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MicroRNAs as Regulatory Elements of Mammalian Spermatogenesis

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Abstract

In recent decades, infertility is becoming a public health issue. Male spermatogenesis failure has been considered a major contributory factor to infertility. Mammalian spermatogenesis is a well-defined process, requiring highly regulation processes in both transcriptional and the posttranscriptional levels. Discovery of microRNAs (miRNAs or miR) as essential class of gene expression regulators has provided new insights into a multitude of biological processes including spermatogenesis. In current review study, we first provide a short overview of spermatogenesis process, and then focus on recent studies that have elucidated the essential role of miRNAs in different steps of sperm production.

Keywords: MicroRNA, Spermatogenesis, Regulation, Gene Expression, Sertoli Cell

1. Background

Male fertility depends on successful production of proper number of functional sperm. The precise process of spermatogenesis requires unique, complex and dynamic patterns of genetics and epigenetics regulation. At the earliest stages of embryogenesis, a small group of cells is induced to become mammalian primordial germ cells (PGCs). PGCs, laterally migrate to the future gonads and become the progenitor population. At this point, PGCs continue to proliferate and then, under control of their microenvironment become committed to a developmental pathway that will direct them to become either eggs or sperms. Male PGCs become known as gonocytes once they cease migration. In males, gonocytes give rise to spermatogonial stem cells (SSC), as early precursor for spermatozoa in the postnatal testis. SSCs are capable of both self-renewal and differentiation. When sexual maturity is reached, initiation of spermatogenesis occurs by differentiation of a SSC subpopulation into spermatocytes, spermatids, and terminally differentiated mature (1). The remaining SSCs proliferate and remain undifferentiated to maintain the spermatogonial pool. In continuous events of spermatogenesis throughout the reproductive lifespan, the pool of SSCs enter meiosis and support the continuous production of spermatozoa (2). Even though, several protein coding genes are have been identified to be involved in production of sperm, the specific mechanisms for regulation of these genes are largely unknown. The discovery of miRNAs shed a new light on understanding of the complexity of gene regulatory networks. MiRNA are a class of small, single-stranded, noncoding RNAs of ~ 22nt in length that bind to their specific target messenger RNAs (mRNAs) and regulate their translation through one of two distinct mechanisms: cleavage of complementarity region of targeted mRNA followed by its degradation or inhibition of mRNA translation. It is estimated that miRNAs account for 1% - 5% of the human genome and regulate more than 50% protein-coding genes (3). This review article provides an overview of current understandings supporting the essential roles of miRNAs in the regulation of spermatogenesis.

2. Methods

Journal databases including PubMed, Science Direct, Google Scholar, Wiley Online Library, and Oxford were searched using key words "microRNA", "spermatogenesis", "spermatogonia" and "Sertoli cells". Researches were included in current study if they met quality and relevance criteria.

3. Results

3.1. Overview of Spermatogenesis

Spermatogenesis accrues in sequential events of mitotic, meiotic and post-meiotic phases in the seminifer-

ous epithelium of the testis. SSCs are the foundation of spermatogenesis located along the basement membrane of seminiferous tubules. SSCs can either undergo self-renewal divisions to renew the stem cell population or divide and stay together as a pair (Apr spermatogonia) that are committed to differentiation. Apr spermatogonia undergo a series of mitotic divisions, forming chains of 4, 8, and occasionally, up to 32 A aligned (Aal) spermatogonia. They then give rise to several generations of differentiating A1-A4, intermediate and B spermatogonia (3). Type B spermatogonia undergo another mitotic division to produce diploid, preleptotene primary spermatocytes. These cells represent the beginning of long-lasting meiosis I, where homologous recombination occurs and chromosome number is reduced. During this stage, a primary spermatocyte generates two secondary spermatocytes, which undergo meiosis II and devides into two equal haploid spermatids. The round spermatids are matured into spermatozoa (sperm) by the process called spermatogenesis.

3.2. miRNAs in Different Steps of Mammalian Spermatogenesis

Not surprisingly several microRNAs are indicated to play crucial roles in the events of spermatogenesis, which involved complex epigenetic modifications and transcriptional regulation. This regulatory milieu includes the expression of components involved in miRNA biogenesis (DGCR8, Drosha, Dicer, Risc, Exportin-5) along with transcription of primary miRNA.

