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Novel dimensions of piRNAs in cancer

Yuping Mei[1], **David Clark**[2], and **Li Mao**[1]

[1]Department of Oncology and Diagnostic Sciences, University of Maryland School of Dentistry

[2]Marlene and Stewart Greenebaum Cancer Center, University of Maryland, 650 W Baltimore St, Baltimore, MD, 21201, USA

Abstract

Piwi-interacting RNAs (piRNAs), a newly identified class of small non-coding RNAs, direct the Piwi-dependent transposon silencing, heterochromatin modification and germ cell maintenance. Owing to our limited knowledge regarding their biogenesis, piRNAs are considered as the most mysterious class of small regulatory RNAs, particularly in pathogenesis such as tumorigenesis. Recently, several lines of evidence have emerged to suggest that piRNAs may be dis-regulated and play crucial roles in tumorigenesis in previously unsuspected ways. In this prospective piece, we will discuss the emerging insights into the potential novel roles of piRNAs in carcinogenesis and highlight their potential implications in cancer detection, classification and therapy.

Keywords

PIWI-interacting RNAs; cancer; diagnosis; therapy

1. Introduction

It has been recently noted that the genomes of all studied eukaryotes are transcribed almost entirely. In fact, >90% of the human genome is likely to be transcribed; however, only ~2% of this is subsequently translated [1,2]. The remaining regulatory noncoding RNAs (ncRNAs) can be roughly categorized into large and small ncRNAs, which mainly include small interfering RNA (siRNA), microRNA (miRNA), small nucleolar RNA (snoRNA) and PIWI-interacting RNA (piRNA). Among these small regulatory ncRNAs, piRNAs are the newest class and the least investigated.

The first hint indicating that piRNAs exist was the study that *Stellate* protein-coding gene repeats were silenced in the *Drosophila melanogaster* germ line [3], and subsequently, repeat-associated small interfering RNAs (rasiRNAs) derived from repetitive genomic elements were found in the *D. melanogaster* testes and early embryos [4]. However, it was not known, at that time, that these small non-coding RNAs were, in fact, piRNAs. In 2006, a class of small RNAs named piRNAs binding to Argonaute proteins in mammalian germ line

Corresponding author: Li Mao, M.D., Department of Oncology and Diagnostic Sciences, University of Maryland School of Dentistry, 650 W Baltimore St, Baltimore, MD, 21201, USA; lmao@umaryland.edu.

Conflict of Interest Statement
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cells, was purified [5–8]. piRNAs have been shown to act mainly in the Piwi-dependent transposon silencing, heterochromatin modification, and in germ cell maintenance [9–12]. Piwi proteins play a key role in spermatogenesis. For example, Miwi, a Piwi subfamily protein, null mice displayed spermatogenic arrest [13], and piRNAs were found to accumulate at the onset of meiosis or during spermatogenesis [5, 7]; it is easy to understand that piRNAs demonstrated critical regulation in reproduction and fertility [11, 14–18]. However, the expression status and roles of piRNAs as well as other factors involved in functions and mechanisms of piRNAs remain poorly understood in tumorigenesis.

Despite the common assumption that piRNAs play a key role in germline development, recent evidences have suggested that piRNAs had important function in tumorigenesis. For instance, independent studies have shown that aberrantly expressed piRNAs suggested their roles in the process of cancer development [19–21]. Furthermore, one study observed that piRNAs might function as a tumor suppressor in human gastric cancer [22]. Here, we will discuss the emerging insights into the potential roles of piRNAs in carcinogenesis, and the possible applications for cancer diagnosis and treatment.

2. Biogenesis of piRNAs

piRNAs are produced independently of Dicer ribonuclease, which is required for double-stranded precursors to generate 21 to 24 nt small regulatory ncRNAs such as siRNAs and miRNA [23–25]. Unlike siRNAs, piRNAs neither show any phasing within a cluster sequence nor do they overlap with each other [26]; Unlike miRNAs, no significant secondary structures of the stem-loop structures in their precursors have been detected in regions surrounding piRNAs [26, 27]. However, like these extensively studied small regulatory ncRNAs, precursors of piRNAs need further post transcriptional processing to become fully matured.

