SCIENCE CHINA PRESS

SCIENCE CHINA Life Sciences
life.scichina.com link.springer.com
https://doi.org/10.1007/s11427-023-2491-7

REVIEW

The burgeoning importance of PIWI-interacting RNAs in cancer progression

Xinpei Deng[1†], Tianle Liao[2†], Jindong Xie[1†], Da Kang[1], Yiwei He[3], Yuying Sun[1], Zhangling Wang[1], Yongluo Jiang[1], Xuan Miao[1], Yixuan Yan[4], Hailin Tang[1], Lewei Zhu[5*], Yutian Zou[1*] & Peng Liu[1*]

[1]State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-sen University Cancer Center, Guangzhou 510060, China;
[2]School of Medicine, Sun Yat-sen University, Shenzhen 518107, China;
[3]Key Laboratory of Cardiovascular and Cerebrovascular Medicine, Nanjing Medical University, Nanjing 211166, China;
[4]Guanghua School of Stomatology, Sun Yat-sen University, Guangzhou 510062, China;
[5]The First People's Hospital of Foshan, Foshan 528000, China

†Contributed equally to this work
*Corresponding authors (Lewei Zhu, email: zlwei@fsyyy.com; Yutian Zou, email: zouyt@sysucc.org.cn; Peng Liu, email: liupeng@sysucc.org.cn)

Received 19 October 2023; Accepted 13 November 2023; Published online 29 December 2023

PIWI-interacting RNAs (piRNAs) are a class of small noncoding RNA molecules that specifically bind to piwi protein family members to exert regulatory functions in germ cells. Recent studies have found that piRNAs, as tissue-specific molecules, both play oncogenic and tumor suppressive roles in cancer progression, including cancer cell proliferation, metastasis, chemoresistance and stemness. Additionally, the atypical manifestation of piRNAs and PIWI proteins in various malignancies presents a promising strategy for the identification of novel biomarkers and therapeutic targets in the diagnosis and management of tumors. Nonetheless, the precise functions of piRNAs in cancer progression and their underlying mechanisms have yet to be fully comprehended. This review aims to examine current research on the biogenesis and functions of piRNA and its burgeoning importance in cancer progression, thereby offering novel perspectives on the potential utilization of piRNAs and piwi proteins in the management and treatment of advanced cancer.

PIWI-interacting RNAs/piRNAs | cancer progression | metastasis | chemoresistance | stemness

Background

PIWI-interacting RNAs (piRNAs) are a recently discovered group of small noncoding RNAs consisting of 18–35 nucleotides and a subset of piRNAs exhibit a 5′-terminal uridine or tenth position adenosine bias. These RNAs lack clear secondary structure motifs and interact with proteins belonging to the PIWI clade of the Argonaute family, including Aubergine (Aub), Argonaute 3 (Ago3), and Piwi in flies, as well as MIWI, MILI, and MIWI2 in mice (Liu et al., 2016; Wang et al., 2023b). Unlike microRNAs (miRNAs) and small interfering RNAs (siRNAs), which are processed by Dicer, an RNase III enzyme, from hairpin-shaped precursors and long double-stranded RNAs, piRNAs are derived from long single-stranded RNA precursors that are transcribed from specific genomic loci and are subsequently processed by various enzymes to produce mature piRNAs. With the exception of *Caenorhabditis elegans*, piRNAs are greater length and are associated with PIWIL3 in golden hamsters and humans are typically 18–20 nucleotides in length and feature a hydroxy group (Lv et al., 2023; Ozata et al., 2019). Upon their generation, piRNAs form a complex with Piwi proteins, which subsequently identifies and binds to complementary sequences within transposable elements. This interaction ultimately impacts various biological processes, including transposon silencing, spermiogenesis, genome rearrangement, epigenetic regulation, protein regulation, and germ stem-cell maintenance (Zamore and Haley, 2005). They are predominantly found in the germline cells of animals, where they play a critical role in silencing the expression of transposable elements and maintaining genomic stability.

As a huge threat to public health, cancer is responsible for millions of death cases around the world (Sung et al., 2021). In contrast to primary tumors, which can be managed through local resection or radiotherapy, systemic metastatic disease often proves fatal for the majority of patients with solid tumors due to the lack of effective treatments (Ganesh and Massagué, 2021; Holohan et al., 2013). This limitation underscores the urgency for the identification of novel biomarkers for advanced cancer diagnosis and prognosis, as well as the development of new targets for efficacious therapeutic interventions. Notably, numerous studies have demonstrated the burgeoning importance of piRNAs in cancer recurrence and metastasis (Tan et al., 2019). This review provides a comprehensive overview of recent research on piRNAs, encompassing their biogenesis, function, and mechanism, as well as their involvement in various cancers and potential utility as biomarkers for cancer progression.

Biogenesis and function

Biogenesis and regulatory mechanism of piRNA

Current studies of piRNAs mainly involve the gonads of organisms such as *Drosophila melanogaster* (Mohn et al., 2014; Yin and Lin, 2007), *Mus musculus* (Aravin et al., 2006; Ding et

al., 2017; Zhang et al., 2017), and *Caenorhabditis elegans* (Grishok, 2005; Tang et al., 2016). piRNA biogenesis differs slightly between species, mostly conserved in germ cells. piRNAs regulate gene expression via interactions with PIWI, including transcriptional gene silencing (TGS), posttranscriptional gene silencing (PTGS) and multiprotein interactions. These complexes conduct TGS mainly through the interaction between histone-modifying enzymes (Goriaux et al., 2014) or DNA methyltransferases (DNMTs) (Aravin et al., 2008; Kuramochi-Miyagawa et al., 2008) and achieve PTGS by directing cleavage of mRNAs (Gou et al., 2014; Vourekas et al., 2016; Watanabe and Lin, 2014), pseudogenes (Wang and Lin, 2021; Watanabe et al., 2015) and long noncoding RNAs (lncRNAs) (He et al., 2015).

Precursor piRNA generation from piRNA cluster

Based on their origin and function, piRNAs mainly have been confirmed to be classified into three groups: transposon-derived, mRNA-derived and lncRNA-derived (Jensen et al., 2020). Transposon-derived precursor piRNAs are produced from end-to-end sequences termed piRNA clusters in the nucleus (Brennecke et al., 2007). Most precursor piRNAs are derived from uni-strand clusters in somatic cell in *Drosophila melanogaster*, which are generated in a similar transcription manner as coding genes (promoters carried with H3K4me2 marks, transcription by RNA polymerase II, and the same capping, splicing and polyadenylation processes) (Czech et al., 2018). To the contrary, dual-strand clusters are predominant in both male and female germline cell of *Drosophila melanogaster* (Mohn et al., 2014), which do not strictly undergo canonical transcription processes by escaping splicing and polyadenylation. RDC complex (Rhino, Deadlock, and Cutoff), which is specific to dual-strand clusters, licenses the transcription of precursor piRNAs and suppress premature termination (Andersen et al., 2017; Chen et al., 2016; Mohn et al., 2014). After transcription, precursor piRNAs required to be transported to the cytoplasm with the guidance of nuclear RNA export Factor 1/NTF2-related export protein 1 (Nxf1/Nxt1) (Dennis et al., 2016) or DEAD-box helicase U2AF65-associated protein (UAP56) are then (Zhang et al., 2012).

Mature piRNA/PIWI complex formation via two pathways

In the primary pathway of *D. melanogaster*, precursor piRNAs are conveyed to the nuage, where the Zucchini (Zuc) endonuclease and its cofactor Minotaur (Mino) cleave them to generate piRNAs with uridine predominantly located at the 5′-end (Czech et al., 2018; Nishimasu et al., 2012). In *D. melanogaster*, 3′-end to 5′-end trimming is dispensable while PARN-1 in *C. elegan* and PNLDC-1 in *M.musculus* are responsible for the trimming to form mature piRNAs (Ding et al., 2017; Tang et al., 2016; Zhang et al., 2017). Later, the 2′-hydroxy group at the 3′-end is methylated by Hua Enhancer 1 (HEN1) to yield a mature piRNA/PIWI complex (Han et al., 2015; Kirino and Mourelatos, 2007). In the secondary pathway of *D. melanogaster*, Aub, as an endonuclease, binds to antisense piRNAs and cleaves transposon elements and then forms secondary sense piRNAs that interact with Ago3 complexes. These piRNA/Ago3 complexes with endonuclease activity subsequently cleave new complementary antisense piRNAs (Tushir et al., 2009). This reciprocal cleavage forms an amplification loop that increases the abundance of piRNAs, also called the ping-pong pathway. piRNA/Aub complexes feature a preference for 5′-end uridine, while piRNA/Ago3 complexes show a bias for an adenosine at position 10 (Tushir et al., 2009). Besides Aub, Ago3 and PIWI can also initiate the secondary pathway to produce piRNAs. The current understanding implies that these two pathways may not be distinctly independent (Czech et al., 2018) (Figure 1).

