cell types.[120]

***Stra8*, *Dmc1* and *Sycp3* are meiotic marker genes.** *Stra8* is a vertebrate-specific gene that encodes a cytoplasmic protein whose expression is induced by retinoic acid.[84,121] It is required for premeiotic DNA replication, chromosome condensation and subsequence events of meiotic prophase in germ cells of embryonic ovaries.[122] Located only in both male and female premeiotic germ cells (specifically in the Spg and preleptotene-stage spermatocytes in males), *Stra8* may play a role in the premeiotic phase of spermatogenesis.[121,123]

In male mice, *Stra8* is expressed postnatally, in the mitotically active cells of the Spg and their immediate descendants (preleptotene spermatocytes).[121,124] The peak of *Stra8* mRNA expression coincides with the onset of meiosis in postnatal testes. *Stra8* is detected as early as 5 dpc and its expression in the neonatal testes was not uniform among Spg. In adult testes, the highest level of *Stra8* mRNA and protein was found in seminiferous epithelial stages VI–VIII. In normal adult testes, RA stimulated *Stra8* mRNA expression. *Stra8* expression in adult Spg is induced by RA stimulation, suggesting its role in spermatogonial differentiation.[84,124] Retinoic acid also increases the number of preleptotene spermatocytes exhibiting 5-bromo-2-deoxyuridine incorporation, indicating a more synchronized premeiotic DNA replication.[124] Mice lacking *Stra8* function produces no sperm; most spermatogenic cells undergo apoptosis at a developmental stage when they normally would have progressed through meiotic prophase.[122] **Figure 3** concludes the possible network of genes controlling the normal Spg differentiation towards meiosis.

Conclusion and Prospect of Spg Studies

Mechanisms controlling spermatogenesis is mammals are still unclear. So far, we have known that the SSCs proliferate by stimulation of soluble growth factors (GDNF and FGF etc.,) and cell structural gene products (*Plzf, Nanos, Bcl6b, Oct, Neurogenin 3* and *Sox 3*, etc.*)*. Competence of entering of meiosis should be obtained (expression of *Dazl*) before the SSCs could respond the meiosis-promoting factors such as *Syp3*, *Dmc1* and *Stra8*. Meiosis is regulated not only by the meiosis-promoting factors, but also by meiosis-inhibiting factors (such as *Cyp26b1*). The prospect of Spg researches should be focused on the mechanisms driving SSCs differentiation toward a haploid male germ cell which will help those infertile men who can't produce sperm.

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References

1. Kai T, Spradling A. Differentiating germ cells can revert into functional stem cells in *Drosophila melanogaster* ovaries. Nature 2004; 428:564-9.
2. Brawley C, Matunis E. Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. Science 2004; 304:1331-4.
3. Barroca V, Lassalle B, Coureuil M, Louis JP, Le Page F, Testart J, et al. Mouse differentiating spermatogonia can generate germinal stem cells in vivo. Nat Cell Biol 2009; 11:190-6.
4. Mauduit C, Hamamah S, Benahmed M. Stem cell factor/c-kit system in spermatogenesis. Hum Reprod Update 1999; 5:535-45.
5. Hayashi K, de Sousa Lopes SM, Surani MA. Germ cell specification in mice. Science 2007; 316:394-6.
6. Schrans-Stassen BH, de Kant HJ, v de Rooij DG, van PAM. Differential expression of c-kit in mouse undifferentiated and differentiating type A spermatogonia. Endocrinology 1999; 140:5894-900.
7. Shinohara T, Avarbock MR, Brinster RL. beta1- and alpha6-integrin are surface markers on mouse spermatogonial stem cells. Proc Natl Acad Sci USA 1999; 96:5504-9.
8. Shinohara T, Orwig KE, Avarbock MR, Brinster RL. Spermatogonial stem cell enrichment by multiparameter selection of mouse testis cells. Proc Natl Acad Sci USA 2000; 97:8346-51.
