Novel Gene Regulation in Normal and Abnormal Spermatogenesis

Li Du 1,†, Wei Chen 1,†, Zixin Cheng 1, Si Wu 1, Jian He 1, Lu Han 2, Zuping He 1,* and Weibing Qin 2,*

1 The Key Laboratory of Model Animals and Stem Cell Biology in Hunan Province, School of Medicine, Hunan Normal University, 371 Tongziipo Road, Changsha 410013, China; duli0809@126.com (L.D.); cweixy3@126.com (W.C.); chengzizili9@126.com (Z.C.); ws912731052@163.com (S.W.); hj04195132@126.com (J.H.)
2 The NHC Key Laboratory of Male Reproduction and Genetics, Family Planning Research Institute of Guangdong Province, Guangzhou 510600, China; sysuhanlu@126.com
* Correspondence: zupinghe@hunnu.edu.cn (Z.H.); guardqin@163.com (W.Q.);
Tel.: +86-731-88912446 (Z.H.); +86-20-87692825 (W.Q.)
† These authors contributed equally to this work.

Abstract: Spermatogenesis is a complex and dynamic process which is precisely controlled by genetic and epigenetic factors. With the development of new technologies (e.g., single-cell RNA sequencing), increasingly more regulatory genes related to spermatogenesis have been identified. In this review, we address the roles and mechanisms of novel genes in regulating the normal and abnormal spermatogenesis. Specifically, we discussed the functions and signaling pathways of key new genes in mediating the proliferation, differentiation, and apoptosis of rodent and human spermatogonial stem cells (SSCs), as well as in controlling the meiosis of spermatocytes and other germ cells. Additionally, we summarized the gene regulation in the abnormal testicular microenvironment or the niche by Sertoli cells, peritubular myoid cells, and Leydig cells. Finally, we pointed out the future directions for investigating the molecular mechanisms underlying human spermatogenesis. This review could offer novel insights into genetic regulation in the normal and abnormal spermatogenesis, and it provides new molecular targets for gene therapy of male infertility.

Keywords: genes; regulation; spermatogenesis; functions and mechanisms; male infertility

1. Introduction

Spermatogenesis is an elaborately organized process in which diploid spermatogonial stem cells (SSCs) differentiate into spermatocytes and haploid spermatozoa. This process is collaborated by somatic cells in the testis, including Sertoli cells, peritubular myoid cells, and Leydig cells. In the past decades, we and others have revealed the molecular mechanisms underlying rodent spermatogenesis. In recent years, several new technologies, e.g., single-cell RNA sequencing and RNA deep sequencing, have been developed, making it feasible to identify more and more novel genes that are involved in the regulation of rodent and human spermatogenesis. In the current review, we addressed the functions and mechanisms of key novel genes in controlling the mitosis and meiosis of rodent and human male germ cells. We also discussed the roles of genes from the normal and abnormal niche of the testis and the perspectives in this field.

2. Novel Gene Regulation in the Fate Decisions of Human SSCs

Human SSCs self-renew to maintain the pool of stem cells in the testis and differentiate into mature spermatozoa. The fate determinations of SSCs, including the self-renewal, differentiation, and apoptosis, are essential for the maintenance of human spermatogenesis [1]. Notably, human and rodent SSCs have the great plasticity, as evidenced by the findings that they are able to become embryonic stem cell-like cells that differentiate into all cell types...
of three germ layers and can be transdifferentiated to other cell lineages [2–6]. As such, human SSCs could have significant applications in both reproductive and regenerative medicine. Human SSCs have the phenotypic characteristics of SSEA4+, CD49+, GPR125+, and c-Kit+/GFRA1low, which makes it feasible for human SSC enrichment, self-renewal, clonal expansion, and differentiation [7].

