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Small Non-Coding RNAs in Mammalian Male Germ Cells and Their Implications for Male Infertility

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Abstract

Global rise in male infertility as a result of dysfunction of male germ cells has been pointed out by many investigations. Therefore, it is essential to elucidate the molecular mechanisms involving in the development of sperm, which rely on phase-specific gene expression that is regulated by myriads of small non-coding RNAs at transcriptional, post-transcriptional and epigenetic level. Recent advancement in small non-coding RNAs mainly including siRNAs, miRNAs and piRNAs has determined several pathways, which synergically regulate the process of male germ cell development. However, the aberrant expression of them is associated with dysfunction of male germlines, such as sperm arrest or apotosis, which further leads to male infertility. In this review, we focus mainly on the biogenesis and functions of these transcripts in the regulation of mammalian male germ cell development, elucidating the mechanisms between their dysregulation and related dysfunctions of sperm and providing some basic informations for diagnosis and treatment of male infertility.

Keywords: Small non-coding RNAs; male germ cells; gene expression; male infertility

Introduction

Development of male germ cells is a complex differentiation process that depends on a well-coordinated gene expression patterns [1,2]. During the whole process, epigenetic factors, such as small non-coding RNAs (sncRNAs), are dynamics to regulate gene expression temporally and spatially, faciliating the formation of viable healthy spermatozoa. Consequently, male germ cells are vulnerable to epigenetic defects that may further result in sperm arrest, apotosis and even cause male infertility. With the capability of sperm to transmit the genetic and epigenetic information to offspring, it is critical to investigate the molecular mechanisms that regulate the complex processes of sperm proliferation and differentiation at various male germ cell phases [3].

The rapid development of high-throughput analytical techniques has detailed a high diversity of sncRNAs specific existing in mammalian testis [4]. These sncRNAs presenting a wide variety of sizes are essential regulators for gene expression at transcriptional, post-transcriptional and epigenetic level, giving rise to a profound insight into the regulation of sperm development [5]. sncRNAs investigations in male germ cell developmental process is becoming an attractive new area.

Fast-increasing amounts of evidence indicate that mammalian male germ cells express a wide range of sncRNAs that comprise a well characterized class of non-coding RNAs [6-9]. The proper sncRNAs processing is of great significance for normal sperm development, but disruption of their regulation may result in some reproductive diseases [10]. As a result, a thorough understanding of the signaling pathways in these biological processes may be a novel attractive target for diagnosis and therapy of reproductive associated diseases. Based on recent advances in sncRNAs regulation, we mainly present an overview of the biogenesis, mechanisms, and functions of different types of sncRNAs in the whole process of male germ cell development, with special focus on miRNAs and piRNAs, and then the appilication of siRNAs in exploring the roles of specific gene will also be discussed.

Development of Male Germ Cells

Origin of male germ cells

The progenitors of Primordial Germ Cells (PGCs) derived from the pluripotent cells of epiblast are the origin of male germ cells. PGCs begin to express blimp protein when they are identifiable [11], which could promote the expression of germ-cell-specific markers and pluripotency genes, leading to the formation of the germ cell lineage [12]. At the phase of gastrulation, once stimulated by BMP4 and under the guidance of steel factor [13], PGCs start to migrate toward the hindgut during its anterior extension and then they move into the mesoderm and bilaterally migrate to the genital ridges [3]. The dynamic movement of PGCs is dependent on E-cadherin (CDH1) and b1-intergrin (Itgb1) and is directed by cxcl12 [14,15]. During these processes, mammalian germ cells initiate proliferation and differentiation, eventually forming gonocytes within seminiferous cords that are surrounded by Sertoli cells [16,17] (Figure 1). Gonocytes show a transitory mitotic activity, and then arrest in the G0/G1 phase of the cell cycle [18].