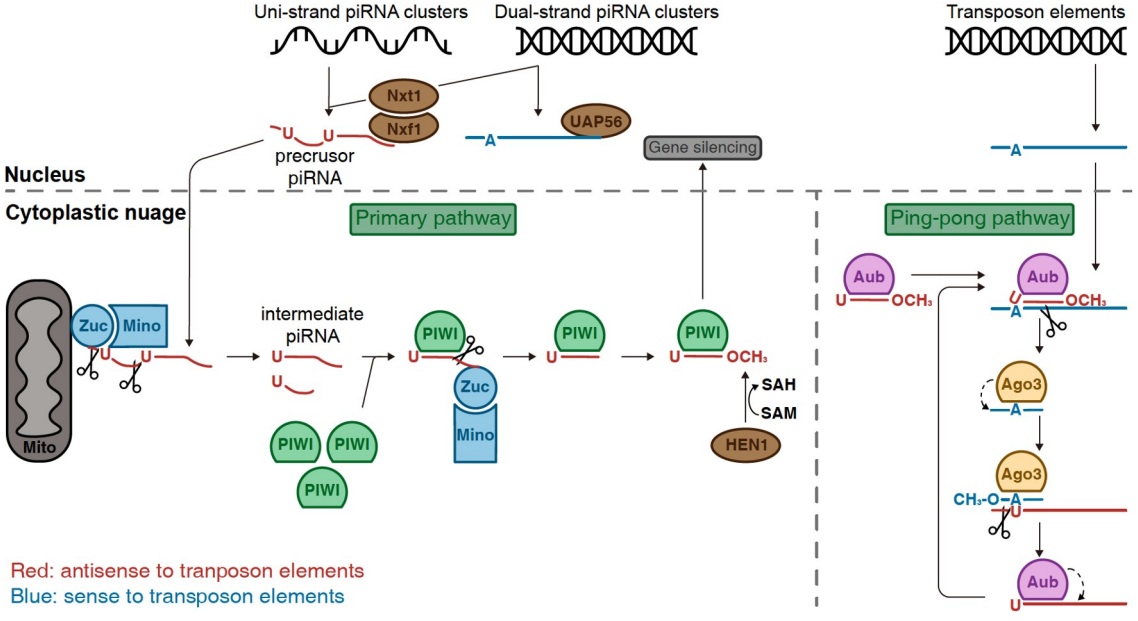

Figure 1. Biogenesis pathway of piRNA. Precursor piRNA from uni-strand and dual-strand piRNA clusters is transported to the cytoplasm through Nxf1-Nxt1 and UAP56. Zuc and its cofactor Mino, which are located on the outer mitochondrial membrane, mediate the cleavage of precursor piRNA and produce intermediate piRNA. Following PIWI binding, 3′–5′ trimming by Zuc and 2′ hydroxyl group methylation by HEN1 take place successively, termed the primary pathway. In the secondary pathway, the piRNA/Aub complex recognizes and binds transposon RNA. Endonuclease Aub cleaves target RNA followed by 3′–5′ trimming by Ago3 and 2′ hydroxyl group methylation. Similarly, the piRNA/Ago3 complex assists the formation of the piRNA/Aub complex to amplify the abundance of piRNA.

https://doi.org/10.1007/s11427-023-2491-7

piRNA/PIWI complex-guided transcriptional gene silencing

Mature nuclear-localized piRNA/PIWI complexes bind target precursor mRNA with the assistance of Asterix (Arx) and Panoramix (Panx) to start TGS. Subsequently, the incorporation of repressive histone 3 lysine 9 di- or tri-methylation (H3K9me2/3) is facilitated by the recruitment of the histone methyltransferase Eggless (Egg) and its cofactor Windei (Wde). However, mere H3K9me2/3 marks are not sufficient for TGS. The recruitment of heterochromatin protein 1 (HP1) leads to the formation of heterochromatin (Post et al., 2014). Histone 3 lysine 4 dimethylation, or H3K4me2 marks, on the promoter, which is the activating sign for gene expression, is removed by lysine-specific demethylase 1 (Lsd1) before Egg methylation. In epigenetic regulation, piRNA/PIWI complex-mediated gene methylation is achieved by recruitment of the canonical DNMTs, such as DNMT1, DNMT3a and DNMT3b (Kuramochi-Miyagawa et al., 2008; Lyko, 2018). For instance, the piR-31470/PIWIL4 complex mediates glutathione S-transferase pi 1 (GSTP1) silencing by CpG hypermethylation of DNMT1 and 3a in prostate cancer, and piR-823 reduces the tumor suppressor $p16^{INK4A}$ via the recruitment of DNMT3a and 3b in multiple myeloma (Yan et al., 2015; Zhang et al., 2020).

piRNA/PIWI complex-guided posttranscriptional gene silencing

The ping-pong amplification not only provides sufficient silencing triggers against active elements but also plays an important role in PTGS. It can directly cleave transposon RNA. Most studies have indicated, piRNA and PIWI are assembled into the piRNA-induced silencing complex (piRISC). piRISC completes PTGS by either mediating cleavage or deadenylation of mRNA. To deadenylate mRNA, piRISC recruits CCR4-NOT complex in *Drosophila melanogaster* (Barckmann et al., 2015; Rouget et al., 2010) while CAF1 in *Mus. musculus* (Gou et al., 2014). A study of lung adenocarcinoma showed that piR-55490 can cleave and degrade mTOR mRNA through the interaction of the 3′-UTR of mTOR (Peng et al., 2016). Similarly, piR-57125 binds the 3′-UTR of C-C Motif Chemokine Ligand 3 (CCL3) mRNA to decrease CCL3 expression levels via a PIWIL4-dependent RISC mechanism in clear cell renal cell carcinoma (ccRCC) proliferation and metastasis (Ding et al., 2021a).

piRNA/PIWI complex-guided multiprotein interactions

In addition, multiprotein interactions and modifications via the piRNA/PIWI complex serve as the third regulatory mechanism. For instance, the piR-823/PIWI complex exhibits a direct binding affinity towards heat shock Factor 1 (HSF1), thereby promoting the phosphorylation of the Ser^{326} residue on the HSF1 protein. This event facilitates the proliferation of colorectal cancer (CRC) cells concurrently suppressing their apoptosis (Yin et al., 2017). Similarly, the piR-54265/PIWIL2 complex interacts with STAT3 and phosphorylated SRC by the PIWIL2 PAZ domain, thereby facilitating STAT3 phosphorylation and cancer chemoresistance in CRC cells (Mai et al., 2018) (Figure 2).

piRNA in cancer progression

Previous studies have already revealed that dysregulated piRNAs play a role in the pathogenesis of many diseases including cancer, cardiac hypertension, and myocardial infarction (Rayford et al., 2021). Cancer is the process in which cancer cells gain the ability to grow and spread without control in the host body. Though there are lots of anti-cancer therapies, cancer progression remains a disturbing problem (Cai et al., 2023; Su, 2023). Proliferation, metastasis, chemoresistance, and stemness are four key biological functions in cancer progression. Currently, a great number of piRNAs have been shown to be related to cancer progression (Figure S1 in Supporting Information). Further studies have revealed the oncogenic and tumor suppressive roles of dysregulated piRNAs in these four biological functions of cancer progression (Tables 1 and 2). Herein, we discuss the regulatory mechanisms of piRNAs in cancer proliferation, metastasis, chemoresistance and stemness.

piRNA in cancer proliferation

Uncontrolled growth and immortality are outstanding features of cancer cells (Feitelson et al., 2015). Cancer cells modulate their cell cycle, change their metabolism strategy, facilitate angiogenesis, inhibit apoptosis and resist oxidative stress. Below, we discuss how piRNAs regulate cancer cell growth, angiogenesis, apoptosis and oxidative stress by modifying multiple cellular signaling pathways and molecules.

Cyclin and CDK pathways

Cyclin and the cyclin-dependent kinase (CDK) family are the main regulators of the cell cycle (Icard et al., 2019). Cyclin D1 and CDK 4, which regulate the transition from G1 phase to S phase, are upregulated by piR-651 in both non-small cell lung carcinoma (NSCLC) and breast cancer (Li et al., 2016; Liu et al., 2021). Thus, cell cycle assays show a decreased percentage of arrested G0/G1 phase cells and an increased percentage of G2/S phase cells. However, the underlying mechanism by which piR-651 upregulates cyclin D1 and CDK4 levels remains unclear. Similarly, in multiple myeloma, antagomir-823 (piR-832 antagonist) has been found to shrink tumor size and downregulate the expression of cyclin D1 and CDK4 *in vivo* (Yan et al., 2015). In addition, PIWIL2 upregulates the expression of cyclin A and CDK2 at both the mRNA and protein levels in NSCLC, and PIWIL2 interference arrests cells at the G2/M phase (Qu et al., 2015). These findings show that piRNAs affect cancer cell growth and modify the cell cycle through the regulation of the cyclin and CDK family.