As a novel method, single-cell RNA sequencing has been employed to reveal the key genes and critical cell signaling pathways of human SSCs. The testicular cells were obtained from male aged from 2 days [8] to 60 years old [9] and separated by MACS (c-Kit+ cells [10], c-Kit+/SSEA4cells [11], ITGA6+ cells [8]), by FACS (GPR125+/DDX4 cells [9]), or StaPut [12], and they were analyzed by 10X Genomics [8,10,12,13] or/and Fluidigm C1 [11,12]. These studies have revealed the phenotypic characteristics of human spermatogonia from infancy to adulthood, indicating the heterogeneous features of these cells. Three neonatal human SSC clusters—the primordial germ cell (PGC)-like cells, PreSPG (prespermatogonia)-1, and PreSPG-2—are the most undifferentiated cells, and they do not proliferate or under go slow proliferation [8]. Infant spermatogonia are most similar to adult SSCs State 0 (FGFR3high/TSPAN3high/SSEA4low), and they specifically express TBX3 and HOXA3 [10]. Puberty spermatogonia can be classified to the undifferentiated SPG (spermatogonia) and the differentiating SPG, which are marked by UTF1 (undifferentiated embryonic cell transcription factor 1) and KIT, respectively [13]. Meanwhile, distinct adult human SSC clusters (2–5) have been identified, which provides clear evidence for the heterogeneity of human SSCs [8–11]. The KIT (differentiation marker) and MKI67 (proliferation marker) are specifically expressed during or after State 2, indicating State 0 and State 1 (UTF1high/GFRA1low or GFRA1high/UTF1low) represent the quiescent SSC states, whereas State 2 indicates an initial differentiation and self-renewal state. TCF3 is expressed in States 0 and 1, suggesting that it may contribute to retain the undifferentiated state of human SSCs [10]. Similarly, 2% of AdVac, a small subpopulation of Adark spermatogonia with nuclear rarefaction zone, seems to be entirely quiescent spermatogonia with high expression of UTF1 and lacking GFRA1 [14]. The undifferentiated spermatogonia remain dormant or slowly self-renew during infancy and pre-puberty, and they develop to the limited and incomplete SSC differentiation in early puberty and then establish a balance between the self-renewal and differentiation in the phases of adulthood.

As shown in Figure 1, single-cell RNA sequencing also reveals numerous signaling pathways for human SSCs, including FGF pathways (e.g., FGFR1 (fibroblast growth factor receptor 1), FGFR2, FGFR3, KRA5, MAP2K2, MAPK1, and MAPK3) and BMP pathways (e.g., BMP7, BMP2, BMP1B, SMAD1, SMAD5, SMAD9, ID1, ID2, and ID4) [9]. Specifically, the surface markers FGFR3, DSG2 (desmoglein 2), PLPPR3 [15,16], and other genes have been identified as the novel signatures. It has been reported that PLZF (promyelocytic leukaemia zinc finger) inhibits the differentiation of mouse SSCs via binding to the promoter regions of differentiation-associated genes (Kit, Stra8, Sohlh2, and Dmrt1) [17]. Nevertheless, the roles and mechanisms of these genes in the fate decisions of human SSCs remain to be explored.

Interestingly, DNA hypomethylation at embryonic developmental genes (SOX2, KLF4, SALL4, TCF3, MBD3, STAT3, and KLF2) supports their epigenetic “poising” in human SSCs for embryonic expression, while the levels of core pluripotency genes (OCT4 and NANOG) are transcriptionally and epigenetically repressed. ATAC sequencing reveals top 12 motifs, including CTCF, DMRT1/6, CTCFL, NFY, PGR (progesterone receptor), SOX9, FOXP1, SOX3, FOXA2, SMAD2, and AR (androgen receptor), are enriched in human SSCs. Through further analysis, stem cell transcription and signaling factors promote the transfer of glucose into cells, causing mitochondrial activation and transforming human SSCs from static condition to the differentiated state [11]. Beyond the coding genes, transposable elements (TE) and lncRNA (e.g., LINC01030) contribute to the balance of human SSCs as well [10].
Recently, we have demonstrated that FOXP3 variants cause male infertility and FOXP3 stimulates the proliferation and inhibits the apoptosis of human SSCs [18]. We have also revealed that PAK1 regulates the proliferation, DNA synthesis, and apoptosis of human SSCs through the PDK1/KDR/ZNF367 and ERK1/2 and AKT signaling pathway [19]. Additionally, we have found that JAZF1 silencing decreases cell proliferation and DNA synthesis as well as increase the apoptosis of human SSCs [20]. In contrast, we have reported that the silencing of microRNA targets, namely, KLF2 (kruppel-like...
factor 2) [21], CBL [22], and NFIX [23], results in the increase of proliferation and DNA synthesis as well as the reduction of apoptosis of human SSCs. Notably, we have shown the PAK1/PDK1/miRNA-31–5p network in mediating the self-renewal and apoptosis of human SSCs, which illustrates the genes/miRNAs (genetics and epigenetics) for the regulation of human SSCs [19,20]. Collectively, our studies highlight the important functions of genes in determining the fate decisions of human SSCs and male fertility, and offer novel endogenous targets for gene therapy for male infertility.