The overall essential of miRNA regulatory cascades for regulation of spermatogenesis process has been demonstrated via approaches that conditionally knockout (cKOs) the factors involved in miRNA biogenesis. Notably, by using different promoters to drive germline-specific gene inactivation at various time points, it has been indicated that the timing of knockout for each component of miRNA biogenesis affects type and extent of spermatogenesis failure.

For instance, knockout of Dicer in mice was associated with early decrease in germ cell number, followed by impaired differentiate as well as abnormal motility (4). In other hand, the early disruption of Dicer in PGCs at embryonic day 10 led to reduced number of neonatal spermatogonia (5). Moreover, inactivation of Dicer, DGCR8 or Drosha at later time points (E18 or postnatal day 3), led to failure of meiosis completion (due to impaired transition from the leptotene/zygotene stage during prophase I to the pachytene) (6), leading to elimination of spermatogenesis at pachytene (7).

However, in all of these approaches miRNA biogenesis apparatus was disrupted and several miRNAs and signaling pathways were affected simultaneously; Thus, although they do not give much insight into the identities of the specific miRNAs related to phenotypes, nor do they reveal their specific functional roles. Moreover, most of miRNAs have several direct or indirect targets; thus, dysregulation of even a single miRNA may lead to great consequences. Below, we briefly summarize the specific contribution of different miRNAs in the regulation of spermatogenesis.

3.2.1. MiRNA and SSCs Cell Renewal

Spermatogenesis initiates once a subset of SSCs commit to differentiation rather than remaining in the self-renewing phase. MiRNAs, have gained significant attention as endogenous regulators for SSCs fate and behavior.

The global expression of miRNAs in the murine testis have been investigated in different animal; But few have investigated profile of miRNAs expression in a specific subset of testicular cells, particularly the SSC population or PGCs (5, 8). Studies on Thy1+ fraction of testis cells, enriched for SSC and PGCs have revealed that a large number of miRNAs, including miR17–92 cluster (9), miR-106b-25 (Mirc3) cluster (9), miR290-295 cluster (10-12), miR146 (13), miR20, miR106a (14), miR21, miR-34c (15), miR221, miR222 (16), miR135a (17) miR302-367 cluster (5) are highly expressed in these cell populations.

Mir-17-92 (Mirc1) cluster is a bona fide oncogene, overexpressed in various cancerous cells (18); thus, it could be essential for maintaining the tumor cells in an undifferentiated status.

High expression of Mir-17-92 (Mirc1) cluster in Thy1+ cells is in agreement with available findings that identified this cluster as preferentially expressed throughout development of mouse neonatal PGCs (14). During induction of spermatogonial differentiation by retinoic acid (RA) the expression of Mir-17-92 (Mirc1) cluster as well as its paralog Mir-106b-25 (Mirc3) cluster is reported to be downregulated (9). This suggests that both of these clusters seem to play a role in SSC self-renewal and proliferation. Putative target genes for these clusters were suggested to be Bcl2l11 (also known as Bim) (19), Kit (20), Socs3 (21), and Stat3 (22) that are suggested to be involved in spermatogonial development.

Interestingly, even though male specific knockout of Mir-17-92 (Mirc1) in male mice germ cell led to smaller testes and reduced numbers of epididymal sperms, it was not associated with significant defects in spermatogenesis in comparison with their littermates. It potentially suggests that the paralog of Mir-17-92 (Mirc1) cluster, Mir-106b-25 (Mirc3) cluster can compensate its depletion. In agreement with this supposition, the absence of Mir-17-92 (Mirc1) is indicated to be associated with upregulation of the Mir-106b-25 (Mirc3) cluster miRNAs in the germ cells (9).

Using miRNA mimics and inhibitors, it has been revealed that three highly expressed miRNAs in mouse SSCs including miRNA-20, miRNA-106a and miR-21 promote renewal of SSCs and contribute to maintenance of mouse SSC homeostasis (14, 15). MiR-20 and miR-106a play regulatory roles via targeting Stat3 and Ccnd1 (14). Furthermore, miR2, highly enriched microRNAs in SSCs, is regulated by the transcription factor ETV5, which is required for continuous spermatogenesis (15).

