Role of microRNAs in mammalian spermatogenesis and testicular germ cell tumors

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Abstract

microRNAs (miRNAs) are a class of small endogenous RNAs, 19–25 nucleotides in size, which play a role in the regulation of gene expression at transcriptional and post-transcriptional levels. Spermatogenesis is a complex process through which spermatogonial stem cells (SSCs) proliferate and differentiate into mature spermatozoa. A large number of miRNAs are abundantly expressed in spermatogenic cells. Growing evidence supports the essential role of miRNA regulation in normal spermatogenesis and male fertility and cumulative research has shown that this form of regulation contributes to the etiology of testicular germ cell tumors (TGCTs). In this review, we addressed recent advancements of miRNA expression profiles in testis and focused on the regulatory functions of miRNA in the process of SSC renewal, spermatogonial mitosis, spermatocyte meiosis, spermiogenesis, and the occurrence of TGCTs.

Introduction to spermatogenesis

Mammalian spermatogenesis starts from the self-renewal and differentiation of spermatogonial stem cells (SSCs). SSCs can divide into either new stem cells (As) or paired (Apr) spermatogonia that are committed to differentiation. Apr spermatogonia produce Aaligned (Aal) spermatogonia mitotically, which then give rise to several generations of spermatogonia, including A1–A4, intermediate, and type B spermatogonia (He et al. 2009). Type B spermatogonia divide by mitosis to form preleptotene spermatocytes, which initiate the long-lasting meiosis I, in which homologous recombination between sister chromatids occurs before they separate. In meiosis II, spermatocytes undergo a reduction division to split the sister chromosomes into two cells to generate
secondary spermatocytes, which divide without replicating their DNA to form haploid round spermatids. The round spermatids commence the differentiation phase (spermiogenesis) to develop into mature spermatozoa (Kotaja 2014). Throughout spermatogenesis, germ cells maintain cytoplasmic bridges to facilitate synchronized cell division and differentiation (McIver et al. 2012a). Besides, spermatogenesis is regulated by hypothalamic–pituitary–gonadal axis. Gonadotropin-releasing hormone from the hypothalamus regulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland. Then FSH and LH stimulate the Leydig cells to release testosterone (Kotaja 2014).

miRNAs and spermatogenesis

Spermatogenesis is a complex and highly regulated process that supports the daily production of millions of sperm, which takes place within the seminiferous tubules of the testis of the sexually mature male. This developmental process requires the coordination of both somatic and germ cells through the phases of proliferation, meiosis, and differentiation to generate mature spermatozoa that are responsible for delivery of the paternal genome (Grimaldi et al. 2013, Fok et al. 2014, Goudarzi et al. 2014).

Emerging evidence has shown that miRNAs are essential for spermatogenesis and may play an important role during mitotic, meiotic, and post-meiotic stages of spermatogenesis by regulating the expression of the target gene (Tang et al. 2007, Hayashi et al. 2008, Bouhallier et al. 2010). Dicer, an RNAse III endonuclease, is essential for the biogenesis of miRNAs. Several studies have shown that testes were reduced in size and the process of spermatogenesis was disrupted after ablation of Dicer (Romero et al. 2011). In addition, these studies demonstrated that removal of Dicer1 at the early onset of male germ cell development led to infertility, due to multiple cumulative defects in the meiotic and post-meiotic stages (Wu et al. 2012). Specifically, the progression of spermatocyte meiosis and spermiogenesis was delayed, the number of haploid cells decreased, the number of apoptotic spermatocytes increased, and the low number of mature sperm in epididymides exhibited abnormal morphology and motility. In addition, sperm lacking Dicer1 were rarely able to fertilize WT eggs to generate viable offspring (Maatouk et al. 2008, Korhonen et al. 2011, Romero et al. 2011).

Expression profile of miRNA in the testis

Over the past several decades, a number of expression profile studies using miRNA microarrays, RT-PCR, or small RNA sequencing have demonstrated that numerous miRNAs are exclusively or preferentially expressed in the testis or germ cells of humans and mice (Barad et al. 2004, Ro et al. 2007, Yan et al. 2007, Buchold et al. 2010, Smorag et al. 2012). A total of 770 known and five novel human miRNAs were detected in normal human testes by Solexa sequencing technology (Yang et al. 2013b).