2.1. Transcription mechanism

Initial analysis of genomic sequences revealed extremely high diversity of piRNAs which have been mapped to a relatively small number of genomic regions called piRNA clusters [5, 6, 10]. Recently, in a series of well-executed compelling experiments, the specific mechanism involved in matured 21U-RNA piRNAs transcription in *C. elegans* was logically and persuasively explained by observing that capped small RNAs (csRNAs) were precursors of piRNAs [28]. There are two types of loci producing csRNAs: type 1 has a feature of CTGTTTCA motif upstream of transcription start site [28, 29], and type 2 locus has no typical motif governing tendency to produce csRNAs. The precursors of csRNAs share some characteristics: (1) ~26nt in length; (2) initiate precisely 2nd nt upstream of mature piRNAs; (3) exhibit a strong bias for a 2 nt motif of pyrimidine (Y) purine (R), or YR; and (4) a U at the csRNA +3 position for piRNA processing or stability [28]. These precursors are then processed into matured piRNAs (Fig.1a), of which with 1U bias in 5’ ends and 2'-O-methylated at their 3' ends.

Consistent with previous observations [9, 10, 30], these csRNAs were mapped to both genomic strands, suggesting bidirectional transcription. Also, single-stranded RNAs were indeed transcribed from piRNA clusters [9, 10, 28, 30]. Different from other observations, these studies did not show that long RNA molecules spanning the whole piRNA cluster were processed into mature piRNAs [10, 31]. One of the possibilities for the difference was that the transcriptional tendency was dependent on specific species. However, additional biochemical experiments are needed to determine the structure and processing of csRNAs caps, and how these caps could be formed or added to 5’ end of the original transcripts. Additionally, it is possible that other motif of loci could govern the tendency to produce precursors other than pol II-based transcription.

2.2. Amplification mechanism

After a primary piRNA is generated, piRNA's accumulation requires amplification by the so-called ping-pong mechanism involving at least two distinct Piwi proteins, a process which occurs in the cytoplasm (Fig. 1b) [10]. This mechanism is similar to secondary siRNA generation [32] in which primary piRNAs recognize their complementary targets: antisense piRNA associates with PIWI/AUB complex, while sense piRNA associates with AGO3 protein. Cleavage of transcript targeted by AUB-bound 1U primary piRNA leads to the generation of the 5' end of new secondary piRNA, which has a 10A bias and then accordingly can be loaded onto AGO3. Finally, piRNA-AGO3 complex binds to the retro-transposon transcript, creating another set of anti-sense piRNA [10, 26, 33–36]. Ping-pong signatures have been identified in *zebrafish* and *D. melanogaster* as well as in very primitive animals such as sponges, suggesting the existence of the ping-pong cycle a somewhat early stage evolutionarily [37].

piRNAs biogenesis during adult spermatogenesis in mice, however, is independent of the ping-pong mechanism [38]. Only Piwi proteins were detectable in the adult and post natal testes and no interaction was observed between Mili and Miwi or piRNA sequence features for the ping-pong mechanism. Additionally, both populations of piRNAs were biased for 5' Uracil but not for Adenine on the 10th nucleotide position, and displayed no complementarity. Furthermore, in Miwi mutants, Mili-associated piRNAs were upregulated, but not downregulated. Together, these results showed that piRNAs biogenesis during adult spermatogenesis in mice did not use ping-pong ball cycle at all.

3. Involvement of piRNAs in cancer development

Cancer cells and germ cells, as well as stem cells, share important biological features such as infinite self-renewal and rapid proliferation. Therefore, it is reasonable to assume that the newly developed cancer cells appropriate the self-renewing division machineries used factors specific for germ cell regulation [39,40]. In fact, studies have shown that many of these transiently expressed genes in developing germcells are proto-oncogenes or oncogenes. For example, cancer/testis antigens (CTAs), a category of tumor antigens whose normal expression was restricted to male germ cells in the testis, were also expressed in various human tumor types including melanoma, breast cancer, bladder cancer, gastric cancer, lung cancer, sarcoma, ovariancancer, prostate cancer and hepatocellular carcinoma, among others [41–44]. Furthermore, CTAs profiles in cancers have been linked to neoplastic phenotype including immortality, invasiveness, immune evasion, and metastatic capacity, thus, CTAs may sever as biomarkers, new targets, and predictors of biochemical recurrence for these cancers [41–44]. These studies are consistent with the concept that tumorigenesis adapts pathways utilized in germ-cell development.

As a class of germline-expressed small regulatory ncRNAs, piRNAs are mainly linked to epigenetic programming, which might confer some of the central characteristics of malignancy.

