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Review

Piwi-interacting RNAs (piRNAs) and cancer: Emerging biological concepts and potential clinical implications

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ABSTRACT

Piwi-interacting RNAs (piRNAs) are a very recently discovered class of small non-coding RNAs (ncRNAs), with approximately 20,000 piRNA genes already identified within the human genome. These short RNAs were originally described as key functional regulators for the germline maintenance and transposon silencing. However, due to our limited knowledge regarding their function, piRNAs were for a long time assumed to be the "dark matter" of ncRNAs in our genome. However, recent evidence has now changed our viewpoint of their biological and clinical significance in various diseases, as newly emerging data reveals that aberrant expression of piRNAs is a unique and distinct feature in many diseases, including multiple human cancers. Furthermore, their altered expression in cancer patients has been significantly associated with clinical outcomes, highlighting their important biological functional role in disease progression. Functionally, piRNAs maintain genomic integrity by silencing transposable elements, and are capable of regulating the expression of specific downstream target genes in a post-transcriptional manner. Moreover, accumulating evidences demonstrates that analogous to other small ncRNAs (*e.g.* miRNAs) piRNAs have both oncogenic and tumor suppressive roles in cancer development. In this article, we discuss emerging insights into roles of piRNAs in a variety of cancers, reveal new findings underpinning various mechanisms of piRNAs-mediated gene regulation, and highlight their potential clinical significance in the management of cancer patients.

1. Introduction

With the realization that only ~2–3% of the human genome is transcribed and subsequently translated into protein, the majority of noncoding RNAs (ncRNAs) have garnered a lot of attention for their role in cellular functions and disease pathogenesis. In fact, a wealth of studies have elegantly demonstrated the pivotal role of ncRNAs in mediating human carcinogenesis [1]. Based on their molecular size, all regulatory ncRNAs can be broadly categorized into small and large ncRNAs. Large ncRNAs, for instance, mainly include long non-coding RNAs (lncRNAs), where the transcripts are longer than 200 nucleotides (nts) [2]. Small ncRNAs, on the other hand, are heterogeneous, including microRNAs (miRNAs), transfer RNAs (tRNAs), small nucleolar RNAs (snoRNAs), short interfering RNAs (siRNAs), ribosomal RNAs (rRNAs), and Piwi-interacting RNAs (piRNAs) [3]. In this regard, miRNAs and lncRNAs have been extensively investigated in regulating the expression of various gene targets, and how this relates to the causation or development of various diseases, including cancer. In spite of an extensive body of active research on this topic, the voyage into the world of ncRNAs and their associations with various diseases remains in its infancy, and a lot more work is currently underway to gain additional insights into the biology of these short transcripts in human health and disease. Among the various small ncRNAs, the P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs), have emerged as newest members of the family that are being recognized as important mediators of cell biology. The piRNAs are ~26 to 30 nt in length, and are very similar in size to the miRNAs. Intriguingly, as per the current database searches, there are about 23,439 piRNAs identified in the human genome, a number that is quite comparable to that of protein-coding mRNAs (~20,000), but far exceeds the total number of miRNAs

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(~2000) [4,5], suggesting that piRNAs may have several critical roles in gene regulation, that remain to be yet explored.

The first evidence illustrating the existence of piRNAs was reported by Aravin, et al [6], wherein, they discovered that a repeat-associated small interfering RNA, derived from repetitive genomic elements, could silence *Stellate* protein-coding gene repeats in the *D.melanogaster* testes and early embryos. However, the biological significance of this group of small ncRNAs were still not fully understood at that time. Up until 2006, the biologists were merely able to successfully isolate and purify piRNAs distinctly from other small ncRNAs, but their actual functionality remained elusive. Subsequently, evidence began to accrue that this new class of small ncRNAs often bind to PIWI subfamily of Argonaute proteins, and utilize this epigenetic machinery to exert their functionality in mammalian germ line cells [7–9]. This recognition for the interaction of piRNAs with PIWI proteins became the paragon for the role of highly conserved small RNA-guided mechanism of gene regulation. More specifically, through a sequence recognition of piRNAs, the piRNA/PIWI complex is able to induce transposon silencing and heterochromatin modification [10,11]. Although the piRNA/PIWI complex was originally described to be primarily a key player in germ cell maintenance [10], recent studies have indicated their seminal role in cancer biology. Nonetheless, this is a fairly recent knowledge, and expression patterns of piRNAs and their specific functions in human cancers still remain poorly understood, but are an intense area of research investigations.

In this review article, we will discuss the current understanding for the biogenesis of piRNAs, their functions and underlying molecular mechanisms, and their emerging role in carcinogenesis. Furthermore, we will discuss their potential applications as disease biomarkers for cancer diagnosis and treatment.

2. Biogenesis of piRNAs

Mature piRNAs are 26–30 nt in length, with a 2′-*O*-methylation at the 3′end – a unique and distinguishing feature of all piRNAs. Unlike miRNAs and siRNAs, the precursors of piRNAs are single stranded transcripts without any prominent secondary hairpin structures. These precursors are usually generated from specific genomic locations containing repetitive elements, a process that is typically orchestrated *via* a Dicer-independent pathway. The nascent piRNAs require additional post-transcriptional modifications prior to becoming as mature piRNAs. The biogenesis of piRNAs include 2 major pathways: a primary and a secondary amplification cycle - also often referred to as the 'ping-pong cycle' (Fig. 1A).