MiR135a has been indicated to play role in maintenance of the spermatogonial stem cell via modulation of transcription factor Foxo1 activity that enhances the elevation of Ret protein on the cell surface of SSCs (17). Besides, miR-544 is indicated to contribute to regulation of goat SSCs self-renewal through targeting the PLZF, a well-stablished transcription factor involved in SSC self-renewal (23). Similarly, miR-224 was reported tp control mouse SSC proliferation by modulating PLZF and GFRα1 (23). MiR-204 was also identified to be involved in the regulation of dairy goat SSC self-renewal via targeting Sirt1(24). Interestingly, in goat SSCs miR-34c is indicated to be highly expressed and regulate SSC population by induction of their apoptosis in a p53-depemdent manner (25).

MiR-202-3p and -5p are among other highly expressed miRNAs in the testis and spermatogenic cells. They are oppositely regulated by GDNF and RA, two crucial regulatory factors involved in self-renewal and differentiation of SSCs (26). Functioning as gatekeepers, these two miRNAs prevent premature differentiation of SSCs via modulation of the expression of various target genes, including cell cycle regulators and RNA binding proteins. Interestingly the knockdown of Rbfox2, a direct target for miR-202 blocks meiosis initiation of cultured SSCs, suggesting that Rbfox2 is one of the members of miR-202-centered regulatory network.

3.2.2. The Roles of MiRNAs in Meiosis and Spermatogenesis

Available studies have indicated that in addition to miRNAs involved in SSCs self-renewal, a several miRNAs are reported to be preferentially expressed in spermatocytes and spermatids. Majority of these miRNAs are indicated to play roles in the regulation of genes involved in meiotic and post-meiotic processes.

The members of MiR-449 cluster are indicated to be exclusively expressed in spermatocytes and spermatids and their expression is significantly increased upon meiotic initiation in spermatogenesis. The stimulation of miR-449 cluster expression in mouse testes accurse by binding of two transcription factors CREMτ, an crucial regulatory element in developing male germ cells (27), and SOX5, a major post-meiotic transcriptional regulator (28, 29) to two highly conserved cis-elements of the members of this cluster (30).

The miR-34c has been indicated to be highly expressed in germ cells (31). Besides, the significant role of this miRNA in the regulation of SSCs status has been reported. However, because miR-34c is also highly expressed in isolated pachytene spermatocytes and round spermatids it seems that this specific miRNA plays a dual role in both SSCs maintenance and spermatogonia differentiation (25, 32). It is noteworthy to mention that two direct targets of miR34c, TGIF2 and NOTCH2, are known to play roles in spermatogenesis (31, 33). TGIF2 contributes to spermatogenesis by inhibition of the TGFβ pathway, as a major player in this process (34, 35); thus, miR-34c potentially play rolls during spermatogenesis via the down-regulation of TGIF2 and subsequent inhibition of TGFβ pathway (36).

Another target of miR-34c, NOTCH2, is scarcely expressed in germ cells, playing an important role during testis somatic cell differentiation (37-39). It suggests that miR-34c potentially plays a significant role in germ cells differentiation via down-regulation of NOTCH2 (31).

MiR-34c also promotes SSC differentiation and meiosis by targeting a male-specific gene, called NANOS2. The product of this gene plays a significant role in keeping or SSC or spermatogonia in an undifferentiated state via inhibiting NANOS3, SCP3, DAZL, and Stra8 expression (40-42).

It is noteworthy to mention that miR-34b/c and miR-449 cluster share the identical seed regions (30, 43), suggesting that they regulate germ cell differentiation and survival via same pathways such as E2F-pRb (44-46). Interestingly, knockout of either miR-449 or miR-34 paralogs alone is not associated with discernible defect in male germ cell development. However, miR-34b/c; miR-449 double knockout mice displayed severe spermatogenic imparment and male infertility. These findings strongly suggest that these two miRNA clusters function redundantly in the regulation of spermatogenesis (30, 43).

Another highly expressed miRNA in spermatocytes is miR-18, one of the miR-17-92 cluster (47). MiR-18 targets Hsf2, which is a critical transcription factor for spermatogenesis (47). Heat shock proteins are also targets of miR-214, which plays a key role in meiosis of pachytene spermatocytes (48, 49).