9. Yoshinaga K, Nishikawa S, Ogawa M, Hayashi S, Kunisada T, Fujimoto T, Nishikawa S. Role of c-kit in mouse spermatogenesis: identification of spermatogonia as a specific site of c-kit expression and function. Development 1991; 113:689-99.
10. Dym M, Jia MC, Dirami G, Price JM, Rabin SJ, Mocchetti I, Ravindranath N. Expression of c-kit receptor and its autophosphorylation in immature rat type A spermatogonia. Biol Reprod 1995; 52:8-19.
11. Packer AI, Besmer P, Bachvarova RF. Kit ligand mediates survival of type A spermatogonia and dividing spermatocytes in postnatal mouse testes. Mol Reprod Dev 1995; 42:303-10.
12. Ohta H, Yomogida K, Dohmae K, Nishimune Y. Regulation of proliferation and differentiation in spermatogonial stem cells: the role of c-kit and its ligand SCF. Development 2000; 127:2125-31.
13. Yan W, Suominen J, Toppari J. Stem cell factor protects germ cells from apoptosis in vitro. J Cell Sci 2000; 113:161-8.
14. Guerif F, Cadoret V, Rahal-Perola V, Lansac J, Bernex F, Panthier JJ, et al. Apoptosis, onset and maintenance of spermatogenesis: evidence for the involvement of Kit in Kit-haplodeficient mice. Biol Reprod 2002; 67:70-9.
15. Prabhu SM, Meistrich ML, McLaughlin EA, Roman SD, Warne S, Mendis S, et al. Expression of c-Kit receptor mRNA and protein in the developing, adult and irradiated rodent testis. Reproduction 2006; 131:489-99.
16. Runyan C, Schaible K, Molyneaux K, Wang Z, Levin L, Wylie C. Steel factor controls midline cell death of primordial germ cells and is essential for their normal proliferation and migration. Development 2006; 133:4861-9.

17. Gu Y, Runyan C, Shoemaker A, Surani A, Wylie C. Steel factor controls primordial germ cell survival and motility from the time of their specification in the allantois, and provides a continuous niche throughout their migration. Development 2009; 136:1295-303.
18. Orth JM, Qiu J, Jester, WF Jr, Pilder S. Expression of the c-kit gene is critical for migration of neonatal rat gonocytes in vitro. Biol Reprod 1997; 57:676-83.
19. Schnabel D, Ramirez L, Gertsenstein M, Nagy A, Lomeli H. Ectopic expression of KitD814Y in spermatids of transgenic mice, interferes with sperm morphogenesis. Dev Dyn 2005; 233:29-40.
20. Morimoto H, Kanatsu-Shinohara M, Takashima S, Chuma S, Nakatsuji N, Takehashi M, Shinohara T. Phenotypic plasticity of mouse spermatogonial stem cells. PLoS One 2009; 4:7909.
21. Bokemeyer C, Kuczyk MA, Dunn T, Serth J, Hartmann K, Jonasson J, et al. Expression of stem-cell factor and its receptor c-kit protein in normal testicular tissue and malignant germ-cell tumours. J Cancer Res Clin Oncol 1996; 122:301-6.
22. de Rooij DG. Proliferation and differentiation of spermatogonial stem cells. Reproduction 2001; 121:347-54.
23. Chabot B, Stephenson DA, Chapman VM, Besmer P, Bernstein A. The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature 1988; 335:88-9.
24. Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, et al. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 1987; 6:3341-51.
25. Blechman JM, Lev S, Barg J, Eisenstein M, Vaks B, Vogel Z, et al. The fourth immunoglobulin domain of the stem cell factor receptor couples ligand binding to signal transduction. Cell 1995; 80:103-13.
26. Blechman JM, Lev S, Givol D, Yarden Y. Structure-function analyses of the kit receptor for the steel factor. Stem Cells 1993; 11:12-21.
27. Lev S, Yarden Y, Givol D. Dimerization and activation of the kit receptor by monovalent and bivalent binding of the stem cell factor. J Biol Chem 1992; 267:15970-7.