2.1. Primary amplification

The piRNAs are derived from a relatively small number of genomic regions, termed piRNA clusters. Interestingly, most of these clusters consist of various transposable DNA elements, indicating that piRNAs are potentially antisense with respect to the retrotransposon-derived RNAs, and provide clues as to how these might impact the cellular function [7,8]. The long, single-stranded precursor piRNAs, are transcribed from piRNA clusters and subsequently cleaved by PIWI proteins, resulting in the generation of primary piRNAs [12,13].

2.2. Secondary amplification (Ping-pong cycle)

Following the generation of primary piRNAs, the secondary amplification is initiated in the cytoplasm through what is termed as a "Ping-pong" mechanism. Briefly, sense-piRNAs loaded within the PIWI proteins recognize their complementary targets and utilize the slicer activity of the PIWI domain to trim the 5′ends of primary piRNAs, which leads to the production of secondary antisense piRNAs. As the name Ping-pong suggests, the antisense piRNAs are coupled with PIWI proteins and once again target their complementary piRNA precursors to produce sense-piRNAs. Through this sequence-complementary-dependent cycle, the piRNAs are amplified and accumulated in the cytoplasm [14–16].

3. PiRNAs and PIWI complexes in cancer

As mentioned previously, a large number of mature piRNAs are believed to be involved in transposon silencing, since their precursor clusters are located within the transposable elements. Consequently, several studies have shown that mutations of critical proteins present within piRNA pathways caused transcriptional activation of retrotransposon elements leading to increased DNA damage in germ cells [17–19]. Interestingly, in addition to their regulatory function on transposons, recent studies have demonstrated that a subpopulation of piRNAs may significantly influence other epigenetic mechanisms, such as regulation of heterochromatin formation and histone modifications [20–22], other post-transcriptional modifications [12], and polycomb group-mediated transgene silencing [23] - all of which implicate the importance of epigenetic functions of the piRNA-PIWI pathways.

There has been a growing interest in understanding the role of piRNAs in human disease, particular in carcinogenesis. Cancer cells, stem cells and germ cells all share several key biological characteristics including the ability for self-renewal and rapid proliferation. Although piRNA pathway was originally discovered as an important regulator in maintaining germline stem cells, it is reasonable to hypothesize that rapidly dividing cancer cells may very likely adopt and utilize self-renewal machineries similar to those used in germ cells. In support of this hypothesis, a recent surge of studies have revealed a previously unrecognized correlation between piRNAs and human cancers. In the following sections, we will summarize and discuss the involvement of piRNA pathway in cancer development and progression.

3.1. PIWI proteins in cancer

The PIWI protein family is highly conserved across species. In humans, there are four PIWI proteins, which are named PIWI-like protein 1 (PIWIL1, also known as HIWI), PIWIL2 (HILI), PIWIL3 (HIWI3) and PIWIL4 (HIWI2) [24]. Even though the functional findings point to an active role of PIWI proteins in tumorigenesis, this area currently remains an area of active research. Some of the underlying enthusiasm for this stems from the observations that a very strong correlation has been discovered between expression of the PIWI proteins and poor clinical outcomes in cancer patients. Interestingly, high expression of PIWI proteins in colorectal, breast, gastric, ovarian, bladder and non-small lung cancers, as well as melanomas, leukemia, hepatocellular carcinomas, esophageal squamous cell carcinomas, soft-tissue sarcomas, renal cell carcinomas, gliomas and hilar cholangiocarcinomas, all have been correlated with aggressive cancers and poor clinical outcomes (summarized in Table 1) [25–44].

Several studies have also revealed the important biological roles of PIWI proteins in tumorigenesis. For instance, PIWIL1, was found to be a stemness-associated factor. In endometrial cancer, the expression level of PIWIL1 was overexpressed in malignant endometrial tissues, and was positively associated with tumors with greater malignant potential. The subsequent *in vitro* study showed enhanced expression of PIWI1 in endometrial cancer cells significantly promoted stem-like behavior, increased cell viability, cell migration, and sphere-forming activity. When HEC-1B cells were stably transfected with expression plasmids containing PIWIL1, cancer stem cell markers such as CD44, ALDH1 and stem cell markers such as Oct4 and Nanog are significantly up-regulated [45]. The general thought is that mechanisms underlying these findings may involve PIWIL1-mediated epigenomic reprogramming. The activation of PIWL1 can effectively cause global loss of DNA methylation (*i.e.* hypomethylation), while hypermethylation of specific tumor suppressor genes, such as PTEN have also been demonstrated to occur *via* PIWL1 activation facilitated by DNA methyltransferase 1 (DNMT1) [46,47].