3.1. piRNAs-associated Piwi proteins in cancer

Piwi proteins have evolutionarily conserved essential roles in germline development, as their mutations in mice, *Drosophila*, and *Zebrafish* can cause defects in gametogenesis [14–17,45].

There are four Piwi proteins expressed in humans: PiwiL1/Hiwi, PiwiL2/Hili, PiwiL3, and PiwiL4/Hiwi2 [46]. The first report of Piwi expression in cancer was in testicular seminomas-tumors originating from embryonic germ cells, which retained a germ cell phenotype [47]. Hiwi was detected in seminomas, but not in non-seminomas, spermatocytic

seminomas, or testicular tumors originating from somatic cells, indicating Hiwi was specifically expressed in both normal and malignant spermatogenic cells in a maturation stage-dependent pattern and might function in cell proliferation [47]. The report on seminomas was followed by reports on a wide variety of cancers, and Piwi protein expression profiles have recently received much attention for their potential functional involvement in a wide variety of human cancers (Table 1), suggesting Piwi proteins may play a role in oncogenesis and may serve as diagnostic and prognostic biomarkers.

3.1.1. Piwi proteins are involved in cancer cell proliferation andapoptosis— Qiao et al. first reported that Piwi protein was overexpressed in testicular seminomas-tumors originating from embryonic germ cells with retention of germ cell phenotype [47]. PiwiL2 silencing could significantly reduce tumor cell proliferation, colony formation but increased apoptosis in vitro, and inhibited tumor growth in vivo [48]. PiwiL2 was reported to be widely expressed in tumors and acted as an oncogene by inhibiting apoptosis and promoting cell proliferation via Stat3/Bcl-X(L) signaling pathway [49], because induction of high-level expression of the anti-apoptotic gene Bcl-X(L) was observed in cells expressing PiwiL2, whereas an increased Bcl-X(L) expression correlated with increase of signal transducer and activator of transcription 3 (STAT3) expression. Additionally, there was a link between PiwiL2 overexpression and reduced apoptosis, enhanced proliferation and induced transformation of fibroblast cells. Furthermore, PiwiL2 inhibition suppressed STAT3 and Bcl-X(L) expression and induced apoptosis [49]. One more insight into the role of Piwil2 in tumorigenesis was that PiwiL2 might reduce apoptosis in tumor cells possessing P53, which was a positive regulator of STAT3 signaling pathway [50]. PiwiL2 can directly bind to STAT3 protein via its PAZ domain and form a PiwiL2/STAT3/c-Src triple protein-protein complex. STAT3 was phosphorylated by c-Src and translocated to nucleus, then bound to P53 promoter and repressed its transcription [50].

3.1.2. Piwi proteins are involved in virus infection, cancer cell metastasis and invasion—The expression of both Hiwi and Hili has been linked to human papilloma virus infection [51, 52]. The expression rate of PiwiL1 was 75% in cervical squamous cell carcinoma (CSCC) and had a statistically positive correlation with HPV16, and the elevated expression of PiwiL1 has been associated with invasion of CSCC, which supports the interaction between HPV16 and host cells the carcinogenesis of CSCC [51].

PiwiL2 can be detected in various stages of human CSCC and adenocarcinomas, and was also detected in some metaplastic epithelial cells, histologically "normal" appearing tissues adjacent to malignant lesions, a typical glandular cells, low-grade and high-grade squamous intra epithelial lesions [52]. Additionally, significantly higher expression levels of PiwiL2 were observed in primary colon cancer tissue and in lymph node metastasis in comparison with normal colon mucosa. Interestingly, its expression significantly correlated with more aggressive clinical and pathological parameters, such as five-year metastasis-free survival and overall survival [53]. PiwiL2 mediated increase of matrix metallopeptidase 9 (MMP9) transcriptional activity has been suggested to cause an increased migration and invasion of cancer cells [53].

3.1.3. Piwi proteins have prognostic value in human cancers—Taubert et al. first showed that an increased expression of Hiwi mRNA was a significant negative prognostic factor for patients with soft-tissue sarcomas (STS), and a high level of its mRNA identified STS patients at high risk of tumor-related death [48]. Another study showed that Hiwi alterations in mRNA expression were associated with an increased risk of tumor-related death in male patients with ductal adenocarcinoma of the pancreas (PDAC) [54].

