MOV10L1 in piRNA processing and gene silencing of retrotransposons during spermatogenesis

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Abstract

Piwi-interacting RNAs (piRNAs) are a broad group of non-coding small RNAs with important biological functions in germline cells. It is well known that piRNAs can maintain genome integrity via silencing retrotransposons. Previous studies on the animal models harboring gene deletions have shown that the genes involved in piRNA biogenesis and their defective expression can result in the spermatogenic dysfunction. In the past decade, significant progress has been achieved for piRNAs and their roles in male germ cells. This review addresses the advances on piRNAs and piRNA biogenesis-associated genes, with a particular focus on the Moloney leukemia virus 10-like 1 (MOV10L1) gene, whose role in primary piRNA processing and in the ‘ping–pong’ cycle during secondary piRNA processing has been illustrated. The biological characteristics of piRNA has been summarized, and emphasis was laid on the roles of MOV10L1 in the mediation of piRNA biogenesis and retrotransposons silencing by DNA methylation. Furthermore, the association between MOV10L1 gene polymorphisms and complete maturation arrest in men has been discussed. Hence, thorough literature review was conducted in order to obtain a greater understanding of the function of MOV10L1 and its mechanisms underlying spermatogenesis in mice and humans.

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Introduction

Germ cells are highly special cells because they can transmit genetic information across generations. They are set apart from somatic cells during the early stages of embryogenesis. Germline stem cells (GSCs) are the foundation for the generation of haploid gametes, i.e., sperms and oocytes for reproduction. Although it remains controversial whether mammalian females have GSCs, it is well known that GSCs are present in the testes of male mammals to generate sperm throughout their lifespan (Yoshida 2010). In Drosophila, however, germ cells of both sexes are continuously produced in adult gonads (Gilboa & Lehmann 2004). Gametogenesis from GSCs is regulated precisely by both genetic and epigenetic factors. Approximately, 45% of the mammalian genome contains transposable elements, and the majority of these elements are retrotransposons. Retrotransposons can be classified into long-terminal repeat (LTR) retrotransposons, non-LTR retrotransposons, e.g., long interspersed element-1 (LINE1), and Alu elements (Jurka et al. 2007). Although transposable elements play a major role in the diversification of transcriptomes, they also pose a potential threat to genome integrity. The P-element-induced wimpy testis (piwi) genes have been shown to be critical for the maintenance of GSCs (Cox et al. 1998).

Piwi-interacting RNAs (piRNAs) are a broad group of 26–31 nt non-coding small RNAs, which are abundantly expressed in animal gonads (Aravin et al. 2007). They were first discovered in Drosophila and appeared to serve as an endogenous defense mechanism against transposons by silencing gene expression (Aravin et al. 2003). Most small RNAs have a 5' monophosphate and 3' hydroxyl ends, although in some cases, such as in plant siRNAs and microRNAs (miRNAs) or Drosophila siRNAs, the 3' terminal nucleotide is modified with a 2'-O-methyl marker, which is a universal feature of all piRNAs examined to date (Horwich et al. 2007). A characteristic feature in all piRNAs encountered is the presence of repeat-derived sequences. Intergenic regions and genic transcripts also contribute to the piRNA pool (Aravin & Bourc'his 2008, Brennecke et al. 2008).

In Drosophila, piRNAs are expressed in both male and female germelines (Lin & Spradling 1997), whereas they are found to be expressed only in the male gonads in mice (Aravin et al. 2007). The piRNAs that map to retrotransposons have been shown to engage a
‘ping–pong’ amplification loop to suppress retrotransposon activity (Gan et al. 2011). Previous reviews (Klattenhoff & Theurkauf 2008, Yadav & Kotaja 2014) indicated that piRNAs play an essential role in the maintenance of DNA integrity in germline cells.