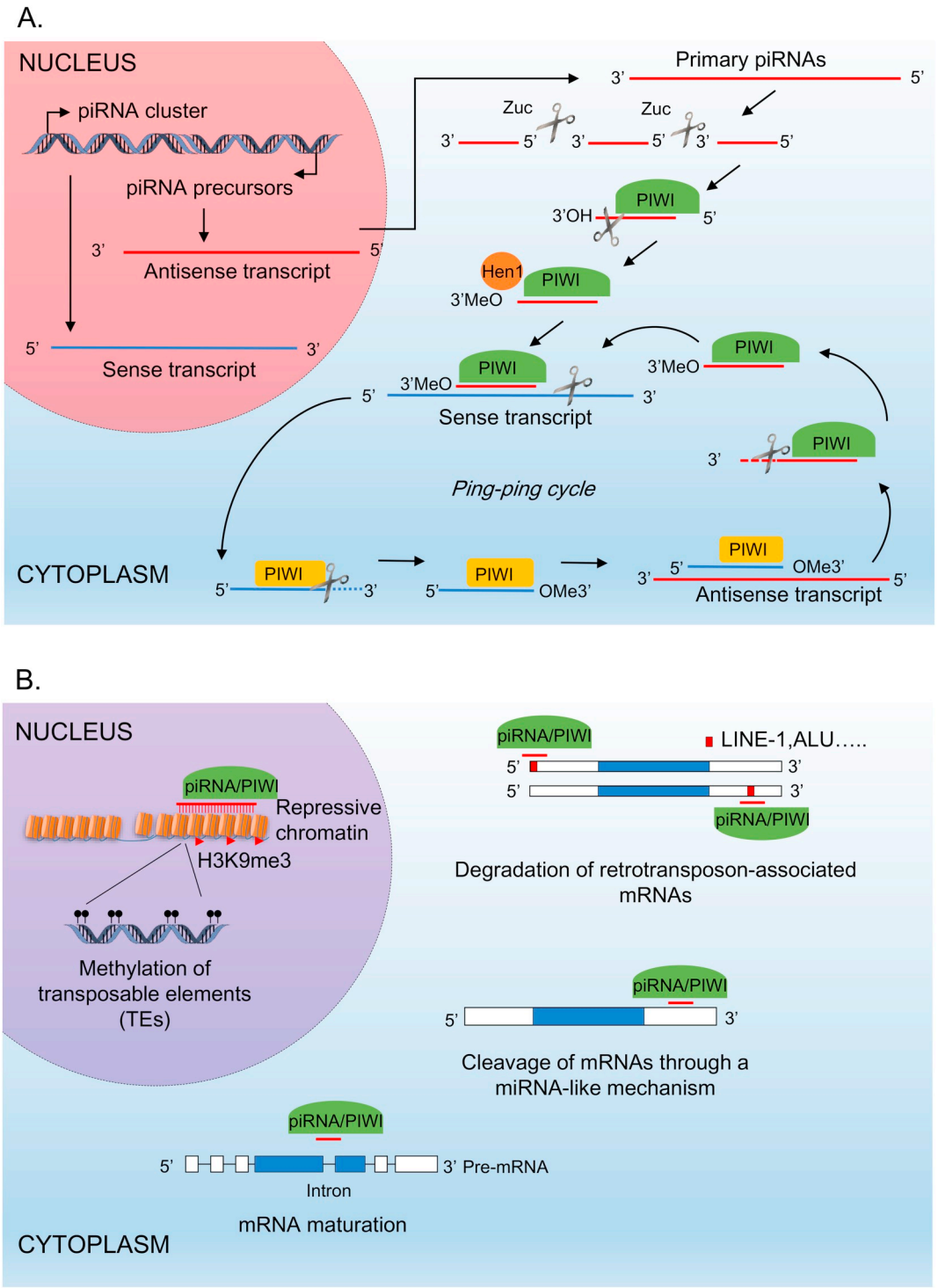

Fig. 1. Biogenesis of piRNAs: A) piRNAs are generated from primary and second pathways (Ping-Pong cycle). In the primary pathway, piRNA precursors are transcribed from piRNA clusters. Antisense primary piRNAs are cleaved, trimmed to short fragments, and its 3′ ends are 2′-*O*-methylated and then loaded onto Piwi family proteins. In the secondary amplification pathways, also known as the Ping-Pong cycle, PIWI proteins associated with antisense piRNA and cleaves piRNA precursors in the sense strand, or PIWI proteins associated with sense piRNA and cleaves antisense piRNA precursors in the sense strand. The incorporated RNA is therefore processed into a mature secondary piRNA by trimming and modification likely by the same mechanisms that generated a primary piRNA. B) The biological functions of piRNAs. In the nucleus, Piwi-piRNAs complexes can repress transposon expression by methylation of transposon region or chromatin modification around transposon region. In the cytoplasm, piRNAs can degrade retrotransposon-associated mRNAs, cause cleavage of mRNAs through a miRNA-like mechanism and facilitate mRNA maturation.

PIWIL2, on the other hand, initially was found to affect outcomes in cancer cells by inhibiting cellular apoptosis and through promotion of cell proliferation. Studies have demonstrated that when expression of PIWIL2 was inhibited, the anti-apoptotic gene Bcl-X_L, as well as signal transducer and activator of transcription 3 (STAT3) expression were suppressed, leading to enhanced cell proliferation [48]. Additionally, PIWIL2 promotes proliferation of cancer cells by inducing c-MYC oncogene, CDK2 and Cyclin A expression [37,49]. In addition, PIWIL2 has been shown to trigger epithelial-mesenchymal transition (EMT) and promote metastasis of cancer cells. Knockdown of PIWIL2 in the PC-3

Table 1
The profile of PIWI proteins in human cancers.

