

Comprehensive landscape and future perspectives of PIWI-interacting RNAs in cancer (Review)

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Received July 7, 2026; Accepted September 22, 2026

DOI: 10.3892/ijo.2026.5946

Abstract. P-element induced wimpy testis (PIWI)-interacting RNAs (piRNAs) are small non-coding RNAs with a length of ~24-32 nucleotides that specifically bind members of the PIWI protein family. Initially identified in germ cells, piRNAs have since been detected in various somatic cells. They regulate multiple physiological and pathological processes and their aberrant expression contributes to human diseases, including cancer. Recent studies indicate that piRNAs

influence key tumour behaviours-including malignant proliferation, invasion and metastasis, angiogenesis, immune evasion and metabolic reprogramming-through epigenetic, transcriptional and post-transcriptional mechanisms. In tumour-bearing mouse models, exogenous knockdown or overexpression of piRNAs using nanodelivery systems can markedly inhibit tumour growth, supporting their potential as therapeutic targets. piRNAs are also stably expressed in

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Abbreviations: piRNAs, PIWI-interacting RNAs; piRISC/piRISCs, PIWI-piRNA-induced silencing complex(es); PIWI, P-element induced wimpy testis; PIWIL1/PIWIL2/PIWIL3, PIWI-like protein 1/2/3; AD, Alzheimer's disease; AGO3, argonaute 3; AI, artificial intelligence; AKT, protein kinase B; Aub, Aubergine; CA19-9, carbohydrate antigen 19-9; ccRCC, clear cell renal cell carcinoma; CCA, cholangiocarcinoma; CEA, carcinoembryonic antigen; CRC, colorectal cancer; CRPC, castration-resistant prostate cancer; ctDNA, circulating tumor DNA; CTCs, circulating tumor cells; DLBCL, diffuse large B-cell lymphoma; DNMT, DNA methyltransferase; DNMT1/3A/3B, DNA methyltransferase 1/3A/3B; EMT, epithelial-mesenchymal transition; ER α , estrogen receptor α ; ERK, extracellular signal-regulated kinase; EVs, extracellular vesicles; FISH, fluorescence in situ hybridization; FOSL1, FOS like 1; FTO, fat mass and obesity-associated protein; GBC, gallbladder cancer; GBM, glioblastoma; G-MDSCs, granulocytic

myeloid-derived suppressor cells; GC, gastric cancer; GPX4, glutathione peroxidase 4; GSDMD, gasdermin D; HA, hyaluronic acid; HCC, hepatocellular carcinoma; HK2, hexokinase 2; hnRNP, heterogeneous nuclear ribonucleoprotein U; H3K9me3, histone H3 lysine 9 trimethylation; IFN- γ , interferon- γ ; IGF2BP, insulin-like growth factor 2 mRNA-binding proteins; IL, interleukin; IL11/IL-6/IL-11, interleukin 11/6/11; INSIG1, insulin-induced gene 1; STAT3, signal transducer and activator of transcription 3; lncRNA, long non-coding RNA; LUAD, lung adenocarcinoma; miRNA, microRNA; MM, multiple myeloma; MMSCs, multiple myeloma stem cells; MOV10L1, Mov10 like RNA helicase 1; m6A, N6-methyladenosine; MUC12, mucin 12; ncRNAs, non-coding RNAs; NEFH, neurofilament heavy chain; NF- κ B, nuclear factor κ B; NLRP3, NLR family pyrin domain containing 3; nt, nucleotide(s); OC, ovarian cancer; OR, odds ratio; OS, overall survival; PD, Parkinson's disease; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PLGA, polylactic-co-glycolic acid; PMLCPIR, PM2.5-induced lung cancer-upregulated piRNA; PNENs, pancreatic neuroendocrine neoplasms; Pol II, RNA polymerase II; PFS, progression-free survival; PTEN, phosphatase and tensin homolog; RT-qPCR, reverse transcription-quantitative PCR; RA, rheumatoid arthritis; RCC, renal cell carcinoma; RNA-seq, RNA sequencing; RNase H, ribonuclease H; ROS, reactive oxygen species; ROC, receiver operating characteristic

Key words: PIWI-interacting RNA, tumour microenvironment, metabolic reprogramming, extracellular vesicles, liquid biopsy, targeted therapy

tumour tissues and bodily fluids, such as serum and urine, either freely circulating or encapsulated within extracellular vesicles; their dysregulation is associated with clinicopathological features of tumours. Accordingly, piRNAs may serve as novel pathological diagnostic markers or liquid biopsy biomarkers. This review summarises piRNA expression patterns and molecular mechanisms, evaluates their clinical potential in cancer diagnosis and treatment and integrates advances in high-throughput sequencing and nanodelivery technologies to provide a theoretical basis and research foundation for the clinical translation of piRNA-related studies.

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1. Introduction

Global Cancer Statistics (GLOBOCAN 2022) estimated nearly 20 million new cancer cases and >10 million cancer-related deaths worldwide in 2022, underscoring cancer as a major global public health burden. Cancer treatment has become increasingly diverse, evolving from conventional surgery, radiotherapy and chemotherapy toward precision approaches, such as targeted therapy, immunotherapy and combination regimens. However, tumour heterogeneity, metastatic capacity, drug resistance and complex tumour-immune interactions continue to limit the broad applicability and long-term effectiveness of current therapies (1-3). Therefore, deeper insight into the mechanisms driving tumour initiation and progression, along with the identification of precise, verifiable molecular biomarkers and therapeutic targets, is essential to improve clinical classification, prognostic assessment and individualised treatment.