3. Novel Gene Regulation in Fate Determinations of Rodent SSCs

The single-cell RNA sequencing analyzed spermatogenic cells of mice, and 7031 genes were found to be involved in spermatogenesis, which shows the expression profiles of the prototypical mouse SSC gene signatures (Ddx4, Gfra1, Id4, Nanos2, and Plzf). Notably, it has identified a panel of novel genes as we summarized in Table 1. As a subset of the type A single spermatogonia, ID4+ cells are the highest population in neonatal mice, which comprise 2% of the undifferentiated SSCs in adulthood [24]. In mice lacking ID4 expression, normal spermatogenesis is impaired due to the gradual loss of the undifferentiated mouse SSCs in adulthood. In vitro, wildtype mouse SSCs survive, but their proliferation ability is abolished due to the reduction of ID4 expression. These results indicate that ID4 is a marker of male germline stem cells and it is critical for the regulation of cell self-renewal [25]. Another gene signature, Nanos2 is expressed in the self-renewing mouse SSCs and it maintains the stem cell property [26]. By contrast, NEDD4 (an E3 ubiquitin ligase) targets NANOS2 in mouse SSCs, which leads to cell differentiation [27].

Table 1. Novel genes involved in mouse germ cells by single-cell RNA sequencing.

| Cells            | Stages          | Novel Genes                                                                 | References |
|------------------|-----------------|------------------------------------------------------------------------------|------------|
| Germ cells       | Ddx4            | Nanos2, Nanos3, Eomes, Pax7, Rhoa10, Tspan8, Sal4, Sdc4, Bcl6, Taf6b, Lhx1, Dusp6, Epha2, Ptpn13, Par, Tdf1 | [12,28]    |
|                  |                 | Uchl1, Tcea3, Crabp1, Dnrh1, Tdx101, Hspa5, Stra8, Syep3, Prdm9, Hormad1, Hormad2, Syep1, Tdx15 | [12,28]    |
| Spermatocytes    | Meioc, Prdm3, Top2a, Smc3 | Piwi1, Ptg1, Insl6, Spag6, Tnp1, Syep1, Syep2, Syep3, Hsfx | [29]       |
| Round spermatids | Acro1, Tsk1, Spaca1, Tsga8, Pkg2, Cd37, Cd63, Cd96, Cd177, Ranbp9, Morf4D1, Catger3, Catger4, Sputn25, Izumo1 | [28–30]    |
| Elongated spermatids | Prm1, Prm2, Prm3, Tnp1, Tnp2, Fshp11, Izumo3, Tsk6, Dnajk3 | [12,28]    |