The global miRNA expression in cell populations from different stages of spermatogenesis, such as spermatogonia, spermatocytes, and spermatids, was conducted by microarray analysis. The results showed that most miRNAs are preferentially expressed in meiotic germ cells (Ro et al. 2007, Marcon et al. 2008). Some studies have demonstrated that miRNA expression patterns differ between immature and mature testes in human. For example, Yan et al. (2007) found 14 up-regulated and five down-regulated miRNAs in immature compared with adult testes. Subsequently, the differences in miRNA expression between immature and mature rhesus monkey (Yan et al. 2009) and porcine testes (Luo et al. 2010) were found. The expression profile of miRNAs in testes is given in Table 1.

miRNA and spermatogonial stem cell renewal

Within the testis, the SSCs reside in a unique micro-environment, or ‘niche’, which includes the surrounding somatic cells. Spermatogenesis originates from SSCs,
which have the dual property of continually renewing and undergoing differentiation into a spermatogonial progenitor that expands and further differentiates (Hess et al. 2006, Ventela et al. 2012, Dove et al. 2013, Silvan et al. 2013, van den Driesche et al. 2014, Guo et al. 2014). In the rodent testis, SSCs are among undifferentiated spermatogonia that include A single (As), A paired (Apr), and A aligned (Aal) spermatogonia (Dym 1994). The regulation of the balance between self-renewal and differentiation of SSCs determines the life-long supply of spermatogonia by maintaining a population of undifferentiated spermatogonial stem cells and ensuring that adequate numbers of spermatogonia undergo spermatogenesis (van den Driesche et al. 2014). miRNAs, as critical endogenous regulators in mammalian cells, play important roles in the regulation of the fate of SSCs. A large number of miRNAs, such as the miR17–92 cluster (Tong et al. 2012), miR290–295 cluster (McIver et al. 2012a,b), miR146 (Huszar & Payne 2013), miR20 (He et al. 2013), miR21 (Niu et al. 2011), miR106a (He et al. 2013), miR221, and miR222 (Yang et al. 2013b), are highly expressed in THY1-enriched undifferentiated spermatogonia. These miRNAs are largely down-regulated during retinoic acid-induced spermatogonial differentiation both in vitro and in vivo, suggesting that they are potentially involved in the regulation of proliferation and differentiation of SSCs during spermatogenesis (Kotaja 2014). For example, miR20 and miR106a promote renewal of SSCs at the post-transcriptional level via targeting Stat3 and Ccnd1 (He et al. 2013). miR135a contributes to SSC maintenance through modulation of Foxo1 activity (Moritoki et al. 2014), and miR21, which is present in SSC-enriched germ cells and is regulated by the transcription factor ETV5, is important in maintaining the SSC population (Niu et al. 2011). The list of miRNAs implicated in maintenance of pluripotency in germ cells is given in Table 2.

The role of miRNAs in spermatocyte meiosis and spermiogenesis

Spermatocyte meiosis and spermiogenesis are unique cellular processes to germ cells in the male. The meiotic phase (chromosomal replication, recombination, and two consecutive meiotic cell divisions of spermatocytes) and haploid phase (also called spermiogenesis, differentiation of spermatids into spermatozoa) of