Biomarkers are essential for early cancer diagnosis, disease monitoring and assessment of therapeutic response (4). An ideal tumour biomarker should offer high specificity and sensitivity, convenient sample collection, good stability and simple detection methods (5). Commonly used biomarkers, including alpha-fetoprotein, carcinoembryonic antigen (CEA), carbohydrate antigen (CA)19-9, human epidermal growth factor receptor 2 and programmed death-ligand 1 (PD-L1), provide clinical value in selected cancer types but remain limited by inadequate sensitivity, high false-positive rates and inconsistent prognostic performance (6,7). Accordingly, tumour biomarker research has expanded beyond traditional protein markers towards multi-omics integration and emerging biomarkers, including non-coding RNAs (ncRNAs) (8,9), circulating tumour DNA (ctDNA) (10), exosomes (11), circulating tumour cells (CTCs) (12) and tumour metabolites (13,14). Among these, ncRNAs, which comprise ~98% of the human genome, exhibit broad yet tissue-specific expression and are closely

associated with diverse diseases, making them a major focus of recent oncology research (15).

P-element induced wimpy testis (PIWI)-interacting RNAs (piRNAs) are a distinct class of small ncRNAs ~24-32 nucleotides in length (16,17). By forming complexes with PIWI proteins, piRNAs suppress transposon activity, preserve genomic stability in germ cells and regulate reproductive development (18,19). Although initially regarded as reproductive system-specific, accumulating evidence indicates that piRNAs may also function as tumour biomarkers and therapeutic targets (20-23). They contribute to tumour regulation through multiple mechanisms, including mRNA degradation, DNA methylation (24-26), histone modifications and post-transcriptional regulation (27). Beyond transposon silencing (28,29), piRNAs influence key signalling pathways involved in tumour initiation and progression (30). Aberrant piRNA expression has been reported in various cancers and is closely associated with tumour stage, subtype, metastasis and prognosis (31-33). Furthermore, their stability in blood and resistance to RNase-mediated degradation support their potential as non-invasive biopsy biomarkers (34,35).

This review summarises the discovery, identification, biogenesis and regulatory mechanisms of piRNAs, examines their associations with malignant tumour phenotypes and evaluates their potential clinical applications. Overall, piRNAs may provide not only a new perspective for understanding cancer development but also valuable tools for next-generation early cancer diagnosis, biomarker screening and individualised therapy.

2. piRNA function and role

piRNA discovery and identification. The discovery and definition of piRNAs developed gradually (Fig. 1). PIWI protein was first identified in *Drosophila melanogaster* in 1997; PIWI mutations disrupted male and female germline stem cell division and caused infertility, demonstrating its essential role in reproductive development (36). In 2000, PIWI was confirmed as a member of the Argonaute protein family, containing a Piwi Argonaut and Zwiille domain for RNA recognition and binding and a PIWI domain with RNase H-like catalytic activity. These features indicate that PIWI proteins participate in RNA binding, RNA interference and gene silencing through endonuclease activity (37).

In 2006, several independent groups identified piRNAs, small RNAs ~24-32 nt long that specifically bind to PIWI proteins, formally establishing the field of piRNA research (38-40). In 2007, Brennecke *et al* (41) clarified that piRNAs are primarily derived from specific piRNA clusters and are closely associated with transposon sequences. That same year, Gunawardane *et al* (42) demonstrated complementarity between sense and antisense piRNAs and confirmed PIWI Slicer activity, proposing a reciprocal cleavage-based amplification mechanism that became central to the classic 'ping-pong' model. Subsequent studies focused on piRNA biogenesis and processing. In 2009, Klattenhoff *et al* (43) discovered that Rhino promotes transcription of dual-strand piRNA clusters, contributing to piRNA production and transposon silencing. In 2012, Nishimasu *et al* (44) identified

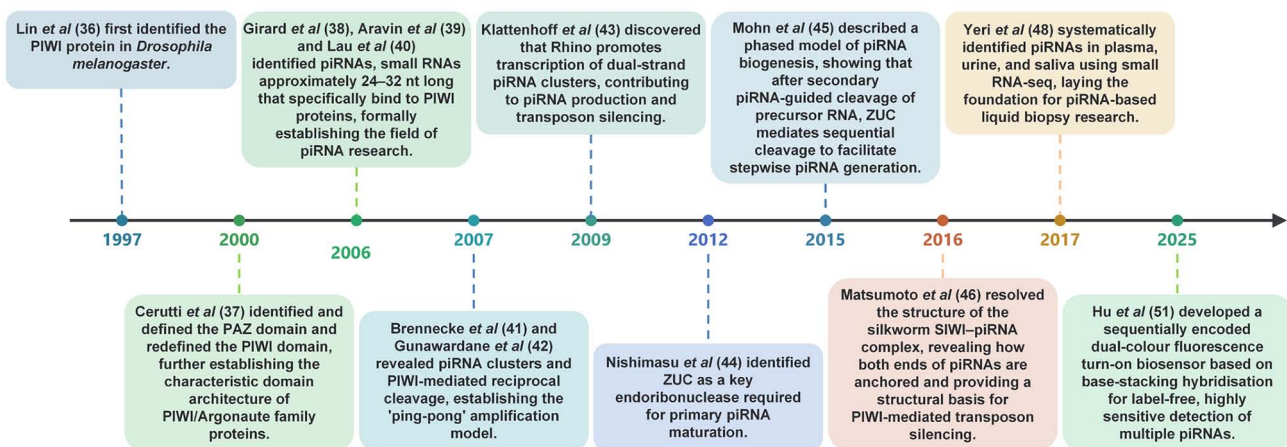


Figure 1. Timeline of important events in the history of piRNA. This timeline illustrates key milestones in the field of piRNA. piRNA, PIWI-interacting RNA; PIWI, P-element induced wimpy testis; ZUC, Zucchini.

Zucchini (ZUC) as a key endoribonuclease required for primary piRNA maturation. In 2015, Mohn *et al* (45) described a phased model of piRNA biogenesis, showing that after secondary piRNA-guided cleavage of precursor RNA, ZUC mediates sequential cleavage to facilitate stepwise piRNA generation. In 2016, Matsumoto *et al* (46) resolved the structure of the silkworm SIWI-piRNA complex, revealing how both ends of piRNAs are anchored and providing a structural basis for PIWI-mediated transposon silencing.