Similarly, Wnt6/β-catenin and p38 MAPK signaling pathways by genes determine the fate of mouse SSCs. The Wnt6/β-catenin pathway specifically promotes mouse SSC proliferation [37], while Eif2s3y regulates the self-renewal of mouse SSCs via Wnt6/β-catenin signaling pathway [31]. In addition, SHISA6 inhibits mouse SSC differentiation through Wnt/β-catenin signaling [32]. P38 MAPK-specific inhibitors decrease the mouse SSC self-renewal ability [33], indicating that the p38 MAPK pathway contributes to the survival of mouse SSCs. FGF9 promotes mouse SSC proliferation by p38 MAPK signaling [34], while we have found that VEGF/VEGFR3 signaling regulates mouse SSC proliferation via the
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activation of AKT/MAPK and Cyclin D1 pathway and mediates the apoptosis by affecting Caspase 3/9 and Bcl-2 [35]. Foxo1 is necessary for SSC homeostasis and spermatogenesis initiation, and the combined deficiency of Foxo1, Foxo3, and Foxo4 results in the severe impairment of mouse SSC self-renewal and complete differentiation disorder [36]. GLIS3 (GLI-similar 3) is expressed in mouse SSCs. In Glis3 knockout mice, nuclear translocation of FOXO1 is inhibited and mouse SSC number is significantly reduced, which causes the severely impaired spermatogenesis [37].

Moreover, a number of genes play critical roles in the differentiation and maintenance of mouse SSCs. Trim28 (tripartite motif-containing 28) promotes the differentiation of mouse SSCs [38], while specific deletion of TRIM71 results in the reduced number of undifferentiated spermatogonia and hinders the transition to differentiated state [39]. DAZL deficiency compromises the expansion and differentiation of spermatogonial progenitor cells by mediating extensive translation programs [40]. In the absence of Pramef12, the number of mouse SSCs is decreased, and low expression of SSC maintenance-related genes and a defective ability of differentiation are observed [41]. The Ras-cyclin D2 pathway regulates the balance between tissue maintenance and tumorigenesis in the mouse SSCs [42]. PAX7+ mouse SSCs self-renew and produce extended clones that differentiate into mature spermatids [43]. Due to losing Rhox10, the number of mouse SSCs is dramatically reduced by mediators spermatogonial differentiation and migration to the mouse SSC niche [44].

The reduced Pou3f1 expression induces male germ cell apoptosis and the impaired mouse SSC maintenance [45]. FXRα establishes and maintains an undifferentiated germ cell pool by regulating the expression of pluripotency factors (e.g., Lin28) [46]. We have found that STAT3 is a target of miRNA-20 and miRNA-106a that regulate the self-renewal of mouse SSCs [47], while DND1 retains the stemness of SSCs by recruiting CCR4-NOT complex [48]. Together, these studies shed a novel light on gene regulatory mechanisms controlling mouse SSC fate decisions.

4. Novel Gene Regulation in Human and Rodent Other Germ Cells

The single-cell RNA sequencing reveals that human germ cells can be classified to several types of cells based upon the biochemical phenotypes, namely, differentiating-SPG (spermatogonia) (PRAME, MKI67, DMRT1, and SOHLH2), differentiated-SPG (STRA8, E2F4, HINFP, and CTCFL), leptothe spermatocytes (SCML1, DPH7, DSG3, DMCT1, RAD51AP2, SYCP3, and ATR), zygotenes spermatocytes (TDRG1, DMCT1, RAD51AP2, and SYCP3), pachytene spermatocytes (CCDC112 and RAD51), diplotene spermatocytes (AYRK3, SPC7, spermatocytes 7) (C9orf116, ACR, CCNA1, CCNA2, TPP3, SCL2A6A3, and SIRPG), and spermatids (TEX29, ACR, NFKBIB, TNP1, PRM1, IQCF3, and LEKL1), as we showed in Figure 1. Specifically, FGFR3, DSG2, E3 ubiquitin ligase c-CBL, CTAG1A/B (cancer/testis antigen NY-ESO-1), UTF1, and SNAP91 (synaptosomal-associated protein 91 kDa homolog) have been regarded as specific biomarkers of human spermatogonia [16]. Some of these genes have been examined for their functions and mechanisms. As examples, PRAMEF12 (preferentially expressed antigen of melanoma family member 12) and DMRT1 (doublesex-related transcription factor) influence the spermiogenesis by regulating the survival of human germ cells. In male mice, Pramef12 gene ablation prevents spermatogenesis and leads to sterility, which can be rescued by transgenic expression of Pramef12, and Pramef12 deficiency leads to overall decrease of spermatogenesis-related gene expression [41]. DMRT1 acts on spermatogonia, restricts retinoic acid response, directly inhibits Stra8 transcription, and activates the transcription of spermatogonial differentiation factor Sohlh1, thus preventing the meiosis and promoting the development of spermatogonia [49].

