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REVIEW ARTICLE

PIWI-interacting RNAs: Mitochondria-based biogenesis and functions in cancer

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Abstract PIWI-interacting RNA (piRNAs), once thought to be mainly functioning in germlines, are now known to play an essential role in somatic and cancerous tissues. Ping-pong cycle initiation and mitochondria-based phased production constitute the core of the piRNA biogenesis and these two processes are well conserved in mammals, including humans. By being involved in DNA methylation, histone marker deposition, mRNA degradation, and protein modification, piRNAs also contribute to carcinogenesis partly due to oncogenic stress-induced piRNA dysregulation. Also, piRNAs play important roles in cancer stemness, drug resistance, and tumor immunology. Results from liquid biopsy analysis of piRNA can be used in both cancer diagnoses and cancer prognoses. A combination of targeting piRNA with other therapeutic strategies could be groundbreaking cancer treatment.

Introduction

P-element-induced wimpy testis (PIWI-interacting RNAs (piRNAs) are the new members of the RNA interference family. They usually comprise of 21–35 nucleotides (nt) with a 2′-O-methyl-modified 3′ termini and interact specifically with the PIWI subfamily of Argonaute proteins.[1–3] Originally described as repeat-associated siRNAs (rasiRNAs),

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piRNAs mediate the silencing of genomic tandem repeats and transposable elements in the *D. melanogaster* germline.[4,5] It wasn't until 2006 when they were found to interact with PIWI proteins in many other organisms were they referred as piRNAs.[1–3] Since then, there have been several impactful discoveries made in the piRNA-PIWI field (Fig. 1A). And their functions have been expanded from the well-known role of transposon silencing in germline cells to epigenetic modification or post-transcriptional gene silencing (PTGS) of protein-coding genes in somatic and cancerous tissues.[6–20]

There are a few major differences that distinguish piRNAs from miRNAs and siRNAs. For example, as Argonaute proteins are divided into two subfamilies, the Argonaute (AGO) and PIWI families, piRNAs bind PIWI subfamily proteins to exert their effects while miRNAs and siRNAs bind the AGO subfamily proteins.[21] In terms of gene regulation, siRNAs, miRNAs, and piRNAs are known to have the ability to silence or trigger the degradation of mRNA. Members of the piRNAs primarily function through epigenetic silencing in the nucleus although they can conduct miRNA-like transcript silencing in the cytoplasm.[22,23] piRNAs, 21 to 35 nucleotides (nt) long, are derived from single strand precursors generated via a Dicer-independent mechanism. In contrast, miRNAs and siRNAs, 20 to 24 nt long, are derived from double-stranded RNA (dsRNA) or stem-loop precursors processed by Dicer RNase III.[24] An additional distinguishing characteristic is that piRNAs possess 2′-O-methylation at the 3′-terminus in a variety of animals while 2′-O-methylation in siRNAs and miRNAs are mainly found in plants, despite a few reports of 2′-O-methylated siRNAs in *Drosophila*.[25–27] Quantitatively speaking, piRNAs far outnumber miRNAs in the eukaryotic genome, for example, the human genome contains ~ 8,400,000 annotated piRNAs while ~ 2000 annotated hairpin miRNA precursors, and ~2600 mature miRNA sequences.[28–30] As a distinguishing characteristic, all piRNAs possess 2′-O-methylation at the 3′-terminus, a unique feature making them distinct from miRNAs and siRNAs. Moreover, both miRNAs and piRNAs predominantly target genes different from their origins by inhibiting translation or enhancing target mRNA degradation (heterosilencing). In contrast, siRNAs target and degrade mRNAs transcribed from the same genes they originated (autosilencing) (Fig. 1B).[31]

Since the discovery of piRNAs in HeLa cells in 2010, the role of piRNAs in malignant cells have also emerged.[10] This review article aimed to discuss the activity of piRNAs at the transcriptional and post-transcriptional levels first followed by the emerging roles of piRNAs in cancer stemness, drug resistance, and tumor immunology. Besides, the applications of piRNAs in liquid biopsy and cancer therapies will also be highlighted.

PIRNA: mitochondria-based biogenesis

piRNA biogenesis

As the root of piRNA dysregulation in cancer is likely the result of disorders in piRNA biogenesis, it is necessary to get insight into the piRNA biogenesis mechanism first. Recent studies of flies and mice,[32–34] and data from an evolutionarily broad range of non-model species,[35] suggest that piRNA biogenesis is a highly conserved process, and in most

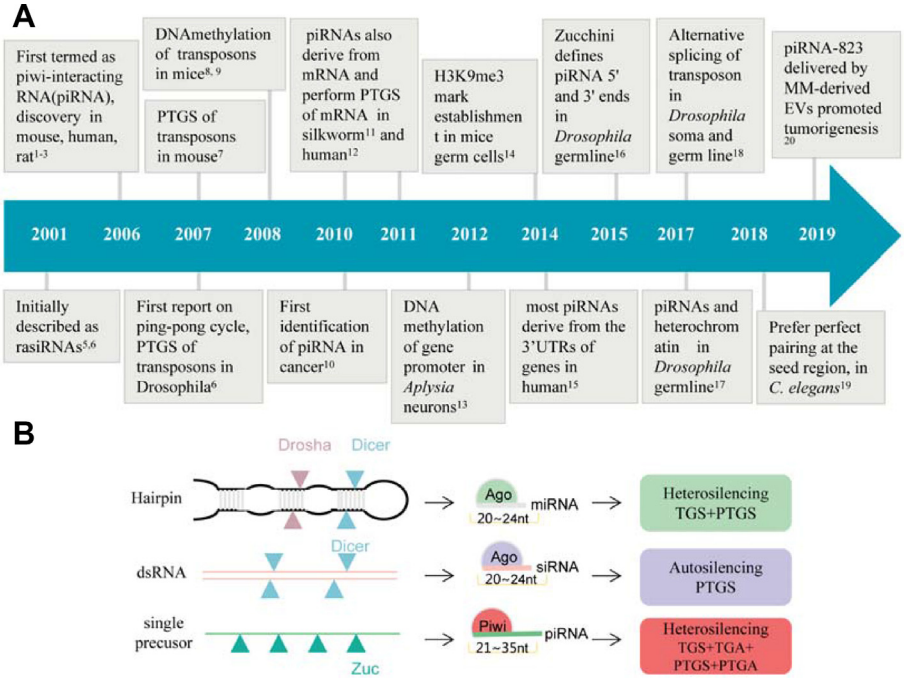

Figure 1 Characteristics and progression of piRNAs. (**A**) The milestone events in the piRNA field. (**B**) piRNAs show distinct characteristics different from miRNAs and siRNAs, from genome source to RISC formulation and gene silencing style. Abbreviation: EV, exosome vesicle; MM, Multiple myeloma; PTGS, post-transcriptional gene silencing; PTGA, post-transcriptional gene activation; TGA, transcriptional gene activation; TGS, transcriptional gene silencing; H3K9me3, trimethylation of H3 lysine 9; UTR, untranslated region; RISC, RNA-induced silencing complex.

animals the ping-pong and phased piRNA pathways collaborate to make complex populations of piRNAs (Fig. 2).

