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 Apaired (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 without further processing (Fig. 1; Okamura et al. 2007, Babiarz et al. 2008, Rosa & Brivanlou 2011, Havens et al. 2012). Increasing evidence has revealed that miRNAs are involved in various biological processes including embryonic development (Yi et al. 2008, Foshay & Gallicano 2009), cell proliferation (Brennecke et al. 2003, Lee et al. 2005), cell differentiation (Chen et al. 2004, Foshay & Gallicano 2009), apoptosis (Ambros 2003, Xu et al. 2003), fat metabolism (Xu et al. 2003), and oncogenesis (Calin et al. 2002, 2004, Esquela-Kerscher & Slack 2006, Blakaj & Lin 2008, Manikandan et al. 2008).

microRNA overview

microRNAs (miRNAs), small non-coding RNAs, play regulatory roles by repressing translation or cleaving RNA transcripts (He et al. 2009, McIver et al. 2012a, b). The first miRNA gene, lin4, was discovered in 1993 and is involved in the normal temporal control of diverse postembryonic developmental events in Caenorhabditis elegans (Lee et al. 1993, Wightman et al. 1993). Subsequently, several hundred miRNAs have been identified in animals and plants. Most of our knowledge of miRNAs comes from the study of canonical miRNAs. In brief, miRNAs are transcribed as longer stem-loop precursors, termed pri-miRNAs, which are further recognized and cleaved by two endonucleases, Drosha/DGCR8 and Dicer, to yield a double-stranded RNA of 21–22 nucleotides (Hutvagner et al. 2001, Carmell & Hannon 2004, Kim et al. 2009). Subsequently, one strand of the duplex is incorporated into the RNA-induced silencing complex, which contains argonaute proteins, and is guided to target sequences by base-pairing (Liu et al. 2004), resulting in degradation of target mRNAs or repression of their translation (Lai 2002, Carrington & Ambros 2003, Kim et al. 2009). The nuclear biogenesis of non-canonical microRNAs appears to bypass Drosha cleavage; rather, upstream processing is performed by splicing machinery and the lariat-de-branching enzyme, which yields pre-miRNA-like hairpins that are directly suitable for Dicer cleavage without further processing (Fig. 1; Okamura et al. 2007, Babiarz et al. 2008, Rosa & Brivanlou 2011, Havens et al. 2012). Increasing evidence has revealed that miRNAs are involved in various biological processes including embryonic development (Yi et al. 2008, Foshay & Gallicano 2009), cell proliferation (Brennecke et al. 2003, Lee et al. 2005), cell differentiation (Chen et al. 2004, Foshay & Gallicano 2009), apoptosis (Ambros 2003, Xu et al. 2003), fat metabolism (Xu et al. 2003), and oncogenesis (Calin et al. 2002, 2004, Esquela-Kerscher & Slack 2006, Blakaj & Lin 2008, Manikandan et al. 2008).
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 microenvironment, 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, Dovere 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 Asingle (As), Apaired (Apr), and Aaligned (Aal) spermatogonia (Dym 1994). The regulation of the balance between self-renewal and differentiation of SSCs determines the lifelong supply of spermatocytes 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>Testis tissue from the immature rhesus monkey, mature rhesus monkey, and mature human</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>Mouse spermatogonia</td>
<td>The miR17–92 and miR290–295 clusters are enriched in the spermatogonia of neonatal mice</td>
<td>Hayashi et al. (2008)</td>
</tr>
<tr>
<td>Human spermatozoa</td>
<td>68 small RNAs have been identified in human spermatozoa</td>
<td>Ostermeier et al. (2005)</td>
</tr>
<tr>
<td>Spermatozoa of patients with varicocele</td>
<td>miR15a was significantly decreased in patients with varicocele compared with the control group</td>
<td>Ji et al. (2014)</td>
</tr>
<tr>
<td>Testes of patients with NOA and normal human testes</td>
<td>154 differentially down-regulated and 19 up-regulated miRNAs were found in testes from NOA patients</td>
<td>Lian et al. (2009)</td>
</tr>
<tr>
<td>Human spermatozoa in patients with different spermatogenic impairments</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>Abu-Halima et al. (2013)</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>
</tbody>
</table>

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, CREM 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). Tgfβ signaling inhibits the second meiotic division in spermatogenesis (Damestoy 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 non-stress conditions, in some cases because of compensatory effects by other functionally related miRNAs (Wu et al. 2014a).