PI3K/AKT/mTOR pathway

The mTOR pathway is a central regulator of mammalian metabolism, and a dysregulated PI3K/AKT/mTOR signaling pathway has been reported to be associated with various diseases, including cancer and type 2 diabetes (Laplante and Sabatini, 2012). Cancer cells can change their metabolic strategy to enhance cell growth by modulating the mTOR pathway. piR-004800 is an oncogenic factor found in multiple myeloma (MM), and downregulated piR-004800 is correlated with cell apoptosis events (Ma et al., 2020). Activation of the sphingosine-1-phosphate receptor (S1PR) signaling pathway leads to overexpression of piR-004800 (Fu et al., 2017; Ma et al., 2020). Subsequently, piR-004800 activates the PI3K/AKT/mTOR pathway and sustains the survival of MM cells both *in vitro* and *in vivo*. Similarly, Peng et al. (2016) found that piR-55490, which is downregulated in lung adenocarcinoma, is negatively correlated

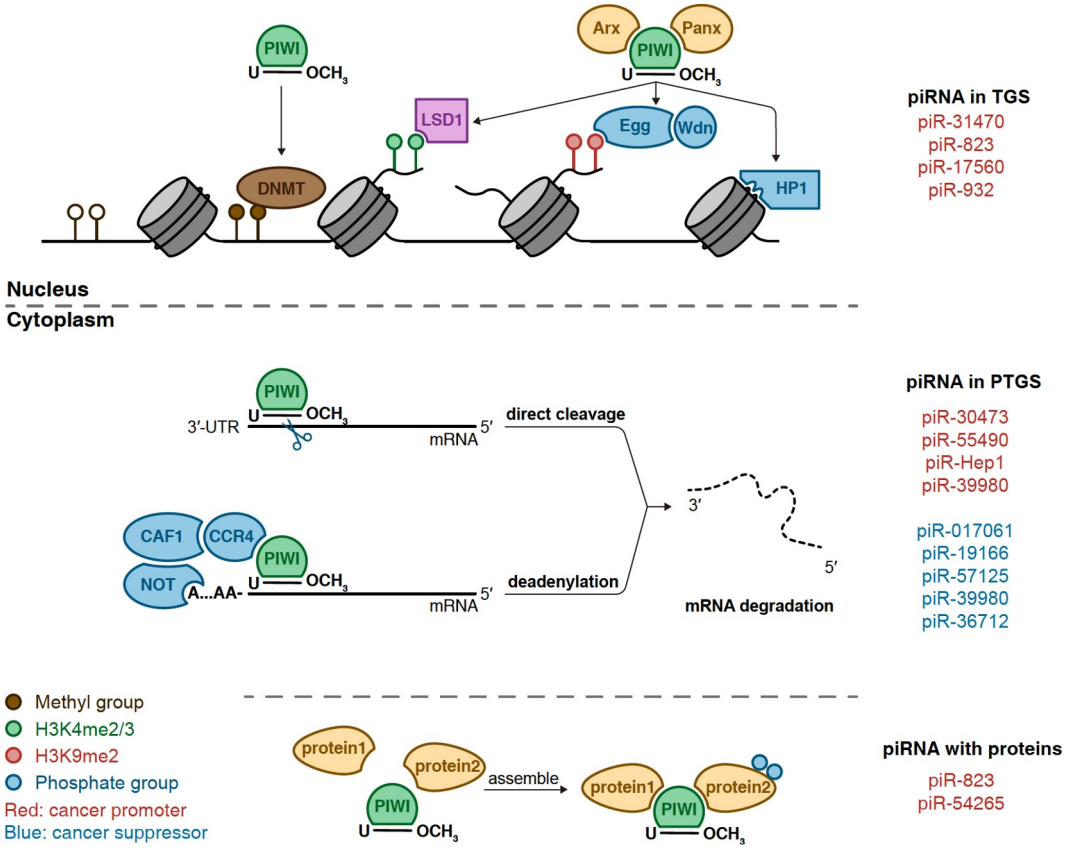

Figure 2. Regulatory mechanisms and functions of piRNA. piRNA mediates DNA methylation through canonical DNMTs (DNMT1, 3a, 3b) and guides histone modification to silence genes at the transcriptional level, such as removal of activating signs H3Kme2/3 by LSD1, application of deactivating signs H3K9me2 by Egg and its cofactor Wdn and heterochromatin formation by HP1. The piRNA/PIWI complex completes PTGS by either cleavage or deadenylation of target RNA, and piRNAs can also interact with proteins to induce phosphorylation.

Table 1. The oncogenic roles of piRNAs in cancer progression[a)]

piRNAs	Cancers	Oncogenic roles	Ref.
piR-823	CRC	Promotes proliferation by regulating cell cycle and suppressing apoptosis	(Yin et al., 2017)
piR-54265		Promotes proliferation, metastasis and chemoresistance by activating STAT3 signaling pathway	(Mai et al., 2020)
piR-30473	DLBCL	Promotes proliferation by regulating cell cycle	(Han et al., 2021)
piR-651	NSCLC	Promotes proliferation by regulating cell cycle and suppressing apoptosis, may promote metastasis	(Li et al., 2016)
piR-823	Multiple myeloma	Promotes proliferation by regulating cell cycle, and reducing apoptosis and angiogenesis; promotes invasion by inducing ICAM-1 and CXCR4 expression	(Li et al., 2019; Yin et al., 2017)
piR-004800		Promotes proliferation by suppressing apoptosis and autophagy, which is regulated by S1PR signaling pathway and regulates mTOR pathway	(Ma et al., 2020)
piR-39980	Osteosarcoma	Promotes proliferation by suppressing apoptosis; promotes invasion and metastasis by targeting SWEPINB1	(Das et al., 2020)
	Fibrosarcoma	Enhances chemoresistance by downregulating RRM2 and CYP1A2	(Das et al., 2021)
	Neuroblastoma	Promotes proliferation by regulating cell cycle and inducing metastasis; enhances chemoresistance and suppresses drug-induced apoptosis by targeting JAK3	(Roy et al., 2020)
piR-1089		Promotes proliferation and migration by inhibiting Cullin 3-based E3 ligase complex	(Wang et al., 2023a)
piR-14633	Cervical cancer	Promotes proliferation by modulating cell cycle; promotes invasion and metastasis by targeting METTL14/CYP1B1 signaling	(Xie et al., 2022)
piR-17560	Breast cancer	Enhances chemoresistance by targeting FTO/ZEB1 signaling	(Ou et al., 2022)
piR-823		Promotes proliferation, enables the acquisition and maintenance of stemness by activating Wnt/β-catenin signaling	(Ding et al., 2021b)
piR-651		Promotes proliferation and migration, inhibits apoptosis by facilitating PTEN promoter methylation	(Liu et al., 2021)

a) DLBCL, diffuse large B-cell lymphoma; NSCLC, non-small cell lung carcinoma.

https://doi.org/10.1007/s11427-023-2491-7

Table 2. The tumor suppressive roles of piRNAs in cancer progression

piRNAs	Cancers	Tumor suppressive roles	Ref.
piR-57125	ccRCC	Inhibits invasion and metastasis by downregulating CCL3 expression	(Ding et al., 2021a)
piR-36712	Breast cancer	Inhibits proliferation by regulating cell cycle and suppressing invasion, metastasis and chemoresistance by interacting with SEPW1P and upregulating p53	(Tan et al., 2019)
piR-2158		Inhibits angiogenesis by inhibiting the IL-11 signaling pathway	(Zhao et al., 2023)
piR-55490	NSCLC	Inhibits proliferation by suppressing the mTOR signaling pathway	(Peng et al., 2016)
piR-31470	Prostate cancer	Inhibits proliferation by increasing vulnerability to oxidative stress	(Zhang et al., 2020)
piR-19166		Inhibits invasiveness and metastasis by modulating the CTTN/MMPs pathway	(Qi et al., 2020)
piR-017061	Pancreatic cancer	Inhibits proliferation by degrading EFNA5 mRNA and inducing cell apoptosis	(Xie et al., 2021)

with the proliferation rate of lung cancer cells. Immunoblotting, luciferase expression and qPCR assays showed that suppression of piR-55490 leads to activation of the AKT/mTOR pathway. Further sequence analysis showed a complementary sequence between the mTOR 3′ UTR and the interaction of piR-55490 with the 3′-UTR of mTOR mRNA to facilitate its degradation in a miRNA-like manner (Peng et al., 2016). Additionally, in hepatocellular carcinoma, attenuation of phosphorylated Akt levels upon silencing piR-Hep1 suggests that piR-Hep1 plays a role in the PI3K/Akt pathway (Law et al., 2013). This finding suggests that piRNAs seem to be upstream regulators of the PI3K/AKT/mTOR pathway and regulate this pathway at the posttranscriptional level.

AMPK pathway

The AMPK pathway plays an important role in cell energy homeostasis to generate more ATP by promoting catalytic pathways and inhibiting analytic pathways when cellular energy is low, which is close to the cancer cell survival strategy (Hsu et al., 2022; Mihaylova and Shaw, 2011; Steinberg and Hardie, 2023). Two upregulated (piR-34871 and piR-52200) and two downregulated (piR-35127 and piR-46545) piRNAs are reported upon overexpression of RASSF1C, which upregulates multiple genes associated with lung cancer cell proliferation (Reeves et al., 2017). Overexpression of RASSF1C decreased the expression levels of p21 and p27, which are downstream of the AMPK pathway, and trichostatin A (AMPK activator) and dorsomorphin (AMPK inhibitor) treatment changed the expression levels of these four piRNAs. Thus, it is believed that these piRNAs are partly involved downstream of the AMPK pathway in cancer proliferation.