Transcription in nuclear

piRNA clusters are a discrete number of genomic loci that stood out as hotspots responsible for producing the vast majority of piRNAs in most species.[36] Based on the ability to generate piRNAs from one or both genomic strands, piRNA clusters are roughly divided into unistrand and dual-strand clusters. Both types of piRNA clusters are transcribed by RNA polymerase II (pol II) (Fig. 2). The products of unistrand clusters depend on canonical genic transcription units, especially the conserved transcriptional factor A-MYB, and will undergo canonical RNA processing in the nucleus, i.e., 5′-capping, splicing, and polyadenylation.[37–41] On the other hand, dual-strand piRNA clusters do not possess their own promoters and are regulated by non-canonical transcriptional mechanisms driven by nuclear transcription factors including Rhino (Rhi), Deadlock (Del), and Cutoff (Cuff).[38,42–45] In flies, piRNA precursors transcribed from genomic clusters are capable of targeting transposons for repression.[6] However, in humans, most genomic piRNAs are derived from human protein-coding genes or long non-coding RNA (lncRNA) genes rather than transposons, indicating that human piRNAs may have functions beyond transposon silencing.[1,3,41,46,47]

Ping-pong cycle

In most animals, piRNA precursors are exported from the nucleus and localize in the nuage where biogenesis via the ping-pong cycle takes place (Fig. 2).[6,48] In the germ cells of *Drosophila*, Argonaute 3 (Ago3) and Aubergine (Aub) play instrumental roles in the ping-pong cycle. Ago3 is biased towards a sense strand and adenine at nucleotide 10, whereas Aub prefers an antisense strand and uridine at nucleotide 1.[6,49] Via their slicer activity, Ago3 and Aub cleave target RNAs at positions 10 and 11 of the guide strand, respectively.[50] More specifically, a trigger piRNA will combine with Ago3 and scan all of the transcripts that exit the nucleus. After identifying a target with a complementary sequence (antisense), the Ago3/piRNA complex binds to the precursor transcript and cleaves it, thereby generating antisense piRNA. The 5′-end of the cleaved transcript then becomes a responder piRNA (antisense) and binds to Aub. This complex can go on to cleave cluster transcripts (sense); the sense piRNA product of this cleavage event can reinitiate the cycle. In mice, ping-pong cycle is primarily driven by PIWIL2/MILI and PIWIL4/MIWI2 in pre-pachytene piRNAs of pre-natal male mice.[8,51,52] The ping-pong signal is also found in bovine, macaque, and human ovarian or testicular piRNA biogenesis.[53] And in fetal human ovaries, ping-pong cycle may be formed by PIWIL2/HILI and PIWIL4/HIWI2.[53,54]

Shuttling process

In *Drosophila*, pre-piRNAs produced in the nuage/Yb bodies are then shuttled by Armitage (Armi) to the outer membrane of mitochondria (OMM) for phased production.[55,56] At the OMM, Armi is anchored to a binding platform by Gasz and Daedalus (Daed).[57] Both Gasz and Daed ensure the proper localization of Armi, proximal to dimerized and active Zuc. In mice, the shuttling protein might be Moloney leukemia virus 10 like-1 (MOV10L1), an RNA helicase analogous to Armi.[58–63] Gasz has also been confirmed to be a component of intramitochondrial cement (IMC) that co-localizes with MILI, TDRD1, and mouse VASA homolog (MVH).[64,65] In humans, MOV10L1 is a testis-specific isoform of MOV10. The latter has been reported to colocalize and interact with Ago1 and Ago2 proteins to mediate miRNA-guided mRNA cleavage in humans.[49,58] There are hints that MOV10L1 gene polymorphisms are associated with male infertility in azoospermic men with complete maturation arrest.[66] Given the critical role of piRNAs in germ cell maturation and infertility, there may be an interaction between MOV10L1 and piRNAs.[67] The exact role of MOV10L1 and any cofactors that may shuttle PIWI-piRNA complex from perinuclear granules to IMC in human piRNA biogenesis is yet to be confirmed, but certainly warrants further investigation.

Mitochondria-dependent phased production

After being shuttled to the OMM, long piRNA precursors will be phased by Zucchini (Zuc)/MitoPLD, a highly conserved mitochondria-anchored endonuclease responsible for generating both the 5′- and 3′-termini of piRNAs.[16,68–72] In *Drosophila* or mice, Zucchini (Zuc)/MitoPLD could cleave

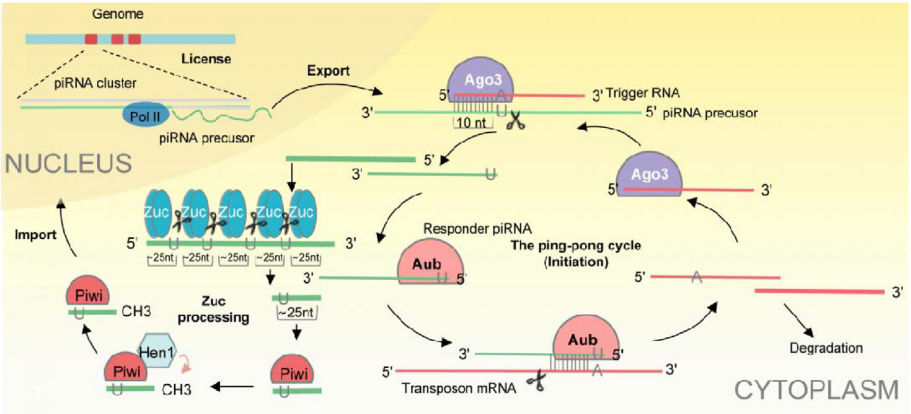

Figure 2 piRNA biogenesis in *Drosophila*. Abbreviations: Ago3, Argonaute3; Aub, Aubergine; Armi, Armitage; CH3, depicts a 2-O-methyl group; Zuc, Zucchini.

long piRNA precursors approximately every 25 or 40 nt, producing shorter trailer piRNAs following one another (Fig. 2).[36,57] As this process occurs, Armi helps to unwind piRNA precursor and PIWI protein helps to position Zuc, thereby dictating the distinct "phasing" of cleavage.[35] In humans, the mitochondria-based piRNA phased production process has been experimentally verified.[73] HIWI2 is enriched in the IMC of the primordial follicles of human oocytes and spermatogonia. Interestingly, HILI is largely excluded from the IMC by HIWI2, suggesting the specific subcellular compartmentalization of PIWI proteins in humans. The exact details of piRNA phased production in humans is not confirmed, but it is thought that human MitoPLD is involved. As a member of the Phospholipase D (PLD) superfamily, human MitoPLD localizes on the OMM.[74,75] Whether or not HIWI2 and MOV10L1 guide human MitoPLD is undiscovered.

Finally, the piRNAs are subjected to 3′ trimming by nibbler and are 2′-O-methylated by Hen1.[76,77] These PIWI-piRNA products are then transferred to the nucleus or remain in the cytoplasm to exert their effects.

piRNA biogenesis and cancer

A variety of cancers involve ectopic piRNA expression (Fig. 3). In essence, the ectopic piRNA expressions are caused by the changes in piRNA biogenesis. Alterations in piRNA biogenesis promote tumorigenesis mainly through the following ways:

(1) The ping-pong amplification loop is a post-transcriptional silencing (PTGS) that combines the silencing of target mRNAs with piRNA production.[36] In *Drosophila* and mice, the ping-pong cycle despair often results in transposon burst.[8,36] In humans, disruption of piRNA biogenesis might result in activation of oncogenes.[78,79]
(2) Mitochondria are well known as the powerhouse of cells for their function of ATP production and suicidal weapon store for signal transduction.[80] The importance of mitochondrial proteins in piRNA biogenesis, especially Zuc/MitoPLD, further supports the mitochondrial regulation of cellular parameters, and possibly carcinogenesis.[36]
(3) Mature piRNAs control target genes via DNA methylation, histone marker deposition, mRNA decay and degradation, and protein modification. However, a disruption in piRNA biogenesis may lead to the disorders of piRNA-mediated transcriptional and post-transcriptional gene regulation; oncogenes can escape silencing, while tumor-suppressive genes are mistakenly silenced.
(4) piRNA biogenesis may be related to cancer stemness. For example, PIWIL2, an important participant in piRNA biogenesis, cannot be detected in most normal adult organs in humans, except in testes, as well as in a wide variety of tumors.[81] These facts suggest that piRNA biogenesis maybe active in germ cells, quiescent in normal tissues, but revive in cancer tissues, consistent with the changing trend of stemness in the corresponding states of organisms. In addition, piRNAs and PIWI proteins are reported to be related to cancer stem-cell maintenance.[82,83] In this regard, piRNA biogenesis may be involved in cancer stemness.

In summary, piRNA biogenesis is a complex process and highly conserved in humans. Even modest changes in this process are expected to influence the piRNA expression level and cellular function profoundly. The following section discusses how the piRNA expression levels are crucial for cancer development.