Overview of spermatogenic process

After birth, gonocytes migrate from the centre to the basal lamina of the seminiferous tubules where they resume mitotic proliferation and become Spermatogonial Stem Cells (SSCs), initiating the spermatogenic process [19]. Subsequently, the SSCs divide into either self-renewal (Asingle spermatogonia) or daughter cells (Apaired spermatogonia)

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that are committed to divide mitotically to produce the type Aaligned spermatogonia [20]. The maintenance of self-renewal was promoted by several factors such as gdnf and etv5, while differentiation process was promoted by steel factor and notch signaling [21,22]. Subsequently, Aaligned spermatogonia produce several generations of spermatogonia, including type A1-A4, intermediate, and type B spermatogonia before entering meiosis. After proliferation phase, type B spermatogonia enter the meiotic stage and divide via mitosis to give rise to several generations of primary spermatocytes, including preleptotene, leptotene, zygotene and pachytene spermatocytes [7]. Pachytene spermatocytes are then divided in meiotic prophase I, and the cells undergo a reduction division to split the sister chromosomes into two cells, generating secondary spermatocytes. The secondary spermatocytes very quickly divide again without replicating their DNA to form haploid round spermatids [23]. Subsequently, haploid spermatids undergo spermiogenesis to develop into mature spermatozoa (Figure 1), which involves several morphological changes such as acrosome formation and nuclear condensation [24]. Finally, spermatozoa are released into the rete testis and the epididymis where a series of additional maturational events take place [25,26].

The proliferation and differentiation of male germ cells undergo throughout the embryogenesis and spermatogenesis. During prenatal stages, the mainly process is the proliferation of PGCs, and then they are differentiated under a series of mediated factors to form gonocytes. After birth, gonocytes resume mitotic proliferation, initiating spermatogenesis that mainly includes three phases. The first phase is the mitosis of spermatogonia; the secnod phase is the meiosis of spermatocytes; and the last phase is the spermatogenesis that involves a series of morphology changes. Modified from He Zuping [7] and Meikar [27].

Biogenesis and Mechanisms of Small Non-coding RNAs

Due to the high requirements for gene regulation, it is therefore not surprised that sncRNAs are involved in the control of sperm production. Male germ lineages express three classes of sncRNAs, including Dicer-dependent microRNAs (miRNAs), small interfering RNAs (siRNAs), as well as Dicer-independent PIWI-interacting RNAs (piRNAs) [27]. These sncRNAs bind to evolutionarily conserved Argonaute (AGO) family proteins that are further classified into AGO and PIWI subclades. The AGO subclades are ubiquitously expressed in multicellular organisms, involving the processes of RNAi and miRNA-mediated gene silencing [28]. The PIWI subclades interacting with piRNAs directly are specifically enriched in the male germ cells [29]. Since sncRNAs are major regulators for male germ cell development [27], we will discuss their proposed biogenesis and mechanisms in details.

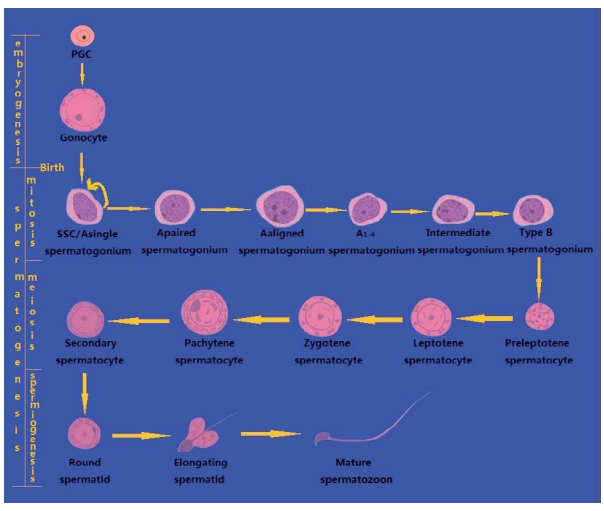

Figure 1: Development of male germ cells

Synthesis of siRNAs

siRNAs, derived from long double-stranded RNA (dsRNA), or a short hairpin RNA (shRNA), are generated into 21~28 nt by Dicer. Owing to this synthetic process, the synthesis of siRNAs does not need Drosha and DGCR8, which are necessary for the processing of the miRNA precursors [30]. After transported to the cytoplasm, siRNAs could recruit a multienzyme complex, forming the siRNA-induced silencing complex (siRISC) that identifies and cleaves the target mRNA with perfect complementarity [31]. As a result, the cognate mRNA is cleaved and protein synthesis is repressed, indicating that siRNAs are essential for post-transcriptional gene silencing [32].