For example, although no discernible 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 EZF–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 the 6-day-old tests, multipotent adult germ cells, and embryonic stem cell</td>
<td>Stat3 and Ccnd1</td>
<td>Zovoilis et al. (2008, 2010) and McIver et al. (2012a,b)</td>
</tr>
<tr>
<td>miR146</td>
<td>Highly expressed in undifferentiated spermatogonia</td>
<td>Me1</td>
<td>Huszar &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></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR21</td>
<td>THY1⁺-enriched undifferentiated spermatogonia</td>
<td>P12</td>
<td>He et al. (2013)</td>
</tr>
<tr>
<td>miR135a</td>
<td></td>
<td></td>
<td>Niu et al. (2011) and Zheng et al. (2011)</td>
</tr>
<tr>
<td>miR302–367 cluster</td>
<td>Primordial germ cells, ES cells</td>
<td>Foxo1</td>
<td>Moritoki et al. (2014)</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 pediatric germ cell tumors</td>
<td>NR2F2</td>
<td>Li et al. (2009)</td>
</tr>
<tr>
<td>miR335</td>
<td></td>
<td></td>
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<tr>
<td>miR367</td>
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</table>

SSCs, spermatogonial stem cells.
Mir34b/Mir34c and Mir449 double knockout (dKO) mice and demonstrated again that these two miRNAs were functionally redundant. The dKO mice were found with severely disrupted spermatogenesis and sterility. The miRNAs that play a regulatory role in spermatocyte meiosis and spermiogenesis can be found in Table 3.

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, Yaniahara 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 subpopulations, 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) Lin28 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-raf 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 LAT52 (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...
miR185 Preferentially expressed in pachytene spermatocytes and spermatids

miR191 Highly expressed in testis, GC, germ cell; mGSCs, male germline stem-cell; SSCs, spermatogonial stem cells.

miR181c Up-regulated in adult testis

Rsbn1

miR34a Up-regulated in mature mouse testis, up-regulated from day 7 to day 14 in mouse testis

miR34b Up-regulated in mature rhesus monkey testis, up-regulated from day 7 to day 14 in mouse testis

miR34c Highly expressed in isolated pachytene spermatocytes and round spermatids

miR184 Localized to the germ cells of mouse testis

miR24 Mainly expressed in pachytene spermatocytes, down-regulated in spermatocytes

miR214 Mainly expressed in pachytene spermatocytes, down-regulated in spermatocytes

miR182a Highly expressed in spermatocytes

miR181b Up-regulated in adult testis

miR181c Up-regulated in adult testis

miR185 Preferentially expressed in pachytene spermatocytes

miR191 Highly expressed in testis, preferentially expressed in beta pachytene spermatocytes, down-regulated in teratozoospermia

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 pre-meiotic, 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

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Table 4 miRNAs implicated in the development of TGCTs.