PIRNAS and cancer: cause and effect

The first report of the role of piRNAs in cancer occurred in 2010 when piRNAs were discovered in HeLa cells.[10] Since that initial discovery, many publications have attempted to elucidate the role piRNAs serve in cancer development and progression (Fig. 3A).[20,55,78,79,82–127] Ectopically expressed piRNAs identified in numerous cancers are shown in Fig. 3A and Table 1. Many piRNAs, especially piR-823 and piR-651, have been reported to be dysregulated in various types of cancers, including but not limited to breast, stomach, lung, prostate, bladder, colorectal, kidney, liver, lymphomas and leukemias (Table 2).

piRNA dysregulation promotes cancer cell malignant phenotypes

Tumor-suppressor piRNAs act as defenders against carcinogenesis by suppressing cell proliferation, increasing apoptosis, disrupting colony formation, decreasing migration and invasion abilities, and arresting cell cycles.[79,84,100,101,106]; Additionally, xenografts with tumor suppressor piRNA overexpression have been found to often show a slower growth rate and exhibit decreased metastasis.[84,100,106] Such reports include cases of increased piR-8014 in human glioblastoma, piR-36712 in breast cancer (BrCa), piRABC in bladder cancer and piR-55490 or piR-L-163 in lung cancer.[79,84,100,101,106]

In contrast, oncogenic piRNAs promote cancer progression by increasing cell proliferation, inhibiting apoptosis, promoting colony formation and angiogenesis, and enhancing migration and invasion[20,90,91,118,121]; Xenografts with oncogenic piRNA overexpression often show a faster rate of growth and exhibit increased angiogenesis and metastasis.[20,91] Such reports include cases of increased piRNA-823 in multiple myeloma, piR-54265 in colorectal adenocarcinoma, piRNA-651 in non-small cell lung cancer, and piR-823 or piR-1245 in colorectal cancer (Fig. 3B).[20,83,90,91,118,121]

Mechanism of piRNA dysregulation and cancer cell malignant phenotypes

To become functional, mature piRNAs usually have to be loaded into PIWI to form the RNA-induced silencing complex.[128] piRNA–PIWI complexes are thought to be primarily involved in target regulation through four mechanisms: transcriptional gene silencing (TGS) or activation (TGA) and PTGS or activation (PTGA). PTGS mechanisms regulate target transcripts directly through cleavage and require the catalytic activity of PIWI proteins. The ping-pong cycle is a

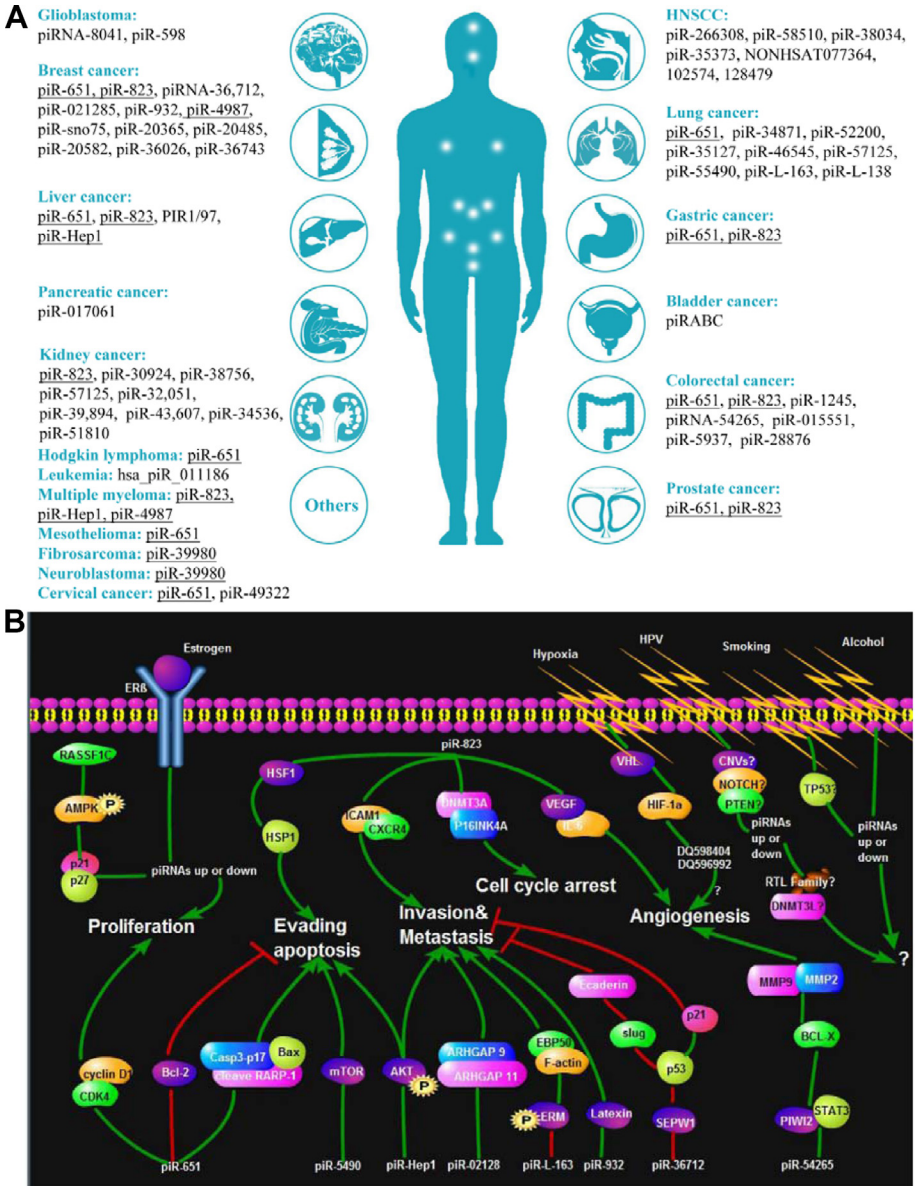

Figure 3 Dysregulated piRNAs and their cellular signaling pathways in cancer. (**A**) Ectopically expressed piRNAs in cancers. The white dots refer to the site of corresponding organs. Among all the piRNAs, piR-651, piR-823, piR-4987, piR-39980, and piR-Hep1 exhibit pan-cancer expression patterns, and they are highlighted by underlines. (**B**) piRNA dysregulation results in the activation or repression of participating molecules in cellular signaling pathways. This leads to malignant cell phenotypes such as cell proliferation, evading apoptosis, metastasis, invasion, and cell cycle arrest. The positive effect is shown as green line, while the negative effect is shown as red line. Abbreviation: HNSCC, head and neck squamous cell carcinoma. ARHGAP, Rho GTPase activating proteins; ERβ, Estrogen receptor; CASP3, caspase 3, CDK4, Cyclin dependent kinase 4; DNMT, DNA methyltransferase; CNV, copy number variation; CXCR4, C-X-C motif chemokine receptor 4; HSP1, heat shock protein 1; HSF1, heat shock transcription factor 1; HPV, human papillomavirus; ICAM1, intercellular adhesion molecule 1; IL6, interleukin 6; mTOR, mechanistic target of rapamycin kinase; PTEN, phosphatase and tensin homolog; SEPW1, selenoprotein W 1; STAT3, signal transducer and activator of transcription 3; TDRD1, tudor domain containing 1; TP53, tumor protein p53; MMP, matrix metallopeptidases; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau tumor suppressor.

representative example of PTGS mechanisms.[32,129] TGS or TGA, in contrast, does not require the cleavage activity of PIWI proteins, although it does require the activity of enzymes such as histone-modifying enzymes or DNA methyltransferases.[129,130] With the contribution of these enzymes, piRNA—PIWI RISC can lead to a change in the mRNA output indirectly via chromatin remodeling. Thus, piRNAs take part in many aspects of tumorigenesis, from epigenetic regulation to mRNA degradation and protein modification (Fig. 4).

TGS and TGA by piRNAs via aberrant DNA methylation

Active genes are normally identified by the absence of promoter DNA methylation with the presence of gene body CpG methylation. In cancer, the methylation status

Table 1 Summary of altered piRNA expression in cancer.