Angiogenesis pathway

A tumor volume greater than approximately 2 mm^3 converts to an angiogenic phenotype that attracts neovascularization or angiogenesis (Hillen and Griffioen, 2007). Vascular endothelial growth factor (VEGF) has been demonstrated to be a major contributor to angiogenesis, and other angiogenic growth factors, such as fibroblast growth factor (FGF), tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6), have also been found to stimulate vessel sprouting (Gopinathan et al., 2015; Viallard and Larrivée, 2017). Multiple cellular signaling pathways are associated with enhanced secretion of VEGF, such as the JAK-STAT and AKT pathways (Karar and Maity, 2011; Xue et al., 2017). MM-derived extracellular vesicle piR-823, which activates the AKT pathway, promotes angiogenesis by increasing VEGF and IL-6 secretion in endothelial cells (Li et al., 2019; Yan et al., 2015).

Intrinsic apoptosis pathway

Cell apoptosis is a type of programmed cell death regulated by both intrinsic and extrinsic apoptosis pathways. Bcl-2 and Bcl-xl are antiapoptotic factors in the intrinsic apoptosis pathway. In MM, piR-823 plays an oncogenic role by inducing the expression of Bcl-2 and Bcl-xl. Therefore, inhibition of cancer cell apoptosis events has been found (Yan et al., 2015). piR-823 also plays an oncogenic role in colorectal cancer by inhibiting cell apoptosis in an unknown way (Yin et al., 2017).

Antioxidative stress pathway

To fight against oxidative stress, glutathione S-transferases (GST) catalyze the conjugation of the reduced form of glutathione (GSH). The upregulated piR-31470/PIWIL4 complex in prostate cancer inhibits GSTP1 at the transcriptional level by hypermethylation of the CpG island of GSTP1 with the help of DNMT1, DNMT3a and methyl-CpG binding domain protein 2 (MBD2) (Zhang et al., 2020) and therefore enhances resistance against oxidative stress in cancer cells. HSF1, as a regulator of the heat shock response pathway, upregulates heat shock proteins against not only heat shock but also oxidative stress. piR-823 can increase the transcriptional activity of HSF1 and directly interact with HSF1 to activate HSF1 by facilitating its Ser^{326} phosphorylation, therefore protecting CRC cells from oxidative stress (Yin et al., 2017).

piRNA in cancer metastasis

Cancer metastasis via local invasion, blood and the lymphatic system are troublesome issues (Gupta and Massagué, 2006; Liu et al., 2017; Quail and Joyce, 2013). Once metastasis occurs, it places a cancer in Stage IV of TNM staging, which indicates a poor prognosis for most patients. Currently, piRNAs have been proven to be involved in cancer metastasis. Therefore, finding the relationship between piRNAs and metastasis and understanding the underlying mechanisms can be of some help for future diagnosis, therapy and prognosis prediction.

AKT/ERK pathway

AKT serves as a central mediator of the PI3K/AKT pathway, while ERK is the end effector of the MAPK/ERK pathway, which regulates multiple cellular events. Aberrant AKT and ERK signaling pathways interact with proteins (Xu et al., 2021), lncRNAs (Qu et al., 2021), and miRNAs (Shen et al., 2016) and have been shown to be correlated with cancer metastasis. The piR-57125/PIWIL4 complex is downregulated in ccRCC and associated with ccRCC cell metastasis (Ding et al., 2021a). piR-57125/PIWIL4 RISC first directly binds and degrades CCL3,

which increases the phosphorylation of AKT and ERK in ccRCC and then negatively modulates the AKT/ERK pathway. Similarly, piR-31115 may activate the PI3K/AKT pathway to promote epithelial-mesenchymal transition (EMT) and therefore increase the invasiveness of ccRCC cells (Du et al., 2021). However, although more evidence shows that the PI3K/AKT pathway plays an oncogenic role in cell growth, invasion and migration (Laplante and Sabatini, 2012), the role of piRNA in the PI3K/AKT pathway remains to be revealed.

ECM remodeling pathway

The process of cancer metastasis includes not only adaptation of cancer cells but also the remodeling of the cancer microenvironment based on the soil and seed hypothesis (Liu et al., 2017). Matrix metalloproteinases (MMPs), which are zinc-containing endopeptidases, facilitate cancer metastasis via their proteinase function (Kleiner and Stetler-Stevenson, 1999). Qi et al. (2020) have reported that piR-19166, a tumor suppressor, is correlated with prostate cancer cell proliferation and metastasis with direct interaction with the 3′-UTR of cortactin (CTTN) to induce its degradation and thus activation of MMPs. Similarly, piR-39980 upregulates MMP-2 to facilitate osteosarcoma cell migration and invasion via degradation of serpin family B member 1 (SERPINB1) mRNA (Das et al., 2020).

piRNA in cancer chemoresistance and stemness

The development of chemoresistance after chemotherapy and stemness in cancer cells has been demonstrated to be related to the poor prognosis of the host. Chemoresistance, referring to the evasion or adaptation of cancer cells in the presence of chemotherapy, usually develops months after chemotherapy initiation *in vitro* (McDermott et al., 2014; Yeldag et al., 2018). Cancer cells can gain and enhance resistance to chemotherapy by building the tumor microenvironment, inducing EMT, expelling and inactivating drugs, repairing DNA damage, and disturbing apoptosis (Ramos et al., 2021; Zhao, 2016). Stemness equips cells with the ability to maintain their lineage and to differentiate into heterogenous cell lineages with high plasticity or high resistance to stressful conditions and to various cancerous events (such as tumor angiogenesis, metastasis, chemoresistance and relapse) (Aponte and Caicedo, 2017; Ayob and Ramasamy, 2018). Herein, we summarize multiple piRNAs that are found to regulate cancer chemoresistance and stemness.

JAK-STAT pathway

Mai et al. (2018) found that the piR-54265/PIWIL2 complex decreases the apoptosis of colorectal cancer cells under 5-fluorouracil (5-FU) treatment and facilitates chemoresistance (Yeldag et al., 2018). To do this, the PAZ domain of PIWIL2 interacts with STAT3 and assembles the piR-54265/PIWIL2/STAT3/phosphorylated-SRC complex in CRC cells. Next, piR-54265 activates the STAT3 pathway by upregulating phosphorylated STAT3 and Bcl-xl and downregulating cleaved CASP9, CASP3 and CASP7, thereby inhibiting apoptosis induced by chemotherapy. Intriguingly, the STAT3 pathway has been shown to regulate exosomal piR-17560 secreted from senescent neutrophils to promote chemoresistance and EMT in breast cancer cells by upregulating ZEB1 through FTO-mediated m6A methylation (Ou et al., 2022). Furthermore, activation of the JAK-STAT pathway was also found to stimulate angiogenesis by upregulating angiogenesis-related growth factors, including VEGF, in ALDH+ breast cancer stem cells (Xue et al., 2017). As IL-11 is proven to be the upstream regulator of the JAK-STAT pathway, piR-2158 in breast cancer can inhibit angiogenesis by inhibiting IL-11 transcription by competitively binding to the AP-1 transcription factor, which upregulates IL-11 by binding to its promoter (Zhao et al., 2023).

CYP pathway

Cytochrome P450 (CYP) is a superfamily of enzymes that plays a major role in drug metabolism. CYP1A2, as a phase I/II metabolizing enzyme, is associated with ovarian cancer chemoresistance (Pavlič et al., 2022). Das et al. (2021) revealed that piR-39980 in fibrosarcoma directly interacts with the 3′-UTR of CYP1A2 to promote doxorubicin (DOX)-induced apoptosis. In addition, piR-39980 also contributes chemosensitivity to neuroblastoma cells, but the underlying mechanism remains unclear (Roy et al., 2020). piR-14633 in cervical cancer has been proven to upregulate CYP1B1 expression by targeting methyltransferase-like 14 (METTL14), which may be related to chemoresistance (Xie et al., 2022). In summary, piRNAs play multiple roles in drug resistance in various cancer types and mainly act as modulators of cell apoptosis, EMT and DNA damage repair based on current knowledge.

Wnt-β-catenin pathway

The Wnt-β-catenin pathway, which promotes migration and inhibits adhesion when activated, is hijacked by cancer stem cells to facilitate metastasis (Liu et al., 2022; Zhang and Wang, 2020). piR-823, the main piRNA in various cancers (Cheng et al., 2012; Ding et al., 2021b; Li et al., 2019; Yan et al., 2015; Yin et al., 2017), activates the Wnt signaling pathway by hypermethylating the adenomatous polyposis coli (APC) promoter in MCF-7 and T-47D luminal-type breast cancer as an oncogenic factor (Cheng et al., 2012). Overexpression of piR-823 in MCF-7 cancer cells upregulates DNMT1, 3a and 3b at both the mRNA and protein levels, thus leading to elevated DNA methylation levels.

piRNAs as cancer biomarkers

Many technologies are used in this field to detect the probability of getting cancer (Gong et al., 2022; Li et al., 2022). piRNAs extensively regulate cancer progression in all human systems and are strongly related in the reproductive system (Figure S2 in Supporting Information). Understanding the precise mechanisms and correlation between piRNAs and cancer benefits both diagnosis and therapy in future clinical application of piRNAs as biomarker. However, to date, there are multiple widely applied biomarkers in clinical work, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) (Henry and Hayes, 2012; Poruk et al., 2013). However, it is seldom that the present biomarkers meet a satisfying standard of both sensitivity and specificity (Das et al., 2017). Precision medicine, as a hotspot in cancer diagnosis and therapy, has been widely researched in multiple cancer types (Wu et al., 2021). Moreover, circulating diagnostic biomarkers and prognostic biomarkers are regarded as the easiest-to-obtain samples in cancer patients without causing tumor implantation and iatrogenic trauma (Shyamala et al., 2014). Currently, numerous piRNAs have been proven to be upregulated or downregulated in various cancers, to be small enough to pass through the cell membrane into circulation (Mei

https://doi.org/10.1007/s11427-023-2491-7

et al., 2013) and to accumulate in exosomes (Gu et al., 2020). Altogether, piRNAs are expected to be novel cancer biomarkers.