Our group has found that a total of 4276 genes are differentially expressed in human undifferentiated spermatogonia and spermatogonia. Among them, 2123 genes are upregulated in the undifferentiated spermatogonia, whereas 2153 genes are upregulated in spermatogonia. Interestingly, seven of these genes belong to the HOX family, suggesting that HOX genes play an important role in mediating the differentiation of mouse germ cells. Gene aggregation and enrichment analysis were used to predict the transcription
factor targets of differentially expressed genes during spermatogenesis. Among them, NFATs, SP1, and TCF3 have been identified in human spermatogonia, spermatocytes, and spermatids, respectively, and these transcription factors are considered to be key regulators of human spermatogenesis [50].

Single-cell RNA sequencing also uncovers the critical regulators (Uchl1, Tcna3, Crabp1, Prdm9, Dmrtb1, Tex101, Hspa5, Stra8, Sycp3, Hormad1, Hormad2, Sycp1, Tex15, and Ly6k) in the regulation of mouse spermatogonia [12,28,30], as we discussed in Table 1. Yet the specific functions and mechanisms of most of these genes remain unknown. It has been reported that Fbxo47, Pparg, and Ccnb3 are involved in mouse spermatogenesis, and male mice lacking Fbxo47 are completely sterile, as spermatogenesis is arrested before meiotic recombination [30]. In addition, Fbxo47 defective spermatocytes are unable to form complete synaptonemal complexes, and the destruction of Fbxo47 destabilizes TRF2, resulting in unstable telomere attachment and slow traversing through the bouquet stage [51], implicating the Fbxo47 regulatory role in the early stages of meiosis prophase I.

On the other hand, multifunctional roles have been identified for DDX5 and the REG-P53-PLZF pathway in spermatogonia. RNA helicase DDX5 is expressed in spermatogonia, which can splice the key genes necessary for spermatogenesis, while it regulates the expression of cell cycle genes in undifferentiated spermatogonia to ensure cell proliferation and survival [52]. Notably, the interaction of DDX5 and PLZF has been shown to be required for germ line maintenance [52]. Ablation of the proteasome activator REGγ leads to male sterility, with a decrease in the number of PLZF-positive spermatogonia [53]. Further studies show that REGγ deletion significantly increases the abundance of testicular P53 protein and directly inhibits the transcription of PLZF, suggesting that the REGγ-P53-PLZF pathway regulates the maintenance of mouse spermatogonia [53].

Furthermore, L3mbtl2, ZMYM3, Bruce, PP6, PHB, SKP1, Claudin 3, and Sam68 have essential roles during metaphase to anaphase transition of mouse spermatogenesis by regulating the fate of pachytene spermatocytes. L3MBTL2 is highly expressed in pachytene spermatocytes, and specific ablation of L3mbtl2 leads to abnormal spermatooza, gradual decrease of sperm counts, and premature testicular failure in mice. In the leptotene spermatocytes, L3mbtl2 deficiency results in an increase of H2AX deposition, crossover and synaptic defects at the pachytene stage of meiosis I, and apoptosis and degradation of male germ cells in aging mice [54]. Knockdown of Zmym3 results in spermatogenesis arrest at the meiosis prophase land the increased number of apoptotic germ cells [55]. Conditional deletion of the Bruce gene in the male germ line causes the impaired spermatogonial maintenance and chromosomal abnormalities during meiosis. DNA fragmentation, the damaged homologous synapses, nonhomologous association, and rearrangement occur in Bruce-deficient spermatocytes [56]. Spermatocytes with PP6 defects are blocked at the pachytene stage with accompanying apoptosis, and DSB repair and cross formation are defective, indicating that PP6 promotes the repair of meiosis double-chain fracture [57,58]. Spermatocytes with SKP1 gene defects assume premature desynapsis [59], while Claudin3 controls the process of early mouse spermatocyte meiosis [60]. The splicing regulator Sam68 is highly expressed in meiotic cells, and Sam68−/− mice produce few spermatids with obvious motor deficit and inability to fertilize eggs [61,62].