28. Roskoski R Jr. Signaling by Kit protein-tyrosine kinase—the stem cell factor receptor. Biochem Biophys Res Commun 2005; 337:1-13.
29. Sorrentino V, Giorgi M, Geremia R, Besmer P, Rossi P. Expression of the c-kit proto-oncogene in the murine male germ cells. Oncogene 1991; 6:149-51.
30. Rossi P, Marziali G, Albanesi C, Charlesworth A, Geremia R, Sorrentino V. A novel c-kit transcript, potentially encoding a truncated receptor, originates within a kit gene intron in mouse spermatids. Dev Biol 1992; 152:203-7.
31. Sette C, Bevilacqua A, Bianchini A, Mangia F, Geremia R, Rossi P. Parthenogenetic activation of mouse eggs by microinjection of a truncated c-kit tyrosine kinase present in spermatozoa. Development 1997; 124:2267-74.
32. Albanesi C, Geremia R, Giorgio M, Dolci S, Sette C, Rossi P. A cell- and developmental stage-specific promoter drives the expression of a truncated c-kit protein during mouse spermatid elongation. Development 1996; 122:1291-302.
33. Sette C, Dolci S, Geremia R, Rossi P. The role of stem cell factor and of alternative c-kit gene products in the establishment, maintenance and function of germ cells. Int J Dev Biol 2000; 44:599-608.
34. Anderson DM, Lyman SD, Baird A, Wignall JM, Eisenman J, Rauch C, et al. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell 1990; 63:235-43.
35. Flanagan JG, Chan DC, Leder P. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the Sld mutant. Cell 1991; 64:1025-35.
36. Toksoz D, Zsebo KM, Smith KA, Hu S, Brankow D, Suggs SV, et al. Support of human hematopoiesis in long-term bone marrow cultures by murine stromal cells selectively expressing the membrane-bound and secreted forms of the human homolog of the steel gene product, stem cell factor. Proc Natl Acad Sci USA 1992; 89:7350-4.
37. Dolci S, Pellegrini M, Di AS, Geremia R, Rossi P. Signaling through extracellular signal-regulated kinase is required for spermatogonial proliferative response to stem cell factor. J Biol Chem 2001; 276:40225-33.
38. Fujisawa M, Kanzaki M, Okuda Y, Okada H, Arakawa S, Kamidono S. Stem cell factor in human seminal plasma as a marker for spermatogenesis. Urology 1998; 51:460-3.
39. Galan JJ, De Felici M, Buch B, Rivero MC, Segura A, Royo JL, et al. Association of genetic markers within the KIT and KITLG genes with human male infertility. Hum Reprod 2006; 21:3185-92.
40. Ebata KT, Yeh JR, Zhang X, Nagano MC. Soluble growth factors stimulate spermatogonial stem cell divisions that maintain a stem cell pool and produce progenitors in vitro. Exp Cell Res 2011; 317:1319-29.
41. Feng H, Sandlow JI, Sandra A. The c-kit receptor and its possible signaling transduction pathway in mouse spermatozoa. Mol Reprod Dev 1998; 49:317-26.
42. Blume-Jensen P, Jiang G, Hyman R, Lee KF, O'Gorman S, Hunter T. Kit/stem cell factor receptor-induced activation of phosphatidylinositol-3'-kinase is essential for male fertility. Nat Genet 2000; 24:157-62.
43. Feng LX, Ravindranath N, Dym M. Stem cell factor/c-kit upregulates cyclin D3 and promotes cell cycle progression via the phosphoinositide-3-kinase/p70 S6 kinase pathway in spermatogonia. J Biol Chem 2000; 275:25572-6.
44. Farini D, La Sala G, Tedesco M, De Felici M. Chemoattractant action and molecular signaling pathways of Kit ligand on mouse primordial germ cells. Dev Biol 2007; 306:572-83.
45. Sette C, Paronetto MP, Barchi M, Bevilacqua A, Geremia R, Rossi P. Tr-kit-induced resumption of the cell cycle in mouse eggs requires activation of a Src-like kinase. EMBO J 2002; 21:5386-95.