**Mutations in the piwi genes**

Eukaryotic Argonaute (Ag0) proteins can be classified into three different subfamilies, including the Ag0, P-element-induced wimpy testis (Piwi), and worm-specific Ag0 (Wago) (Yigit et al. 2006). The members of the Ag0 clade are found in almost all organisms, ranging from prokaryotes to eukaryotes. The Ag0 proteins are ubiquitously expressed and they bind to miRNAs and siRNAs (Meister & Tuschl 2004). On the other hand, the members of the Piwi clade are found exclusively in animal gonads and they are associated exclusively with piRNAs (Aravin et al. 2006, Girard et al. 2006, Lau et al. 2006, Gunawardane et al. 2007). Ago proteins consist of four domains, i.e., N-terminal, PAZ, MID, and PIWI (Ma et al. 2005, Barneza et al. 2012).

In Drosophila, Piwi alone is expressed in gonadal somatic cells (Malone et al. 2009). Lin & Spradling (1997) induced mutations in the piwi gene, and they found that Drosophila individuals with mutations in the piwi gene had a variety of gonadal defects. The ovarioles were found devoid of germline cells, and the mutant testes contained very few bundles of mature sperm without other male germ cells (Lin & Spradling 1997).

**Association between piRNAs and PIWI proteins**

Several studies have reported an association between piRNAs and the Piwi clade of Ag0 proteins. Aravin et al. (2006) showed that the Piwi family of Ag0 proteins is essential for germ and stem cell development in invertebrates. In mice, the Piwi clade consists of Miwi2, Mili, and Miwi. Among them, Miwi is expressed in perinatal germ cells, Miwi2 is detected in fetal and perinatal germ cells (Zheng & Wang 2012), and Mili is present only in pachytcne spermatocytes and round spermatids in the adult testes (Grivna et al. 2006a). Aravin & Bourc’his (2008) discovered an endogenous piRNA-based defense system that silences retrotransposons in germ cells through a combination of both transcriptional and post-transcriptional mechanisms.

Evidence from previous studies in mice has shown that Mili and Miwi are required for spermatogenesis in mice (Carmell et al. 2007). After mouse PIWI proteins were found to bind to a class of small RNAs, all PIWI-associated small RNAs, including repeat-associated siRNAs (rasiRNAs), were designated as piRNAs. The silencing of transposable elements has been considered to occur by the de novo DNA methylation of their regulatory regions (Aravin et al. 2006).

**Mutations in the piRNA pathway genes lead to germline-specific defects**

The Piwi genes and the piRNA biogenesis-associated genes have been shown to be expressed at various stages of germ cell development in the testis (Pillai & Chuma 2012). In mouse, PIWI proteins are expressed throughout spermatogenesis, MIWI2 was detected only in embryonic stages (Aravin & Bourc’his 2008), while Mili was reported to localize in all stages but to be enriched in spermatocytes and MIWI was found from meiotic pachytcne germ cells (Deng & Lin 2002, Kuramochi-Miyagawa et al. 2004). Moloney leukemia virus 10-like 1 (MOV10L1) was clearly expressed in pachytcne spermatocytes but absent in post-meiotic spermatids (Frost et al. 2010). Shoji et al. (2009) detected that Tudor-domain-related protein 9 (TDRD9) was expressed in mitotic spermatagonia, meiotic spermatocytes, haploid spermatids in the mouse testis. By immunofluorescence, MAEL was found to be expressed in spermatocytes and round spermatids (Soper et al. 2008) and MVH was detected to be expressed in the male germ cells from E10.5 to around spermatid (Kuramochi-Miyagawa et al. 2010). Just like most members of the TDRD family, Tdrd12 was found to play an essential role for spermatogenesis and to be expressed in meiotic spermatocytes, postmeiotic round spermatids, and elongating spermatids in mouse testis (Pandey et al. 2013). Studies on animals that harbor these genes’ deletions have consistently shown that the defective expression of one or more of the genes associated with the piRNA pathway result in the development of infertility.