<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>miR301</td>
<td>SS, YST, teratoma</td>
<td></td>
<td></td>
<td>Gillis et al. (2007)</td>
</tr>
<tr>
<td>miR302 cluster</td>
<td>SE, YST</td>
<td></td>
<td></td>
<td>Gillis et al. (2007), Palmer et al. (2010) and Rosa &amp; Brivanlou (2011)</td>
</tr>
<tr>
<td>miR302d</td>
<td>SE, EC, up-regulated in YST</td>
<td>LATS2, LEFTY1, MLL3, DAZAP2, TNFAIP1, BCL11B, and PLAG1</td>
<td>Oncogene, suppressor of apoptosis</td>
<td>Gillis et al. (2007) and Murray et al. (2010)</td>
</tr>
<tr>
<td>miR21</td>
<td>Highly expressed in SE and SS</td>
<td>P12</td>
<td>Oncogene</td>
<td>Gillis et al. (2007)</td>
</tr>
<tr>
<td>miR155</td>
<td>Highly expressed in SE and SS</td>
<td></td>
<td>Oncogene</td>
<td>Gillis et al. (2007)</td>
</tr>
<tr>
<td>miR19a</td>
<td>Overexpressed in SE and SS</td>
<td>LATS2, LEFTY1, MLL3, DAZAP2, TNFAIP1, BCL11B, and PLAG1</td>
<td>Oncogene</td>
<td>Gillis et al. (2007)</td>
</tr>
<tr>
<td>miR29a</td>
<td>Overexpressed in SE and SS</td>
<td>LATS2, LEFTY1, MLL3, DAZAP2, TNFAIP1, BCL11B, and PLAG1</td>
<td>Oncogene</td>
<td>Gillis et al. (2007)</td>
</tr>
<tr>
<td>miR133a</td>
<td>Down-regulated in SE and SS</td>
<td>POU4F1, MEIS2, and FSCN1</td>
<td>Tumor suppressor</td>
<td>Gillis et al. (2007), Kano et al. (2010) and Qiu et al. (2014)</td>
</tr>
<tr>
<td>miR133b</td>
<td>Teratomas</td>
<td>BCL11A, SOX4, and CXCL12</td>
<td>Skeletal development and cartilage maintenance</td>
<td>Nicolas et al. (2008) and Miyakawa et al. (2010)</td>
</tr>
<tr>
<td>Let7</td>
<td>Teratomas</td>
<td>Lin28</td>
<td>Maintenance of pluripotency</td>
<td>Zhong et al. (2010)</td>
</tr>
<tr>
<td>miR9</td>
<td>Teratomas</td>
<td>Lin28</td>
<td>Tumor suppressor</td>
<td>Zhong et al. (2010)</td>
</tr>
<tr>
<td>miR125a</td>
<td>Teratomas</td>
<td>Lin28</td>
<td>Tumor suppressor</td>
<td>Zhong et al. (2010)</td>
</tr>
<tr>
<td>miR145</td>
<td>Down-regulated in SS</td>
<td>PLACL2, E2F3, SOX9, OCT4, SOS2, KLF4, and FSCN1</td>
<td>Represses pluripotency, tumor suppressor</td>
<td>Gillis et al. (2007), Kano et al. (2010), Port et al. (2011) and Qiu et al. (2014)</td>
</tr>
<tr>
<td>miR146</td>
<td>Down-regulated in SE, SS, and even certain type II tumors, such as EC and teratomas</td>
<td>MAFB</td>
<td>Tumor suppressor</td>
<td>Gu et al. (2013)</td>
</tr>
<tr>
<td>miR199a</td>
<td>Expressed at low levels in TGCTs</td>
<td>ZEB1 and TRKB</td>
<td>Tumor suppressor, inhibitor of cell invasion/migration, control of apoptosis</td>
<td>Radisky 2011</td>
</tr>
<tr>
<td>miR200c</td>
<td>Expressed in EC, SE, and various elements of NS</td>
<td>ZEB1 and TRKB</td>
<td>Tumor suppressor, inhibitor of cell invasion/migration, control of apoptosis</td>
<td>Radisky 2011</td>
</tr>
<tr>
<td>miR367</td>
<td>Expressed at low levels in human ES and EC cells</td>
<td>LATS2, KLF4, RUNX1, SYN1, SMAD6</td>
<td>Maintenance of pluripotency, regulation of gene transcription</td>
<td>Gillis et al. (2007) and Li et al. (2009)</td>
</tr>
<tr>
<td>miR368</td>
<td>SE, EC</td>
<td>LATS2, ZIC4, LEFTY1, DAZAP2, TNFAIP1, BCL11B, and PLAG1</td>
<td>Involved in over-ruling cellular senescence</td>
<td>Gillis et al. (2007), Voorhoeve et al. (2006, 2007) and Gillis et al. (2007)</td>
</tr>
<tr>
<td>miR371–373</td>
<td>SE, EC, overexpressed in malignant pediatric germ cell tumors and cisplatin-resistant cell lines derived from GCTs</td>
<td>LATS2, ZIC4, LEFTY1, DAZAP2, TNFAIP1, BCL11B, and PLAG1</td>
<td>Involved in over-ruling cellular senescence</td>
<td>Gillis et al. (2007), Voorhoeve et al. (2006, 2007) and Gillis et al. (2007)</td>
</tr>
<tr>
<td>miR375</td>
<td>Overexpressed in pediatric YSTs</td>
<td>IGR1R</td>
<td>Tumor suppressor, inhibits metastasis</td>
<td>Murray et al. (2010) and Hudson et al. (2013)</td>
</tr>
<tr>
<td>miR142–3p</td>
<td>TGCT cell lines</td>
<td>IGR1R</td>
<td>Represses PTPN23 expression</td>
<td>Tanaka et al. (2013)</td>
</tr>
</tbody>
</table>

SE, seminomas; NS, non-seminomas; EC, embryonal carcinoma; YST, yolk sack tumors; SS, spermatocytic seminoma; TGCTs, testicular germ cell tumors; GCT, germ cell tumor.