46. Mithraprabhu S, Loveland KL. Control of KIT signalling in male germ cells: what can we learn from other systems. Reproduction 2009; 138:743-57.
47. Ginsburg M, Snow MH, McLaren A. Primordial germ cells in the mouse embryo during gastrulation. Development 1990; 110:521-8.
48. Seki Y, Hayashi K, Itoh K, Mizugaki M, Saitou M, Matsui Y. Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev Biol 2005; 278:440-58.
49. Orr-Urtreger A, Avivi A, Zimmer Y, Givol D, Yarden Y, Lonai P. Developmental expression of c-kit, a proto-oncogene encoded by the W locus. Development 1990; 109:911-23.
50. Manova K, Bachvarova RF. Expression of c-kit encoded at the W locus of mice in developing embryonic germ cells and presumptive melanoblasts. Dev Biol 1991; 146:312-24.
51. Matsui Y, Zsebo KM, Hogan BL. Embryonic expression of a haematopoietic growth factor encoded by the Sl locus and the ligand for c-kit. Nature 1990; 347:667-9.
52. De Felici M, Di CA, Pesce M. Role of stem cell factor in somatic-germ cell interactions during prenatal oogenesis. Zygote 1996; 4:349-51.
53. Godin I, Deed R, Cooke J, Zsebo K, Dexter M, Wylie CC. Effects of the steel gene product on mouse primordial germ cells in culture. Nature 1991; 352:807-9.
54. Bashamboo A, Taylor AH, Samuel K, Panthier JJ, Whetton AD, Forrester LM. The survival of differentiating embryonic stem cells is dependent on the SCF-KIT pathway. J Cell Sci 2006; 119:3039-46.
55. Tajima Y, Sawada K, Morimoto T, Nishimune Y. Switching of mouse spermatogonial proliferation from the c-kit receptor-independent type to the receptor-dependent type during differentiation. J Reprod Fertil 1994; 102:117-22.
56. Ohta H, Tohda A, Nishimune Y. Proliferation and differentiation of spermatogonial stem cells in the w/wv mutant mouse testis. Biol Reprod 2003; 69:1815-21.
57. Rossi P, Albanesi C, Grimaldi P, Geremia R. Expression of the mRNA for the ligand of c-kit in mouse Sertoli cells. Biochem Biophys Res Commun 1991; 176:910-4.
58. Tajima Y, Sakamaki K, Watanabe D, Koshimizu U, Matsuzawa T, Nishimune Y. Steel-Dickie (Sld) mutation affects both maintenance and differentiation of testicular germ cells in mice. J Reprod Fertil 1991; 91:441-9.
59. Tajima Y, Onoue H, Kitamura Y, Nishimune Y. Biologically active kit ligand growth factor is produced by mouse Sertoli cells and is defective in SId mutant mice. Development 1991; 113:1031-5.
60. Wilson A, Oser GM, Jaworski M, Blanco-Bose WE, Laurenti E, Adolphe C, et al. Dormant and self-renewing hematopoietic stem cells and their niches. Ann NY Acad Sci 2007; 1106:64-75.
61. Kubota H, Avarbock MR, Schmidt JA, Brinster RL. Spermatogonial stem cells derived from infertile Wv/Wv mice self-renew in vitro and generate progeny following transplantation. Biol Reprod 2009; 81:293-301.
62. Trefil P, Bakst MR, Yan H, Hejnar J, Kalina J, Mucksová J. Restoration of spermatogenesis after transplantation of c-Kit positive testicular cells in the fowl. Theriogenology 2010; 74:1670-6.
63. Izadyar F, Pau F, Marh J, Slepko N, Wang T, Gonzalez R,, et al. Generation of multipotent cell lines from a distinct population of male germ line stem cells. Reproduction 2008; 135:771-84.
64. Heim C, Minniear K, Dann CT. Imatinib has deleterious effects on differentiating spermatogonia while sparing spermatogonial stem cell self renewal. Reprod Toxicol 2011; 31:454-63.