With advances in high-throughput RNA sequencing (RNA-seq) and single-cell transcriptomics, aberrant piRNA expression has been detected in multiple cancer tissues (47). In 2017, Yeri *et al* (48) systematically identified piRNAs in plasma, urine and saliva using small RNA-seq, laying the foundation for piRNA-based liquid biopsy research. In addition, piRNA-PIWI complexes can mediate epigenetic events such as DNA methylation and histone modification, broadening their functional relevance in cancer. Continued advances in techniques including fluorescence *in situ* hybridisation (FISH), RNA pull-down, cross-linking immunoprecipitation sequencing and clustered regularly interspaced short palindromic repeats interference have shifted piRNA research from expression profiling to disease-mechanism studies and potential clinical applications (49,50). In 2025, Hu *et al* (51) developed a sequentially encoded dual-colour fluorescence turn-on biosensor based on base-stacking hybridisation for label-free, highly sensitive detection of multiple piRNAs, offering a novel strategy for clinical piRNA detection.

piRNA biogenesis. piRNA biogenesis differs markedly from that of classical small RNAs, such as microRNAs (miRNAs) and small interfering RNAs. Unlike these pathways, piRNA production does not depend on Drosha or Dicer; instead, it uses a specialised processing system. Based on mode of generation, piRNA synthesis is divided into the primary processing pathway and the secondary amplification pathway, also known as the ping-pong cycle (52,53). By tissue origin, piRNA biogenesis can also be classified into germline pathways, which occur primarily in sperm or oocytes, and somatic pathways, which occur in ovarian somatic cells or other

non-germline tissues. The primary pathway functions in both systems, whereas the secondary pathway is largely restricted to germline cells, constituting the main mechanistic difference between them (54).

piRNA production begins in specific genomic regions called piRNA clusters, often located in heterochromatic, transposon-rich regions, as well as in intergenic or intronic regions. After transcription, precursor piRNAs are transported to the outer mitochondrial membrane, where the endonuclease ZUC generates intermediates with 5' monophosphate ends. These fragments are loaded onto PIWI family proteins, including PIWI-like protein 1 (PIWIL1) and PIWIL2 in humans; their 3' ends are then trimmed by enzymes such as Trimmer and 2'-O-methylated by nescient helix-loop-helix 1 (HEN1) to form mature piRNAs. Mature piRNA-PIWI complexes subsequently act in the cytoplasm or nucleus to silence transposons and regulate gene expression (32,45).

Primary piRNAs typically exhibit a 5'-terminal uridine bias. In germline cells, piRNAs are amplified through the ping-pong mechanism, which relies on coordinated activity between two PIWI proteins: Aubergine (AUB) and argonaute RISC catalytic component 3 (AGO3) in *Drosophila*, and mouse PIWI-like protein and mouse PIWI-like 2 in mice. Antisense piRNAs loaded onto AUB guide cleavage of target transposon mRNAs, producing sense piRNAs that are then loaded onto AGO3 to cleave antisense precursor RNAs. This reciprocal cleavage generates piRNAs with a characteristic 10-nucleotide 5' overlap, the ping-pong signature. The cycle increases piRNA diversity and abundance while strengthening transposon repression, making it a central defence mechanism for germline genomic stability (28,55,56).

piRNA biogenesis is a coordinated process involving multiple regulatory factors. Beyond core processing enzymes such as ZUC, HEN1 and Trimmer, several RNA-binding proteins help preserve pathway integrity. For example, Mov10-like RNA helicase 1 (MOV10L1) regulates precursor piRNA recognition and cleavage-site positioning; MOV10L1 deficiency blocks piRNA production, disrupts transposon control and causes germ cell failure (57). Tudor domain-containing (TDRD) proteins, including TDRD1 and TDRD9, bind dimethylated arginine residues on PIWI proteins