PIWI type	Genomic location	MW (*kDa*)	Cancer type	Expression	Biology/Clinical relevance	Ref
PIWIL1/HIWI	121q24.33	98.5	Colorectal cancer	↑	Proliferation	[25]
			Breast cancer	↑	Prognosis	[26]
			Chronic myeloid leukemia	↑	Growth and migration	[27]
			Hepatocellular carcinoma	↑	Proliferation and migration	[28]
			Glioma	↑	Diagnosis and prognosis	[29]
			Esophageal squamous cell carcinoma	↑	Prognosis	[30]
			Soft-tissue sarcoma	↑	Prognosis	[31]
			Gastric cancer	↑	Diagnosis and prognosis	[32]
			Ovarian cancer	↑	Diagnosis	[33]
			Renal cell carcinoma	↑	Prognosis	[34]
PIWIL2/HILI	8p21.3	110	Colorectal cancer	↑	Diagnosis and prognosis	[35]
			Ovarian cancer	↑	Diagnosis	[33]
			Renal cell carcinoma	↑	Prognosis	[34]
			Glioma	↑	Prognosis	[36]
			Non-small cell lung cancer	↑	Prognosis	[37]
			hilar cholangiocarcinoma	↑	Prognosis	[36]
			Bladder cancer	↑	Prognosis	[39]
PIWIL3/HIWI3	22q11.23	101	Melanoma	↑	diagnosis	[40]
			Ovarian cancer	↑	Diagnosis	[33]
			Breast cancer	↑	Prognosis	[41]
			Colorectal cancer	↑	Prognosis	[42]
			Gastric cancer	↑	Proliferation, migration and invasion	[43]
PIWIL4/HIWI2	11q21	97	Ovarian cancer	↑	Diagnosis	[33]
			Breast cancer	↑	Prognosis	[41]
			Renal cell carcinoma	↑	Prognosis	[34]
			Colorectal cancer	↑	Prognosis	[42]
			Cervical cancer	↑	Diagnosis and prognosis	[77]

MW: Molecular weight.
Ref: Reference.

prostate cancer cells impaired their ability to migrate and invade. This change in metastatic capability of PC-3 cells was accompanied by reduction in the expression of E-cadherin, alongwith concurrent increase in the expression of N-cadherin, Twist, Vimentin and Matrix Metalloproteinase-9 (MMP9) expression [50]. Similarly, in colorectal cancer, a significantly higher expression of PIWIL2 was observed in primary tumor tissues when compared to normal mucosa, and this higher expression of PIWIL2 correlated with poor metastasis-free survival and overall survival [51]. Interestingly, overexpression of PIWIL2 in colon cancer cells up-regulated MMP9 expression, an enzyme that is important in remodeling of the cellular matrix within the tumor microenvironment [51]. Recent studies demonstrated that cancers associated with various viral infections, such as HPV-associated cervical cancer, had a strong correlation with higher expression of PIWIL2 and poor prognosis. Feng et al, found that human papillomavirus (HPV) associated oncoproteins, E6 and E7, can effectively reactivate PIWIL2 expression in HaCaT cells, resulting in the increased acetylation of H3K9 and simultaneously reduced tri-methylation. Such switches in epigenetic reprogramming promote tumorigenesis and cancer stem cell signatures [52].

Compared to PIWIL1 and PIWIL2, there are fewer studies that have investigated the functional roles of PIWIL3 and PIWIL4 in cancer, suggesting they may have limited influence on tumor initiation and progression. However, one research group recently found that PIWIL3 expression was significantly up-regulated in gastric cancer compared to matched normal mucosa. These researchers also discovered that down-regulation of PIWIL3 in gastric cancer cells suppressed their ability to migrate and invade, mediated *via* phosphorylation of the JAK2/STAT3 signal pathway [43]. Likewise, another group confirmed that PIWIL3 overexpression contributes to metastatic melanoma [40]. With regards to PIWIL4, it was found to be overexpressed in cervical cancer and promoted cell growth by suppressing apoptosis through the $p14^{ARF}$/p53 pathway [44]. Collectively, it appears that PIWI family of proteins are functionally involved in a wide variety of human cancers, and the results of these studies highlight the emerging tumorigenic roles of piRNAs in cancer. Given their altered expression in a variety of human cancers, these proteins may be attractive clinical targets for modulating clinical outcomes in cancer patients in future.

3.2. PiRNAs: novel functions and roles in cancer

The evidence presented above highlights the epigenetic functions of PIWI proteins and their importance in carcinogenesis. In contrast, piRNAs on the other hand, can guide PIWI proteins and their associated epigenetic machinery to program the genome or transcriptome through recognition of numerous piRNA-complementary sequences, leading to transcriptional silencing of specific target genes.

3.2.1. The piRNAs maintain genomic integrity by silencing transposable elements

The transposable elements (TEs) can result in genetic diversity as well as genetic instability, providing natural candidates for enhanced pathogenicity in human cancers [53–55]. In general, TEs can be classified based on their mode of replication, including: 1) Class I TEs or retrotransposons, which function through mobilization of reverse transcription, or 2) DNA transposons, which encode for protein transposase and require insertion and excision to be mobilized. When these TEs are active, their repetitive 'copy and paste' or 'cut and paste' actions increase the cancer risk, and their frequent dysregulation maybe one of the major causal mechanisms in cancer [56,57]. In particular, non-long terminal repeat (non-LTR) TEs, such as long interspersed elements (LINEs), short interspersed elements (SINEs), SINE/VNTR(variable number of tandem repeat)/Alu(SVA), and Alu elements have all been implicated in the progression of cancer [58–60].