In addition to the silencing mechanism for post-transcriptional mRNA regulation, siRNAs also have epigenetic effects on chromatin modifications by targeting specific sites that is essential for delivering epigenetic grooming enzymes to particular sites [33]. Firstly, siRNAs are packaged into the RITS complex, and then the siRNA effector molecule, the AGO1 protein, is delivered to the silenced sites. AGO1 interacts with the H3K9 methyltransferase Clr4, recruiting it to the sites of heterochromatin formation [33]. Finally, recruitment of HP1 allows spreading and maintenance of the heterochromatic state[34], leading to gene silencing through histone H3K9 methylation [35]. Moreover, similar to piRNAs, siRNAs could also result in gene silencing through DNA methylation [36].

Biogenesis of miRNAs

miRNAs are well-characterized endogenous RNA molecules. Coding genes for miRNAs are distributed throughout the genome and approximately half of them are located in the introns [37]. The synthesis and mechanism of miRNAs are a multi-step process. In nucleus of cells, miRNA sequences are typically transcribed by RNA Polymerase II as hairpin-loop-folded primary transcripts (pri-miRNAs) [38]. Pri-miRNAs are cleaved by the microprocessor complex (Drosha/DGCR8), generating 70 nucleotide (nt) pre-miRNA that are then transported to the cytoplasm via exportin-5 [39]. In cytoplasm of cells, the pre-miRNA is further processed by Dicer to their mature 20-24 nt double-stranded mature miRNA. The produced mature miRNA is unwound and one of the strand are then incorporated into an AGO2, forming miRNA-induced silencing complex (miRISC), with imperfect complementarity to multiple targets [40].

The mechanisms of miRNAs can be pictured as a cascade of events where miRNA pathway regulates gene expression by repressing or activating the target mRNAs [41]. It is most commonly believed that the miRISC usually mediates the numerous downstream protein-coding target gene silencing by the sequence-specific recognition of their target mRNAs, which leads to either degradation or translationally inhibition of the target sequences [42]. Usually, miRNAs bind to target mRNAs in the 3'UTR to form the RNA duplexes. Also, miRNAs have been demonstrated to bind to the 5'UTR and open reading frame of a subset of target mRNA [43]. Additionally, there is another mechanism by which miRNAs repress the translation by targeting the m7G-cap

recognition [44]. However, under some conditions, miRNAs have also been shown to activate translation. For instance, miR-369-3 expression is up-regulated and binds to the 3'UTR of tumour necrosis factor alpha (TNFa), resulting in enhanced translation of TNFa [45].

piRNAs and PIWI proteins

piRNAs, about 25-33 nt in length, constitute the largest class of sncRNAs, whose coding genes mostly derive from specific genome clusters, terming piRNA clusters that range from a few to hundreds of kilobases in size [46]. The majority of piRNA clusters map typically to only one genome strand, which are located in a limited number of pericentromeric and telomeric sites and are enriched for retrotransposon sequences [47]. The synthesis of piRNAs is a PIWI protein dependent mechanism, which requires primary processing pathway and ping-pong cycle to produce the mature sense and antisense single-stranded piRNAs [48].

Firstly, sense and antisense transcripts are transcribed from piRNA clusters and transported to the cytoplasm for further processing. These transcripts are catalyzed probably by Zucchini protein, a single-strand-specific nuclease, to produce 5'ends with a phosphate group, which are then loaded to MILI [49]. Subsequently, 3'ends are cleaved again by mHEN1, a RNA methyl transferase, to produce 2'-O-methylation with the size of piRNAs being determined by the types of PIWI proteins [50,51]. This pathway is called the primary processing of piRNAs. In postnatal germ cells, sense-strand piRNAs, are produced only by primary processing pathway and are loaded to MILI, MIWI and AGO3, but their functions are less well characterized [52].