In spermatids of Tdrd6 (tudor domain-containing 6)-deficient mice, the chromatid bodies (CBs) are severely damaged, and the elongation of round sperm to elongated sperm, namely, spermiogenesis, is cancelled [63]. Together with Ttdr6, Tdrd7 identifies the key biogenic processes of CBs [64], and the TDRD1/6/7/9 localization in CBs depends on Tdrd5 [65]. Atg5 mutant mice have malformation of sperm head, discontinuous middle appendage structure, abnormal acrosomal formation, spermatozoa individualization loss, which results in about 70% infertility [66]. Germ cell-specific Atg7 KO mice are sterile due to acrosomal biogenesis defects [67], suggesting that Atg7 is necessary for prolongation of sperm development, sperm individualization, and normal fertility in male mice. In addition to Tdrds and Atgs families, Spata6 [68], HIPK4 [69], Cdy1 [70], and TAp73 [71]
are essential regulators for sperm head shaping and motility through the interaction with myosin subunits, F-actin, histone Kcr, and CDKN2B, respectively.

N-6-methyladenosine (m(6)A) is the most common internal modification in eukaryotic mRNA and may ensure the coordinated translation of the different stages of spermatogenesis. Mettl3 or Mettl14 with Vasa-Cre leads to the loss of m(6)A and depletion of SSCs. Deletion of m(6)A distorts the translation of transcripts required for SSC proliferation/differentiation. The removal of Mettl3 in germ cells severely inhibits the differentiation of spermatogonia and hinders the initiation of meiosis [72]. Combined deletion of Mettl3 and Mettl14 with Stra8GFP-Cre in late germ cells disrupts sperm formation [73]. YTHDC2 is a m(6)A binding protein, and its knockout mice are sterile, as male germ cells do not get past the zygotic stage and Ythdc2 is upregulated in testis in the beginning of meiosis [74], suggesting that Ythdc2 may be involved in mouse meiosis. As a m(6)A eraser, ALKBH5 specifically removes m(6)A from target mRNAs and controls male sterility in mice [75]. As such, m(6)A modification is a key mechanism for controlling the mRNA fate of posttranscriptional meiosis and haploid cells.

5. Novel Gene Regulation in Testicular Microenvironment

Spermatogenesis is precisely regulated by the microenvironment or the niche of the testis, which is mainly composed of the somatic cells as well as the growth factors and cytokines produced by the somatic cells. Single-cell transcriptome data of human and mouse testicular microenvironment uncover a set of new genes [8,28] as shown in Figure 2. In human, our group has found that BMP6 accelerates the proliferation and represses the apoptosis of Sertoli cells via DACH1 and TFAP2A activation and Smad2/3 pathway [76] and that BMP4 promotes the proliferation of Sertoli cell through the Smad1/5 and ID2/3 pathway [77]. On the contrary, GLI3 decreases the growth of human Sertoli cells [78]. In rodents, conditional ablation of Mdm2 (murine double minute 2) in Sertoli cells results in a significant increase in apoptosis of these cells and male sterility [79]. Adult mice lacking Insr and Igf1r have the reduced testicular size and daily sperm production by 75% [80]. These studies illustrate that growth factors produced by human and rodent Sertoli cells are required for normal spermatogenesis and that their deficiencies cause male infertility.