<table>
<thead>
<tr>
<th>Origin</th>
<th>Expression</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse testis</td>
<td>141 miRNAs from the mouse testis were detected, of which 29 were novel</td>
<td>Ro et al. (2007)</td>
</tr>
<tr>
<td>Human testis</td>
<td>770 known and five novel human miRNAs were detected by Solexa sequencing technology</td>
<td>Yang et al. (2013a)</td>
</tr>
<tr>
<td>Saanen dairy goat testis</td>
<td>91 novel paired miRNAs were found</td>
<td>Wu et al. (2014b)</td>
</tr>
<tr>
<td>Immature and mature testes (mouse)</td>
<td>14 up-regulated and five down-regulated miRNAs were found in the immature testis compared with the adult testis</td>
<td>Yan et al. (2007)</td>
</tr>
<tr>
<td>Immature and mature testes (pig)</td>
<td>51 miRNAs were significantly up-regulated and 78 miRNAs were down-regulated in mature testes</td>
<td>Luo et al. (2010)</td>
</tr>
<tr>
<td>Testes of patients with NOA and normal human testes</td>
<td>26 miRNAs were shared by the IR and MR, and the IR and MH, although miRNA levels in each pair of samples differed by more than threefold</td>
<td>Yan et al. (2009)</td>
</tr>
<tr>
<td>Testis of patients with cryptorchid and normal testes</td>
<td>154 differentially down-regulated and 19 up-regulated miRNAs were found in testes from NOA patients</td>
<td>Hess et al. (2006), Abu-Halima et al. (2012)</td>
</tr>
<tr>
<td>Testes of patients with NOA and normal human testes</td>
<td>50 up-regulated and 27 down-regulated miRNAs were found in asthenozoospermic males. 42 up-regulated and 44 down-regulated miRNAs were found in oligoasthenozoospermic males compared with normozoospermic males</td>
<td>Lian et al. (2009)</td>
</tr>
<tr>
<td>Cryptorchid and normal testes</td>
<td>miR135a was expressed at a lower level in cryptorchid testes, which contributes to the maintenance of spermatogonial stem cells by regulating Foxo1</td>
<td>Moritoki et al. (2014)</td>
</tr>
<tr>
<td>Human testicular tissues of infertile men with different histopathological patterns</td>
<td>A total of 197, 68, and 46 miRNAs were found to be differentially expressed when comparing the samples from Sertoli cell only, mixed atrophy, and germ cell arrest groups, respectively, with normal spermatogenesis</td>
<td>Abu-Halima et al. (2014)</td>
</tr>
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</table>

Table 1 Expression profile of miRNAs in testes.

NOA, non-obstructive azoospermia.
spermatogenesis are characterized by high transcriptional activity but suppressed translational activity. Post-transcriptional control of gene expression in these phases is a significant feature of mammalian spermatogenesis (Kotaja 2014). A large number of miRNAs are preferentially expressed in spermatocytes and spermatids and are involved in the regulation of meiotic and post-meiotic gene expression (Kotaja 2014). Expression of the miR449 cluster is drastically up-regulated upon meiotic initiation during testicular development and in adult spermatogenesis, both of which are regulated by testes-specific transcription factors, CREMt and SOX5, through binding to two highly conserved cis-elements of the Cdc20b/miR449 cluster (Bao et al. 2012). miR34c is highly expressed in isolated pachytene spermatocytes and round spermatids (Zhang et al. 2012, Li et al. 2013), and Tgif2 and Notch2 (important in spermatogenesis) are the direct targets of miR34c (Bouhallier et al. 2010). Tgfb signaling inhibits the second meiotic division in spermatogenesis (Damestoy et al. 2010). miR34c is up-regulated in the adult testis and is involved in transcriptional regulation in haploid germ cells by targeting Rbsn1 (Yan et al. 2007, Mciver et al. 2012a). miR184 may be involved in the post-transcriptional regulation of miRNAs, such as Ncor2, in mammalian spermatogenesis (Wu et al. 2011). One miRNA can target various mRNAs and one mRNA can be targeted by multiple miRNAs (Peter 2010). Ablation of a single miRNA or miRNA cluster rarely leads to a discernable phenotype in mice under stress-free conditions, in some cases because of compensatory effects by other functionally related miRNAs (Wu et al. 2014a). For example, although no discernable phenotype has been observed in miR449 cluster-knockout mice, studies have suggested that miR34b/miR34c may compensate for the absence of miR449, as both miRNA families function redundantly by targeting the E2F–pRb pathway (Bao et al. 2012). Wu et al. (2014a) constructed the