In fetal male germ cells, piRNAs are also operated by secondary processing to amplify themself, which is essential for transposon gene silencing regulation. In this pathway, primary piRNAs produced by primary processing bind to MILI protein, initiating the ping-pong cycle that could amplify piRNA response. Firstly, AGO3 associates with sense-strand piRNAs to form an AGO3-piRNA complex that catalyzes antisense-strand cleavage and generates antisense piRNAs [47]. Then Aubergine (Aub) and PIWI associate with antisense piRNAs to form a complex, generating mature antisense piRNAs. By contrast, mature antisense piRNAs bound to Aub to form an Aub-piRNA complex that cleaves sense-strand piRNAs and produces sense piRNA precursor. The sense piRNA precursor associates with AGO3 to forms a complex that gives rise to mature sense piRNAs, eventually completing the cycle. piRNAs bind to PIWI proteins and several other functional components, forming PIWI-interacting RNA complex (piRISC), which guides de novo DNA methylation that specializes in targeting sites, resulting in Transposable Elements (TE) silencing [53]. Moreover, PIWI, HP1a, and Su(var)3-9 will also be recruited to these sites, reflecting that piRNAs are essential for the recruitment of epigenetic factors to specific genomic sites [54].

Profile and Functions of Small Non-coding RNAs in Sperm Development

The role of siRNAs in male germ cell development

Sequencing of the mouse testicular RNA have revealed that siRNAs are highly enriched in male germ cells and a total of 73 siRNAs have been identified [55]. The functions of siRNAs are mainly involved in post-transcriptional gene silencing through inducing target mRNA degradation. Moreover, they also have nuclear effects on chromatin modifications due to their high diverse of hits on multiple chromosomes [55]. For instance, siRNA-mediated repression of transcripts is associated with histone or DNA methylation, which targets the promoters of specific genes [56,57]. However, several researchers have found that siRNA targeted genes are first activated and then their transcripts are repressed, and the mechanism of that is not change the state of DNA methylation, but involves in histone demethylation [58].

On the other hand, siRNAs could also be used as a valuable approach to knock down the expression of interest genes that is RNA interference (RNAi) technology. RNAi could effectively repress a certain gene and further analyze the physiological functions of this gene, which is essential for elucidating the effects of individual gene on male germ cells [7]. For example, He et al found that the suppression of *Gfra1* in mouse through siRNAs could result in a switch from self-renewal to differentiation of SSCs [59]. Moreover, siRNAs was also applied to repress some transcription factors, including Bcl6b, Erm and Lhx1, that are essential for SSCs self-renewal regulation [60,61]. Collectively, these studies illustrate that siRNAs are crucial for exploring the roles of particular genes in the regulation of male germ cell development.

miRNAs in male germ cell development

With the stage-specific manner, miRNAs are highly, exclusively or preferentially expressed in male germ cells for the maintenance of their undifferentiated state and the induction of their differentiation [62]. Growing evidence has identified that miRNAs as potential regulators, in cooperation with other epigenetic modifications or not, are clearly involved in the orchestrated and stage-specific regulation of gene expression, which is critical for forming functional spermatozoa [63,64]. Thus the characterization of the profiles and functions of miRNAs in each step of male germ cell development is necessary.