The location of the PIWI-piRNA complex in the nucleus can result in establishing a repressive chromatin state. The ectopic expression of piRNAs has been found to increase heterochromatin-1 (HP1) and its binding substrate, H3K9me3, which when bound, results in a heterochromatin state [9]. Notably, Lee et al, showed that TEs in a euchromatin state can be epigenetically silenced *via* a piRNA-dependent heterochromatin formation [61]. Intriguingly, a recent study showed that wild type p53 protein can restrain transposon mobility through

interaction with PIWI-piRNA complex. Wild type p53 alleles were found to suppress transposons, but mutant p53 alleles from cancer patients were unable to do so. Furthermore, p53 status was observed to correlate with repressive chromatin marks (*e.g.* H3K9me3) in the transcriptional enhancer region in cancer tissues. Considering the oncogenic role of LINE-1 as well as other repetitive elements, the restoration of tumor suppressor p53 and associated piRNAs maybe a promising strategy for cancer treatment [62]. Consistent with the previous observations, an animal model showed that a knockdown of mice PIWI proteins caused de-repression of LINE-1. In addition, the reduced expression of the piRNA cluster was found to be associated with hyperactivity of TEs [10,63,64].

3.2.2. PiRNAs contribute to tumorigenesis through regulation of DNA methylation

DNA methylation is another epigenetic event functionally linked to piRNAs. Recent evidence indicates that piRNAs, together with their PIWI proteins, can promote *de novo* DNA methylation of retrotransposons [10,65,66]. Moreover, piRNAs can direct DNA methylation not only within the context of transposons, but also on non-transposon loci. Recently, epigenome-wide analysis was performed to identify specific genes that may be methylated by piRNAs [67]. Surprisingly, there were relatively few piRNAs that were found to target human transposons. In contrast, a large number of piRNAs that existed in high copy numbers originated predominantly from intronic and intergenic regions. Transfection of human somatic cells with piRNA mimics demonstrated alteration of methylation at numerous genome loci, suggesting that piRNAs are not only essential for transposon methylation but also for methylation of specific genes [67]. It is of note that Fu, et al found piR-021285 associated variant rs1326306 G > T, which was found to be significantly associated with breast cancer. Through comparison of genome-wide methylation profiles in MCF7 cells transfected with either wild type or variant piR-021285, they observed methylation differences in a number of cancer-related genes. Further evidence of the wide scope of piRNA-mediated DNA methylation comes from another study in patients with multiple myeloma [68]. In this study, piR-823 was found to be up-regulated in multiple myeloma patients, and its overexpression was positively correlated with their clinical stage. Furthermore, the mechanisms underlying its oncogenic function showed that the inhibition of piR-823 caused a significant reduction in the expression of DNMT3A and DNMT3B, which resulted in diminished global DNA methylation and caused reactivation of the methylation-silenced $p16^{INK4A}$ tumor suppressor gene. These findings indicate that piRNAs may be one of important regulators of DNA methylation in cancer.

3.2.3. Post-transcriptional regulation of gene expression by piRNAs

The function of piRNAs include, but is not limited to, DNA methylation and chromatin silencing. Several studies have demonstrated that piRNAs can use miRNA-like mechanism to suppress expression of their target genes [69–71]. In human testis, expressed piRNAs have been found to be enriched within the 3′-untranslated regions (UTRs), in comparison to Coding DNA Sequences (CDSs) and 5′-UTRs. It has also been shown that mouse PIWI (MIWI) proteins when in association with piRNAs can cleave mRNA fragments from mouse testes [71]. In the case of *Miwi* mutant mice, the mRNAs targeted by piRNA were over-expressed due to their lack of cleavage in the absence of MIWI.

In addition to enhancing the cleavage of mRNA through miRNA-like mechanisms, piRNAs also post-transcriptionally regulate expression of mRNAs that carry retrotransposon-derived sequences. Retrotransposons are known to facilitate evolution of the genome by causing specific changes within the support structure, providing alternative promoters, splice junctions and exon usage [72,73]. With this tremendous ability for change, gene expression can be easily modulated through insertion of retrotransposons, resulting in eventual disease manifestation [74]. Thus, actions of the retrotransposons are thought to be under strict control, in order to maintain cellular homeostasis. Interestingly, greater than 25% of protein-coding mRNAs have retrotransposon sequences in their 3′-UTR, indicating that their expression could be rather easily regulated post-transcriptionally [75]. Indeed, several studies have revealed that piRNAs have the ability to act as a surveillance system to degrade retrotransposon-associated mRNAs to prevent pervasive diffusion of transposon sequences, highlighting an important tumor suppressive function of piRNAs [76].

One of the recent studies revealed a novel function of piRNAs in mRNA maturation. Zhong, et al found that snoRNA-derived and C (C′)/D′ (D)-box conserved piRNAs are abundant in human CD4 primary T-lymphocytes [77]. One such snoRNA-derived piRNA, piR-30,840, was found to play a critical role in the development of Th2 lymphocytes by suppressing mRNA maturation of interlukin-4. Through sequence complementarity binding to an IL-4 pre-mRNA intron, piR-30,840 recruited PIWI/Ago4 and subsequently interacted with the Trf4-Air2-Mtr4 Polyadenylation (TRAMP) complex, leading to the decay of IL-4 pre-mRNA through nuclear exosomes. Overall, this is a unique mechanism by which piRNAs control expression of genes in somatic cells.

