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 (Girard *et al.* 2006). 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 germlines (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 (Ago) proteins can be classified into three different subfamilies, including the Ago, P-element-induced wimpy testis (Piwi), and worm-specific Ago (Wago) (Yigit *et al.* 2006). The members of the Ago clade are found in almost all organisms, ranging from prokaryotes to eukaryotes. The Ago 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, Bamezai *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 Ago proteins. Aravin *et al.* (2006) showed that the Piwi family of Ago proteins is essential for germ and stem cell development in invertebrates. In mice, the Piwi clade consists of *Miw2*, *Mili*, and *Miwi*. Among them, *Miwi* is expressed in perinatal germ cells. *Miw2* is detected in fetal and perinatal germ cells (Zheng & Wang 2012), and *Miwi* is present only in pachytene spermatocytes and round spermatids in the adult testes (Grivna *et al.* 2006a). Aravin & Bourc'h (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, *Miw2* was detected only in embryonic stages (Aravin & Bourc'h 2008), while *Mili* was reported to localize in all stages but to be enriched in spermatocytes and *Miwi* was found from meiotic pachytene germ cells (Deng & Lin 2002, Kuramochi-Miyagawa *et al.* 2004). Moloney leukemia virus 10-like 1 (MOV10L1) was clearly expressed in pachytene 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 spermatogonia, 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'h (2008) reported that a mutation in the *Mili* and *Miw2* 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 *Miw2* 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 *Mili* 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 Ziwi 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 Ziwi 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*^{-/-} germ cells did not undergo female development, and *zili*^{-/-} 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 (*spn-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 Uracil (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

Figure 1 A model for the role of MOV10. HSPA2 couples with MOV10L1 and then MOV10L1 interacts with PIWI protein (MIWI), which can help pre-piRNAs load onto a PIWI protein. After pre-piRNAs associating with a PIWI protein (MIWI), target RNAs are cleaved to generate secondary piRNAs. MIWI2, TDRD9, and MEAL act together and generate pi-body components. PiRNA-bound MIWI2 enters nucleus to regulate DNA methylation of transposon loci. The green circular arrows indicate intra-Piwi ping-pong.

Figure 2 A model for ping-pong cycle. Ago3 binds to sense-strand piRNAs and produces Ago3-piRNA complexes, which cleave the antisense-strand transcript precursors, producing antisense-strand piRNAs. Piwi and Aub bind to antisense-strand piRNAs and generate Piwi-antisense piRNA complexes and Aub-antisense-strand piRNA complexes. Aub-antisense-strand piRNA complexes target the cleavage of sense transcripts, producing the sense-strand piRNAs that load onto Ago3. Piwi-antisense piRNA complexes enter the nucleus, which results in silencing gene expression and producing sense-strand piRNAs and antisense-strand piRNA precursors.

associations between *Mov10l1* and *Mili*, *Miwi*, and *Miwi2* (Frost *et al.* 2010). The *Mili* and *Miwi* genes are important for the synthesis of piRNAs, as evidenced by the knockdown mutations in these genes, which result in the inhibition of piRNA synthesis (Kuramochi-Miyagawa *et al.* 2004). Moreover, purification of MOV10L1 has revealed the presence of piRNA (Zheng *et al.* 2010).

Liu *et al.* (2001) revealed that the putative RNA helicase encoded by differently sized transcripts in testis (exons 1–26) and heart (exons 15–26). The testis-specific isoform has also been referred to as MOV10L1 (Meister *et al.* 2005), which has been identified to be expressed in the germ cells in mice (Zheng *et al.* 2010). This gene encodes a putative RNA helicase; however, little is known about this helicase and its function. The N-terminal portion of MOV10L1 is not homologous to that in any other mouse proteins, and the C-terminal RNA helicase region of this protein exhibits very low levels of homology (45% amino acid identity) with MOV10. *Mov10l1* was found to be primarily expressed in pachytene spermatocytes. To generate *Mov10l1*^{−/−} mice, the exon that encoded the putative helicase domain was flanked by LoxP sites, and a global deletion of *Mov10l1* was achieved by breeding *Mov10l1*^{+/fl} mice to CAG-Cre transgenic mice. Using these mutant mice, Frost *et al.* (2010) discovered that *Mov10l1* was essential for the piRNA-dependent repression of retrotransposons. Male mice with the *Mov10l1*^{−/−} genotype were sterile due to the defective role of this protein in meiosis, both MILI and TDRD1 were found to be expressed in spermatogonia but not in spermatocytes in *Mov10l1*^{−/−} testis, thus leading to a sharp reduction in testicle size and weight. Female mice with the same genotype

exhibited normal fertility. This suggests that the *Mov10l1* gene is vital for fertility in male mice (Zheng *et al.* 2010). To date, not 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 the 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. Sarkardeh *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* (Rajasethupathy *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 on 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*^{-/-} 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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