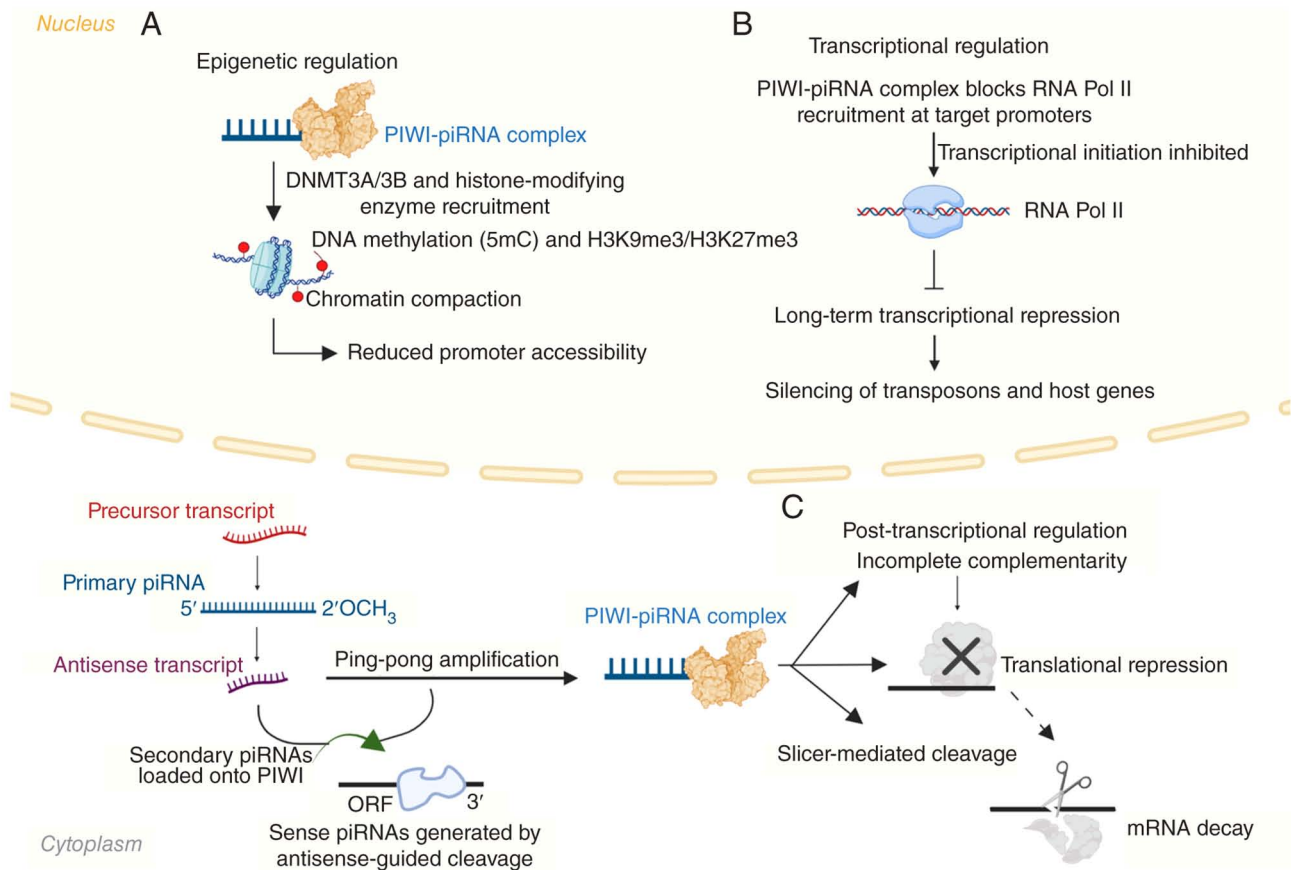


Figure 2. Mechanisms of piRNA-mediated gene regulation. (A) In the nucleus, PIWI-piRNA complexes recruit DNA methyltransferases and histone-modifying enzymes, leading to chromatin compaction and reduced promoter accessibility. (B) PIWI-piRNA complexes inhibit RNA Pol II recruitment, repressing target genes. (C) In the cytoplasm, piRNAs regulate gene expression post-transcriptionally through translational repression or slicer-mediated mRNA degradation. piRNA, PIWI-interacting RNA; PIWI, P-element induced wimpy testis; RNA Pol II, RNA polymerase II; ORF, open reading frame.

and promote stable piRNA-PIWI complex assembly (58). Proteins such as Maelstrom, Vasa and GasZ also support the formation and maintenance of processing sites, including Nuage or Yb bodies, thereby enabling efficient precursor piRNA processing and export (59-62). These factors are evolutionarily conserved, and their loss or dysfunction often leads to infertility or increased transposon activity (57,59-62).

It is also important to distinguish authentic piRNAs from piRNA-like candidates in somatic cells (63,64). Some small RNAs annotated as piRNAs in human somatic tissues lack confirmed PIWI binding or evidence of functional silencing-complex formation (63,65). Given that mature piRNAs typically contain a 3'-terminal 2'-O-methyl group, oxidation-based assays provide stronger support for piRNA identification than sequence length or database annotation alone (66).

Molecular mechanisms of piRNA-mediated gene regulation. piRNAs bind PIWI proteins to form PIWI-piRNA complexes, or PIWI-piRNA-induced silencing complexes (piRISCs), which regulate gene expression across subcellular compartments. Their main mechanisms include epigenetic, transcriptional and post-transcriptional regulation (Fig. 2), with epigenetic control often operating alongside transcriptional regulation. Together, these mechanisms help maintain genome stability, suppress transposon activation and provide

specialised regulatory potential in selected somatic contexts and during tumorigenesis (67-69).

The best-characterised function of piRNAs is nuclear epigenetic silencing. After entering the nucleus, mature piRNAs associate with PIWI proteins to form piRISCs. Through base-pairing complementarity, these complexes are guided to nascent transposon transcripts or RNA-chromatin complexes without direct piRNA-DNA interaction. Once localised, piRISCs recruit DNA methyltransferases (DNMTs), including DNMT3A and DNMT3B, and histone-modifying enzymes, inducing DNA methylation (5-methylcytosine) and heterochromatin marks such as histone H3 lysine 9 trimethylation (H3K9me3) and H3K27me3 at target regions. The resulting chromatin compaction and reduced promoter accessibility promote transposon silencing. This mechanism is particularly critical in germline cells, where it helps preserve hereditary genomic stability (70,71). Emerging evidence suggests that similar mechanisms also occur in somatic cells and tumour models, where piRNA-PIWI complexes localise to host gene promoters and repress transcription by increasing DNA methylation, heterochromatin marks and local compaction (72-75).

In the cytoplasm, piRNAs primarily regulate mRNA stability and translational efficiency through post-transcriptional mechanisms. When piRNAs exhibit high complementarity to their target mRNAs, PIWI proteins with

endonuclease activity cleave the target mRNAs, triggering rapid degradation. When complementarity is insufficient for cleavage, piRISCs can inhibit ribosome loading, repress translation and subsequently promote mRNA decay. Evidence from multiple animal models and emerging disease studies supports both mechanisms (76-78). For example, piR-36712 mediates degradation of target transcripts in breast cancer cells, thereby inhibiting cell growth and invasion (79).

Beyond epigenetic chromatin remodelling, piRNAs can directly regulate transcription in the nucleus. PIWI-piRNA complexes at target regions may prevent RNA polymerase II recruitment to promoters or disrupt transcription-initiation assembly, thereby suppressing transcription and sustaining target-gene repression. Although the full mechanism in somatic tissues remains elusive, growing evidence from tumour studies indicates that piRNA-PIWI complexes can reprogram host gene expression through direct transcriptional regulation. This regulation may occur independently of or in coordination with epigenetic modifications such as DNA methylation and histone modifications, and is closely linked to oncogene activation and tumour-suppressor silencing (80-85). Additionally, PIWI proteins can promote tumorigenesis through piRNA-independent pathways. For example, Shi *et al* (86) reported that PIWIL1 facilitates gastric cancer progression by interacting with the UPF1 RNA helicase and ATPase-mediated nonsense-mediated mRNA decay pathway. These findings support an important role for nuclear piRNA networks in cancer-associated transcriptional dysregulation.