Cancer	piRNA	Expression	Related gene	Mechanical points	Clinical Utility	Sample Type					Detection Technique	Reference
						TCGA	Tissue	cell	serum	mice		
Breast cancer	piRNA-36,712	↓	SEPW1P, P53,P21 and E-cadherin	Inhibits SEPW1 expression, which may suppress P53, leading to the upregulated Slug but decreased P21 and E-cadherin levels.	Prognostic	yes	yes	yes	no	yes	TCGA	[84]
	piR-021285	↑	ARHGAP11A	Attenuated 5′-UTR/first exon methylation of proinvasive ARHGAP11A gene	Diagnostic	yes	no	yes	yes	no	Genome wide screen RT-PCR	[78]
	piR-932	↑	Latexin	piR-932 and PIWIL2 may promote metastasis through Latexin methylation	Theraputic	no	yes	yes	no	yes	piRNA microarray RT-PCR	[82]
	piR-36026	↑	SERPINA1, LRAT	Directly interacts with SERPINA1 or LRAT and induce caspase-3 and PI in the nucleus.	Diagnostic	no	no	yes	no	no	RT-PCR Molecular beacon	[85—87]
	piR-36743	↑	—	—	Diagnostic	no	no	yes	no	no	RT-PCR Molecular beacon	[85,87]
	piR-sno75	↓	TRAIL, MLL3, KDM6A	piR-sno 75/PIWIL1/4 complex upregulates the transcription of tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) by recruiting H3K4 methylase MLL3 and H3K27 demethylase KDM6A	Theraputic	no	yes	yes	yes	no	Small RNA sequencing RT-PCR	[137]
	piR-20365	↑	—	—	Prognostic	no	yes	no	no	no	piRNA microarray RT-PCR	[88]
	piR-20485 piR-20582 17 piRNAs	↑	—	—	Diagnostic	yes	yes	no	no	no	piRNA microarray RT-PCR	[89]
	8 piRNAs	↓										
Bladder cancer	piRABC	↓	TNFSF4	Inhibit cell proliferation, colony formation, and promote cell apoptosis by increasing the expression of TNFSF4 protein	Diagnostic	no	yes	yes	no	no	piRNA microarray RT-PCR	[79]
Cervical cancer	piR-30074	—	—	Regulating transformation of stem cells	Theraputic	no	no	yes	no	no	—	[125]
	piR-49322	↑	LINE1	Suppress LINE1 retrotransposon along with HILI	—	no	no	yes	no	no	high throughput sequencing	[10]
Colorectal cancer	piR-1245	↑	—	An inverse correlation between piR-1245 and a panel of tumor suppressor genes (predicted)	Prognostic	yes	yes	yes	no	yes	TCGA piRNA microarray RT-PCR	[90]

	piRNA-54265	↑	STAT3 signaling pathway	Induce p-STAT3 and BCL-XL, MMP2 and MMP9, reduce cleaved-CASP9, 3 and 7	Diagnostic Theraputic	no	yes	yes	yes	yes	TCGA GEO RT-PCR	91
	piR-015551	↓	—	piR-015551 may be generated from LNC00964-3, which may be involved in the development of CRC	Risk assesment	no	yes	no	no	no	piRNA microarray RT-PCR	92
	piR-5937	↓	—	Differentiate between cancer patients and healthy donors, diagnostic performance in patients with stage I disease; higher sensitivity than currently used biomarkers CEA and CA19-9.	Diagnostic	no	no	no	yes	no	piRNA microarray RT-PCR	93
	piR-28876											
	piR-30074	—	—	Regulating transformation of stem cells	Theraputic	no	no	yes	no	no	—	94
Gastric cancer	93 piRNAs	↓	—	—	—	yes					TCGA	95
	7 piRNAs	↑										
Liver cancer	PIR1/97	↑	—	Involved in cell adhesion.	—	no	yes	yes	no	no	piRNA microarray RT-PCR	96
Lung cancer	piR-34871	↑	RASSF1C	RASSF1C modulate piRNA through attenuation of the AMPK pathway, as over-expression of RASSF1C resulted in reduction of p-AMPK, p21, and p27 protein levels.	Theraputic	no	yes	yes	no	no	piRNA microarray RT-PCR	98
	piR-52200											
	piR-35127	↓										
	piR-46545											
	piR-57125	↓	—	Most significantly associated with distant metastasis	Prognostic							99
	piR-55490	↓	mTOR	Directly binds to 3′-UTR of mTOR mRNA and induce its degradation	Theraputic	no	yes	yes	no	yes	RT-PCR	100
	piR-L-163	↓	ERM proteins, F-actin, EBP50	Binds directly to phosphorylated ERM proteins (p-ERM), which is critical for p-ERM's binding capability to filamentous actin (F-actin) and ERM-binding phosphoprotein 50 (EBP50).	Theraputic	no	no	yes	yes	no	RNA sequencing RT-PCR	101
	piR-L-138	↑	p60-MDM2	piR-L-138 is upregulated by CDDP-based agents and inhibits apoptosis through direct interaction with p60-MDM2.	Theraputic	no	no	yes	no	no	RT-PCR	149
	piR-30074	—	—	Regulating transformation of stem cells	Theraputic	no	no	yes	no	no	—	28
Leukemia	hsa_piR_011186	↑	CDKN2B, DNMT1, Suv39H1, EZH2	hsa_piR_011186 promoted cell-cycle progression, decreased apoptosis by forming complex with DNMT1, Suv39H1 and/or EZH2 proteins, which inhibit CDKN2B gene through modulation of the methylation of DNA and histone H3 in its promoter region.	Diagnostic	no	no	yes	no	no	RT-PCR	126

(*continued on next page*)

Table 1 (*continued*)

Cancer	piRNA	Expression	Related gene	Mechanical points	Clinical Utility	Sample Type					Detection Technique	Reference
						TCGA	Tissue	cell	serum	mice		
Kidney cancer	piR-34536	↓	—	—	Prognostic	no	yes	no	yes	no	RT-PCR	[102]
	piR-51810		—	—								
	piR-30924	↑	—	Associated with tumor recurrence and overall survival.	Prognostic	no	yes	no	no	no	piRNA microarray RT-PCR	[104]
	piR-38756											
	piR-57125	↓										
	piR-32,051	↑	—	Associated with ccRCC metastasis, late clinical stage and poor cancer-specific survival.	Prognostic	no	yes	no	no	no	piRNA microarray RT-PCR	[105]
	piR-39,894											
	piR-43,607											
Glioblastoma	piRNA-8041	↓	Cellular stress and survival pathway	Suppressed the heat shock protein and related DNAJ Protein chaperone families, MAPK/ERK signaling pathway proteins; (PREICTED)	Therapeutic	no	yes	yes	no	yes	piRNA microarray RT-PCR	[106]
	piR-598	↑	—	variant rs147061479 in piR-598 increases glioma risk by abolishing the tumor-suppressive function of piR-598	Diagnostic	yes	no	yes	no	no	genome-wide profiling RT-PCR	[107]
HNSCC	*NONHSAT077364*	↑	HPV, PIWIL4, RTL, DNMT3L, PTEN, NOTCH, HNSCC-associated CNVs	Positively correlated with PIWIL4 and DNMT3L; negatively relative to RTL by direct binding interaction; upregulated by PTEN mutation or NOTCH mutation or downregulated by HNSCC associated CNVs	Diagnostic	yes	no	yes	no	no	TCGA RT-PCR	[109]
	NONHSAT102574											
	NONHSAT128479											
	piR-266308	↑	Alcohol	Low expression of piR-58510 and piR-35373 significantly correlated with improved patient survival. Alcohol consumption may cause dysregulation of piRNA expression and upregulation of human PIWIL 4.	Prognostic	yes	no	yes	no	no	RT-PCR	[110]
	piR-58510											
	piR-38034											
	piR-35373											
	13 smoking-related piRNAs	↑/↓	Smoking, PIWIL1, TP53	Associated with tumor stage, patient survival, and smoking-altered PIWIL1 protein expression. correlated with TP53 mutation, TP53—3p co-occurrence, and 3q26, 8q24, and 11q13 amplification.	Prognostic	yes	no	no	no	no	TCGA	[111]

Hypoxic cancers	40 hypoxia regulated piRNAs		Hypoxia, HIF-1α and VHL	Hypoxia-regulated piRNAs are regulated by VHL/HIF signaling in vitro, HIF-1α stabilization induced by VHL knockdown was responsible for the increase in piRNA expression.	Prognostic	yes	no	yes	no	no	TCGA RT-PCR	[112]
	36 piRNAs	↑										
	4 piRNAs	→										
Pancreatic cancer	piR-017061	↓	—	—	Theraputic	no	yes	no	no	no	Next-generation sequencing	[113]