### Table 2 miRNAs implicated in maintenance of pluripotency in germ cells.

<table>
<thead>
<tr>
<th>Name of miRNA</th>
<th>Expression</th>
<th>Targets (predicted and confirmed)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR17–92 cluster</td>
<td>THY1⁺-enriched undifferentiated spermatogonia</td>
<td>Bcl2l11, Kit, Socs3, and Stat3</td>
<td>Tong et al. (2012)</td>
</tr>
<tr>
<td>miR106b-25</td>
<td>THY1⁺-enriched undifferentiated spermatogonia</td>
<td>Bcl2l11, Kit, Socs3, and Stat3</td>
<td>Tong et al. (2012)</td>
</tr>
<tr>
<td>miR290–295 cluster</td>
<td>Highly enriched in the germ cell population of</td>
<td>Stat3 and Ccnd1</td>
<td>Zovoilis et al. (2008, 2010) and</td>
</tr>
<tr>
<td></td>
<td>the 6-day-old tests, multipotent adult germ</td>
<td></td>
<td>McIver et al. (2012a,b)</td>
</tr>
<tr>
<td></td>
<td>cells, and embryonic stem cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR146</td>
<td>Highly expressed in undifferentiated spermatogonia</td>
<td>Med1</td>
<td>Luo &amp; Payne (2013)</td>
</tr>
<tr>
<td>miR221 and miR222</td>
<td>THY1⁺-enriched undifferentiated spermatogonia</td>
<td></td>
<td>Yang et al. (2013b)</td>
</tr>
<tr>
<td>miR20 and miR106a</td>
<td>Thy1⁺-enriched undifferentiated spermatogonia</td>
<td>P12</td>
<td>He et al. (2013)</td>
</tr>
<tr>
<td>miR21</td>
<td>Thy1⁺-enriched undifferentiated spermatogonia</td>
<td></td>
<td>Niu et al. (2011) and Zheng et al.</td>
</tr>
<tr>
<td>miR135a</td>
<td>SSCs</td>
<td>Foxo1</td>
<td>Moritoki et al. (2014)</td>
</tr>
<tr>
<td>miR302-367 cluster</td>
<td>Primordial germ cells, ES cells</td>
<td>NR2F2</td>
<td>Hayashi et al. (2008), Dyce et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2010) and Rosa &amp; Brivanlou (2011)</td>
</tr>
<tr>
<td>miR376a</td>
<td>Down-regulated in mature mouse testes</td>
<td>CDK2 and AGO2</td>
<td>Wang et al. (2011)</td>
</tr>
<tr>
<td>miR302 cluster</td>
<td>Overexpressed in adult (undifferentiated) and</td>
<td></td>
<td>Liu et al. (2009), Murray et al.</td>
</tr>
<tr>
<td></td>
<td>pediatric germ cell tumors</td>
<td></td>
<td>(2010), Palmer et al. (2010) and</td>
</tr>
<tr>
<td>miR335</td>
<td>Down-regulated in mature mouse testes</td>
<td>CCNT2, CCD2, RBSN1, and RUNX2</td>
<td>Rosa &amp; Brivanlou (2011)</td>
</tr>
<tr>
<td>miR367</td>
<td>Embryonal carcinoma, ES cells</td>
<td>LATS2, KLF4, RUNX1, SYNJ1, and SMAD6</td>
<td>Li et al. (2009)</td>
</tr>
</tbody>
</table>

SSCs, spermatogonial stem cells.
Mendell et al. 2007, and as such, could assist in the diagnosis from the normal tissue obtained from the same source expression profiles in tumor tissue significantly differed.

A high-throughput microarray showed that miRNA & Shiekhattar 2005, Calin & Croce 2006, Costinean involved in occurrence and evolution of tumors (Gregory may play a similar role as proto-oncogenes, as both are number of studies have demonstrated that miRNAs

Role of miRNA expression in Sertoli cells

Sertoli cells play a central and essential role in coordinating spermatogenesis by structurally and nutritionally supporting germ cells and secreting factors that control the survival and progression of germ cells, without which the production of normal sperm would be hindered (Papaioannou et al. 2011, Panneerdoss et al. 2012). Dicer is absolutely essential for Sertoli cells to mature, survive, and ultimately sustain germ cell development. In fact, ablation of Dicer leads to infertility suggesting that miRNA expression in Sertoli cells is important for supporting germ cell development (Nicholls et al. 2011, Papaioannou et al. 2011). Panneerdoss et al. (2012) had identified miRNAs as one group of testosterone-dependent trans-acting factors in postnatal Sertoli cells that may play a crucial role in androgen-mediated events during spermatogenesis by targeting Sertoli cell/germ cell-specific genes. A large number of miRNAs (e.g., miR471, miR470, miR463, miR465, miR743a/miR743b, miR883, miR880, miR201, and miR547) expressed in Sertoli cells have been demonstrated to play a coordinated role in androgen-dependent spermatogenic events (Panneerdoss et al. 2012). In addition, a subset of miRNAs expressed in Sertoli cells, including miR23b, miR30c, miR30d, and miR690, are stimulated upon FSH and androgen suppression, which are critical regulators of target proteins associated with junction restructuring and spermiation (Nicholls et al. 2011). Thus, FSH and androgens act on Sertoli cells at stage VIII to regulate the expression of miRNAs that operate in a coordinated manner to control cell adhesion pathways and male fertility.