microRNAs and spermatogenesis


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Tong MH, Mitchell DA, McGowan SD, Ewanski R & Grissow MD 2012 Two miRNA clusters, Mir-17~92 (Mirc1) and Mir-106b-25 (Mirc3), are involved in the regulation of spermatogonia differentiation in mice. Biology of Reproduction 86 72. (doi:10.1095/biolreprod.111.096313)


volinia s, calin ga, liu cg, ambs s, cimmino a, petrocca f, visone r, ione m, rolfo c, ferracin m et al. 2006 a microRNA expression signature of human solid tumors defines cancer gene targets. PNAS 103 2257–2261. (doi:10.1073/pnas.0510651103)


voorhoeve pm, le sc, schrier m, gilliss ai, stoop h, nagel r, liu y, van duijse j, drost j, griekspoor a et al. 2007 a genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Advances in Experimental Medicine and Biology 604 17–46.


wu q, song r, ortogero n, zheng h, evanoff r, small cl, griswold md, namekawa sh, royo h, turner jm et al. 2012 the RNAse III enzyme DROSHA is essential for microRNA production and spermatogenesis. Journal of Biological Chemistry 287 25173–25190. (doi:10.1074/jbc.M112.362053)

wu j, bao j, kim m, yuan s, tang c, zheng h, mastick gs, xu c & yan w 2014a two microRNA clusters, miR-34b/c and miR-449, are essential for normal brain development, motile ciliogenesis, and spermatogenesis. PNAS 111 E2851–E2857. (doi:10.1073/pnas.1407777111)

wu j, zhu h, song w, li m, liu c, li n, tang f, mu h, liao m, li x et al. 2014b Identification of conservative microRNAs in Saanen dairy goat testis through deep sequencing. Reproduction in Domestic Animals 49 32–40. (doi:10.1111/rda.12217)


yan n, lu y, sun h, tao d, zhang s, liu w & ma y 2007 a microarray for microRNA profiling in mouse testis tissues. Reproduction 134 73–79. (doi:10.1530/REP-07-0056)


yanaihara n, caplen n, bowman e, seike m, kumamoto k, yim m, stepsen rm, okamoto a, yokota j, tanaka t et al. 2006 unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 9 189–198. (doi:10.1016/j.ccr.2006.01.025)

yang q, hua j, wang l, xu b, zhang h, ye n, zhang z, hu d, cooke hj, zhang y et al. 2013a MicroRNA and piRNA profiles in normal human testes detected by next generation sequencing. PLoS ONE 8 e66809. (doi:10.1371/journal.pone.0066809)

yang qe, racicot ke, kaucher av, oatley mj & oatley jm 2013b microRNAs 221 and 222 regulate the undifferentiated state in mammalian male germ cells. Development 140 280–290. (doi:10.1242/dev.087403)

yi r, poy mn, stoelf m & fuchs e 2008 a skin microRNA promotes differentiation by repressing ‘stemness’. Nature 455 225–229. (doi:10.1038/nature06642)


yu m, mu h, niu z, chu z, zhu h & hua j 2014 miR-34c enhances mouse spermatogonial stem cells differentiation by targeting Nanos2. Journal of Cellular Biochemistry 115 232–242. (doi:10.1002/jcb.24655)

zhang s, yu m, liu c, wang l, hu y, bai y & hua j 2012 miR-34c regulates mouse embryonic stem cells differentiation into male germ-like cells through RA-R. Cell Biochemistry and Function 30 623–632. (doi:10.1002/cbf.2922)

zheng j, xue h, wang t, jiang y, liu b, li j, liu y, wang w, zhang b & sun m 2011 miR-21 downregulates the tumor suppressor P12 CDK2AP1 and stimulates cell proliferation and invasion. Journal of Cellular Biochemistry 112 872–880. (doi:10.1002/jcb.22995)

zhong x, li ni, liang s, huang q, coukos g & zhang l 2010 Identification of microRNAs regulating reprogramming factor LIN28 in embryonic stem cells and cancer cells. Journal of Biological Chemistry 285 41961–41971. (doi:10.1074/jbc.M110.169607)

zoviliis a, nolte j, drusenheimer n, zechner u, hada h, guan k, hasenfuss g, nayernia k & engel w 2008 Multipotent adult germline stem cells and embryonic stem cells have similar microRNA profiles. Molecular Human Reproduction 14 521–529. (doi:10.1093/molehr/gan144)

zoviliis a, pantazi a, smorag l, opitz l, riester gs, wolf m, zechner u, holubowska a, stewart cl & engel w 2010 Embryonic stem cell-related miRNAs are involved in differentiation of pluripotent cells originating from the germ line. Molecular Human Reproduction 16 793–803. (doi:10.1093/molehr/gan053)

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