Abbreviation: ARHGAP, Rho GTPase activating proteins; CNV, copy number variation; ccRCC, clear cell renal cell carcinoma; CRC, colorectal cancer; CDKN2B, cyclin dependent kinase inhibitor 2B; ERβ, estrogen receptor; CASP3, caspase 3; CNV, copy number variation; GEO, gene expression omnibus; HPV, human papillomavirus; mTOR, mechanistic target of rapamycin kinase; LRAT, lecithin retinol acyltransferase; PTEN, phosphatase and tensin homolog; PIWIL, piwi like RNA-mediated gene silencing 2; RT-PCR, quantificational real time-polymerase chain reaction; RRM2, ribonucleotide reductase regulatory subunit; RTL, Retrotransposon Gag like; SEPW1, selenoprotein W 1; SERPINA1, serpin family A member 1; STAT3, signal transducer and activator of transcription 3; TCGA, The Cancer Genome Atlas; TDRD1, tudor domain containing 1; TNFSF4, TNF superfamily member 4; TP53, tumor protein p53; MMP, matrix metallopeptidases; VHL, von Hippel-Lindau tumor suppressor; UTR, untranslated region.

is reversed with promoter hypermethylation or genome hypomethylation (Fig. 4A).[131,132] piRNAs are hypothesized to have the ability to both inactivate tumor suppressor genes via aberrant promoter hypermethylation and activate oncogenes via gene body hypomethylation (Fig. 4A).[78,124,133] After direct sequence-specific binding to target CpG sites, piRNAs recruit methyltransferases such as DNMT1, DNMT3A, and DNMT3B to the respective region (Fig. 4A).[124,126,133] Since binding of transcription factors and DNA methylation are mutually exclusive, CpG methylation in promoter regions by piRNAs has been found to result in transcriptional repression of tumor suppressor genes p15 and p16.[124,126,134] In terms of hypomethylation the exact mechanism is still unclear. It is thought to involve a TET methylcytosine dioxygenase (TET)-dependent manner, as TET in collaboration with transcription factors may lead to DNA demethylation and thus gene activation (Fig. 4A).[131] Interestingly, piRNA mutations may contribute to cancer if the variant fails to bind to target regions and fails to form the piRNA-PIWI-DNMT complex. The exact roles of piRNA in DNA methylation and hypomethylation are worth further investigation.

TGS and TGA by piRNAs via histone modification

The N-terminal of histone proteins undergoes extensive modifications including but not limited to methylation, acetylation, phosphorylation, ubiquitination, and SUMOylation.[135] Activating histone markers include histone H3 lysine 4 trimethylation (H3K4me3), H3 and H4 acetylation, and histone H3 lysine 36 (H3K36me2 and H3K36me3). These activating markers help to shape the three-dimensional structure of the nucleosome into a euchromatin conformation with open chromatin available for transcription. Repressive histone markers such as trimethylation of H3 lysine 9 and 27 (H3K9me3 and H3K27me3) create a heterochromatin state with condensed chromatin (Fig. 4B).[136] piRNA can switch the chromatin conformation freely from heterochromatin to euchromatin by recruiting different histone-modifying enzymes. Such enzymes include mixed lineage leukaemia 3 (MLL3) for H3K4 methylation, suppressor of variegation 3–9 homolog 1 (SUV39H1) for H3K9 methylation, enhancer of zeste homolog 2 (EZH2) for H3K27 methylation, lysine demethylases 1A and 5B (KDM1A and 5B) for H3K4 demethylation, as well as KDM6A for H3K27 demethylation (Fig. 4B).[126,137,138] It has been reported that the piR-011186/Ago3 complex represses the expression of CDKN2B by recruiting Suv39H1 and/or EZH2 to modify the methylation levels of H3K9 and H3K27 in its promoter region.[126] On the other hand, by complexing with PIWIL1/4 and subsequently recruiting MLL3 and KDM6A, piR-sno75 is capable of simultaneously enhancing H3K4 methylation and H3K27 demethylation, and ultimately upregulates the expression of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL).[137] These observations demonstrate the flexibility of different piRNA/PIWI complexes in the regulation of gene expression through specific chromatin modification.

PTGS by piRNAs via mRNA decay or degradation

piRNA-induced repression may be mediated at the PTGS level via direct binding followed by PIWI protein-mediated cleavage or deadenylation and degradation of the target

Table 2 Summary of piRNAs showing pan-cancer expression patterns.

piRNA	Cancer	Expression	Related gene	Mechanical points	Clinical Utility	Sample Type					Detection Technique	Reference
						TCGA	Tissue	cell	serum	mice		
piR-651	Breast cancer	↑	Estrogen and Androgen	Modulated by estrogen and androgen hormone levels	Theraputic	no	no	yes	no	no	RT-PCR	[114]
		↑	—	—	Diagnostic	no	yes	yes	no	no	piRNA microarrayRT-PCR	[115]
	Cervical cancer	↑	—	—	Diagnostic	no	no	yes	no	no	piRNA microarrayRT-PCR	[115]
	Gastric cancer	↓	—	The piR-651 level in gastric adenocarcinoma was higher than that in gastric signet ring cell carcinoma.	Diagnostic Theraputic	no	no	no	yes	no	RT-PCR	[116]
		↑	—	Promote cancer cell growth	Diagnostic	no	no	yes	no	no	piRNA microarrayRT-PCR	[115]
	Hodgkin lymphoma	↓	—	—	Diagnostic	no	yes	yes	yes	no	RT-PCR	[117]
	Liver cancer	↑	—	—	Diagnostic	no	no	yes	no	no	piRNA microarrayRT-PCR	[115]
	Lung cancer	↑	Caspase-3-p17, cleaved-PARP-1, Bcl-2	piR651 inhibitor induced cell apoptosis, with caspase-3-p17 and cleaved-PARP-1 increased, whereas Bcl-2 expression decreased	Diagnostic	no	no	yes	no	no	RT-PCR	[118]
		↑	—	—	Diagnostic	no	no	yes	no	no	piRNA microarrayRT-PCR	[115]
		↑	Cyclin D1 and CDK4	piR-651 induces NSCLC progression through the cyclin D1 and CDK4 pathway	Diagnostic Theraputic	no	yes	yes	no	yes	RT-PCR	[119]
		↑	—	promote cell proliferation, apoptosis, migration and invasion	Theraputic	no	no	yes	no	no	RT-PCR	[120]
	Mesothelium	↑	—	—	Diagnostic	no	no	yes	no	no	piRNA microarrayRT-PCR	[115]
	Prostate cancer	↑	Estrogen and Androgen	Modulated by estrogen and androgen hormone levels	Theraputic	no	no	yes	no	no	RT-PCR	[114]
piR-823	Breast cancer	↑	Estrogen and Androgen	Modulated by estrogen and androgen hormone levels	Theraputic	no	no	yes	no	no	RT-PCR	[114]
	Colorectal cancer	↑	HSF1 (heat shock protein (HSP) 27, 60, 70)	Upregulate phosphorylation and transcriptional activity of HSF1	Theraputic	no	yes	yes	no	no	RT-PCR	[121]
	Gastric cancer	↓	—	The piR-823 level was positively associated with tumor-node-metastasis stage and distant metastasis.	Diagnostic Theraputic	no	no	no	yes	no	RT-PCR	[116]