MiR-355 and miR-181b/c, two other up-regulated microRNAs in adult testis, are indicated to target rsbn1, a novel homeobox-like gene involved in transcriptional regulation of haploid germ cells (11, 44, 50). MiR-320, which is expressed in all germ cells, is predicted to target important mediators of cell-adhesion known as protocadherins. These molecules play important in cell adhesions between sertoli and germ cells (46).

Two testis-expressed microRNA, miR-469 and miR122a,

are indicated to target transition protein2 (TNP) and protamines (PRM) in pachytene spermatocytes and round spermatids. As the timely regulated expression of these two important chromatin remodelers play significant roles in sperm maturation and spermatogenesis, dysregulation of miR-469 and miR122a seems to be involved in failure of spermatogenesis (49, 51).

The expression of let-7 family miRNAs is indicated to be induced upon RA induced spermatogonial differentiation. The down-regulation of let-7 family was showed to be associated with down-regulation of let-7 family targets including Mycn, Ccnd1, and Colla2 (52). It has been indicated that the upregulation of let-7 is mediated by decrease in the levels of an RNA-binding protein LIN28, which is known to be involved in inhibition of let-7 miRNA biogenesis (53). LIN28 is expressed in mouse undifferentiated spermatogonia, and plays regulatory role in cyclic expansion of spermatogonial progenitor population (54). Interestingly Lin28 itself is a target gene for let7 family, suggesting that they function together as a regulatory feed-forward loop.

3.2.3. Role of miRNA Expression in Sertoli Cells

Sertoli cells are unique as they are non-growing terminally differentiated cell types in adult testis being active for the reproductive lifetime. Several distinctive specialized junctions are formed by these cells to provide a well-organized scaffold and environment for germ cell development. Besides, these cells secrete several substances crucial for the maintenance of the spermatogenesis synchrony, development and survival of germ cells.

The majority of investigations on human sertoli cells' miRNAs activity have been conducted by comparing their expression level of in men with sertoli cells-only syndrome (SCOS) and in men with normal spermatogenesis (55, 56). Available findings have suggested the miR-133b and miR-202 are potentially involved in pathogenesis of azoospermia or SCOS (55, 57). The expression of MiR-133b is reported to be increased in human sertoli cells of SCOS patients compared to obstructive azoospermia (OA) patients with normal spermatogenesis. MiR-133b plays its role in promotion of human sertoli cells proliferation through targeting GLI3 and regulating expression of Cyclin B1 and Cyclin D1 (57). In contrast to miRNA-133b, miRNA-202-5p is indicated to be selectively expressed in sertoli cells of fertile men and totally absent in sertoli cells in this aforementioned condition (55).

The results of in vitro studies using porcine models as an alternative showed that miR-762 and miR-1285 play significant roles in stimulation of sertoli cells proliferation through distinct pathways in the cell cycle (58, 59).

MiR-762 was identified to be upregulated in Large White boar immature testes (45). In sertoli cells, miR-762 directly binds the 3′UTR of RNF4, leading to downregulation of its expression. RNF4 is a coregulator of androgen receptor (AR)-dependent transcription (58, 60, 61) and its decreased expression was associated with weakening of the AR transcriptional regulatory activity. Thus, miR-762 seems to affect immature sertoli cells by partially enervating the AR transcriptional regulatory activity via targeting RNF4 (58).

In vitro experiments conducted on boar showed that the miR-1285 mimics directly downregulated the expression of AMPK via a 3'UTR target site, resulting in promotion of sertoli cell proliferation. Moreover, miR-1285 mimics led to increased levels of mRNA and protein ATP, phosphorylated mTOR (mammalian target of rapamycin) and Skp2 (S-phase kinase-associated protein 2); meanwhile, reduced the expression of p53 and p27. These findings suggested the miR-1285 plays significant role in proliferation of immature sertoli cell (59).

4. Conclusions

Extensive and precise regulation of gene expression is essential for governing accurate spermatogenesis process. The discovery of miRNAs has opened new horizons in the unravelling the mechanisms involved in different steps of spermatogenesis. It is now clear that dysregulation of miRNAs, either in the germ lineage or supporting cells of the testis, has detrimental impact on spermatogenesis male fertility. The emergence of powerful set of technologies for studying single cell small RNA would be very beneficial to further explore the functions of germ-cell/supporting cells specific miRNAs and identify their potential targets.

Footnotes

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