Conclusions and perspectives

Current knowledge shows that piRNAs, newly found noncoding RNAs, are responsible for regulating a wide range of downstream genes by heterochromatin formation, DNA methylation, mRNA cleavage and protein interactions. piRNA dysregulation has been proven to be associated with various diseases, especially cancer. In this review, we first summarize the biogenesis and three regulatory mechanisms of the piRNA/PIWI complex. Specifically, the piRNA/PIWI complex guides TGS mainly by regulating canonical DNMTs (DNMT1, 3a, and 3b), while it conducts mRNA cleavage to finish PTGS and modulates protein phosphorylation via direct multiprotein interactions. Next, we elaborate on the multiple mechanisms of dysregulated piRNAs in cancer progression, including cancer cell proliferation, metastasis, chemoresistance and stemness. For example, dysregulated piRNAs are able to disturb the percentage of G0/G1 and G2/M phase cells via Cyclin D and the CDK4 pathway (Li et al., 2019; Yan et al., 2015). As piRNAs are abundantly presented in the blood of cancer patients, some piRNAs have been proven to be potential diagnostic and prognostic biomarkers for future clinical application (Cheng et al., 2012; Cordeiro et al., 2016; Cui et al., 2011; Feng et al., 2020; Gu et al., 2020; Iliev et al., 2016; Li et al., 2019; Mai et al., 2020; Markert et al., 2021; Sabbah et al., 2021; Vychytilova-Faltejskova et al., 2018) (Table 3). Further investigation into piRNAs may lead to their emergence as a novel class of small molecule markers for cancer progression, thereby facilitating the advancement of diagnosis, treatment, and prognosis for patients with advanced cancer.

Because of the extremely low abundance of piRNAs in human cancer cell and the "camouflage" of other small RNAs that resemble the structure of piRNAs, it has long been considered controversial that how to detect trace, bona fide piRNAs in cancer cell and whether these piRNAs are really the causation of cancer progression or not (Genzor et al., 2019; Shi et al., 2020). piRNA, as its name suggested, interacts with PIWI proteins to conduct its biological functions. Thus, the initial investigation of bona fide piRNAs should focus on examining the expression of PIWI proteins, followed by an assessment of other components involved in piRNA biogenesis. Moreover, thorough scrutiny should be applied to investigate whether PIWI can effectively interact with the "alleged piRNAs." This investigation warrants careful examination, utilizing a PIWI knock-out (KO) cell clone as a stringent systematic control for accuracy. In conclusion, it is crucial not to excessively extrapolate or magnify the role of piRNAs in cancer until we confirm whether these small RNAs are bona fide piRNAs.

Given the vast number of species with low abundance, individual piRNA species' naming conventions do not adhere to typical piRNA naming convention. Transposon elements, from Arthropoda to Mammalia, are regarded as hazards to genome integrity. Compared to miRNA-AGO interaction of PTGS, piRNA-PIWI interaction seems to design more specific for these evolving threats. Furthermore, the recent discovery of stringent piRNA pairing rules, requiring a minimum of 15 continuous nt base pairing between piRNAs and their targets, suggests that each species likely does not have a unique function but target transposon elements (Anzelon et al., 2021; Gainetdinov et al., 2023).

While research on piRNAs has predominantly centered on tumorigenesis, limited attention has been given to their role in

Table 3. piRNAs as diagnostic and prognostic biomarkers in cancer from peripheral blood samples[a)]

piRNAs	Cancer	Expression in blood	AUC sensitivity and specificity	Diagnostic biomarker	Prognostic biomarker	Ref.
piR-823	Gastric cancer	down	/	+	–	(Cheng et al., 2012; Cui et al., 2011)
piR-651		down	0.709 0.813			(Cui et al., 2011)
piR-5937	CRC	down	0.718 0.725	+	–	(Vychytilova-Faltejskova et al., 2018)
piR-28876		down	0.753 0.700	+	–	(Vychytilova-Faltejskova et al., 2018)
piR-823		up	/	+	–	(Feng et al., 2020)
piR-823		up	/	–	+	(Sabbah et al., 2021)
piR-54265		up	0.857 0.651	+	–	(Mai et al., 2020)
piR-020619		up	0.843 0.764	+	–	(Wang et al., 2020)
piR-020450		up	0.814 0.750	+	–	(Wang et al., 2020)
piR-10506469	cholangiocarcinoma and gallbladder carcinoma	up	/	+	–	(Gu et al., 2020)
piR-823	Renal cell carcinoma	up	/	+	–	(Iliev et al., 2016)
piR-651	Classical Hodgkin lymphoma	down	/	–	+	(Cordeiro et al., 2016)
piR-018849	Prostate cancer	up	0.900 (combined)	+	–	(Markert et al., 2021)
piR-019324		up	0.700 (combined)	+	–	(Markert et al., 2021)
piR-823	Multiple myeloma	up	/	+	+	(Li et al., 2019)

a) "/" stands for no related data, "+" or "–" marks that certain piRNA is or is not proven to be diagnostic or prognostic biomarker, respectively.

tumor progression. The exploration of piRNA posttranslational modification through further research holds immense potential in elucidating the pathogenesis of cancer progression. Furthermore, due to the nascent stage of piRNA research, certain functions and biosynthesis mechanisms have yet to be fully elucidated. Notably, the upregulated expression of numerous piRNAs in blood samples indicates their potential utility as biomarkers for cancer detection and monitoring. In contrast to conventional tumor markers, piRNAs exhibit greater precision and sensitivity, although their practicality necessitates further investigation (Mai et al., 2023). Moreover, the utilization of siRNA, antisense oligonucleotides, and CRISPR–Cas9-mediated genome editing by piRNAs has the potential to impede the proliferation and mitosis of neoplastic cells while also inducing apoptosis. Nevertheless, there is a dearth of research and practical implementation of piRNAs in targeted therapy, and the underlying mechanism of piRNA expression alteration in cancer progression remains unclear. This review aims to inspire further research on the fundamental biological mechanisms and importance of piRNAs in cancer progression, as well as their potential as clinical tools for managing and treating advanced cancer.

Compliance and ethics

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgement

This work was supported by the Basic and Applied Basic Research Foundation of Guangdong Province (2022A1515220184) and the Natural Science Foundation of Guangdong Province (2021A1515010547). We thank Biorender for providing scientific researchers with exquisite materials and convenient drawing platforms. All the figures in this article were made by Biorender (biorender.com).

Supporting information

The supporting information is available online at https://doi.org/10.1007/s11427-023-2491-7. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

References

Andersen, P.R., Tirian, L., Vunjak, M., and Brennecke, J. (2017). A heterochromatin-dependent transcription machinery drives piRNA expression. Nature 549, 54–59.

Anzelon, T.A., Chowdhury, S., Hughes, S.M., Xiao, Y., Lander, G.C., and MacRae, I.J. (2021). Structural basis for piRNA targeting. Nature 597, 285–289.

Aponte, P.M., and Caicedo, A. (2017). Stemness in cancer: stem cells, cancer stem cells, and their microenvironment. Stem Cells Int 2017, 1–17.

Aravin, A., Gaidatzis, D., Pfeffer, S., Lagos-Quintana, M., Landgraf, P., Iovino, N., Morris, P., Brownstein, M.J., Kuramochi-Miyagawa, S., Nakano, T., et al. (2006). A novel class of small RNAs bind to MILI protein in mouse testes. Nature 442, 203–207.

Aravin, A.A., Sachidanandam, R., Bourc'his, D., Schaefer, C., Pezic, D., Toth, K.F., Bestor, T., and Hannon, G.J. (2008). A piRNA pathway primed by individual transposons is linked to *de novo* DNA methylation in mice. Mol Cell 31, 785–799.

Ayob, A.Z., and Ramasamy, T.S. (2018). Cancer stem cells as key drivers of tumour progression. J Biomed Sci 25, 20.

Barckmann, B., Pierson, S., Dufourt, J., Papin, C., Armenise, C., Port, F., Grentzinger, T., Chambeyron, S., Baronian, G., Desvignes, J.P., et al. (2015). Aubergine iCLIP reveals piRNA-dependent decay of mRNAs involved in germ cell development in the early embryo. Cell Rep 12, 1205–1216.

Brennecke, J., Aravin, A.A., Stark, A., Dus, M., Kellis, M., Sachidanandam, R., and Hannon, G.J. (2007). Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. Cell 128, 1089–1103.

Cai, J., Xu, X., and Saw, P.E. (2023). Nanomedicine targeting ferroptosis to overcome anticancer therapeutic resistance. Sci China Life Sci doi: 10.1007/s11427-022-2340-4.