		↓	—	Inhibited cell growth *in vitro* and *in vivo*.	Theraputic	no	yes	yes	no	yes	piRNA microarray RT-PCR	[122]
	Kidney cancer	↑/↓	—	piR-823 is down-regulated in tumor tissue, but positively correlated with worse outcome, indicating its complex role in RCC pathogenesis (↑blood serum and urine↓tumor tissue)	Diagnostic	no	yes	no	yes	no	RT-PCR	[123]
	Liver cancer	↑		Gradually increased from cirrhosis to low- and high-grade dysplastic nodules and to hepatocellular carcinoma (HCC)	Diagnostic	no	no	yes	no	no	RT-PCR	[55]
	Multiple myeloma	↑	VEGF, IL-6, ICAM-1, CXCR4, Bax, Bcl2	Promoted cell proliferation, tube formation, and invasion by enhancing VEGF, IL-6, ICAM-1, CXCR4 and attenuating apoptosis. (inhibition of Caspase-3 activation, downregulation of Bax, and upregulation of Bcl2) and production of ROS and NO	Prognostic Therapeutic	no	no	yes	yes	yes	RT-PCR	[20]
		↑	DNMT3B	Myeloid-derived suppressor cells confer stem-like qualities to multiple myeloma cells by upregulating piRNA-823 expression and activating DNMT3B activation	Theraputic	no	yes	yes	no	yes	RT-PCR	[83]
		↑	DNMT3A and 3B, $p16^{INK4A}$	induce *de novo* DNA methyltransferases, DNMT3A and 3B and inhibit methylation-silenced tumor suppressor, $p16^{INK4A}$.	Theraputic	no	yes	yes	no	no	RT-PCR	[124]
	Prostate cancer	↑	Estrogen and Androgen	Modulated by estrogen and androgen hormone levels	Theraputic	no	no	yes	no	no	RT-PCR	[114]
piR-4987	Breast cancer	↑	—	Up-regulated piR-4987 expression was associated with lymph node positivity	Prognostic	no	yes	no	no	no	piRNA microarray RT-PCR	[88]
	Multiple myeloma	↑	—	Upregulated in stage III MM patients compared with healthy individuals	Diagnostic	no	yes	no	no	no	RT-PCR	[124]

(*continued on next page*)

Table 2 (*continued*)

piRNA	Cancer	Expression	Related gene	Mechanical points	Clinical Utility	Sample Type					Detection Technique	Reference
						TCGA	Tissue	cell	serum	mice		
piR-Hep1	Liver cancer	↑	AKT	Promotes cell viability, motility, and invasiveness by activation of AKT phosphorylation	—	no	yes	yes	no	no	RT-PCR	[97]
	Multiple myeloma	↑	—	Upregulated in stage III MM patients compared with healthy individuals	Diagnostic	no	yes	no	no	no	RT-PCR	[124]
piR-39980	Fibrosarcoma	↓	RRM2	piR-39980 promotes apoptosis and inhibits proliferation by repressing RRM2 through direct targeting at its 3′-UTR through extensive sequence complementary binding	Therapeutic	no	no	yes	no	no	RT-PCR	[108]
	Neuroblastoma	↑	JAK3	Inhibition of piRNA induces NB cells senescence by modulating JAK3 through target binding. piR-39980 desensitizes the effect of doxorubicin and inhibit drug-induced apoptosis.	Theraputic	no	no	yes	no	no	piRNA profiling RT-PCR	[127]

Abbreviation: BCL2, BCL2 apoptosis regulator; CDK4, Cyclin dependent kinase 4; CXCR4, C-X-C motif chemokine receptor 4; DNMT, DNA methyltransferase; JAK3, Janus kinase 3; HSP, heat shock protein; HSF1, heat shock transcription factor 1; VEGF, vascular endothelial growth factor; IL6, interleukin 6; ICAM1, intercellular adhesion molecule 1; MM, Multiple myeloma; RRM2, ribonucleotide reductase regulatory subunit M2; RT-PCR, quantificational real time-polymerase chain reaction; TCGA, The Cancer Genome Atlas; UTR, untranslated region.

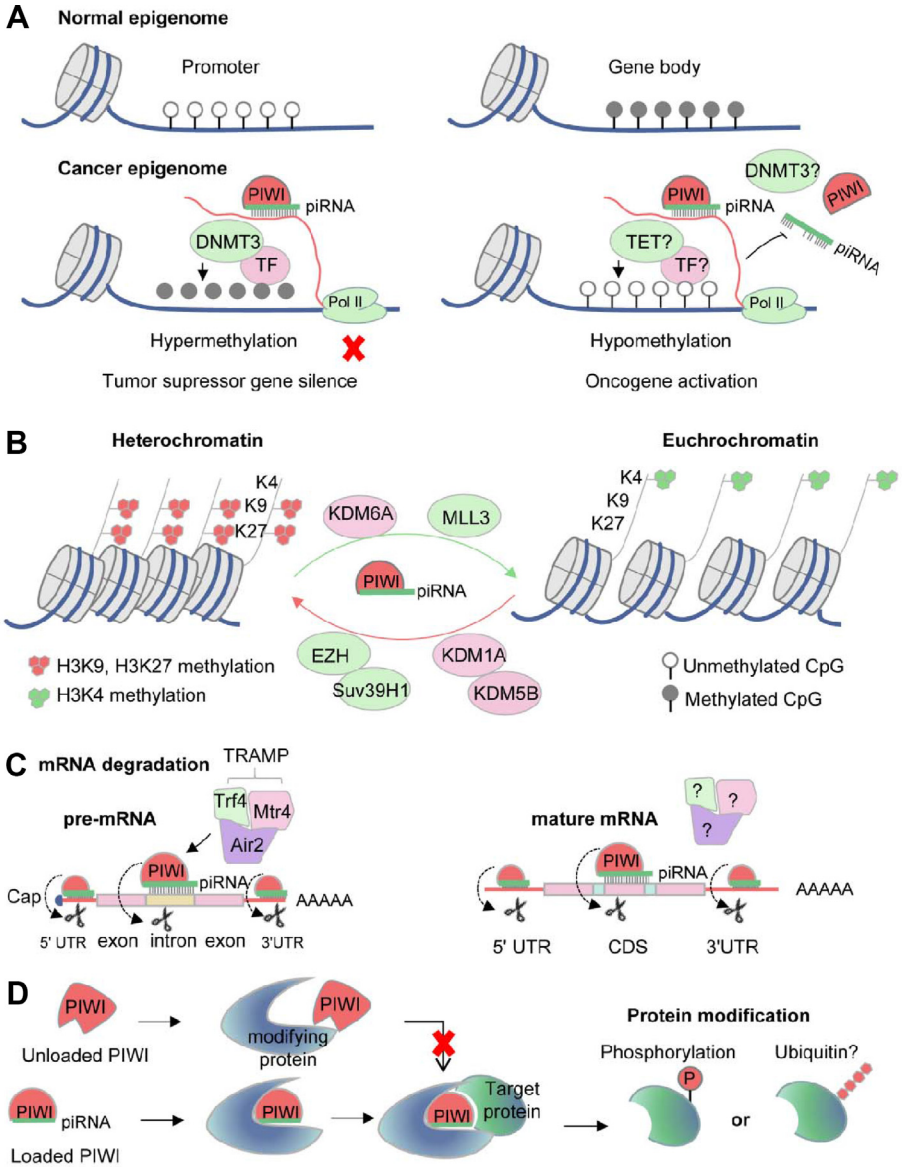

Figure 4 Mechanism of piRNAs in cancer. (**A**) TGS or TGA by piRNAs via aberrant DNA methylation: In cancer, the piRNA—PIWI complex represses tumor-suppressor genes (TSGs) via recruiting DNA methyltransferases to the promoter and activates oncogenes by recruiting TET to the gene body. piRNA variants fail to bind to target regions and to form a piRNA—PIWI—DNMT complex, resulting in gene body hypomethylation and oncogene activation. (**B**) TGS or TGA by piRNAs via histone modification: the piRNA—PIWI complex induces the transformation between heterochromatin (condensed) and euchromatin (open) by recruiting methyltransferases or demethylases to histones and depositing active (H3K4me3) or repressive histone markers (H3K9me3 or H3K27me3). (**C**) PTGS by piRNAs via mRNA decay or deregulation: the piRNA—PIWI complex recruits the TRAMP complex and leads to mRNA decay. The piRNA—PIWI complex results in mRNA degradation via the slicer activity of PIWI. Whether or not other factors are needed is unknown. (**D**) PTGS or PTGA by piRNAs via protein modification: piRNA loading induces a conformational change in PIWI and enhances its interaction with proteasomes, resulting in protein phosphorylation or ubiquitination. Abbreviation: CDS, coding DNA sequence; DNMT, DNA methyltransferase; H3K9, H3 lysine 9; H3K27, H3 lysine 27; H3K4, H3 lysine 4; KDM, lysine demethylase 6A; PTGS, post-transcriptional gene silencing; PTGA, post-transcriptional gene activation; TF, transcription factor; SUV39H1, suppressor of variegation 3—9 homolog 1; TET, tet methylcytosine dioxygenase 1; TGA, transcriptional gene activation; TGS, transcriptional gene silencing; UTR, untranslated region.