miRNA and germ cell tumors

miRNAs are essential for cell proliferation and differentiation by regulating cell cycle-related factors. A growing number of studies have demonstrated that miRNAs may play a similar role as proto-oncogenes, as both are involved in occurrence and evolution of tumors (Gregory & Shiekhattar 2005, Calin & Croce 2006, Costinean et al. 2006, Hwang & Mendell 2007, Manikandan et al. 2008). A high-throughput microarray showed that miRNA expression profiles in tumor tissue significantly differed from the normal tissue obtained from the same source (Murakami et al. 2006, Wang & Wang 2006, Hwang & Mendell 2007), and as such, could assist in the diagnosis and treatment of cancer according to this differential expression pattern (Mattie et al. 2006, Volinia et al. 2006, Yaniaihara et al. 2006). Human germ cell tumors comprise a heterogeneous group of neoplasms, all with a defined histological appearance. They have specific epidemiological characteristics, clinical behavior, and pathogenesis (Looijenga et al. 2014). In the testis, there are three types of testicular germ cell tumors (TGCTs) that occur at distinct ages in men, namely, the teratomas–yolk sac tumors of the infantile testis (type I), the seminomas and non-seminomas of adolescents and adults (type II), and the spermatocytic seminomas of the elderly (type III) (Oosterhuis & Looijenga 2005, Looijenga et al. 2006, 2014, Gillis et al. 2007). The expression profiles of miRNAs in these different types of TGCTs vary. For example, Gillis found that the miR302 cluster, which plays a role in the maintenance of embryonic stem cells pluripotency, is elevated in seminoma tumors. miR21 and miR155, as oncogenic miRNAs, are highly expressed in seminomas and spermatocytic seminomas. The up-regulation of miR19a and miR29a and down-regulation of miR133a and miR145 are found in seminomas and spermatocytic seminomas. miR146 expression is lower in seminomas, spermatocytic seminomas, and even different type II tumors such as embryonic carcinomas and teratomas compared with normal testes (Gillis et al. 2007). Analysis of the 156 miRNAs expressed in normal human testes, type II and type III TGCTs, and cell lines derived from TGCTs, verified that terminally differentiated histological subgroups (e.g., normal testicular tissue) expressed most discriminating miRNAs at a higher level than poorly differentiated tumor tissue sub-populations, such as seminoma, dysgerminoma, and embryonal carcinomas (Gillis et al. 2007). These results support the model that miRNAs are involved in the regulation of differentiation of stem cells retained in germ cell tumor (GCT). Lin 28 is thought to be involved in the maintenance of pluripotency. Let7, miR125a, and miR9 are implicated in the formation of testicular teratomas by targeting Lin28 (Zhong et al. 2010). miR199a expression is significantly lower in TGCTs compared with normal testicular germ cells. The v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (MAFB) transcription factor was identified as a putative target of miR199a-5p in TGCTs and mediates the tumor suppression activity of miR199a (Gu et al. 2013). miR372 and miR373 neutralize p53-mediated cyclin-dependent kinase (CDK) inhibition, possibly through direct inhibition of expression of the tumor suppressor LATS2 (Voorhoeve et al. 2006, 2007, Gillis et al. 2007). Studies have demonstrated that these miRNAs are potential novel oncogenes that participate in the development of human TGCTs by inhibiting the Trp 53 pathway, thus allowing tumorigenic growth in the presence of WT Trp 53 (Costinean et al. 2006). The hsa-miR371–373 cluster is involved in counteracting cellular
senescence induced by oncogenic stress, allowing cells to become malignant (Gillis et al. 2007). Protein tyrosine phosphatase non-receptor type 23 (PTPN23) is one of the important tumor suppressor candidates and is involved in the tumorigenesis of various organs. Relatively higher miR142-3p expression can be found in the absence of PTPN23 protein expression in human TGCTs, suggesting that miR142-3p plays an important role in the pathogenesis of TGCTs by repressing PTPN23 expression (Tanaka et al. 2013). Together, the data presented in this review indicate that aberrant miRNA expression is related to human germ cell tumor occurrence. The miRNAs implicated in the development of TGCTs are shown in Table 4.