Figure 2. Expression of new genes in somatic cells of testis by single-cell RNA sequencing. Left panel: schematic illustration of major somatic cell types in the testis; right panel: new genes specifically expressed in specific types of somatic cells in human and rodent testis.
The tight junctions between Sertoli cells in the blood–testis barrier (BTB) are essential for the migration and maturation of male germ cells during spermatogenesis. Conditional knockout of Cx43 (connexin-43) mice assume the downregulated genes critical for mitosis and meiosis, e.g., Stra8, Dazl, and members of the DM (dsxand map-3) gene family, and the upregulated genes related to Sertoli cell maturation and proliferation [81,82]. The expression levels of cross-epithelial resistance and tight junctions are significantly increased in primary Sertoli cells of mice lacking Cx43. These results reflect the role of Cx43 in regulating the function, composition, and dynamics of the BTB [83]. Inactivation of Wt1 (Wilms tumor gene 1), specifically expressed in Sertoli cells, results in germ cell death and Sertoli cell-onlysynrome (SCOS). The BTB is disrupted in Wt1-deficient testes. Meanwhile, polarity maintenance in Sertoli cells is controlled by Wt1 via Wnt signaling pathway [84], and Wnt/β-Catenin signaling controls the spermatogenesis via Sertoli cell maturation [85]. The conditional deletion of Utx [86], Tspan8 [87], Activin A [88], GATA4 [89], and Cldn11 [90] in Sertoli cells results in the loss of male germ cells and incomplete structure of BTB, the smaller testis size, the reducedweight, and the eventually impaired spermatogenesis [87].

A network necessary for communication of niches is regulated by some new genes. Selective ablation of AR in mouse Sertoli cells completely blocks spermatogenesisisat the meiosis stage [91]. Besides, GDNF (glial cell line-derived neurotrophic factor) produced by Sertoli cells and peritubular myoid(PM) cells is also critical for male germ cell development. Mice with specific knockout of Gdnf in PM cells results in sterility due to spermatogenesis disorder and the loss of undifferentiated spermatagonia [92]. Ablation of RNA-binding protein Pthbp2 in germcells leads to the disorder of filamentous actin cytoskeleton in Sertolicells [93]. The luteinizing hormone testosteron pathway regulates the self-renewal of mouse SSCs by inhibiting the expression of WNT5A in Sertolcells [94]. In addition, the translocation of genes over time is also necessary for spermatogenesis. RNA sequencing reveals that about 2939 genes in the Sertolicells assume dynamic stage-specific profiles, including cell cycle regulation, metabolism, and energy generation, RA synthesis, and biogenesis of the blood–testosterone barrier, which reflects the evolutionary role of Sertoli cells in controlling spermatogenesis [95]. Prior to puberty, MAST4 is localized to the Sertoli cells, and it is transferred to Leydig cells and spermatids throughout puberty [96]. Moreover, Mast4 depletion leads to the increase of germ cellapoptosis, germ cell, and tubular structure loss and testis size reduction by the FGF2/ERM pathway [96].

6. Novel Gene Regulation in Abnormal Human Spermatogenesis

Around 10% of men suffer from infertilityworldwide, and the common causes of male infertility, e.g., idiopathic nonobstructiveazoospermia (NOA) andSCOS, may be derived from genetic defects [97,98]. Deletions or changes in the expression levels of genes have been shown to cause NOA, as shown in Table 2. There is a close association between NOA risk in Chinese Han males and common variations of PRMT6, PEX10, and SOX5 [99]. Bi-allelic recessive loss-of-function variants in FANCM [100] or missense mutation of WT1 [84] result in the NOA. In addition, NOA patients have a disorder of genomic methylation modification in testicular cells, with significant difference in the expression of reproduction-related genes [101]. Transcripts of RARA, RXRβ, and RXRγ are significantly reduced in patients with SCOS and maturation arrest (MA), but not in patients with spermatogenesis hypogenesis, suggesting that decreased levels of these genes are closely associated with the failure of SCOS and spermatogenesis MA [102]. New mutation in USP26 is related toSCOS patients [103]. Our team has found differential expression of LRP6 and Cyclin D1 in Sertoli cells between SCOS and OA patients with normal spermatogenesis [104].
Table 2. Novel genes involved in abnormal human spermatogenesis.