mRNA (Fig. 4C).[79,84,86,90,100,109] Typically, piRNAs target the 3′-untranslated region (3′-UTR) of an mRNA, which may be attributed to the fact that most human genomic piRNAs are derived from 3′-UTR (Fig. 4C).[15,79,84,86,90,100] However, the coding sequence or 5′-UTR has been identified as other possible binding regions (Fig. 4C).[86,90] In mice, a large increase in the expression of targeted RNAs in PIWIL1 slicer mutants indicates the piRNA-mediated degradation of mRNA depends on the slicer activity of PIWIL1. Furthermore, piRNAs can also trigger mRNA deadenylation and decay in human somatic cells; a similar report has been made in *Drosophila* and mice.[139,140]

In CD4 primary T-lymphocytes, piR-30840 binds to the pre-mRNA intron via sequence complementarity.[141] After that, the complex acts together with PIWIL4 and Ago4 to recruit the Trf-Air2-Mtr4 polyadenylation (TRAMP) complex to this region. This induces the decay of targeted pre-mRNA through nuclear exosomes. In this way, piR-30840 significantly downregulates interleukin-4 (IL-4) and subsequently inhibits the development of Th2 $CD4^+$ T-lymphocytes (Fig. 4C).[141] This mechanism portrays the versatility of piRNA downregulating mechanisms, specifically in post-transcriptional gene silencing.

PTGS and PTGA by piRNAs via protein modification

Dysregulation of phosphorylation or ubiquitination of proteins is a defining feature of numerous cancers.[142] piRNAs may promote tumor formation in this way by upregulating the phosphorylation and transcriptional activity of signaling proteins such as heat shock transcription factor 1 (HSF1), Akt, or signal transducer and activator of transcription 3 (STAT3).[91,97,121,143] piR-Hep1 overexpression in hepatocellular carcinoma (HCC) promotes cell viability, motility, and invasiveness by inducing AKT phosphorylation and thus results in augmentation of PI3K/AKT signaling pathway.[97] By binding to the PIWI domain of PIWIL2, piR-54265 facilitates the formation of the PIWIL2/STAT3/phosphorylated-SRC (p-SRC) complex, thereby inducing the phosphorylating activation of STAT3 by p-SRC and promoting malignancy of CRC cells.[91] It has been proposed that the molecular mechanism may be that piRNA loading leads to a conformational change in PIWI which enhances its interaction with proteasomes (Fig. 4D).[144]

Although piRNAs can act as either tumor suppressors or tumor promoters, the general mechanisms used by piRNA-mediated inhibition or activation are the following four-step process. (1) Formation of the PIWI-piRNA complex because a specific PIWI protein is essential for a functional piRNA[128]; (2) Searching and interacting with their specific targets; (3) Recruiting of specific enzymes such as DNA methyltransferases and/or histone/protein-modifying enzymes[129,145]; (4) Silencing or activating their targets. However, there are some exceptions. For example, in piRNA-mediated mRNA degradation, the PIWI-piRNA complex themselves are fully functional without any additional enzymes.

Emerging role of piRNAs in cancer stemness and drug resistance

Cancer stem cells (CSCs) are regarded as small and unique sources of cells with self-renewal and differentiation capacity and can generate a variety of cell types that constitute a tumor.[146] These cells, despite making up a small proportion of the total number of tumor cells, may serve as the primary contributors to progression, metastasis, recurrence, and drug resistance.[147] In recent studies researchers have been investigating the role of piRNAs in cancer stem or stem-like cells and the various mechanisms they may be involved in. It has been reported that the expression of piR-932 and PIWIL2 is significantly higher in breast CSCs which have been induced to epithelial-to-mesenchymal transition (EMT) by transforming growth factor-β1 (TGF-β1).[82] Further functional study shows that the piR-932-PIWIL2 complex depresses the Latexin tumor suppressor gene via the aberrant methylation of its promoter region CpG island, thus contributing to the progression of breast cancer.[82] In another instance, granulocytic-myeloid-derived suppressor cells (G-MDSCs) have been found to confer stem-like properties to multiple myeloma cells by upregulating piR-823 expression and activating DNMT3B activation.[83] Interestingly, the knockdown of piR-823 reverses the upregulation of CSC marker genes and proteins induced by G-MDSCs in multiple myeloma cells. This suggests that piR-823 is an essential factor in the maintenance of multiple myeloma stem cells (MMSC) stemness.

Drug resistance is an important feature of CSCs and one of the primary reasons for therapeutic failure.[148] The biological functions of piRNAs have been found to play an important roles in chemoresistance.[84,91,149] piR-L-138 inhibits apoptosis in lung squamous cell carcinoma cells and has been shown to provide chemoresistance toward cisplatin-based chemotherapy via interaction with p60-mouse double minute 2 homolog (MDM2).[149] Additionally, increased levels of piR-54265 binds to PIWIL2 and activates STAT3 signaling, thereby conferring chemoresistance to 5-fluorouracil (5-FU) and oxaliplatin in colorectal cancer cells.[91] A separate study conducted by the same authors suggests that a lower level of piR-36712 induces chemoresistance towards paclitaxel or doxorubicin in breast cancer.[84] piR-39980 decreases the effects of doxorubicin and inhibits drug-induced apoptosis by modulating Janus Kinase 3 (JAK3) through target binding.[127] Collectively, piRNAs may play a crucial role in maintaining the stem-cell properties of cancer cells, especially in drug resistance.

The uncertain meaning of piRNAs in tumor immunology

Studies on the involvement of piRNAs in the mammalian immune system are few, let alone tumor immunology. The limited number of reports indicate that piRNAs are abundant in human CD4 primary T-lymphocytes, monocytes or dendritic cells (DCs), and they play an important role in regulating gene expression in these highly differentiated somatic cells.[141,150] piR-30840 significantly decreases interleukin-4 (IL-4) expression via sequence complementarity and binding to its pre-mRNA intron, subsequently restraining the development of Th2 T-lymphocytes. The piR-30840/PIWIL4/Ago4 complex further interacts with the TRAMP complex, and induces IL-4 decay in nuclear exosomes.[141] Another study suggests that tRNA-Glu-derived piRNA [td-piR(Glu)] depresses CD1A transcription by inducing H3K9 methylation on monocytes/dendritic cells. The depression can be reversed by IL-4, inhibiting the biogenesis of td-piR(Glu) and upregulating CD1a molecules. As CD1a is a specific DC marker responsible for the presentation of lipid antigen to T-lymphocytes,[151] it suggests that piRNAs can act as a mediator signal for lipid Ag presentation in monocytes/dendritic cells.[150] These novel mechanisms provide evidence for the importance of piRNAs in immune cells. Given the impact of immune cells in tumor progression, piRNAs may contribute to the cancer immune

system via IL-4 decay or CD1a dysregulation or other unidentified methods.

Potential clinical applications of piRNAs in cancer

Diagnostic and prognostic value of piRNAs

piRNAs have been proven to be effective diagnostic indicators in cancer, and appear to have higher sensitivity than currently used biomarkers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9).[93,116] Due to their distinct expression in postoperative and preoperative patients, peripheral blood piRNAs can be applied in postoperative long term monitoring and disease progression.[93,116] Patients with increased levels of onco-piRNAs or low levels of tumor-suppressive piRNAs often show worse progression-free survival (PFS) or overall survival (OS) correlated with overall unfavorable prognosis.[84,90,91,110,102] For example, PFS time and OS time are significantly shortened in patients with colorectal cancer (CRC) who have high piR-54265 levels compared with patients who have low piR-54265 levels.[91] Additionally, piR-36,712 levels contain an independent prognostic value in breast cancer in that low levels are associated with worse clinical outcomes, shorter PFS, and higher rates of axillary lymph node metastasis.[84] These clear pieces of evidence provide support for the idea that piRNAs are promising indicators for prognostic value in cancer.