3.2.4. PiRNAs have tumorigenic or suppressive roles in cancer development

A growing body of literature highlights that piRNAs function as either oncogenes or tumor suppressors in cancer development (Table 2). In addition, they may also contribute to the proliferation and metastasis of cancer cells. For example, the up-regulation of piR-651 was found to be associated with cancer progression in patients with non-small cell lung carcinoma (NSCLC). Notably, the overexpression of piR-651 in the A549 lung cancer cell line significantly augmented tumor growth and metastasis. Furthermore overexpression of piR-651 impacted cell cycle arrest, with a corresponding increase in the expression of cyclin D1 and CDK4, suggesting that piR-651 is a potential oncogene in this malignancy [78,79]. PiR-Hep1, another well-known oncogenic piRNA, promotes cell proliferation, migration and invasion in liver cancer [80]. In contrast, piR-823 serves as a tumor suppressor in gastric cancer, as its expression was dramatically decreased in gastric cancer tissues. Restoration of piR-823 in gastric cancer cells, however, inhibited cancer cells growth both *in vitro* and *in vivo* [81].

Collectively, the current picture for the function of the PIWI/piRNA complex in human cancer is still evolving. The PIWI/piRNA complex is likely to be engaged in more than just the repression of transposon elements, but may have repressive interactions with DNA methylation or target RNAs. Nevertheless, all the evidence points in the direction that altered expression of piRNAs in cancer has profound consequences for the biological behavior of cancer, indicating these may serve as promising targets for diagnostic and therapeutic clinical applications.

3.2.5. The role of piRNAs in the maintenance of cancer stemness and chemoresistance

Although PIWI proteins and piRNAs were originally shown to possess conserved role in repressing the expression of transposable elements in the germ cell cells, spermatogenesis, or germ stem-cell maintenance; a growing number of recent studies have now provided evidence that the PIWI pathway is also linked to the maintenance of cancer stemness, challenging the dogma in the piRNA field. In breast cancer, Zhang, et al [82] isolated CD44 (+)/CD24 (−) tumor cells (Cancer stem cell, CSC) from 1086 clinical specimens, and discovered that PIWIL2 and piR-932 were expressed at higher levels in CSC cells compared to the control cells. Furthermore, the authors suggested that the piR-932 PIWIL2 complex might contribute towards the breast cancer stem cell maintenance through promotion of aberrant methylation of the Latxin gene.

Chemoresistance is one of hallmark characteristics of cancer stem cells. Several studies have shown that disruption of piRNA and PIWI complex leads to impaired tumor sphere formation and enhanced chemo responsiveness. For instance, overexpression of Hiwi (PIWI proteins in human) expression in cervical cancer cells resulted in

Table 2
The clinical application of piRNAs in cancer.

Cancer type	piRNA ID	Sample type	Expression	Potential clinical utility	Ref
Breast cancer	piR-008114	Tissue	↓	Therapeutic targets	[41]
	piR-019676				
	piR-000552				
	piR-020548				
	piR-008113				
	piR-016735				
	piR-020450				
	piR-017033				
	piR-020365	Tissue	↑	Therapeutic targets	[41]
	piR-019675				
	piR-019914				
	piR-015249				
	piR-009294				
	piR-021032				
	piR-009051				
	piR-000753				
	piR-008112				
	piR-020814				
	piR-001318				
	piR-006426				
	piR-017184				
	piR-020829				
	piR-019912				
	piR-018780				
	piR-018849				
	piR-021285	Blood	↑	Diagnostic marker	[85]
	piR-932	Tissue	↑	Therapeutic target	[82]
Prostate cancer	piR-651	Cell lines	NA	Therapeutic target	[86]
	piR-823				
Kidney cancer	piR-30,924	Tissue	↑	Prognostic markers	[87]
	piR-38,756				
	piR-32,051	Tissue	↑	Prognostic markers	[88]
	piR-39,894				
	piR-43,607				
	piR-823	Tissue	↓	Prognostic markers	[89]
Gastric cancer	piR-651	Blood	↓	Diagnostic markers	[90]
	piR-823				
	piR-823	Tissue	↓	Therapeutic target	[81]
Colorectal cancer	piR-015551	Tissue	↓	Risk assesment	[91]
	piR-1245	Tissue	↑	Prognostic markers	[95]
Lung cancer	piR-651	Tissue	↑	Therapeutic target	[78,79]
	piR-55,490	Cell lines	NA	Therapeutic target	[92]
	piR-L-163	Blood	↓	Diagnostic marker	[93]
Liver cancer	piR-Hep1	Tissue	↑	Prognostic marker	[80]
Pancreatic cancer	piR-017061	Tissue	↓	Therapeutic target	[94]
Multiple myeloma	piR-823	Tissue	↑	Therapeutic target	[68]

Ref: Reference.

increased chemoresistance, tumor sphere formation and enhanced tumorigenicity *in vivo*, together with the activation of several stem-associated genes [83]. Interestingly, in a cisplatin-induced chromatin relaxation model of mouse embryonic fibroblasts (MEF), Wang, et al. [84] found that the up-regulation of Piwil2 endowed cancer stem cells with cisplatin resistance, possibly due to reinforced chromatin condensation to strengthen the DNA repair ability. Collectively, PIWI proteins and their associated piRNAs play a crucial role in maintaining the stem-cell properties of cancer cells; hence targeting the PIWI pathway may be a promising strategy for chemotherapy.