piRNAs may also function within multilayered RNA networks, including indirect or competitive interactions with other ncRNAs, such as miRNAs and long ncRNAs (lncRNAs), thereby modulating downstream signalling pathways. However, expression correlations, target prediction or network construction alone cannot establish direct regulation within lncRNA-miRNA-piRNA networks. Without evidence of direct molecular interaction, PIWI dependence and functional validation, these networks should be considered as potential mechanisms rather than confirmed causal pathways (63). Overall, piRNAs form a sophisticated regulatory system centred on PIWI-piRNA complexes, integrating nuclear epigenetic and transcriptional regulation with cytoplasmic post-transcriptional control. These networks have been implicated in key biological processes, including cell proliferation, apoptosis, migration and metabolism (87-91).

piRNAs and human diseases. piRNAs are present in a wide range of somatic tissues, where they appear to participate in physiological and pathological regulation and may influence the onset and progression of human diseases (92-99) (Table I). These diseases include autoimmune disorders such as rheumatoid arthritis, neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease and type 2 diabetes with diabetic nephropathy. PiRNAs regulate immune responses, support neural homeostasis and participate in glucose metabolism at the somatic level, suggesting their potential as biomarkers and therapeutic targets in these diseases (92-107). For example, in brain tissues and patient-derived neurons from individuals with PD, piRNAs and piRNA-like molecules exhibit widespread

aberrant expression. A subset of these differentially expressed piRNAs is consistently detected in both sample types, suggesting possible diagnostic value (101).

With advances in high-throughput sequencing, multiple databases and bioinformatics tools have been developed for piRNA sequence collection, functional annotation, target-gene prediction and disease-association analysis. These platforms provide systematic resources for exploring piRNA roles in diverse biological processes and diseases (108-116) (Table II). Several tools are now used in human disease research, particularly in oncology. For instance, piRBase integrates piRNA sequences, expression profiles, target information and disease annotations, and has been widely used to screen for cancer-related piRNAs in breast, gastric, hepatocellular, renal and colorectal cancer (CRC) (108,117).

3. piRNAs regulate cancer hallmarks

Cancer initiation and progression involve complex biological processes that can be organised into 14 established hallmarks (118,119). Emerging evidence shows that piRNAs regulate tumour biology by affecting malignant proliferation, invasion, metastasis, angiogenesis, immune evasion, metabolic reprogramming and resistance to cell death (Fig. 3).

piRNAs and malignant tumour cell proliferation. Malignant proliferation describes the ability of tumour cells to acquire and sustain abnormal proliferative signals, bypass normal cell-cycle control and proliferate uncontrollably. piRNAs regulate the expression of cell-cycle-related genes by forming complexes with PIWI proteins.

Liu *et al* (120) showed that piR-651 forms a complex with PIWIL2 and recruits the DNA methyltransferase DNMT1 to the phosphatase and tensin homolog (*PTEN*) promoter, inducing promoter hypermethylation and transcriptional silencing, thereby enhancing breast cancer cell proliferation and migration. Ding *et al* (121) reported that piR-823 is upregulated in luminal breast cancer stem cells, where it enhances stemness and tumour growth by increasing DNMT expression, promoting the methylation of the *APC* regulator of Wnt signaling pathway gene and activating Wnt signalling. In CRC, piR-1245 enhances proliferation by downregulating tumour suppressors such as B-cell translocation gene 1 and FAS cell surface death receptor, with higher expression associated with advanced and metastatic disease (122). Similarly, piR-36026 promotes breast cancer cell proliferation by inhibiting the tumour suppressors serpin family A member 1 ($\alpha 1$ antitrypsin) and lecithin retinol acyltransferase (121). A molecular beacon probe targeting piR-36026 enabled both imaging and functional inhibition, suppressing oncogenic activity *in vitro* and *in vivo* and highlighting its theranostic potential.

Complex piRNA-mediated regulatory networks have also been reported in other malignancies. Liu *et al* (123) identified a PIWIL3/piR-30188/OIP5-AS1/miR-367-3p/CCAAT enhancer binding protein α positive-feedback axis as a critical driver of glioma progression through coordinated regulation of TNF receptor-associated factor 4 expression; disrupting this pathway reduced tumour growth and improved survival in mouse models, suggesting therapeutic potential.

Table I. Relationship between piRNA expression and human diseases.

Disease type	piRNA name	Expression level	Function	(Refs.)
Abdominal aortic aneurysm	piR-011324	Up	Promotes the phenotypic transformation in human aortic vascular smooth muscle cells, boosting the activity of matrix metalloproteinases, inflammation (IL-1 β /TNF- α) and apoptosis	(92)
AD	piR-019324	Down	Detects AD and predicts the conversion of mild-cognitive impaired patients to AD dementia	(93)
	piR-020364	Up		
	piR-019949			
Aortic dissection	piR-0228	Down	Attenuates receptor-interacting protein kinase 1 inhibition, promotes stimulator of interferon genes pathway-mediated macrophage inflammation and vascular smooth muscle cell necrosis and accelerates the progression of aortic dissection	(94)
Diabetes mellitus	piR-112710	Up	Attenuates diabetic cardiomyopathy through inhibiting the thioredoxin-interacting protein/NLRP3-mediated pyroptosis	(95)
	piR-019825		Associated with diabetic nephropathy	(96)
RA	piR-27620	Up	Regulation of the immune signaling pathways to affect the pathogenesis of RA	(97)
	piR-27124			
	piR-ENOX2	Up	Promotes M1 polarization of macrophages through the ALKBH5/ITGA4/PI3K-AKT axis, driving the progression of RA	(98)
Tumors	piR-1742	Up	Directly binds to heterogeneous nuclear ribonucleo-protein U to regulate the stability of ubiquitin specific peptidase 8 mRNA to modulate MUC12 ubiquitination and influence the progression of malignant ccRCC	(99)

piRNA, PIWI-interacting RNA; PIWI, P-element induced wimpy testis; AD, Alzheimer's disease; RA, rheumatoid arthritis; ccRCC, clear cell renal cell carcinoma.