Chen, Y.C.A., Stuwe, E., Luo, Y., Ninova, M., Le Thomas, A., Rozhavskaya, E., Li, S., Vempati, S., Laver, J.D., Patel, D.J., et al. (2016). Cutoff suppresses RNA polymerase II termination to ensure expression of piRNA precursors. Mol Cell 63, 97–109.

Cheng, J., Deng, H., Xiao, B., Zhou, H., Zhou, F., Shen, Z., and Guo, J. (2012). piR-823, a novel non-coding small RNA, demonstrates *in vitro* and *in vivo* tumor suppressive activity in human gastric cancer cells. Cancer Lett 315, 12–17.

Cordeiro, A., Navarro, A., Gaya, A., Díaz-Beyá, M., Gonzalez-Farré, B., Castellano, J.J., Fuster, D., Martínez, C., Martínez, A., and Monzó, M. (2016). PiwiRNA-651 as marker of treatment response and survival in classical Hodgkin lymphoma. Oncotarget 7, 46002–46013.

Cui, L., Lou, Y., Zhang, X., Zhou, H., Deng, H., Song, H., Yu, X., Xiao, B., Wang, W., and Guo, J. (2011). Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using piRNAs as markers. Clin Biochem 44, 1050–1057.

Czech, B., Munafò, M., Ciabrelli, F., Eastwood, E.L., Fabry, M.H., Kneuss, E., and Hannon, G.J. (2018). piRNA-guided genome defense: from biogenesis to silencing. Annu Rev Genet 52, 131–157.

Das, B., Jain, N., and Mallick, B. (2021). piR-39980 mediates doxorubicin resistance in fibrosarcoma by regulating drug accumulation and DNA repair. Commun Biol 4, 1312.

Das, B., Jain, N., and Mallick, B. (2020). piR-39980 promotes cell proliferation, migration and invasion, and inhibits apoptosis via repression of SERPINB1 in human osteosarcoma. Biol Cell 112, 73–91.

Das, V., Kalita, J., and Pal, M. (2017). Predictive and prognostic biomarkers in colorectal cancer: A systematic review of recent advances and challenges. Biomed Pharmacother 87, 8–19.

Dennis, C., Brasset, E., Sarkar, A., and Vaury, C. (2016). Export of piRNA precursors by EJC triggers assembly of cytoplasmic Yb-body in *Drosophila*. Nat Commun 7, 13739.

Ding, D., Liu, J., Dong, K., Midic, U., Hess, R.A., Xie, H., Demireva, E.Y., and Chen, C. (2017). PNLDC1 is essential for piRNA 3′ end trimming and transposon silencing during spermatogenesis in mice. Nat Commun 8, 819.

Ding, L., Wang, R., Xu, W., Shen, D., Cheng, S., Wang, H., Lu, Z., Zheng, Q., Wang, L., Xia, L., et al. (2021a). PIWI-interacting RNA 57125 restrains clear cell renal cell carcinoma metastasis by downregulating CCL3 expression. Cell Death Discov 7, 333.

Ding, X., Li, Y., Lü, J., Zhao, Q., Guo, Y., Lu, Z., Ma, W., Liu, P., Pestell, R.G., Liang, C., et al. (2021b). piRNA-823 is involved in cancer stem cell regulation through altering DNA methylation in association with luminal breast cancer. Front Cell Dev Biol 9, 641052.

Du, X., Li, H., Xie, X., Shi, L., Wu, F., Li, G., Lai, C., and Heng, B. (2021). piRNA-31115 promotes cell proliferation and invasion via PI3K/AKT pathway in clear cell renal carcinoma. Dis Markers 2021, 1–8.

Feitelson, M.A., Arzumanyan, A., Kulathinal, R.J., Blain, S.W., Holcombe, R.F., Mahajna, J., Marino, M., Martinez-Chantar, M.L., Nawroth, R., Sanchez-Garcia, I., et al. (2015). Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. Semin Cancer Biol 35, S25–S54.

Feng, J., Yang, M., Wei, Q., Song, F., Zhang, Y., Wang, X., Liu, B., and Li, J. (2020). Novel evidence for oncogenic piRNA-823 as a promising prognostic biomarker and a potential therapeutic target in colorectal cancer. J Cell Mol Medi 24, 9028–9040.

Fu, D., Li, Y., Li, J., Shi, X., Yang, R., Zhong, Y., Wang, H., and Liao, A. (2017). The effect of S1P receptor signaling pathway on the survival and drug resistance in multiple myeloma cells. Mol Cell Biochem 424, 185–193.

Gainetdinov, I., Vega-Badillo, J., Cecchini, K., Bagci, A., Colpan, C., De, D., Bailey, S., Arif, A., Wu, P.H., MacRae, I.J., et al. (2023). Relaxed targeting rules help PIWI proteins silence transposons. Nature 619, 394–402.

Ganesh, K., and Massagué, J. (2021). Targeting metastatic cancer. Nat Med 27, 34–44.

Genzor, P., Cordts, S.C., Bokil, N.V., and Haase, A.D. (2019). Aberrant expression of select piRNA-pathway genes does not reactivate piRNA silencing in cancer cells. Proc Natl Acad Sci USA 116, 11111–11112.

Gong, C., Cheng, Z., Yang, Y., Shen, J., Zhu, Y., Ling, L., Lin, W., Yu, Z., Li, Z., Tan, W., et al. (2022). A 10-miRNA risk score-based prediction model for pathological complete response to neoadjuvant chemotherapy in hormone receptor-positive breast cancer. Sci China Life Sci 65, 2205–2217.

Gopinathan, G., Milagre, C., Pearce, O.M.T., Reynolds, L.E., Hodivala-Dilke, K., Leinster, D.A., Zhong, H., Hollingsworth, R.E., Thompson, R., Whiteford, J.R., et al. (2015). Interleukin-6 stimulates defective angiogenesis. Cancer Res 75, 3098–3107.

Goriaux, C., Desset, S., Renaud, Y., Vaury, C., and Brasset, E. (2014). Transcriptional properties and splicing of the *flamenco* piRNA cluster. EMBO Rep 15, 411–418.

Gou, L.T., Dai, P., Yang, J.H., Xue, Y., Hu, Y.P., Zhou, Y., Kang, J.Y., Wang, X., Li, H., Hua, M.M., et al. (2014). Pachytene piRNAs instruct massive mRNA elimination during late spermiogenesis. Cell Res 24, 680–700.

Grishok, A. (2005). RNAi mechanisms in *Caenorhabditis elegans*. FEBS Lett 579, 5932–5939.

Gu, X., Wang, C., Deng, H., Qing, C., Liu, R., Liu, S., and Xue, X. (2020). Exosomal piRNA profiling revealed unique circulating piRNA signatures of cholangiocarci-