4. Potential clinical applications of piRNAs as cancer biomarkers

As mentioned previously, and as illustrated in Table 2 and Fig. 2, aberrant expression of piRNAs and their correlation with various features in cancer patients suggests these may have important clinical significance as diagnostic biomarkers or druggable targets [41,68,78,80–82,85–94].

In gastric cancer, the level of circulating piR-651 and piR-823 were significantly lower in gastric cancer patients compared to healthy controls (AUC of 0.841, 0.812, and 0.860 for piR-651, piR-823, and their combination, respectively), suggesting that these piRNAs may serve as valuable biomarkers for detection of gastric cancer [90]. Ectopic expression of piR-823 in gastric cancer cells remarkably inhibited tumor growth in a dose-dependent manner, highlighting its potential clinical value for treatment [81].

In clear cell renal cancer (ccRCC), several studies have revealed a specific piRNA signature that could serve as prognostic predictors. By using piRNA microarray and further confirming candidate piRNAs in a larger cohort, Busch, et al identified 3 piRNAs (piR-30,924, piR-57,125, and piR-38,756) that were significantly associated with tumor recurrence and overall survival [87]. Interestingly, another research group used the same strategy to explore potential biomarkers and discovered 46 metastasis-related piRNAs. Among these, 3 piRNAs (piR-32,051, piR-39,894 and piR-43,607) were derived from the same piRNA cluster at chromosome 17. Further validation of these 3 piRNAs in a 68 FFPE ccRCC tissues showed their positive correlation with metastasis, advanced stage and poor overall survival [88].

In breast cancer, the expression profiles of piRNAs from tumor tissues and matched normal tissues were determined using deep sequencing. The eight selected piRNAs (piR-34,736, piR-36,249, piR-35,407, piR-36,318, piR-34,377, piR-36,743, piR-36,026, and piR-31,106)