Conversely, certain piRNAs act as tumour suppressors. piR-MW557525 is significantly downregulated in neuroblastoma, whereas its overexpression induces apoptosis and inhibits proliferation, migration and invasion by activating the P53-P21-cyclin-dependent kinase 2-Cyclin E pathway and triggering G0/G1 arrest (124).

Together, these observations indicate that piRNAs can either promote or suppress tumorigenesis through diverse mechanisms that affect tumour-cell proliferation, stemness and malignant progression.

piRNAs and tumour invasion and metastasis. Invasion and metastasis occur when tumour cells breach local tissue barriers, migrate to distant organs, survive and colonise in new microenvironments and form secondary tumours. Growing evidence indicates that piRNAs are important regulators of these processes.

In castration-resistant prostate cancer (CRPC), piR-4447944 is significantly overexpressed and associated with increased proliferation, migration and invasion, as well as reduced apoptosis (125). Mechanistically, piR-4447944 forms a complex with PIWIL2 and suppresses post-transcriptional expression of the tumour-suppressor neurofilament

heavy chain (*NEFH*), thereby promoting androgen-independent growth and CRPC progression. In CRC, high piR-54265 expression activates STAT3 signalling through PIWIL2/STAT3/phospho-SRC complex formation, thereby promoting proliferation, metastasis and chemoresistance; inhibiting piR-54265 blocks this pathway, reducing tumour growth and metastasis (23). Additionally, in oral squamous cell carcinoma, cancer-associated fibroblast-derived exosomal piR-35462 increases expression of the fat mass and obesity-associated protein, which stabilises *Twist1* mRNA by inhibiting N⁶-methyladenosine (m⁶A) RNA methylation, thereby promoting epithelial-mesenchymal transition and tumour progression (126).

Overall, piRNAs may regulate tumour invasion and metastasis by modulating signalling pathways, transcriptional networks and tumour-stroma interactions, supporting their potential as therapeutic targets.

piRNAs and tumour angiogenesis. Angiogenesis enables tumours to generate new blood vessels and secure oxygen and nutrients required for sustained growth and progression. Emerging evidence suggests that piRNAs contribute to the regulation of tumour angiogenesis.

Table II. PiRNA-related databases and tools.

Database	Functions	URL	(Refs.)
piRBase	Provides comprehensive tools for piRNA sequencing, annotation, target gene prediction and functional analysis, covering datasets of multiple species.	http://www.regulatoryrna.org/database/piRNA/	(108)
piRNA clusterDB	Provides comprehensive data on piRNA clusters across multiple species, tissues and developmental stages, based on small RNA sequencing data.	https://www.smallrnagroup.uni-mainz.de/piRNAclusterDB	(109)
TarpiD	Provides comprehensive information on piRNAs and their targets, including expression, verification methods, tissue types and disease associations	https://tarpid.nitrkl.ac.in/tarpid_db/	(110)
PUTrans GCN	A tool to identify potential associations between piRNAs and diseases using heterogeneous graph convolutional networks, supporting in-depth research on piRNA-disease relationships.	https://github.com/chengqihao/PUTransGCN	(111)
iPiDA-SWGCN	A piRNA-disease association prediction tool based on graph convolutional networks, which provides predictions of potential associations between piRNAs and diseases.	http://bliulab.net/iPiDA-SWGCN/	(112)
piRNA Quest V.2	Provides information on piRNA expression profiles, target gene predictions and associations with disease systems, supporting data analysis for multiple species.	http://dibresources.jcbose.ac.in/zhumur/pirnaquest2	(113)
LSTM4 piRNA	Efficient piRNA detection in large-scale genomic databases using deep learning-based LSTM networks.	https://lstm4pirna.ee.ncyu.edu.tw	(114)
piRNA Bank	Web resources for the classification and clustering of piRNAs.	http://pirnabank.ibab.ac.in/	(115)
proTRAC	A software for probabilistic piRNA cluster detection, visualization and analysis.	https://sourceforge.net/projects/protrac/	(116)

piRNA, PIWI-interacting RNA; PIWI, P-element induced wimpy testis; LSTM, long short-term memory.

In multiple myeloma (MM), granulocytic myeloid-derived suppressor cells (G-MDSCs) increase piR-823 expression, promoting DNA methylation and enhancing the stemness and tumorigenic capacity of MM stem cells (MMSCs). Ai *et al* (127) reported that piR-823 knockdown weakens G-MDSC-mediated maintenance of stemness and reduces tumour growth and angiogenesis *in vivo*, highlighting the G-MDSC/piR-823/DNA methylation axis. Yan *et al* (26) similarly reported that piR-823 upregulates DNMT3A and DNMT3B, increases global DNA methylation, silences the tumour suppressor gene *P16* and promotes MM. Silencing piR-823 inhibits cell proliferation and angiogenesis, supporting its key role in the epigenetic regulation of MM. Li *et al* (128) further showed that myeloma-derived extracellular vesicles (EVs) deliver piR-823 to endothelial cells, where it upregulates vascular endothelial growth factor and interleukin (IL)-6,

thereby promoting endothelial proliferation, tubule formation, invasion and angiogenesis.

Furthermore, piR-2158 is downregulated in breast cancer and competitively interacts with the *IL11* promoter together with FOS-like 1, suppressing *IL11* expression and STAT3 signalling (129). This reduces cancer cell stemness, angiogenesis and breast tumour proliferation. Similarly, piR-31115 is upregulated in MDA-MB-231 triple-negative breast cancer (TNBC) cells and induces methyltransferase 3 expression in endothelial cells (130). This increases m⁶A modification and stabilises Yes1-associated transcriptional regulator through insulin-like growth factor-2 mRNA-binding protein, thereby promoting HMEC-1 cell proliferation, migration and tumour angiogenesis (130).

Overall, current evidence suggests that piRNAs regulate tumour angiogenesis through both pro- and anti-angiogenic mechanisms, thereby modulating tumour progression.