Increasing evidence showed that the specific prognostic value of a subfamily Piwi protein was variable for different cancers or different stages of cancers. PiwiL1 has been shown as an independent prognostic factor in gastric cancer, and both PiwiL1 and PiwiL2 expression in gastric cancer tissue predicted poorer overall survival [55]. PiwiL2 expression was relatively higher in colorectal carcinomas and has been correlated with various clinic-pathologic parameters and a poor prognosis [56]. Hiwi could be used as a potential molecular marker for pathological diagnosis and prognostic indicator for malignant gliomas [57], whereas in hepato-cellular carcinoma (HCC), Hiwi played a key role in proliferation and metastasis, and could be a potential prognostic factor for HCC after curative resection, particularly with well-differentiated HCC [58]. In colorectal cancer, Hiwi was a potential prognostic biomarker especially for those at early stages or without lymph node metastasis [59].

Additionally, cellular distribution of a Piwi protein influenced its function. Expression level of Hiwi in cytoplasm of esophageal cancer cells was significantly associated with histological grade, clinical stage and poorer clinical outcome. However, there is no correlation between the nuclear Hiwi expression and clinic-pathological features [60]. Interestingly, PiwiL2 expression patterns were different in different stages of breast cancers: the cytoplasm, nucleus or both cytoplasm and nucleus expression patterns were observed in invasive and metastatic breast cancers, while nucleus pattern was less common in breast pre-cancers, indicating that PiwiL2 was expressed in various stages of breast cancers and has the potential to be used a novel biomarker [61].

3.2. piRNAs play a key role in epigenetic regulation for genome programming

All organisms balance the need to maintain genetic variation against the danger of accumulating potentially deleterious genesor pathogenic sequences [62,63]. In *C. elegans* and *Drosophila*, the piRNAs have been shown to be necessary for silencing transposons during germline development by RNA degradation at the post transcriptional level [9, 26, 31, 64]. piRNAs function in silencing transposons by DNA methylation also leaded to transcriptional gene silencing. For example, piRNAs expressed in the central nervous system (CNS) and other somatic tissues in *Aplysia* could mediate CpG methylation and transcriptional silencing of a key plasticity-related gene, CREB2 [65]. These observations indicate that piRNAs have function in epigenetic regulation, as transposons are ubiquitous genome pathogens that can mobilize and induce mutations that alter gene expression, cause disease, and drive evolution [64, 66, 67]. In *Oxytricha*, piRNAs can protect against loss during genome rearrangement [68], showing that piRNAs are powerful trans-generational carriers of epigenetic information for genome programming. The same observation of the piRNAs' function has been further confirmed in multi generational epigenetic memory in *C. elegans* [69], in which piRNAs were found to initiate an epigenetic memory of nonself RNA [70]. Furthermore, piRNAs guide both Piwi and Piwi-associated epigenetic factors to program the genome by binding to numerous piRNA-complementary sequences, which explained that how functions of specific genomic sites were regulated by epigenetic factors [71].

3.3. piRNA regulates its host genes and its interacting proteins

In contrast to the mechanism known for miRNA-mediated modulation of gene expression, piRNA can regulate its host gene expression. The human melatonin receptor 1A gene (MTNR1A) gene, expression of which has been shown altered in prostate cancer cells, is located on the chromosomalregion 4q35.2 with two exons. piR_015520 is in intron 1of MTNR1A gene, 14 kb downstream of the ATG start codon. This piRNA negatively regulated MTNR1A gene expression by binding to its genomic region, as overexpression of piR_015520 resulted in a repression of MTNR1A expression in a concentration-dependent

manner [65]. This finding suggests that changes in individual piRNA levels could influence the expression of the gene in which the piRNA is located, offering a new perspective for piRNAs functioning as gene regulators in humans.

Also, piRNAs have function in regulating its interacting proteins stability. For example, during mouse sperm maturation, its Piwi protein Miwi ubiqutination was regulated by piRNAs through enhancing Miwi interaction with an APC/C substrate-binding subunit [18]. This observation not only identified how piRNAs-interacting protein was regulated by its binding piRNAs, but also expanded our knowledge in piRNAs regulation processes [72].