65. Yoshida S, Sukeno M, Nakagawa T, Ohbo K, Nagamatsu G, Suda T, Nabeshima Y. The first round of mouse spermatogenesis is a distinctive program that lacks the self-renewing spermatogonia stage. Development 2006; 133:1495-505.
66. Suzuki H, Sada A, Yoshida S, Saga Y. 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 2009; 336:222-31.
67. Nakagawa T, Sharma M, Nabeshima Y, Braun RE, Yoshida S. Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. Science 2010; 328:62-7.
68. Di CAD, Travia G, De Felici M. The meiotic specific synaptonemal complex protein SCP3 is expressed by female and male primordial germ cells of the mouse embryo. Int J Dev Biol 2000; 44:241-4.
69. Vincent S, Segretain D, Nishikawa S, Nishikawa SI, Sage J, Cuzin F, Rassoulzadegan M. Stage-specific expression of the Kit receptor and its ligand (KL) during male gametogenesis in the mouse: a Kit-KL interaction critical for meiosis. Development 1998; 125:4585-93.
70. Pellegrini M, Filipponi D, Gori M, Barrios F, Lolicato F, Grimaldi P, et al. ATRA and KL promote differentiation toward the meiotic program of male germ cells. Cell Cycle 2008; 7:3878-88.
71. Lufkin T, Lohnes D, Mark M, Dierich A, Gorry P, Gaub MP, et al. High postnatal lethality and testis degeneration in retinoic acid receptor alpha mutant mice. Proc Natl Acad Sci USA 1993; 90:7225-9.
72. Rossi P, Dolci S, Sette C, Geremia R. Molecular mechanisms utilized by alternative c-kit gene products in the control of spermatogonial proliferation and sperm-mediated egg activation. Andrologia 2003; 35:71-8.

73. Sikarwar AP, Reddy KV. siRNA-mediated silencing of c-kit in mouse primary spermatogonial cells induces cell cycle arrest. Oligonucleotides 2008; 18:145-60.
74. Rossi P, Lolicato F, Grimaldi P, Dolci S, Di Sauro A, Filipponi D, Geremia R. Transcriptome analysis of differentiating spermatogonia stimulated with kit ligand. Gene Expr Patterns 2008; 8:58-70.
75. Muciaccia B, Sette C, Paronetto MP, Barchi M, Pensini S, D'Agostino A, et al. Expression of a truncated form of KIT tyrosine kinase in human spermatozoa correlates with sperm DNA integrity. Hum Reprod 2010; 25:2188-202.
76. Feng HL, Sandlow JI, Zheng LJ. c-kit receptor and its possible function in human spermatozoa. Mol Reprod Dev 2005; 70:103-10.
77. Livera G, Rouiller-Fabre V, Pairault C, Levacher C, Habert R. Regulation and perturbation of testicular functions by vitamin A. Reproduction 2002; 124:173-80.
78. Bowles J, Knight D, Smith C, Wilhelm D, Richman J, Mamiya S, et al. Retinoid signaling determines germ cell fate in mice. Science 2006; 312:596-600.
79. Vogel G. Embryonic stem cells. Scientists make sperm in a dish. Science 2003; 302:1875.
80. Eguizabal C, Shovlin TC, Durcova-Hills G, Surani A, McLaren A. Generation of primordial germ cells from pluripotent stem cells. Differentiation 2009; 78:116-23.
81. Vernet N, Dennefeld C, Rochette-Egly C, Oulad-Abdelghani M, Chambon P, Ghyselinck NB, Mark M. Retinoic acid metabolism and signaling pathways in the adult and developing mouse testis. Endocrinology 2006; 147:96-110.
82. Snyder EM, Small C, Griswold MD. Retinoic acid availability drives the asynchronous initiation of spermatogonial differentiation in the mouse. Biol Reprod 2010; 83:783-90.
83. Wang Y, Culty M. Identification and distribution of a novel platelet-derived growth factor receptor beta variant: effect of retinoic acid and involvement in cell differentiation. Endocrinology 2007; 148:2233-50.