Table 3 miRNAs that play a regulatory role in spermatocyte meiosis and spermiogenesis.

<table>
<thead>
<tr>
<th>Name of MiRNA</th>
<th>Expression</th>
<th>Targets (predicted and confirmed)</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR449</td>
<td>Up-regulated in mature rhesus monkey and mouse testis, preferentially expressed in mouse testes and localized to spermatocytes and spermatids</td>
<td>MECP2, AS1B, BCL2, NOTCH1, CASP2, KITLG, VCL, FOXJ2, INHBB, SOX11, CCNE2, GMFB, and DLL1</td>
<td>Represses the proliferation of a germ cell line, GC-1spg</td>
<td>Bao et al. (2012)</td>
</tr>
<tr>
<td>miR34a</td>
<td>Up-regulated in mature mouse testis, up-regulated from day 7 to day 14 in mouse testis</td>
<td>CCND2, BLC2, GMFB, and SIRT1</td>
<td>Represses proliferation, promotes apoptosis</td>
<td>Yan et al. (2007), Buchold et al. (2010) and Ito et al. (2010)</td>
</tr>
<tr>
<td>miR34b</td>
<td>Up-regulated in mature rhesus monkey testis, up-regulated from day 7 to day 14 in mouse testis</td>
<td>NOTCH1, LGR4, VEZT, MAN2A2, and FOXJ2</td>
<td>Regulates the germ cell proliferation and survival</td>
<td>Yan et al. (2009), Buchold et al. (2010) and Vogt et al. (2011)</td>
</tr>
<tr>
<td>miR34c</td>
<td>Highly expressed in isolated pachytyne spermatocytes and round spermatids</td>
<td>CCND3, CCNG1, CCNB1, CCNC, CCNE1, CDK4, CDK6, E2F5, FOS, CDC2, TGF2, NOTCH2, STRBP LGR4 KLF4, NOTCH1 PIP1IC, GALT, KITLG, SPAG4, CCNL, ZFP148, and GMFB</td>
<td>Cycle regulator, promoted mGSC apoptosis, SSC differentiation, enhances the germline phenotype of cells already committed to this lineage</td>
<td>Yan et al. (2009), Buchold et al. (2010), Li et al. (2013) and Yu et al. (2014)</td>
</tr>
<tr>
<td>miR184</td>
<td>Localized to the germ cells of mouse testis</td>
<td>Ncor2</td>
<td>Promotes the proliferation of a germ cell line, GC-1spg</td>
<td>Marcon et al. (2008) and Wu et al. (2011)</td>
</tr>
<tr>
<td>miR24</td>
<td>Pachytyne spermatocytes</td>
<td>MBD6 and H2AX</td>
<td>Potential role in meiosis</td>
<td>Marcon et al. (2008)</td>
</tr>
<tr>
<td>miR214</td>
<td>Mainly expressed in pachytyne spermatocytes</td>
<td>WDTC1, heat shock proteins</td>
<td>Potential role in meiosis</td>
<td>Marcon et al. (2008) and Dai et al. (2011)</td>
</tr>
<tr>
<td>miR320</td>
<td>Expressed in all germ cells</td>
<td>Protocadherins</td>
<td>Cell adhesions</td>
<td>Marcon et al. (2008) and Dai et al. (2011)</td>
</tr>
<tr>
<td>miR18</td>
<td>Highly expressed in spermatocytes</td>
<td>HSF2</td>
<td>Male germ cell maturation</td>
<td>Bjork et al. (2010)</td>
</tr>
<tr>
<td>miR122a</td>
<td>Enriched in late-stage male germ cells</td>
<td>TNP2</td>
<td>Regulates the chromatin remodeling, plays a role in spermiogenesis</td>
<td>Yu et al. (2005), Yu &amp; Hecht (2008) and Liu et al. (2013b)</td>
</tr>
<tr>
<td>miR469</td>
<td>Pachytyne spermatocytes and round spermatids</td>
<td>TP2 and PRM2</td>
<td>Regulates the chromatin remodeling, plays a role in spermiogenesis</td>
<td>Dai et al. (2011)</td>
</tr>
<tr>
<td>miR355</td>
<td>Up-regulated in adult testis</td>
<td>Rsbn1</td>
<td>Transcriptional regulation in haploid germ cells</td>
<td>Yan et al. (2007) and McIver et al. (2012a)</td>
</tr>
<tr>
<td>miR181b</td>
<td>Up-regulated in adult testis</td>
<td>Rsbn1</td>
<td>Transcriptional regulation in haploid germ cells</td>
<td>Yan et al. (2007) and McIver et al. (2012a)</td>
</tr>
<tr>
<td>miR181c</td>
<td>Up-regulated in adult testis</td>
<td>Sox5, Sox6, and Rsbn1</td>
<td>Transcriptional regulation in haploid germ cells</td>
<td>Yan et al. (2007) and McIver et al. (2012a)</td>
</tr>
<tr>
<td>miR185</td>
<td>Preferentially expressed in pachytyne spermatocytes</td>
<td>RHOA and CDC42</td>
<td>Cell cycle regulator</td>
<td>Marcon et al. (2008)</td>
</tr>
<tr>
<td>miR191</td>
<td>Highly expressed in tests, preferentially expressed in beta pachytyne spermatocytes, down-regulated in teratozoospermia</td>
<td>BNC2</td>
<td>Required for normal sperm morphology</td>
<td>Marcon et al. (2008)</td>
</tr>
</tbody>
</table>