| Types                  | Novel Genes                                                      | References          |
|------------------------|-----------------------------------------------------------------|---------------------|
| Maturation arrest       | CDY2, HSFY, Prmef12, H3t, PLK4, CARF, FGF9, IGF1, ETV5, HnRNPL, PRPS2 | [105]               |
| NOA                    | SCOS TDRD7, TDRD9, TEX15, DMCI, DGCR8, FANCM, DDR1, SAM68, RanBP3, RNF212, STAG3, NPAS2 | [41,106–113]        |
| Unclassified NOA       | TDRD7, TDRD9, TEX15, DMCI, DGCR8, FANCM, DDR1, SAM68, RanBP3, RNF212, STAG3, NPAS2 | [100,114–125]       |

Notably, we have revealed that 10 of 300 NOA (3.3%) patients have FOXP3 variants [18], which is 10 time higher than other gene variants in NOA patients, suggesting that FOXP3 mutation is closely associated with male infertility. With regards to cryptorchidism, the NR5A1 mutation appears to cause more severe forms of male infertility [126,127]. Exome sequencing of infertile men reveals three heterozygous SYCP2 transcoding variants in cryptospermia and azoospermia [128]. These results may contribute to the development of new molecular indicators for spermatogenesis dysfunction and provide novel therapeutic targets for male infertility [102].

Meiotic arrest and abnormal morphology and movement of sperm caused by genetic abnormality are important factors, leading to spermatogenesis failure. Total exome sequencing shows that M1AP biallele mutation is a common cause of male infertility due to the cessation of meiosis and severe spermatogenesis damage [129]. In mutant homozygous patients, male germ cells are deficient in SCAPER expression, with early spermatogenesis defects and azoospermia, which leads to the complete loss of meiotic cells [130]. Expression of 1700102P08Rik is downregulated in men with spermatocyte arrest [131]. Multiple morphological abnormalities of the sperm flagella (MMAF) is a severe form of asthenoteratozoospermia. Variants of several genes, including CFAP family members (CFAP43, CFAP44 [132,133], CFAP58 [134], CFAP69 [135], and CFAP251 [136]), DNAH8 [137], ARMC2 [138], TTC21A [139], and QRICH2 [140], lead to multiple morphological abnormalities of the sperm flagella and primary male infertility. Mutation in PMFBP1 [141] is involved in asthenospermia syndrome. Biallelic SUN5 mutations [142] contribute to a severe teratozoospermia. The mutation of DNAH17, which enforces the heavy chain of sperm-specific outer dyneinarms and leads to flagellum instability and asthenospermia [143,144]. Besides the morphology, motility abnormalities of sperm caused by mutations in DNAJB13 result in male infertility [145]. Meta-analysis of 6570 mutations indicates that germline methylation affects mutation rates. The mutation rate of each cell division is higher during early embryogenesis and primordial germ cell differentiation, while it is decreased significantly during post-pubertal spermatogenesis [146]. It is worth investigating whether the mutation in the above causes of abnormal spermatogenesis in men is mediated by the methylation of the genes.

7. Perspectives and Future Directions

With the development of technologies, especially single-cell RNA sequencing, a number of novel genes critical for regulating spermatogenesis have been identified. Sperm-seq, a new way of simultaneously analyzing the genomes of thousands of individual sperm [147], may also offer new insights into gene regulation in spermatogenesis. Numerous genes are specifically expressed in male germ cells or somatic cells, but their specific functions and mechanisms remain to be explored further. The changes in the expression of these genes in time and location and the locus of gene expression are also important ways to investigate the roles and signaling pathways of spermatogenesis-related genes. After identifying the key genes, it remains to be determined how these genes are regulated by DNA or RNA methylation and other epigenetic regulators (e.g., miRNAs). Abnormal gene variants or mutation in male germ cells between different species, especially human,
also warrant further exploration. These studies would provide new genetic regulators for human spermatogenesis and could offer novel targets for gene therapy of male infertility.

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