84. Zhou Q, Li Y, Nie R, Friel P, Mitchell D, Evanoff RM, et al. Expression of stimulated by retinoic acid gene 8 (Stra8) and maturation of murine gonocytes and spermatogonia induced by retinoic acid in vitro. Biol Reprod 2008; 78:537-45.
85. Snyder EM, Davis JC, Zhou Q, Evanoff R, Griswold MD. Exposure to Retinoic Acid in the Neonatal but Not Adult Mouse Results in Synchronous Spermatogenesis. Biol Reprod 2011.
86. Carlomagno G, van Bragt MP, Korver CM, Repping S, de Rooij DG, van Pelt AM. BMP4-induced differentiation of a rat spermatogonial stem cell line causes changes in its cell adhesion properties. Biol Reprod 2010; 83:742-9.
87. Pellegrini M, Grimaldi P, Rossi P, Geremia R, Dolci S. Developmental expression of BMP4/ALK3/SMAD5 signaling pathway in the mouse testis: a potential role of BMP4 in spermatogonia differentiation. J Cell Sci 2003; 116:3363-72.
88. Pesce M, Gioia KF, De Felici M. Derivation in culture of primordial germ cells from cells of the mouse epiblast: phenotypic induction and growth control by Bmp4 signalling. Mech Dev 2002; 112:15-24.
89. Baleato RM, Aitken RJ, Roman SD. Vitamin A regulation of BMP4 expression in the male germ line. Dev Biol 2005; 286:78-90.
90. Ying Y, Liu XM, Marble A, Lawson KA, Zhao GQ. Requirement of Bmp8b for the generation of primordial germ cells in the mouse. Mol Endocrinol 2000; 14:1053-63.
91. Zhao GQ, Deng K, Labosky PA, Liaw L, Hogan BL. The gene encoding bone morphogenetic protein 8B is required for the initiation and maintenance of spermatogenesis in the mouse. Genes Dev 1996; 10:1657-69.
92. Kumar TR, Palapattu G, Wang P, Woodruff TK, Boime I, Byrne MC, Matzuk MM. Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. Mol Endocrinol 1999; 13:851-65.
93. Vaskivuo TE, Aittomäki K, Anttonen M, Ruokonen A, Herva R, Osawa Y, et al. Effects of follicle-stimulating hormone (FSH) and human chorionic gonadotropin in individuals with an inactivating mutation of the FSH receptor. Fertil Steril 2002; 78:108-13.
94. Kumar TR, Wang Y, Lu N, Matzuk MM. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat Genet 1997; 15:201-4.
95. Dierich A, Sairam MR, Monaco L, Fimia GM, Gansmuller A, LeMeur M, et al. Impairing follicle-stimulating hormone (FSH) signaling in vivo: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. Proc Natl Acad Sci USA 1998; 95:13612-7.
96. Abel MH, Wootton AN, Wilkins V, Huhtaniemi I, Knight PG, Charlton HM. The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. Endocrinology 2000; 141:1795-803.
97. Krishnamurthy H, Danilovich N, Morales CR, Sairam MR. Qualitative and quantitative decline in spermatogenesis of the follicle-stimulating hormone receptor knockout (FORKO) mouse. Biol Reprod 2000; 62:1146-59.
98. Santos NC, Kim KH. Activity of retinoic acid receptor-alpha is directly regulated at its protein kinase A sites in response to follicle-stimulating hormone signaling. Endocrinology 2010; 151:2361-72.
99. Buaas FW, Kirsh AL, Sharma M, McLean DJ, Morris JL, Griswold MD, et al. Plzf is required in adult male germ cells for stem cell self-renewal. Nat Genet 2004; 36:647-52.
100. Costoya JA, Hobbs RM, Barna M, Cattoretti G, Manova K, Sukhwani M, et al. Essential role of Plzf in maintenance of spermatogonial stem cells. Nat Genet 2004; 36:653-9.