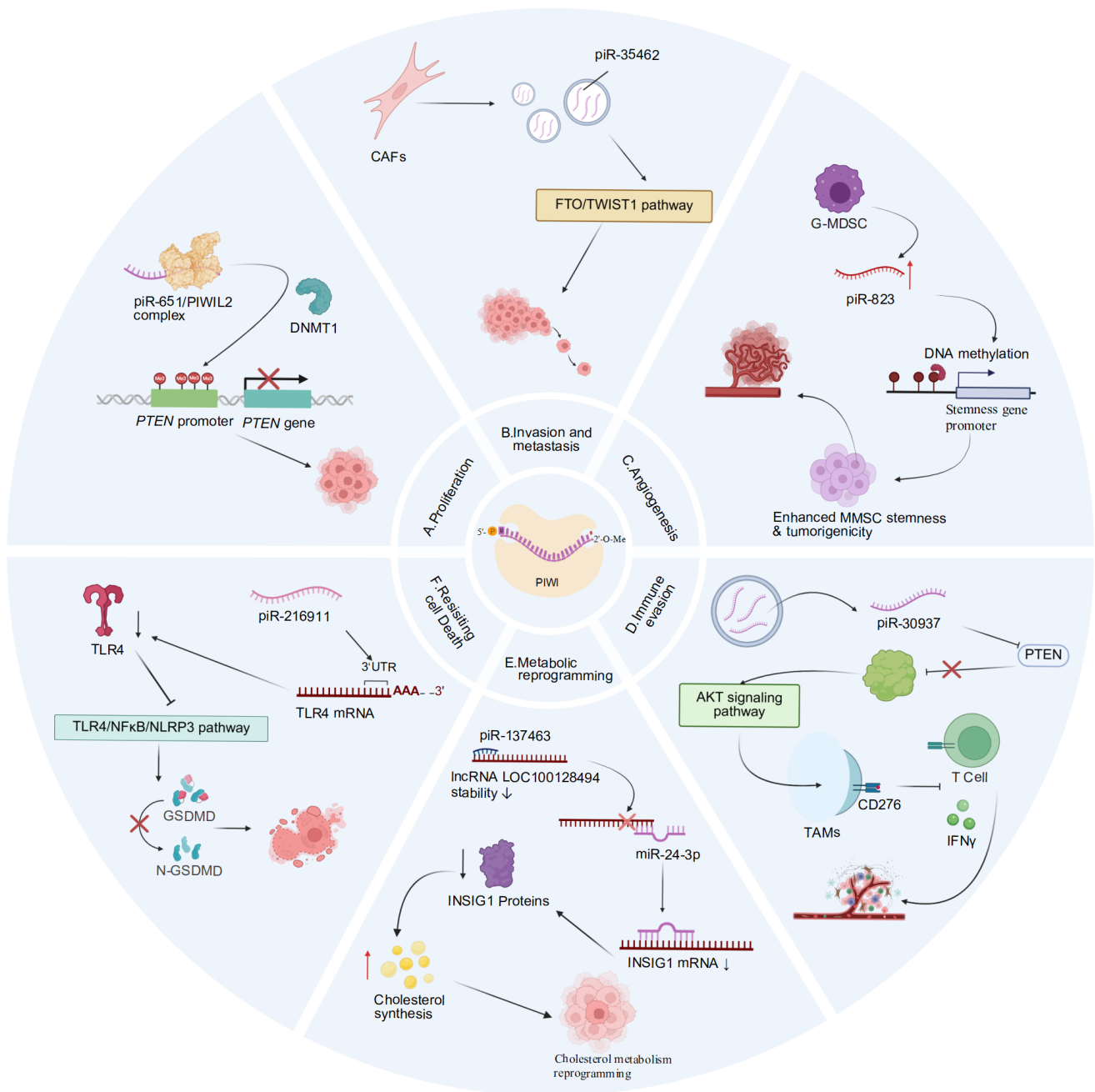


Figure 3. PiRNAs can regulate tumour progression. (A) piR-651 promotes tumour proliferation in breast cancer. (B) piR-35462 promotes tumour invasion and metastasis in oral squamous cell carcinoma. (C) piR-823 promotes tumour angiogenesis in multiple myeloma. (D) piR-30937 promotes tumour immune evasion in pancreatic neuroendocrine neoplasms. (E) piR-137463 promotes tumour metabolic reprogramming in lung adenocarcinoma. (F) piR-216911 promotes tumour resistance to cell death in hepatocellular carcinoma. piRNA, PIWI-interacting RNA; PIWI, P-element induced wimpy testis; CAF, cancer-associated fibroblast; TAM, tumor-associated macrophage; TLR, Toll-like receptor; GSDMD, gasdermin D; PTEN, phosphatase and tensin homolog; lncRNA, long non-coding RNA; DNMT1, DNA methyltransferase 1; FTO, Fat mass and obesity-associated protein; TWIST1, Twist family bHLH transcription factor 1; G-MDSC, Granulocytic myeloid-derived suppressor cell; MMSC, multiple myeloma stem cells; IFN γ , Interferon-gamma; INSIG1, Insulin-induced gene 1; NF- κ B, Nuclear factor κ -light-chain-enhancer of activated B cells; NLRP3, NLR family pyrin domain containing 3.

piRNAs and tumour immune responses. Immune evasion allows tumour cells to avoid immune clearance by reducing immune recognition or suppressing anti-tumour effector functions. piRNAs are increasingly recognised as regulators of tumour-immune interactions.

In high-risk myelodysplastic syndromes, increased piRNA expression has been linked to disease progression via suppression of transposable elements (TEs), thereby disrupting energy metabolism, cell-cycle regulation and

immune pathways. Elevated PIWIL2 expression is also associated with poor prognosis, suggesting an important role for the TE/piRNA axis and potential prognostic value (131). Zhong *et al* (132) showed that small EVs (sEVs) from pancreatic neuroendocrine neoplasms (PNEs) carry piR-30937, which activates AKT signalling by inhibiting *PTEN* and upregulates CD276 in tumour-associated macrophages (TAMs). CD276⁺ TAMs suppress T-cell proliferation and interferon- γ production, promoting immune evasion and

tumour progression through the piR-30937/PTEN/AKT/CD276 axis (132). Lu *et al* (133) further demonstrated that Cyclin D1 alters ncRNA secretion by increasing miR-21, miR-93 and piRNAs such as piR-016658 and piR-016975. This promotes stem cell expansion and immune evasion by enhancing PIWIL2 expression and activating Toll-like receptor 8 (TLR8)-related inflammatory signalling in oestrogen receptor α -positive breast cancer.