3.4. piRNAs have oncogenic or tumor suppressor roles in cancer development

Recently, several independent lines of evidence have indicated that the piRNAs were involved in cancer development (Table 2). The relationship between piRNAs and carcinogenesis has been suggested by results of microarray screening, next generation sequencing, and real-time quantitative reverse transcription-polymerase chain reaction analyses. The up-regulated expression of piR-651, piR-4987, piR-20365, piR-20485 and piR-20582 was observed in cancer cell lines and in primary tumors compared to matched non-cancerous tissues [20, 21]. piR-Hep1 was found to be up-regulated in hepatocellular carcinoma tumors compared to their corresponding adjacent non-cancerous liver tissues [73]. On the other hand, the level of piR-823 in gastric cancer tissues was found significantly lower than that in the non-cancerous tissues [21]. These phenomena of dys-regulated piRNA expression implied that piRNA could act as an oncogene or a tumor suppressor in carcinogenesis and these piRNAs might be involved in regulating cancer cell activities in the following ways:

3.4.1. Cell proliferation and viability—The growth of gastric cancer cells such as MGC-803 and SGC-7901was significantly inhibited by a piR-651 inhibitor in a dose-dependent manner [20]. The mechanism of the decreased proliferation ability by the piR-651 inhibitor was that the cells transfected with the piR-651 inhibitor were arrested at G(2)/M phase [20]. However, cell growth was significantly inhibited by piR-823 overexpression in these two cell lines [22], and the effect of growth inhibition by ectopic piR-823 expression was further confirmed by *in vivo* experiments. Both tumor volume and weight were decreased significantly in cells treated with more ectopic piR-823 level, indicating that elevation of piR-823 level resulted in inhibition of tumor formation. Also, in hepatocellular carcinoma cell line HKCI-8, cells transfected with piR-Hep1 inhibitor displayed reduced cell viability by knockdown piR-Hep1 when compared with the control inhibitor [73].

3.4.2. Cell invasion and transwell motility—Cell invasion and trans-well motility decreased when inhibiting piR-Hep1in Hepatocellular carcinoma HKCI-8 cells, whereas overexpression of piR-Hep1 cells could result in profound augmentations on cell migrationin immortalized hepatocyte line (MIHA) cells [73]. These observations suggested that piR-Hep1played a key role in enhancing cells' capability to invade and migrate [73]. Although overexpression of piR-Hep1 in MIHA cells showed no apparent effect on cell viability, the authors thought that functional effects on motility and invasiveness might be more evident in non-tumorigenic cells than in carcinoma cells, as pre-malignant cells displayed a more modest genetic background [73].

3.5. Other possibilities of piRNAs contributing to tumorigenesis

One of the most important functions of piRNAs is to repress transposon, a kind of transposable elements or "jumping genes" which are pieces of DNA that insert themselves into other locations in the genome. These transposable elements of somatic insertions were present in multiple tumor types [74]. There were transposons insertion occurred in genes

that were frequently mutated in cancer, suggesting that transposon actively contributed to the development of cancer by introducing mutations that disrupted gene expression [74–76].

4. piRNAs are potential biomarkers for cancer diagnosis and prognosis

Given the important role of piRNAs and their interacting proteins in diverse cellular processes, defining different expression profiles of cancer type-specific piRNAs should allow development of novel cancer biomarkers. Indeed, we have seen the emergence of certain piRNAs as potential regulators of cancer cell development in the past two years, suggesting that piRNAs can serve as potential markers for cancer diagnosis. For example, the level of piR-651 was associated with tumor-node-metastasis (TNM) stage in gastric cancer [20]; and up-regulated piR-4987 expression was associated with lymph node positivity in breast cancer [21]. These findings suggested that a fraction of piRNAs would be expressed in a highly cancer-specific way, and the quantitative differences in the expression level of these piRNAs may allow determine tissue-origin of cancers. Furthermore, the possible molecular mechanisms by which piRNAs may carry out the regulatory functions are also partially explained: the growth of gastric cancer cells was inhibited by a piR-651 inhibitor and arrested at the G(2)/M phase [20], and Silencing of piRHep1 inhibited cell viability, motility and invasiveness with a concomitant reduction in the level of active AKT phosphorylation in hepatocellular carcinoma [73]. Although the proposed mechanisms require further testing and may not provide a complete explanation for piRNAs as biomarkers for cancer, the emerging evidences demonstrated that piRNAs might serve as potential biomarkers for both diagnosis and prognosis of malignancies. In addition, piRNAs are 26–32 nt in length, and such a short fragment of RNA means that piRNAs are not so easily degraded as other long RNAs, and also means piRNAs can pass through cell membrane easily. These characteristics imply that piRNAs can be detected in patient samples, and potentially isolated from easily obtained bodily fluids, such as blood plasma and serum, saliva, sputum, and urine. Therefore, piRNAs have attracted a great deal of attention to serve as a potential noninvasive approach to improve diagnosis of human cancers, and the exploitation of this knowledge is already presenting potential opportunities for advances in cancer diagnosis. Thus, elucidating the relationship between piRNAs and cancer is a promising new field of cancer research.