101. Filipponi D, Hobbs RM, Ottolenghi S, Rossi P, Jannini EA, Pandolfi PP, Dolci S. Repression of kit expression by Plzf in germ cells. Mol Cell Biol 2007; 27:6770-81.
102. Sonoda J, Wharton RP. Recruitment of Nanos to hunchback mRNA by Pumilio. Genes Dev 1999; 13:2704-12.
103. Suzuki H, Tsuda M, Kiso M, Saga Y. Nanos3 maintains the germ cell lineage in the mouse by suppressing both Bax-dependent and -independent apoptotic pathways. Dev Biol 2008; 318:133-42.
104. Asaoka-Taguchi M, Yamada M, Nakamura A, Hanyu K, Kobayashi S. Maternal Pumilio acts together with Nanos in germline development in Drosophila embryos. Nat Cell Biol 1999; 1:431-7.
105. Lolicato F, et al. Potential role of Nanos3 in maintaining the undifferentiated spermatogonia population. Dev Biol 2008; 313:725-38.
106. Tsuda M, et al. Conserved role of nanos proteins in germ cell development. Science 2003; 301:1239-41.
107. Scholer HR, Ciesiolka T, Gruss P. A nexus between Oct-4 and E1A: implications for gene regulation in embryonic stem cells. Cell 1991; 66:291-304.
108. Kehler J, et al. Oct4 is required for primordial germ cell survival. EMBO Rep 2004; 5:1078-83.
109. Durcova-Hills G, Capel B. Development of germ cells in the mouse. Curr Top Dev Biol 2008; 83:185-212.
110. Buehr M, Gu S, McLaren A. Mesonephric contribution to testis differentiation in the fetal mouse. Development 1993; 117:273-81.
111. Francavilla S, Zamboni L. Differentiation of mouse ectopic germinal cells in intra- and perigonadal locations. J Exp Zool 1985; 233:101-9.
112. McLaren A. Meiosis and differentiation of mouse germ cells. Symp Soc Exp Biol 1984; 38:7-23.
113. Trautmann E, et al. Retinoic acid prevents germ cell mitotic arrest in mouse fetal testes. Cell Cycle 2008; 7:656-64.
114. Cook MS, Munger SC, Nadeau JH, Capel B. Regulation of male germ cell cycle arrest and differentiation by DND1 is modulated by genetic background. Development 2011; 138:23-32.
115. Mol CD, et al. Structure of a c-kit product complex reveals the basis for kinase transactivation. J Biol Chem 2003; 278:31461-4.
116. Biermann K, et al. c-KIT is frequently mutated in bilateral germ cell tumours and downregulated during progression from intratubular germ cell neoplasia to seminoma. J Pathol 2007; 213:311-8.
117. Koubova J, et al. Retinoic acid regulates sex-specific timing of meiotic initiation in mice. Proc Natl Acad Sci USA 2006; 103:2474-9.
118. Lin Y, Gill ME, Koubova J, Page DC. Germ cell-intrinsic and -extrinsic factors govern meiotic initiation in mouse embryos. Science 2008; 322:1685-7.
119. Ruggiu M, et al. The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. Nature 1997; 389:73-7.
120. Schrans-Stassen BH, Saunders PT, Cooke HJ, de Rooij DG. Nature of the spermatogenic arrest in Dazl$^{-/-}$ mice. Biol Reprod 2001; 65:771-6.
121. Oulad-Abdelghani M, et al. Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. J Cell Biol 1996; 135:469-77.
122. Baltus AE, et al. In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. Nat Genet 2006; 38:1430-4.
123. Menke DB, Koubova J, Page DC. Sexual differentiation of germ cells in XX mouse gonads occurs in an anterior-to-posterior wave. Dev Biol 2003; 262:303-12.
124. Zhou Q, et al. Expression of stimulated by retinoic acid gene 8 (Stra8) in spermatogenic cells induced by retinoic acid: an in vivo study in vitamin A-sufficient postnatal murine testes. Biol Reprod 2008; 79:35-42.