noma and gallbladder carcinoma. Acta Biochim Biophys Sin 52, 475–484.
Gupta, G.P., and Massagué, J. (2006). Cancer metastasis: building a framework. Cell 127, 679–695.
Han, B.W., Wang, W., Li, C., Weng, Z., and Zamore, P.D. (2015). piRNA-guided transposon cleavage initiates Zucchini-dependent, phased piRNA production. Science 348, 817–821.
Han, H., Fan, G., Song, S., Jiang, Y., Qian, C., Zhang, W., Su, Q., Xue, X., Zhuang, W., and Li, B. (2021). piRNA-30473 contributes to tumorigenesis and poor prognosis by regulating m^{6}A RNA methylation in DLBCL. Blood 137, 1603–1614.
He, X., Chen, X., Zhang, X., Duan, X., Pan, T., Hu, Q., Zhang, Y., Zhong, F., Liu, J., Zhang, H., et al. (2015). An LncRNA (GAS5)/SnoRNA-derived piRNA induces activation of *TRAIL* gene by site-specifically recruiting MLL/COMPASS-like complexes. Nucleic Acids Res 43, 3712–3725.
Henry, N.L., and Hayes, D.F. (2012). Cancer biomarkers. Mol Oncol 6, 140–146.
Hillen, F., and Griffioen, A.W. (2007). Tumour vascularization: sprouting angiogenesis and beyond. Cancer Metastasis Rev 26, 489–502.
Holohan, C., Van Schaeybroeck, S., Longley, D.B., and Johnston, P.G. (2013). Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 13, 714–726.
Hsu, C.C., Peng, D., Cai, Z., and Lin, H.K. (2022). AMPK signaling and its targeting in cancer progression and treatment. Semin Cancer Biol 85, 52–68.
Icard, P., Fournel, L., Wu, Z., Alifano, M., and Lincet, H. (2019). Interconnection between metabolism and cell cycle in cancer. Trends Biochem Sci 44, 490–501.
Iliev, R., Fedorko, M., Machackova, T., Mlcochova, H., Svoboda, M., Pacik, D., Dolezel, J., Stanik, M., and Slaby, O. (2016). Expression levels of PIWI-interacting RNA, piR-823, are deregulated in tumor tissue, blood serum and urine of patients with renal cell carcinoma. Anticancer Res 36, 6419–6424.
Jensen, S., Brasset, E., Parey, E., Roest Crollius, H., Sharakhov, I.V., and Vaury, C. (2020). Conserved small nucleotidic elements at the origin of concerted piRNA biogenesis from genes and lncRNAs. Cells 9, 1491.
Karar, J., and Maity, A. (2011). PI3K/AKT/mTOR pathway in angiogenesis. Front Mol Neurosci 4.
Kirino, Y., and Mourelatos, Z. (2007). The mouse homolog of HEN1 is a potential methylase for Piwi-interacting RNAs. RNA 13, 1397–1401.
Kleiner, D.E., and Stetler-Stevenson, W.G. (1999). Matrix metalloproteinases and metastasis. Cancer Chemother Pharmacol 43, S42–S51.
Kuramochi-Miyagawa, S., Watanabe, T., Gotoh, K., Totoki, Y., Toyoda, A., Ikawa, M., Asada, N., Kojima, K., Yamaguchi, Y., Ijiri, T.W., et al. (2008). DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. Genes Dev 22, 908–917.
Laplante, M., and Sabatini, D.M. (2012). mTOR signaling in growth control and disease. Cell 149, 274–293.
Law, P.T.Y., Qin, H., Ching, A.K.K., Lai, K.P., Co, N.N., He, M., Lung, R.W.M., Chan, A.W.H., Chan, T.F., and Wong, N. (2013). Deep sequencing of small RNA transcriptome reveals novel non-coding RNAs in hepatocellular carcinoma. J Hepatol 58, 1165–1173.
Li, B., Hong, J., Hong, M., Wang, Y., Yu, T., Zang, S., and Wu, Q. (2019). piRNA-823 delivered by multiple myeloma-derived extracellular vesicles promoted tumorigenesis through re-educating endothelial cells in the tumor environment. Oncogene 38, 5227–5238.
Li, D., Luo, Y., Gao, Y., Yang, Y., Wang, Y., Xu, Y., Tan, S., Zhang, Y., Duan, J., and Yang, Y. (2016). piR-651 promotes tumor formation in non-small cell lung carcinoma through the upregulation of cyclin D1 and CDK4. Int J Mol Med 38, 927–936.
Li, G., Li, X., Zhuang, S., Wang, L., Zhu, Y., Chen, Y., Sun, W., Wu, Z., Zhou, Z., Chen, J., et al. (2022). Gene editing and its applications in biomedicine. Sci China Life Sci 65, 660–700.
Liu, J., Xiao, Q., Xiao, J., Niu, C., Li, Y., Zhang, X., Zhou, Z., Shu, G., and Yin, G. (2022). Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Sig Transduct Target Ther 7, 3.
Liu, P., Dong, Y., Gu, J., Puthiyakunnon, S., Wu, Y., and Chen, X.G. (2016). Developmental piRNA profiles of the invasive vector mosquito *Aedes albopictus*. Parasit Vectors 9, 524.
Liu, Q., Zhang, H., Jiang, X., Qian, C., Liu, Z., and Luo, D. (2017). Factors involved in cancer metastasis: a better understanding to "seed and soil" hypothesis. Mol Cancer 16, 176.
Liu, T., Wang, J., Sun, L., Li, M., He, X., Jiang, J., and Zhou, Q. (2021). Piwi-interacting RNA-651 promotes cell proliferation and migration and inhibits apoptosis in breast cancer by facilitating DNMT1-mediated PTEN promoter methylation. Cell Cycle 20, 1603–1616.
Lv, X., Xiao, W., Lai, Y., Zhang, Z., Zhang, H., Qiu, C., Hou, L., Chen, Q., Wang, D., Gao, Y., et al. (2023). The non-redundant functions of PIWI family proteins in gametogenesis in golden hamsters. Nat Commun 14, 5267.
Lyko, F. (2018). The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. Nat Rev Genet 19, 81–92.
Ma, H., Wang, H., Tian, F., Zhong, Y., Liu, Z., and Liao, A. (2020). PIWI-interacting RNA-004800 is regulated by S1P receptor signaling pathway to keep myeloma cell survival. Front Oncol 10, 438.
Mai, D., Ding, P., Tan, L., Zhang, J., Pan, Z., Bai, R., Li, C., Li, M., Zhou, Y., Tan, W., et al. (2018). PIWI-interacting RNA-54265 is oncogenic and a potential therapeutic target in colorectal adenocarcinoma. Theranostics 8, 5213–5230.
Mai, D., Ye, Y., Zhuang, L., Zheng, J., and Lin, D. (2023). Detection of piRNA-54265 in human serum: evidence and significance. Cancer Commun 43, 276–279.
Mai, D., Zheng, Y., Guo, H., Ding, P., Bai, R., Li, M., Ye, Y., Zhang, J., Huang, X., Liu, D., et al. (2020). Serum piRNA-54265 is a new biomarker for early detection and clinical surveillance of human colorectal cancer. Theranostics 10, 8468–8478.
Markert, L., Holdmann, J., Klinger, C., Kaufmann, M., Schork, K., Turewicz, M., Eisenacher, M., and Savelsbergh, A. (2021). Small RNAs as biomarkers to differentiate benign and malign prostate diseases: An alternative for transrectal punch biopsy of the prostate? PLoS ONE 16, e0247930.
McDermott, M., Eustace, A.J., Busschots, S., Breen, L., Crown, J., Clynes, M., Oâ €™Donovan, N., and Stordal, B. (2014). *In vitro* development of chemotherapy and targeted therapy drug-resistant cancer cell lines: a practical guide with case studies. Front Oncol 4.
Mei, Y., Clark, D., and Mao, L. (2013). Novel dimensions of piRNAs in cancer. Cancer Lett 336, 46–52.
Mihaylova, M.M., and Shaw, R.J. (2011). The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat Cell Biol 13, 1016–1023.
Mohn, F., Sienski, G., Handler, D., and Brennecke, J. (2014). The Rhino-Deadlock-Cutoff complex licenses noncanonical transcription of dual-strand piRNA clusters in *Drosophila*. Cell 157, 1364–1379.
Nishimasu, H., Ishizu, H., Saito, K., Fukuhara, S., Kamatani, M.K., Bonnefond, L., Matsumoto, N., Nishizawa, T., Nakanaga, K., Aoki, J., et al. (2012). Structure and function of Zucchini endoribonuclease in piRNA biogenesis. Nature 491, 284–287.
Ou, B., Liu, Y., Gao, Z., Xu, J., Yan, Y., Li, Y., and Zhang, J. (2022). Senescent neutrophils-derived exosomal piRNA-17560 promotes chemoresistance and EMT of breast cancer via FTO-mediated m6A demethylation. Cell Death Dis 13, 905.
Ozata, D.M., Gainetdinov, I., Zoch, A., O'Carroll, D., and Zamore, P.D. (2019). PIWI-interacting RNAs: small RNAs with big functions. Nat Rev Genet 20, 89–108.
Pavlič, R., Gjorgoska, M., and Rižner, T.L. (2022). Model cell lines and tissues of different HGSOC subtypes differ in local estrogen biosynthesis. Cancers 14, 2583.
Peng, L., Song, L., Liu, C., Lv, X., Li, X., Jie, J., Zhao, D., and Li, D. (2016). piR-55490 inhibits the growth of lung carcinoma by suppressing mTOR signaling. Tumor Biol 37, 2749–2756.
Poruk, K.E., Gay, D.Z., Brown, K., Mulvihill, J.D., Boucher, K.M., Scaife, C.L., Firpo, M. A., and Mulvihill, S.J. (2013). The clinical utility of CA 19-9 in pancreatic adenocarcinoma: diagnostic and prognostic updates. Curr Med Med 13, 340–351.
Post, C., Clark, J.P., Sytnikova, Y.A., Chirn, G.W., and Lau, N.C. (2014). The capacity of target silencing by *Drosophila* PIWI and piRNAs. RNA 20, 1977–1986.
Qi, T., Cao, H., Sun, H., Feng, H., Li, N., Wang, C., and Wang, L. (2020). piR-19166 inhibits migration and metastasis through CTTN/MMPs pathway in prostate carcinoma. Aging 12, 18209–18220.
Qu, S., Niu, K., Wang, J., Dai, J., Ganguly, A., Gao, C., Tian, Y., Lin, Z., Yang, X., Zhang, X., et al. (2021). LINC00671 suppresses cell proliferation and metastasis in pancreatic cancer by inhibiting AKT and ERK signaling pathway. Cancer Gene Ther 28, 221–233.
Qu, Xiaohan, Liu, Jinlu, Zhong, Xinwen, Li, Xi, and Zhang, Qigang (2015). PIWIL2 promotes progression of non-small cell lung cancer by inducing CDK2 and Cyclin A expression. J Transl Med 13, 301.
Quail, D.F., and Joyce, J.A. (2013). Microenvironmental regulation of tumor progression and metastasis. Nat Med 19, 1423–1437.
Ramos, A., Sadeghi, S., and Tabatabaeian, H. (2021). Battling chemoresistance in cancer: root causes and strategies to uproot them. Int J Mol Sci 22, 9451.
Rayford, K.J., Cooley, A., Rumph, J.T., Arun, A., Rachakonda, G., Villalta, F., Lima, M. F., Pratap, S., Misra, S., and Nde, P.N. (2021). piRNAs as modulators of disease pathogenesis. Int J Mol Sci 22, 2373.
Reeves, M.E., Firek, M., Jliedi, A., and Amaar, Y.G. (2017). Identification and characterization of RASSF1C piRNA target genes in lung cancer cells. Oncotarget 8, 34268–34282.
Rouget, C., Papin, C., Boureux, A., Meunier, A.C., Franco, B., Robine, N., Lai, E.C., Pelisson, A., and Simonelig, M. (2010). Maternal mRNA deadenylation and decay by the piRNA pathway in the early *Drosophila* embryo. Nature 467, 1128–1132.
Roy, J., Das, B., Jain, N., and Mallick, B. (2020). PIWI-interacting RNA 39980 promotes tumor progression and reduces drug sensitivity in neuroblastoma cells. J Cell Physiol 235, 2286–2299.
Sabbah, N.A., Abdalla, W.M., Mawla, W.A., AbdAlMonem, N., Gharib, A.F., Abdul-Saboor, A., Abdelazem, A.S., and Raafat, N. (2021). piRNA-823 is a unique potential diagnostic non-invasive biomarker in colorectal cancer patients. Genes 12, 598.