Recently, Miwi has been found to be exclusively expressed in mouse testes and essential for spermatogenesis (Deng & Lin 2002). Aravin & Bourc’his (2008) reported that a mutation in the Mili and Miwi2 genes led to the elimination of transposable elements in DNA methylation and sterility in male rats. Another Piwi pathway gene, namely Mael, was found to be vital for the correct differentiation of GSC in Drosophila and vertebrates (Pek et al. 2009). It has been indicated that Miwi and Mili were both essential for the meiotic process and play a significant role in controlling transposons in the male germline (Kuramochi-Miyagawa et al. 2008). Similar to the case observed in flies, mutations in the Miwi2 gene resulted in the accumulation of DNA damage (Carmell et al. 2007). Kuramochi-Miyagawa et al. (2004) conducted a study on Mili-deficient mice and found that Miwi played an important part in the timing of this de novo methylation of the LINE1 and IAP regulatory regions. Houwing et al. studied the Piwi pathway proteins Zwi and Zili in zebrafish. In their study, Zili was found to be present with subcellular localization at all stages during germ cell differentiation and has a dynamic distribution in the nucleus and cytoplasm. Both Zili and Zwi were found...
to bind to piRNA populations. Zili played a vital role in meiosis, and eggs of female zebrafish with mutations in the zili gene displayed defects in the meiotic process. Interestingly, zili<sup>-/-</sup> germ cells did not undergo female development, and zili<sup>-/-</sup> fish are always phenotypically male (Houwing et al. 2008). To date, however, the molecular mechanisms that underlie piRNA-induced DNA methylation remain to be elucidated. Piwi-piRNA complexes are localized in the nucleus, and piRNA protein has been found to be localized in the nucleus in Drosophila (Brennecke et al. 2007), reflects a potential role of piRNA proteins in the regulation of DNA transcription and cell cycle progression.

**Genes involved in piRNA generation**

Numerous genes are involved in the generation of piRNAs. In particular, Piwi proteins are required for their biogenesis and function. Piwi proteins interact with armitage (armi) and zucchini (zuc) in Drosophila to regulate piRNA biogenesis (Olivieri et al. 2010, Saito et al. 2010). In addition, genes such as mov10, Spindle-E (spr-E), and Maelstrom (mael) have been implicated in the biogenesis of piRNAs (Meister et al. 2005, Soper et al. 2008, Patil & Kai 2010). The depletions of Piwi, Zuc, and Arm levels in the cells have been found to reduce the piRNA levels. There are two piRNA biogenesis pathways, known as the primary processing pathway and the ping–pong amplification cycle, as we illustrated in Fig. 1. Tudor-domain-containing proteins or TDRDs have been increasingly studied in relationship with Piwi proteins (Chen et al. 2009). These proteins belong to the Tudor protein family and have also been found to play a key role in piRNA biogenesis.

**Generation of piRNAs**

Two pathways have been proposed for the generation of piRNAs. In the first pathway, termed the primary pathway, antisense piRNA precursor transcripts are processed by Zuc, a putative endonuclease (Nishimasu et al. 2012). The resulting products have an U at the first nucleotide position, and are loaded on Piwi or Aub (Klattenhoff & Theurkauf 2008). The 3’ end of this product is then trimmed and methylated to obtain mature piRNAs (Brennecke et al. 2007). On the whole, piRNAs tend to have a very strong sequence bias toward Uridine (77.6%) at their 5’ end (Grivna et al. 2006b), which suggests that they may be generated by Dicer-like cleavage. As Dicer produces 21- to 22-nt products from double-stranded precursors and the length of the piRNAs ranges from 26 to 31 nt, they may be produced via a Dicer-independent mechanism (Klattenhoff & Theurkauf 2008).