5. Potential use of piRNAs in cancer therapies and future directions

Although our understanding of the molecular mechanisms of piRNA function in tumorigenesis is still very limited, some features of piRNAs would make them ideal candidates for therapeutic intervention. For instance, piRNAs functioning in RNA degradation at the post transcriptional level could be of therapeutic benefit, or its function in DNA methylation leading to transcriptional gene silencing could be used to inhibit certain oncogenes expression. Furthermore, RNAi-mediated gene silencing for cancer treatment could be applied to selectively silence oncogenic piRNAs. Moreover, although piRNAs are discovered to be restricted to germ cells and germline tissues, recent findings have showed that piRNAs are also expressed in the central nervous system (CNS) and other somatic tissues as well as cancer cells and tumor tissues [20–22, 65, 73, 77]. This indicates that there are undiscovered piRNAs-interacting proteins depending on tissue- or cancer- specific manner; because the advantage that piRNAs indeed have protein-binding ability provides a means of therapeutic intervention. Although piRNAs are discovered to be restricted to bind to Piwi proteins, there are observations have showed that piRNAs also bind to other proteins. For example, two complexes other than PIWI proteins could bind to piR_015520 in cytoplasmic extract from HEK 293 by electrophoretic mobility shift assay, suggesting that other RNA-binding proteins, which could bind piRNAs, not yet identified [65].

The fields of piRNAs and their interacting proteins in cancer have yet to be fully explored, and this area has the potential to yield many promising leads. Future use of comprehensive and high throughput techniques could identify more informative piRNA-biomarkers and their interacting proteins specific to different type or stage of cancers. If the function and mechanism were explored for all of them, piRNAs and their interacting proteins would attract a great deal of attention as effective biomarkers for diagnosis as well as novel targets for capable therapeutic manipulation.

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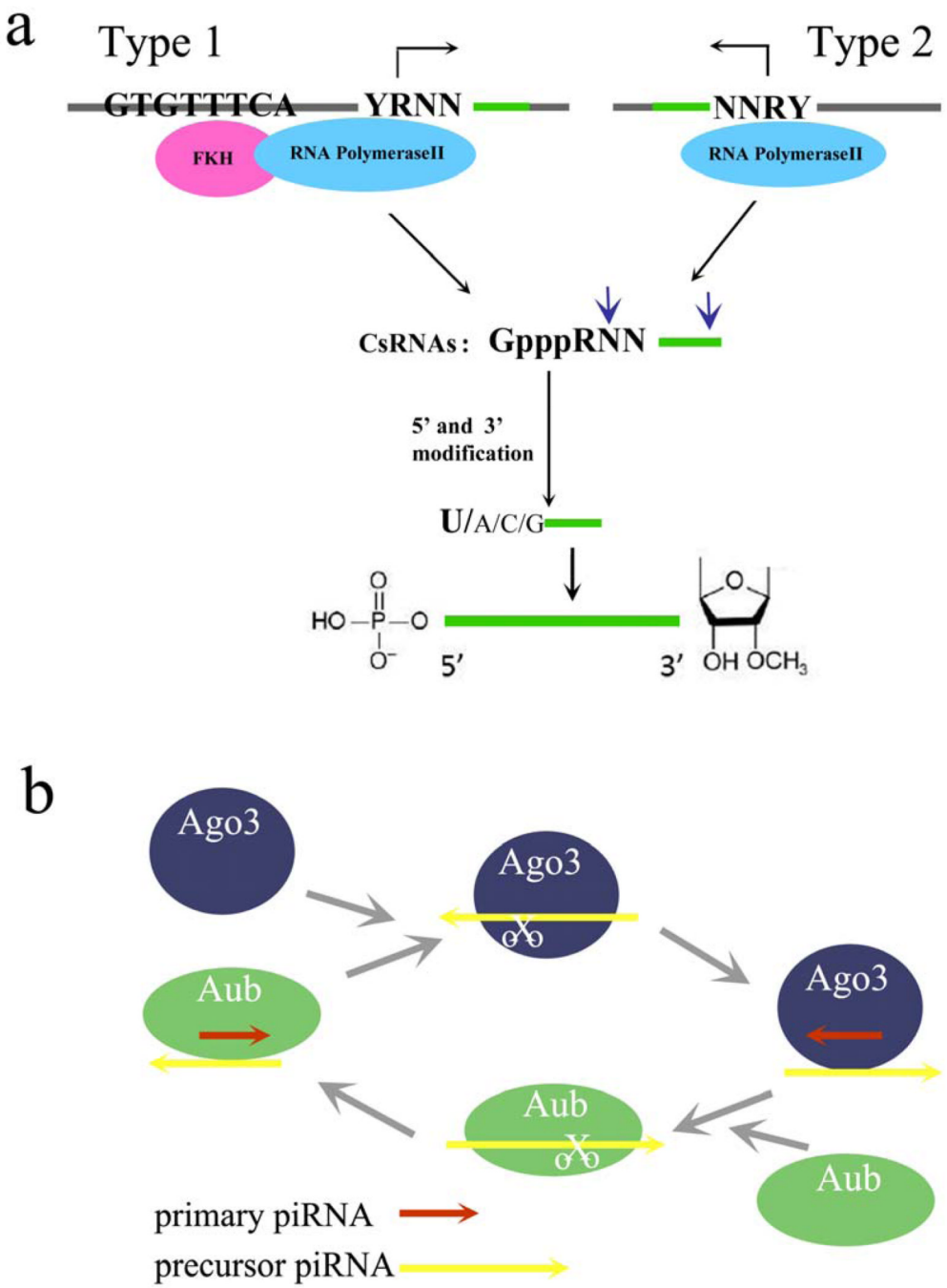