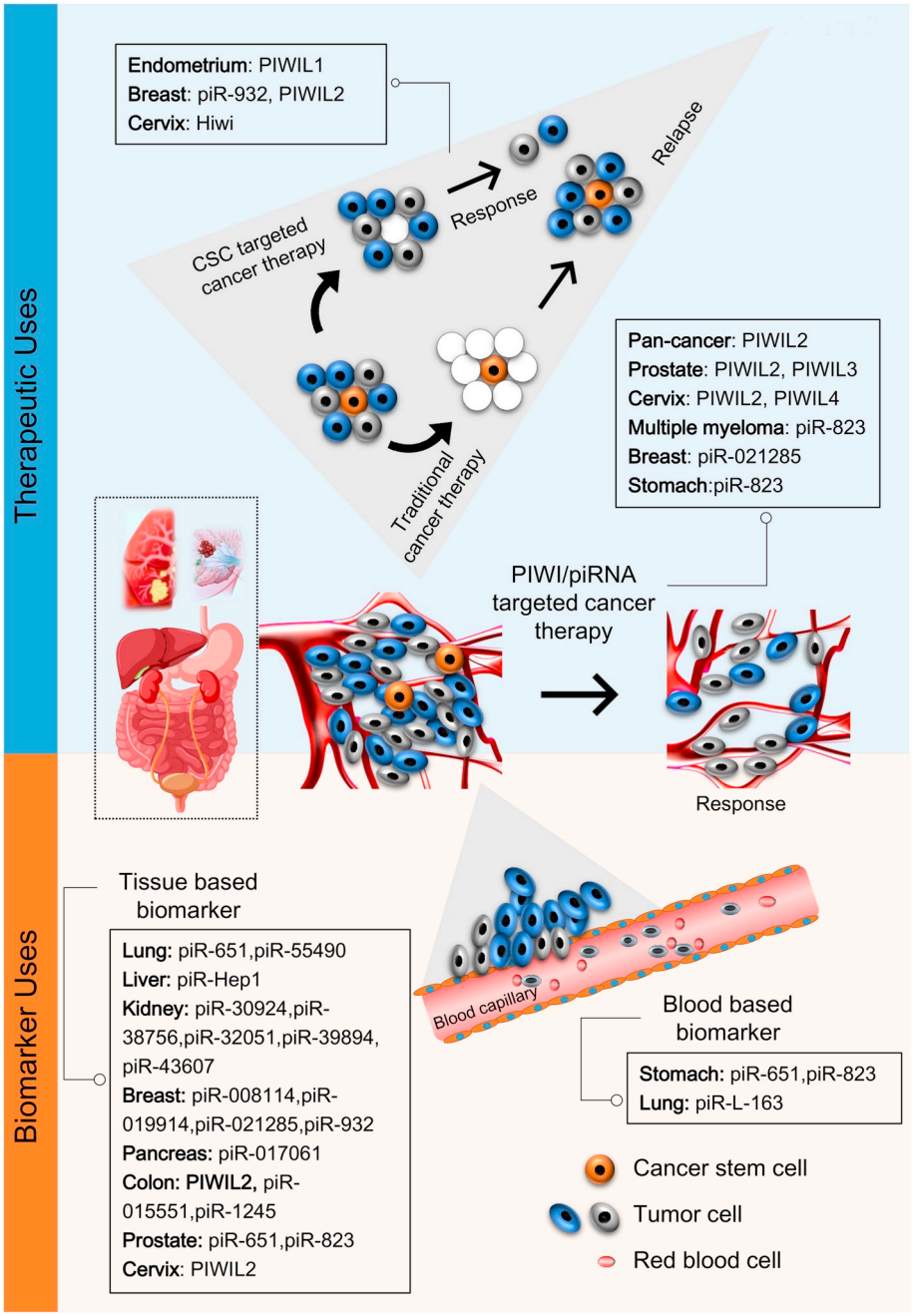

Fig. 2. Potential clinical applications of PIWI-piRNAs pathway: The dysregulation of PIWI-piRNAs pathway and their correlation with various features in cancer patients suggests these may have important clinical significance as biomarkers or therapeutic targets. For treatment purposes, manipulations of PIWI proteins or piRNAs expression can cause inhibition of tumor growth or metastasis by affecting several key biological processes such as proliferation, apoptosis, or invasion capability. Since stem cell subpopulations contribute to tumor chemoresistance and ultimately recurrent disease, targeting stemness-associated PIWI proteins or piRNAs maybe a promising strategy for chemotherapy. In spite of their roles as druggable targets, it is worthy to note that PIWI proteins or piRNAs could serves as either tissues-based biomarkers or circulating markers for prognostic or diagnostic purpose.

revealed novel independent prognostic characteristics, and their correlation with overall survival was further confirmed in an independent TCGA cohort. Interestingly, Zhang, et al sorted breast cancer stem cells CD44(+)/CD24(−) from tumor specimens by using flow cytometry. They further identified piR-932 as a novel stemness associated-piRNA, and subsequent experiments confirmed that overexpression of piR-932 effectively induced an EMT phenotype through demethylation of the Latexin gene [82].

In addition to above cancers, the clinical relevance of piRNAs in prostate, lung, colon, liver, and pancreatic cancers, along with multiple myeloma are described and summarized in Table 2. In particular, Weng et al. [95] identified piR-1245 as a potential prognostic biomarker in colorectal cancer. Furthermore, piR-1245 functions was shown to act as an oncogene and contributed to tumor progression. In spite of their roles as biomarkers, it is worthy to note that the aforementioned studies showed that since piRNAs can induce epigenetic silencing of target genes, these may serve as potential therapeutic tool as well in future.

5. Database for piRNAs and functional predictions

The piRNABank was first established as a web resource on classified and clustered piRNAs by an Indian scientific group, Lakshmi et al [4]. This database provides comprehensive information on piRNAs in Human, Mouse, Rat and Drosophila. It compiles all the possible clusters of piRNAs, and also depicts piRNAs along with the associated genomic elements, such as genes and repeat elements on a genomewide map (http://pirnabank.ibab.ac.in/).

A newly developed piRNA database-piRBase provides a more powerful tool for piRNA functional studies by Chinese scientists Wang, et al [96] and Zhang et al [97]. This database integrated 264 datasets from 21 organisms, and the number of collected piRNAs has reached more than 173 million. Moreover, it incorporates potential information of piRNA targets and disease-related piRNAs. In particular, repeat-derived and gene-derived piRNAs, which may have regulatory relevance, are also logged into this database. Furthermore, epigenetic data and

reported piRNA targets are also collected. Therefore, these databases integrates epigenetic and post-transcriptional regulation data to support piRNA functional analysis (http://www.regulatoryrna.org/database/piRNA/).

6. Conclusions

With the development of high-throughput sequencing technologies, crucial roles of ncRNAs in health and disease are gradually emerging. In particular, piRNAs are extensively being studied for their roles in cancer biology. In this review article, we illustrate how piRNAs are aberrantly expressed in a cancer-specific manner. Furthermore, the expression of some piRNAs was associated with pathological variables or clinical outcomes. With regards to potential limitations, most of the studies till date have been performed in clinical specimens that were retrospective in nature; suggesting that these conclusions should be further validated in future, prospective, multi-center clinical trials. In addition, some of the clinical parameters, treatment or prognosis information were not consistently or appropriately evaluated, which may be easier to address in a future well-defined patient cohort.

Although we have presented many mechanistic studies in our article, revealing molecular functions of piRNAs in carcinogenesis, there still is a somewhat early and unclear understanding for the precise roles of piRNAs in cancer. Of the many fascinating questions that remain, a few stand out. For example, how does the piRNAs/PIWI complex function in somatic cells, as there are obviously few piRNAs present? Which downstream targets are directly regulated by the piRNAs/PIWI complex in cancer? Is the aberrant expression of piRNAs the causative effect in the initiation and progression of cancer or just a by-product of other driven molecular events? Finally, the development of piRNAs for use as therapeutic tools also raises questions and challenges. Addressing the aforementioned questions would go a long way in substantially advancing this area of cancer biology.

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Conflicts of interest

None of the authors have any potential conflicts to disclose.

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