In the second pathway, namely the ‘ping–pong’ pathway, Ago3 binds to sense-strand piRNAs, which catalyzes the cleavage of the antisense strand at an A:U base pair. This in turn leads to the generation of the 5’ end of antisense piRNA. The 5’ ends of the resulting cleavage products are then loaded on to Aub or Piwi, and trimmed and methylated at the 3’ end to yield the mature antisense piRNA. Piwi-antisense piRNA complexes enter the nucleus, thus silencing gene expression and producing sense-strand piRNAs and antisense strand piRNA precursors that exported from the nucleus to nuage. The mature antisense piRNA–Ago3 complexes are then proposed to bind to and cleave sense-strand RNAs (Brennecke et al. 2007, Klattenhoff & Theurkauf 2008). A model for ping–pong cycle is shown in Fig. 2.

**Role of Mov10l1**

The armitage gene encodes a homolog of SDE3, an RNA helicase that is involved in RNAi in Arabidopsis (Tomari et al. 2004). A previous study showed that the putative RNA helicase encoded by armitage is vital for the Piwi function in Drosophila (Malone et al. 2009). Mice and humans have two genes that are analogous to the armitage gene of Drosophila, namely MOV10 and MOV10L1 in human (Wang et al. 2001, Zheng et al. 2010). Frost et al. analyzed the expression levels of these two genes in mouse testis postnatally during the development of the first spermatogenic waves. The expression pattern of Mov10l1 was found to be similar to that of Mili, another murine Piwi gene that has been found vital for piRNA synthesis and function. The study in a mice model has demonstrated that there were
Male mice with the Mov10l1 allele exhibited normal fertility. This suggests that the Mov10l1 gene is vital for fertility in male mice (Zheng et al. 2010). To date, no any correlation studies can explain that the Mov10l1−/− mice is still fertile.

In female mice, TAF4B is a gonad-enriched subunit of the TFIID complex required for fertility (Voronina et al. 2007). Lovasco et al. reported the development of premature reproductive senescence in young Taf4b-null female mice. In brief, they evaluated the levels of gene expression in these mice in comparison with WT controls. Interestingly, Mov10l1 was found to be expressed in mouse oocytes, and their expression levels were significantly reduced in the Taf4b-null mouse (Lovasco et al. 2010). Although the Mov10l1−/−; mutants contained the reduced transcription levels of the Mili protein, they also lacked piRNAs binding to this protein. In other words, Mov10l1 was found to be indirectly associated with piRNAs and play a major role in the process of piRNA biogenesis by interacting with the above-mentioned PIWI proteins (Zheng et al. 2010). The importance of MOV10L1 has been documented in patients at risk of azoospermia. In the group of cryptorchid boys at high risk of infertility, the expression of DDX4, MAEL, MOV10L1, PIWIL2, PIWIL4, and TDRD9 was impaired, which indicated that gene instability induced by impaired expression of transposon silencing genes contribute to the development of azoospermia (Hadziselimovic et al. 2011). The MOV10L1 gene is thus named because it is homologous to MOV10. The effect of Mov10l1 inactivation on two retrotransposons, LINE1 and IAP, were shown in mice testes. In brief, both LINE1 and IAP transcripts increased dramatically in Mov10l1−/− testes (Zheng et al. 2010).

Apart from the Mov10l1 transcript, two smaller transcripts, namely Champ and Csm, have been described previously. Liu et al. reported a novel MEF2C-dependent gene that encodes a cardiac-restricted protein. This gene, designated Champ (for cardiac helicase activated by MEF2 protein), contained seven conserved motifs which are characteristic of helicases involved in RNA processing, DNA replication, and transcription. Champ plays a role in a cardiac-specific regulatory pathway for RNA processing and/or transcriptional control (Liu et al. 2001). Ueyama et al. discovered a Mov10l1 isoform that encodes a putative RNA helicase, which was downregulated in the hearts of Nkx2.5-null mice embryos. This gene was designated Csm (for cardiac-specific isoform of Mov10l1), and it was found to be identical to 3′ region of the Mov10l1 gene (Ueyama et al. 2003). Thus, as outlined in the previous section, a considerable body of evidence indicates that MOV10L1 is vital for the generation of piRNAs. A previous study has investigated the association between MOV10L1 and male infertility in humans. Sarkaradeh et al. conducted a PCR single-strand conformation polymorphism (PCR-SSCP) study on 30 infertile men with complete maturation arrest in their...
spermatocyte levels. Their results suggest that MOV10L1 gene polymorphisms may be linked to infertility in men with complete maturation arrest (Sarkardeh et al. 2014).