Figure 1.

Biogenesis of piRNAs. (a) Model for transcriptional biogenesis of piRNAs. There are two types of loci producing csRNAs, which are modified at 5' and 3' to be matured ones. A strong bias for a U at 5' end of matured piRNA is indicated in bold letter. (b) Amplification ping-pong mechanism of piRNAs. It involves two distinct Piwi proteins. The number of piRNAs is increased after cycle.

Table 1

Piwi proteins profiles in human cancers

Piwi types	Disease	Material	Reference
PiwiL1/Hiwi	Seminomas	Tissue	[47] Qiao,et al. 2002
	Gastric cancer	Tissue and cell lines	[55] Wang,et al. 2012
	Sarcoma	Tissue	[48]Taubert,et al. 2007
	Pancreatic cancer	Tissue	[54] Grochola, et al.2008
	Livwe cancer	Tissue and cell lines	[58] Zhao,et al. 2012
	Glioma	Tissue and cell lines	[57] Sun, et al.2011
	Esophageal cancer	Tissue and cell lines	[60] He,et al. 2009
	Colorectal cancer	Tissue and cell lines	[59] Zeng, et al. 2011;
PiwiL2/Hili	Breast cancer	Tissue and cell lines	[61] Liu, et al. 2010
	Cervical	Tissue and cell lines	[52] He, et al. 2010
	Seminoma cancer	Tissue and cell lines	[49] Lee,et al. 2006
	Colon cancer	Tissue	[53] Li, et al. 2012
PiwiL3	Gastric cancer	Tissue	[55] Wang, et al. 2012
PiwiL4/Hiwi2	Gastric cancer	Tissue	[55] Wang, et al. 2012

Table 2

piRNAs expression in human cancers

SpeificpiRNA	Expression Level	Disease	Refence
piR-651	up-regulated	gastric, colon, lung, breast, cervical mesothelium, and liver cancer	[20] Cheng, et al. 2011
piR-823	downregulated	gastric cancer	[22] Cheng, et al. 2012
piR-4987	upregulated	ductal carcinoma of breast	[21] Huang, et al.2012
piR-20365	upregulated	ductal carcinoma of breast	[21] Huang, et al.2012
piR-20485	upregulated	ductal carcinoma of breast	[21] Huang, et al.2012
piR-20582	upregulated	ductal carcinoma of breast	[21] Huang, et al.2012
piR-19825	upregulated	ductal carcinoma of breast	[21] Huang, et al.2012
pi-17458	downregulated	ductal carcinoma of breast	[21] Huang, et al.2012
piR-Hep1	upregulated	liver cancer	[73] Law, et al. 2013
piR-015520	upregulated	Tissues and HEK293	[65] Esposito, et al. 2011