PGCs arise as a small cohort of cells in early embryogenesis, and the levels of miRNAs are higher in PGCs than somatic cells [65]. Disruption of miRNA pathway has demonstrated that miRNAs are critical for male germ cell development. For example, the germ cell-specific *Dicer* knockout mouse shows the defects of PGCs in proliferation and post-natal spermatogenesis [63,64]. Signatures of miRNAs were also identified in populations of SSCs, spermatocytes and spermatids [66,67]. These cell types seem to share several common miRNAs, although some miRNAs are expressed in the certain cell types. MiR-21 is exclusively expressed in SSCs population, which is essential for the maintenance of SSCs undifferentiated state via working with etv5 [68]. Meanwhile, miR-135a has also been demonstrated to play important roles in self-renewal of SSCs by FoxO1 [69]. On the other hand, miR-34c targets the Nanos2 that could trigger SSCs differentiation, and then it also involves in the differentiation of spermatogonia through targeting Notch-signalling pathways [21,70]. Moreover, miR-146 is highly expressed in SSCs and the presence of this miRNA is important for their differentiation through retinoic acid signaling pathway [71]. However, the clusters of miR-17-92, highly expressed in SSCs, are drastically down-regulated by retinoic acid induction, reflecting that miR-17-92 clusters are essential for promoting the survival and proliferation of SSCs [72]. Other miRNAs such as miR-221/222 have also been shown to play potential roles in maintaining the state of SSCs [73].

Sequencing of miRNAs revealed that miRNA gene clusters on the X chromosome seem to have a higher expression levels in meiotic spermatocytes and haploid spermatids than that in somatic cells [74]. Likewise, Liu et al found that several miRNAs such as miR-34b-5p and miR-34c-5p were up-regulated in human spermatocytes compared

to spermatids, reflecting their roles in the regulation of meiosis [75]. However, X chromosomal genes undergo epigenetic silencing in mid-to-late pachytene spermatocytes by the process of Meiotic Sex Chromosome Inactivation (MSCI). Interestingly, numerous X-linked miRNAs could escape the silencing by MSCI, indicating that the miRNA gene duplications on the X chromosome were selectively favored during evolution to allow their expression in spite of sex chromosome inactivation, which is necessary for the differentiation of spermatocytes and spermatids [76]. miR-449, under the regulation of transcription factors CREMtau and SOX5, is significantly up-regulated upon meiotic initiation, which is involved in the regulation of gene expression in spermatocytes and spermatids [77]. Coincidentally, miR-34c exhibits a similar effect to that of miR-449 [78]. Both of miR-34c and miR-449 have been shown to share some target genes that belong to the E2F transcription factor-retinoblastoma regulatory network, reflecting a functional complementation [79].

The presence of miRNAs is also necessary for spermatogenesis. The correct timing expression of transition proteins (TPs) and protamine's (Prms) are essential for sperm chromatin compaction through the process of histone-protamine transition [80]. TPs and Prms are subjected to extensive translational control that involves in miRNA-mediated mechanisms. For example, miR-469, a testis-specific miRNA, has been demonstrated to target TP2 and Prm2 mRNAs, inhibiting their transcripts at the translation level with a minor effect on mRNA degradation [81]. On the other hand, miR-122a, predominately enriched in late-stage male germ cells, has been shown to repress the transcription of TP2 directly through binding to and inducing TP2 mRNA cleavage [82]. Furthermore, there are another miRNA mediated pathway that regulate gene expression through targetting the mRNA of heat shock factor 2 (HSF2), a transcription factor that regulates the expression of numerous genes required for normal spermatogenesis [83]. For instance, miR-18 that belongs to the miR-17-92 clusters is abundantly expressed in the testis, which could directly target HSF2 mRNA, influencing a wide range of developmental processes including gametogenesis [83].

piRNAs in male germ cell development

The complex procedure of male germ cell development requires an accurate gene expression and regulation. In contrast to miRNAs that are expressed abundantly throughout the process of sperm development, piRNAs are expressed predominantly in fetal and neonatal male germ cells, pachytene spermatocytes as well as round spermatids, which are necessary for the proliferation of SSCs and the differentiation of male germ cells [84,85]. In mammals, piRNAs are divided mainly into three categories (foetal/pre-natal, post-natal pre-pachytene and pachytene) on the basis of their specific phases of expression patterns [86]. Moreover, piRNA functions are supported by binding to PIWI proteins and other functional adjuncts. There are three PIWI subfamily members, including MILI, MIWI and MIWI2, are primarily expressed in male germ cells with distinct expression patterns [87,88]. Each PIWI protein, binding a specific subset of piRNAs or not, is crucial for male germ cell development through guiding TE silencing and mRNA repression [89].