Liquid biopsy of circulating piRNAs

Liquid biopsy is a commonly used technique that has been developed to detect cancer at an early stage and allows for tracking of tumor progression over time. In 2011, it was discovered that piRNAs could be a promising factor of analysis in liquid biopsy.[122] Area under the receiver operating characteristic curves (AUCs) of piR-651, piR-823, and combinations of the two were 0.841, 0.822, 0.860 for gastric cancer detection in that 2011 study.[122] Since then, a variety of circulating piRNAs have been reported as excellent candidates for liquid biopsy of cancer.[91,93,102,117] Interestingly, exosomal piRNAs in the serum of cancer patients have also been quantified and are often more stable than miRNAs for the protection of PIWI proteins.[20] Specifically, a higher level of piRNA-823 has been detected in extracellular vesicles secreted from multiple myeloma than from healthy controls and is positively associated with the stage of the disease. This indicates that the quantification of piRNAs in exosomes is also an attractive and promising inquiry.[20]

Therapeutic targeting of piRNAs

Molecular beacon (MB)-based bioimaging systems, which simultaneously combine the diagnosis and therapy of cancer, have attracted considerable attention in the cancer theragnosis field. Specific advantages include non-invasiveness, low cost, easy accessibility, and real-time quantitative imaging. MBs have also been applied in piRNA bioimaging.[86,87] For example, endogenous piR-36026 in breast cancer cells has been successfully visualized by using a double stranded piR-36026 MB labeled with Cy5.5 fluorescent dye and black hole quencher 2 (BHQ2).[86] In addition, the labeled piR-36026 displays a therapeutic response by binding to endogenous piR-36026 and blocking its hybridization with TSGs serpin peptidase inhibitor, clade A, member 1 (SERPINA1) and lecithin retinol acyltransferase (LRAT).[86] This results in the upregulation of SERPINA1 and LRAT followed by caspase-3 activation and eventually cancer cell death.[86]

Moreover, labeling piRNA MBs with different Cyanine (Cy) fluorescent dyes allows the simultaneous visualization of multiple endogenous piRNAs in a single cell. For example, the dual transfection of Cy5.5 labeled piR-36026 MB and Cy3 labeled piR-36743 MB allows for the detection of both piRNAs in breast cancer cells.[87] Furthermore, the quantitative measurement of different Cy5.5 and Cy3 signal intensities can successfully distinguish between the subtypes of breast cancer.[87] In general, piRNA MBs may be a potential cancer theragnosis platform that allows for piRNA visualization, subtype identification, and TSG activation.

piRNA targeted therapy in combination with standard chemotherapeutic drugs has demonstrated promising anticancer effects in both *in vitro* and *in vivo* experiments. Both *in vitro* and *in vivo* trials have achieved greater efficacy in combined treatment of breast and colorectal cancer cells. Using a piR-54265 inhibitor (CRC) or a piR-36,712 analog (BrCa) in combination with chemotherapeutic agents such as 5-FU, paclitaxel, and doxorubicin were found to be more efficacious than treating with each agent alone.[84,91] In addition, the combination of piRNA- and miRNA-targeting particles has been shown to achieve a satisfactory anticancer effect. After incubation with nano-sized complexes of miRNA-152 and piRNA-30074, CaCo2 colorectal adenocarcinoma cells is transformed into $CD4^{+}$ cells. Increased levels of apoptotic markers caspase 9 and mechanistic target of rapamycin kinase (mTOR) and decreased levels of marker of proliferation ki-67 (MKI-67) has been detected in these cells.[94] The same effect has been observed in A-549 lung adenocarcinoma cells and Girardi heart cells (cervical cancer combined with right atrium cancer) after incubation with the piRNA-30074 and antago-miR-155 complex[103,125] These findings support the concept that the incorporation of piRNA-targeting particles with chemotherapy or miRNA-targeted therapy may lead to new pathways for cancer treatment research. In the future, additional combination therapeutic strategies, including the combination of piRNAs with immunotherapies such as immune checkpoint inhibitors [Programmed Death 1 (PD1)/cytotoxic T-lymphocyte-associated protein 4 (CTLA4)], can be designed to provide novel opportunities for addressing cancer treatment and drug resistance.

Conclusions and future perspectives

The last two decades have brought to light the true potential of piRNAs in liquid biopsy, prognosis, prediction of treatment response, and target therapy in cancer. piRNAs contribute to carcinogenesis via aberrant DNA methylation, histone marker deposition, mRNA degradation, and protein

modification. However, most of the current researches focus on the downstream effects of piRNA dysregulation, and only a few studies investigated the upstream factors that cause piRNA dysregulation. The root of piRNA dysregulation in cancer is likely the result of disorders in piRNA biogenesis. Although the process of piRNA biogenesis in *Drosophila* and mice is well defined, the exact mechanism in humans remains unclear. Additionally, it is unknown whether or not piRNA biogenesis relevant proteins, such as MitoPLD or MOV10L1, is associated with piRNA dysregulation in cancer.

What's more, if mitochondria-mediated phased production is the final stage of piRNA biogenesis, then how do mature piRNAs return from mitochondria to nucleus to exert transcriptional regulation? In flies, most of the mature piRNAs produced on OMM are loaded into Piwi, one step essential for piRNAs nuclear translocation.[16,33,72] piRNA loading triggers conformational change in Piwi and exposes a nuclear localization signal (NLS) at Piwi's N terminus which leads to importin-α-mediated transportation to the nucleus.[152] Piwi could also interact with nucleoporin 358 (Nup358) to enter the nucleus.[153] In mice, piRNAs are loaded with MIWI2 and subsequently translocated to the nucleus.[6] In humans, however, the nucleus translocation mechanism remains elusive. Is PIWI loading necessary for piRNA nuclear entry in a similar way as in *Drosophila* and mice? If so, which protein is involved? It has been reported that HIWI/HIWI2-piR-sno75 complex could activate TRAIL gene by recruiting histone methytransferases MLL3 and KDM6A to its promoter. Since histone modification happens, it indicates that HIWI/HIWI2 may be relevant to nucleus import of piRNAs.[137] However, the PIWI proteins which could be detected in nucleus are in the form of HIWI/HIWI2 and HILI.[141,154,155] So, HILI may also be related in this process. Therefore, more systematic research on piRNA biogenesis and nuclear translocation machinery in humans is needed before we could better understand the piRNA dysregulation in cancer. Only then can we combat cancer by targeting piRNA imbalance-mediated carcinogenesis.

Despite the well-established PIWI-piRNA pathway during normal or cancer development, there are emerging hints that PIWI could function in a piRNA independent way. Li et al demonstrate that in the absence of piRNAs, human PIWIL1 plays an oncoprotein role in activating the anaphase promoting complex/cyclosome (APC/C) E3 complex, which then results in proteolysis of a critical cell adhesion-related protein, Pinin, and finally enhances the metastasis of pancreatic ductal adenocarcinomas (PDACs).[156] Another study also shows that piRNA-unloaded PIWIL1 promotes gastric cancer through interaction with the UPF1- mediated nonsense-mediated mRNA decay (NMD) mechanism. Furthermore, mutating piRNA-binding residues of PIWIL1 does not compromise its oncogenic function. These piRNA-independent manner of PIWIL1 are consistant with an earlier report which failed to detected potentially functional piRISCs formed by PIWIL1 and piRNAs in a colon cancer cell line (COLO205).[157] These lines of evidence support an piRNA-independent manner of oncogenic PIWIL1 in carcinogenesis. More importantly, all these studies uncover an unexpected fact, that is piRNAs are barely detectable in a series of cancer cells.[156,157] It raises the question that whether the previous dysregulated piRNAs identified in various types of cancer cells are authentic piRNAs. A recent report indicates that ubiquitous background of small RNA-sequencing (RNA-seq) data may contaminate databases and abundant RNA fragments have sometimes been misinterpreted as piRNAs.[158] So the authenticity of some of the reported somatic and cancerous piRNAs and their interaction between PIWI proteins deserve further investigation.

Authors contribution

Zhou Fuyou: Conceptualization, Supervision, Validation, Writing-review & editing. Zhang Hao: Conceptualization, Supervision, Validation, Writing-review & editing. Anthony Concilla: Investigation, Data curation, Writing-review & editing. Zhang Dianzheng: Investigation, Data curation, Writing-review & editing. Su Jingfen: Investigation, Data curation, Writing-original draft. Zhao Fang: Investigation, Data curation. Shen Fangfang: Investigation, Data curation.

Conflict of Interests

The authors declare that they have no competing interests.

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