Thus far, the function of Piwi–piRNA complexes has not been fully elucidated. However, some studies on the complexes have suggested that they may play a role in the epigenetic regulation of transposable elements in GSCs (Brennecke et al. 2008, Kuramochi-Miyagawa et al. 2008). Deep sequencing of piRNA libraries led to the revelation that piRNAs have a very complex nature of sequences, corresponding to several million individual piRNA clusters as opposed to miRNA clusters, which are numbered in the hundreds (Grivna et al. 2006b, Aravin & Bourc’his 2008). Lee et al. (2011) previously reported the presence of piRNAs in the mice neurons. In another study, neuronally expressed piRNAs were subjected to deep sequencing and validation by Northern blotting. In that study, a total of 372 distinct piRNA clusters were identified from the CNS tissue of Aplysia californica (Rajaseethapathy et al. 2012). Serotonin exposure increased the methylation of Cpg islands in the CREB2 promoter, which leads to long-term downregulation of the CREB2 RNA and protein levels. They studied the regulatory role of these piRNAs in this memory-related synaptic plasticity by determining changes in the expression levels of these piRNAs followed by serotonin exposure. Interestingly, their findings revealed that Piwi–piRNA complexes actively demethylated the CREB2 promoter region in A. californica neurons.

Summary

The piRNAs have a wide range of functionality and are not always easy to identify; therefore, they have not been thoroughly studied beyond their functions in germ cells. Numerous studies have found the associations between piRNA biogenesis genes and fertility; however, the exact mechanism of action of Piwi–piRNA complexes remains to be elucidated. Moreover, the mechanism of piRNA-associated gene silencing also remains to be determined. Overall, based on all the reviewed studies, it has been well established that MOV10L1 is vital for the generation of piRNAs and spermatogenesis. As mentioned previously, some studies have humans have also reported an association between MOV10L1 gene polymorphisms and infertility (Sarkardeh et al. 2014). This may indicate that the MOV10L1 gene mutations with loss-of-function may cause male infertility in human. Although MOV10L1 is also expressed as a cardiac-specific isoform in the heart and plays a role in a cardiac-specific regulatory pathway for piRNA processing and/or transcriptional control, full-length Mov10l1 seems to be specifically found in the mouse male germline and Mov10l1<sup>−/−</sup> mice impaired only spermatogenesis as we referred to above (Frost et al. 2010). Studies in mice have identified the presence of piRNAs in neurons and play a role in spine morphogenesis (Lee et al. 2011). Thus, on the basis of the reviewed evidence, it can be proposed that piRNAs play much broader roles than it have been previously appreciated. A study by Hadziselimovic et al. (2011) reported the lack of expression of MOV10L1 in cryptorchid boys at high risk of developing azoospermia. The lower level of testosterone and free-androgen was observed in cryptorchid infants, indicating insufficient gonadotropin and testosterone stimulation (Pierik et al. 2009). Thus, mutations in the MOV10L1 gene may be linked to low gonadotropin levels in humans. Although there are interesting hints supporting it, further studies still need to be confirmed on molecular details of MOV10L1 gene’s function in hormone secretion. Therefore, several questions remain to be answered before MOV10L1 obtain a greater insight into its function, and we also believed that more and more researchers will dedicate to the study of MOV10L1 and other piRNA-associated genes functions.

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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