In fetal male germ cells, the majority of fetal piRNAs that bind to MILI and MIWI2, are involved in the TE silencing at epigenetic and post-transcriptional level [90,91]. MILI is a cytoplasmic protein that first expresses in PGCs at around the time of sex differentiation and then continues its expression throughout the spermatogenesis until the round spermatid stage. MIWI2 can be detected in a very narrow expression window that from embryonic pro-spermatogonia to the very early post-natal spermatogonia, and then it is diminished a few days after birth [6]. Moreover, both MILI and MIWI2 localize in germ granules, which are loaded with foetal piRNAs and are important for the proper function of the piRNA pathway in transposon silencing [92]. In addition, there is another piRNAs existing before the pachytene stage,that is pre-pachytene piRNAs [86]. Consistent with foetal piRNAs, pre-pachytene piRNAs play a similar role in TE silencing [46]. However, MILI is the only PIWI protein that is expressed at this stage, and there is one protein, namely MAEL, may be involved in the regulation of the meiotic TE silencing through the PIWI-piRNA pathway [93].

On the other hand, the pachytene piRNAs associated with MILI and MIWI are produced later in pachytene spermatocytes and round spermatids in exceptionally large amounts, reflecting their roles in these germ cells [94]. The pachytene piRNA expression starts when pachytene spermatocytes appear, peaks in postmeiotic round spermatids and disappears during spermatogenesis [27]. Meanwhile, MIWI2 expression ceases and MIWI expression starts in the pachytene stage in meiosis and continues throughout the phase of round spermatid differentiation [86]. Although there is a large amount of pachytene piRNAs massively increase in pachytene spermatocytes and round spermatids, the exact functions of them remain to be characterized. Interestingly, Vourekas et al have found that piRNAs had no complementary larger RNA targets, but MILI and MIWI seem to have other piRNA-independent functions as well [95]. For example, MIWI has been demonstrated to bind directly to spermatogenic mRNAs, involving in the formation of mRNP complexes or the translational repression of spermatogenic mRNAs, which established a novel role of the piRNA pathway that shed light on this mechanism in the regulation of male germ cell development [95,96].

Alterations of sncRNAs in sperm development and the biomarkers for male diseases

Sperm development is prone to mistakes as demonstrated by the falling sperm quality. The increasing incidence of male infertility reflects a dysfunction of male germ cell development, and thus the characterization of the epigenetic factors that negatively affect sperm quality is of great importance for a better understanding of the etiology of sterility. Since sncRNAs act as regulatory factors of gene expression and epigenetic events during sperm development, the exploration of the functions and the potential targets of sncRNAs, and the identification for the profiles of cell- and stage-specific distribution of sncRNAs in male germ cells would be helpful for more insights into the molecular control of sperm development, and may provide a potent tool for the diagnosis of infertility and gene therapy for male reproductive diseases.

Dysregulation of miRNAs and Male Infertility

Male infertility is a world-wide disease, and approximates half of all cases result from abnormal male germ cell development [97]. It is well known that miRNAs, a versatile regulator, can down-regulate the expression of genes and control a wide range of biological processes [98]. However, the miRNA-mediated mechanisms in male infertility are limited. There are some basic assumptions that any disorder or failure in miRNA biogenesis, deregulation in expression of certain miRNAs and single nucleotide polymorphism in the miRNA binding site or related genes may lead to male infertility[10]. Dysregulation of pacific miRNAs in sperm development is associated with certain male

infertility, which may serve as effective biomarkers for diagnosis of this disease. Sequencing of miRNAs in azoospermia and asthenozoospermia revealed that the levels of miR-34c-5p, miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509-5p, and miR-513a-5p are down-regulated in azoospermia and increased in asthenozoospermia [99]. Likewise, miR-34c-5p was lower in seminal plasma of azoospermic patients and higher in seminal plasma of asthenozoospermia patients [99]. In addition, miR-19b and let-7a are highly expressed in non-obstructive azoospermia and oligozoospermia [100].