GC, germ cell; mGSCs, male germline stem-cell; SSCs, spermatogonial stem cells.

Epigenetics of miRNA in spermatogenesis and TGCTs

Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence and is an important mechanism that underlies the ability of environmental chemicals to influence health and disease (Singh & Li 2012). The association of DNA methylation, histone modification, and miRNA with environmental exposure and heritable phenotypes has been widely established. Cigarette smoke, one of the most prevalent and significant global chemical carcinogens, induces differential miRNA expression in the spermatozoa of smokers compared with non-smokers. These altered miRNAs predominantly mediate pathways...
vital for healthy sperm and normal embryonic development, particularly in cell death (Marczylo et al. 2012). Epigenetics can also affect miRNA expression through DNA methylation and histone modifications and can inversely regulate epigenetic inheritance through DNA methylation transferase, thereby maintaining DNA methylation levels and altering histone modifications (Sato et al. 2011, Liu et al. 2013a, Udali et al. 2013, Nana-Sinkam & Choi 2014). For example, expression of miR34b and let-7a-3 is regulated by DNA methylation (Lu et al. 2007, Toyota et al. 2008), and miRNAs with tumor suppressor functions are often silenced by DNA hypermethylation, leading to tumor formation. miR199a expression, which is significantly lower in TGCTs compared with normal testicular germ cells, is controlled by epigenetic changes such as DNA methylation and histone modification; demethylation with 5-aza can restore miR199a expression (Gu et al. 2013).

### Future perspectives

The most current miRNA research has demonstrated the biological significance of these molecules in the regulation of spermatogenesis and TGCTs. There are a large number of miRNAs and their expression during spermatogenesis is phase or cell specific. In other words, some miRNA expression differs from SSCs, and premeiotic, meiotic, post-meiotic, and spermatid cells. The differential expression pattern of these miRNAs demonstrates that they play a regulating role in a specific phase during spermatogenesis. In addition, studies demonstrate that some serum miRNAs could act as biomarkers to detect germ cell tumors. However, future studies...
concerning the stage- and cell-specific expression of miRNAs during spermatogenesis and the potential roles of miRNA in TGCT occurrence are still required for addressing more thoroughly. The in-depth study of miRNAs in testes would lead to a better understanding of the etiology of male infertility and testicular cancer and would probably result in the design and development of a number of biological agents for the diagnosis and treatment of male infertility and testicular cancer. This would lead to an improved quality of life, ultimately resulting in enormous economic and social benefits.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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