Shen, B., Yu, S., Zhang, Y., Yuan, Y., Li, X., Zhong, J., and Feng, J. (2016). miR-590-5p regulates gastric cancer cell growth and chemosensitivity through RECK and the AKT/ERK pathway. Onco Targets Ther Volume 9, 6009–6019.
Shi, S., Yang, Z.Z., Liu, S., Yang, F., and Lin, H. (2020). PIWIL1 promotes gastric cancer via a piRNA-independent mechanism. Proc Natl Acad Sci USA 117, 22390–22401.
Shyamala, K., Girish, H.C., and Murgod, S. (2014). Risk of tumor cell seeding through biopsy and aspiration cytology. J Int Soc Prev Communit Dent 4, 5.
Steinberg, G.R., and Hardie, D.G. (2023). New insights into activation and function of the AMPK. Nat Rev Mol Cell Biol 24, 255–272.
Su, S. (2023). Effector immune cell deployment: a revolutionized concept in cancer immunotherapy. Sci China Life Sci 66, 1930–1933.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71, 209–249.
Tan, L., Mai, D., Zhang, B., Jiang, X., Zhang, J., Bai, R., Ye, Y., Li, M., Pan, L., Su, J., et al. (2019). PIWI-interacting RNA-36712 restrains breast cancer progression and chemoresistance by interaction with SEPW1 pseudogene SEPW1P RNA. Mol Cancer 18, 9.
Tang, W., Tu, S., Lee, H.C., Weng, Z., and Mello, C.C. (2016). The RNase PARN-1 trims piRNA 3′ ends to promote transcriptome surveillance in *C. elegans*. Cell 164, 974–984.
Tushir, J.S., Zamore, P.D., and Zhang, Z. (2009). SnapShot: fly piRNAs, PIWI proteins, and the Ping-Pong cycle. Cell 139, 634–634.e1.
Viallard, C., and Larrivée, B. (2017). Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis 20, 409–426.
Vourekas, A., Alexiou, P., Vrettos, N., Maragkakis, M., and Mourelatos, Z. (2016). Sequence-dependent but not sequence-specific piRNA adhesion traps mRNAs to the germ plasm. Nature 531, 390–394.
Vychytilova-Faltejskova, P., Stitkovcova, K., Radova, L., Sachlova, M., Kosarova, Z., Slaba, K., Kala, Z., Svoboda, M., Kiss, I., Vyzula, R., et al. (2018). Circulating PIWI-interacting RNAs piR-5937 and piR-28876 are promising diagnostic biomarkers of colon cancer. Cancer Epidemiol Biomarkers Prev 27, 1019–1028.
Wang, C., and Lin, H. (2021). Roles of piRNAs in transposon and pseudogene regulation of germline mRNAs and lncRNAs. Genome Biol 22, 27.
Wang, H., Shi, B., Zhang, X., Shen, P., He, Q., Yin, M., Pan, Y., and Ma, J. (2023a). Exosomal hsa-piR1089 promotes proliferation and migration in neuroblastoma via targeting KEAP1. Pathol Res Pract 241, 154240.
Wang, X., Ramat, A., Simonelig, M., and Liu, M.F. (2023b). Emerging roles and functional mechanisms of PIWI-interacting RNAs. Nat Rev Mol Cell Biol 24, 123–141.
Wang, Z., Yang, H., Ma, D., Mu, Y., Tan, X., Hao, Q., Feng, L., Liang, J., Xin, W., Chen, Y., et al. (2020). Serum PIWI-interacting RNAs piR-020619 and piR-020450 are promising novel biomarkers for early detection of colorectal cancer. Cancer Epidemiol Biomarkers Prev 29, 990–998.
Watanabe, T., Cheng, E., Zhong, M., and Lin, H. (2015). Retrotransposons and pseudogenes regulate mRNAs and lncRNAs via the piRNA pathway in the germline. Genome Res 25, 368–380.
Watanabe, T., and Lin, H. (2014). Posttranscriptional regulation of gene expression by Piwi proteins and piRNAs. Mol Cell 56, 18–27.
Wu, S.Y., Wang, H., Shao, Z.M., and Jiang, Y.Z. (2021). Triple-negative breast cancer: new treatment strategies in the era of precision medicine. Sci China Life Sci 64, 372–388.
Xie, J., Xing, S., Shen, B.Y., Chen, H.T., Sun, B., Wang, Z.T., Wang, J.W., and Lu, X.X. (2021). PIWIL1 interacting RNA piR-017061 inhibits pancreatic cancer growth via regulating EFNA5. Hum Cell 34, 550–563.
Xie, Q., Li, Z., Luo, X., Wang, D., Zhou, Y., Zhao, J., Gao, S., Yang, Y., Fu, W., Kong, L., et al. (2022). piRNA-14633 promotes cervical cancer cell malignancy in a METTL14-dependent m^{6}A RNA methylation manner. J Transl Med 20, 51.
Xu, J., Chen, T., Liao, L., Chen, T., Li, Q., Xu, J., Hu, J., Zhou, P., and Zhang, Y. (2021). NETO2 promotes esophageal cancer progression by inducing proliferation and metastasis via PI3K/AKT and ERK pathway. Int J Biol Sci 17, 259–270.
Xue, C., Xie, J., Zhao, D., Lin, S., Zhou, T., Shi, S., Shao, X., Lin, Y., Zhu, B., and Cai, X. (2017). The JAK/STAT3 signalling pathway regulated angiogenesis in an endothelial cell/adipose-derived stromal cell co-culture, 3D gel model. Cell Proliferation 50, e12307.
Yan, H., Wu, Q.L., Sun, C.Y., Ai, L.S., Deng, J., Zhang, L., Chen, L., Chu, Z.B., Tang, B., Wang, K., et al. (2015). piRNA-823 contributes to tumorigenesis by regulating *de novo* DNA methylation and angiogenesis in multiple myeloma. Leukemia 29, 196–206.
Yeldag, G., Rice, A., and Del Río Hernández, A. (2018). Chemoresistance and the self-maintaining tumor microenvironment. Cancers 10, 471.
Yin, H., and Lin, H. (2007). An epigenetic activation role of Piwi and a Piwi-associated piRNA in *Drosophila melanogaster*. Nature 450, 304–308.
Yin, J., Jiang, X., Qi, W., Ji, C., Xie, X., Zhang, D., Cui, Z., Wang, C., Bai, Y., Wang, J., et al. (2017). piR-823 contributes to colorectal tumorigenesis by enhancing the transcriptional activity of HSF1. Cancer Sci 108, 1746–1756.
Zamore, P.D., and Haley, B. (2005). Ribo-gnome: the big world of small RNAs. Science 309, 1519–1524.
Zhang, F., Wang, J., Xu, J., Zhang, Z., Koppetsch, B.S., Schultz, N., Vreven, T., Meignin, C., Davis, I., Zamore, P.D., et al. (2012). UAP56 couples piRNA clusters to the perinuclear transposon silencing machinery. Cell 151, 871–884.
Zhang, L., Meng, X., Pan, C., Qu, F., Gan, W., Xiang, Z., Han, X., and Li, D. (2020). piR-31470 epigenetically suppresses the expression of glutathione S-transferase pi 1 in prostate cancer via DNA methylation. Cell Signal 67, 109501.
Zhang, Y., Guo, R., Cui, Y., Zhu, Z., Zhang, Y., Wu, H., Zheng, B., Yue, Q., Bai, S., Zeng, W., et al. (2017). An essential role for PNLDC1 in piRNA 3′ end trimming and male fertility in mice. Cell Res 27, 1392–1396.
Zhang, Y., and Wang, X. (2020). Targeting the Wnt/β-catenin signaling pathway in cancer. J Hematol Oncol 13, 165.
Zhao, J. (2016). Cancer stem cells and chemoresistance: The smartest survives the raid. Pharmacol Ther 160, 145–158.
Zhao, Q., Qian, L., Guo, Y., Lü, J., Li, D., Xie, H., Wang, Q., Ma, W., Liu, P., Liu, Y., et al. (2023). IL11 signaling mediates piR-2158 suppression of cell stemness and angiogenesis in breast cancer. Theranostics 13, 2337–2349.