On the other hand, the dysfunction of miRNAs could also result in cells apoptosis. For example, the most abundant sperm miRNA in the human is miR-34c and its inhibition in primary spermatocytes seems to prevent germ cell from testosterone deprivation-induced apoptosis, while its overexpression could trigger cultured germ cells apoptosis [101,102]. High-throughput sequencing has identified miR-21, along with miR-34c, -182, -183, and -146a, are preferentially expressed in SSCs-enriched germ cell cultures. When miR-21 was inhibited transiently in SSCs-enriched population, the number of germ cells undergoing apoptosis is increased,suggesting that miR-21 is essential for maintaining the SSCs population [68].

Mutations of PIWI proteins and dysfunction of male germ cell development

The PIWI proteins including MILI, MIWI and MIWI2 are crucial components in the piRNA pathway, which are essential for the maturation and functions of piRNAs. Moreover, they also could cleave target nucleic acids as small RNA-guided nucleases, which play important roles in the successful completion of sperm production, reflecting an essential role for piRNAs in this process [103,104]. It is therefore not surprised that mutations of PIWI proteins could result in the dysfunction of male germ cell development.

In fetal male germ cells, there is a dramatic resetting of de novo DNA methylation, MILI and MIWI2 establish the repression of TE to enforce genomic stability through this pathway. In MILI and MIWI2 mutants, the biogenesis and profiles of fetal piRNAs are severely disrupted, which further results in the hypo methylation in transposon genes [88]. It is the hypo methylation that later activates the retro transposon genes in spermatocytes, wherein gross cell death occurs. These testicular phenotypes resemble remarkably that in the Dnmt3L mutant mouse, indicating that DNA demethylation may be a major cause of MILI and MIWI2 mutant phenotypes [105]. In addition, it is revealed that the increased activities of retro-transposons could also lead to a high extent of DNA Double-Strand Breaks (DSBs), which results in the defect synapsis of homologous chromosomes in meiotic spermatocytes and spermatogenesis stops with meiotic arrest [106].

After birth, there is another mechanism that consisting of MILI, MIWI and other epigenetic factors involving in the silence of TE. For example, MILI and H3K9me2 exist in meiotic male germ cells and MIWI can cleave transposon RNAs directly in another aspect, which may be essential for post-transcriptionally TE silencing [107]. In the MILI knockout mouse models, there is a notably testicular phenotype with spermatogenesis blocked at the pachytene spermatocyte stage, which possibly results from the genomic instability due to an abnormal TE expression [88]. On the other hand, in the MIWI knockout testis, the expression of pachytene piRNAs is significantly reduced, which results in round spermatid arrest with no apparent defects in meiotic progression and complete lack of elongating spermatids, reflecting that spermatogenesis arrested at the round spermatid stage is not capable to start the elongation of spermatids [87,88]. In addition, the MOV10L1, an RNA helicase, has been shown to act upstream of PIWI proteins in the primary processing of pachytene piRNAs [108], and its disruption may induce a complete loss of pachytene piRNAs, which further leads to the chromatoid body fragmentation and severe DNA damage [109].

Conclusion

Given the increasing cases of idiopathic infertility that results mainly from falling sperm quality, the study of regulation in male germ cell development is of great importance for elucidating the etiology of male infertility. It is already apparent that the combined actions of siRNAs, miRNAs and piRNAs have major contribution to the highly orchestrated expression of specific genes. It is obvious that the dysregulation of specific sncRNAs will result in certain male infertility or male germ cell tumors, which may serve as biomarkers for the diagnosis and treatment of these reproductive diseases. Moreover, we can also assess the fertility potential of specific sperm and select high quality sperm used for assisted reproductive treatment through sequencing specific sncRNAs. In addition, spermatozoa RNAs can be transmitted into oocyte through fertilization process, and we can predict the risk of embryo development based on the profiles of specific RNAs. On the other hand, sncRNAs could also interact with other epigenetic factors, such as DNA methylation and histone modifications, to regulate certain genes expression. In the future, it is necessary to characterize the regulatory network that involves in the interaction between sncRNAs and other epigenetic modifications, which will fetch more functional surprises in the event of male germ cell development.

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