Current evidence indicates that piRNAs modulate the tumour immune microenvironment, potentially affecting immune evasion, macrophage polarisation, and T-cell suppression, although the underlying mechanisms warrant further clarification.

piRNAs and tumour metabolic reprogramming. Metabolic reprogramming enables tumour cells to adapt nutrient use and energy metabolism-including glucose, glutamine, and lipid pathways-to support rapid proliferation under hypoxia, nutrient limitation and other pressures within the tumour microenvironment.

Emerging evidence suggests that piRNAs affect tumour-cell metabolism and survival by regulating key genes within metabolic pathways. In lung adenocarcinoma (LUAD), piR-137463 downregulates insulin-induced gene 1 (*INSIG1*) by destabilising lncRNA LOC100128494 and reducing its competitive binding to miR-24-3p, thereby disrupting cholesterol metabolism and promoting LUAD progression. Inhibiting piR-137463 activates the LOC100128494/miR-24-3p/INSIG1 axis, reduces PD-L1 expression, enhances T-cell cytotoxicity, and improves responses to anti-programmed cell death 1 (PD-1) therapy (134). In TNBC, piR-35410 is highly expressed and promotes aerobic glycolysis by directly binding the amino acid residues 470-780 of ATP-dependent 6-phosphofructokinase, liver type, thereby enhancing its enzymatic activity and increasing ATP and lactate production, thereby driving TNBC progression (135). Conversely, Yan *et al* (136) identified piR-26441 as a potential tumour suppressor in ovarian cancer (OC), where low expression is associated with malignant progression and poor prognosis. piR-26441 inhibits OC growth by downregulating the mitochondrial translation elongation factor through YTH domain-containing protein 1-mediated m⁶A modification, thereby suppressing mitochondrial oxidative phosphorylation and activating reactive oxygen species-mediated apoptosis.

Together, these studies indicate that piRNAs contribute to tumour metabolic reprogramming and may influence tumour growth and therapeutic response by regulating energy metabolism and nutrient utilisation.

piRNAs and tumour resistance to cell death. Resisting cell death enables tumour cells to evade clearance by immune surveillance or tissue homeostasis, often by suppressing programmed cell death pathways such as apoptosis. piRNAs have been implicated in regulating apoptosis, pyroptosis and related survival pathways, thereby contributing to tumour progression and therapeutic resistance.

In hepatocellular carcinoma (HCC), piR-216911 acts as an oncogenic piRNA that promotes invasion and survival by suppressing pyroptosis. piR-216911 binds the 3'UTR of *TLR4* mRNA, silencing *TLR4* and inhibiting the TLR4/nuclear

factor κ B and nucleotide-binding domain and leucine-rich repeat-containing family, pyrin domain containing 3 pathway, thereby blocking gasdermin D-mediated pyroptosis (137). Similarly, piR-823 is highly expressed in MM and promotes proliferation, suppresses apoptosis and enhances chemoresistance through the TGF- β 1/AKT/ERK pathway. *In vitro* studies in U266 and RPMI-8226 cells have shown that piR-823 overexpression increases proliferation and reduces apoptosis, whereas knockdown inhibits proliferation and induces apoptosis by decreasing BCL-2 and increasing cleaved caspase 3 (138). In CRPC, piR-4447944 is highly and specifically expressed, forms a complex with PIWIL2 and suppresses *NEFH* expression, thereby promoting proliferation, migration and invasion under castration conditions, while inhibiting apoptosis and driving CRPC progression (125).

Collectively, these findings suggest that piRNAs regulate tumour-cell apoptosis and pyroptosis pathways and may influence the therapeutic efficacy of malignancies.

4. Clinical value and applications of piRNAs in tumours

Accumulating evidence demonstrates that piRNAs play important roles in cancer initiation and progression, supported by PIWI-binding, methylation, and *in vitro* and *in vivo* validation studies (64,66,121,139). As components of ncRNA regulatory networks, piRNAs are emerging as key molecules in cancer mechanism research and precision medicine. Increasing evidence suggests that they may serve as both diagnostic biomarkers and therapeutic targets in cancer (140-142).

piRNAs as novel biomarkers in cancer. Given their high specificity, stability and close association with tumour progression, piRNAs are considered promising biomarkers for liquid biopsy and pathological diagnosis. Aberrant piRNA expression has been observed in multiple cancers, including CRC, clear cell renal cell carcinoma (ccRCC), HCC and breast cancer (Fig. 4).

Liquid biopsy detects tumour-derived materials in body fluids, including ctDNA, exosomes and CTCs, and has become a powerful non-invasive approach for cancer biomarker discovery (143). Studies have shown that piRNAs in body fluids can achieve promising sensitivity and specificity (122,144). Their abnormal expression in patients with cancer supports their potential as liquid-biopsy biomarkers for tumour diagnosis (22,23,34,35,145-157) (Table III). In CRC, Qu *et al* (145) identified five serum piRNAs-piR-001311, piR-004153, piR-017723, piR-017724 and piR-020365-that differed between patients and healthy controls. Serum piR-017724 was further identified as an independent prognostic factor for overall survival (OS) and progression-free survival (PFS), suggesting that serum piRNA profiles may aid CRC detection and prognosis. Sabbah *et al* (146) similarly showed that serum piR-823 has diagnostic value for CRC and correlates positively with tumour stage, particularly in advanced stages (III and IV). Additionally, serum piR-54265 is CRC-specific and may support non-invasive screening, early detection and clinical monitoring (22). Wang *et al* (147) identified serum piR-020619 and piR-020450 as potential biomarkers for early, specific detection of CRC; a two-piRNA panel showed high diagnostic accuracy and detected small lesions, early-stage CRC and colorectal adenomas. Vychy

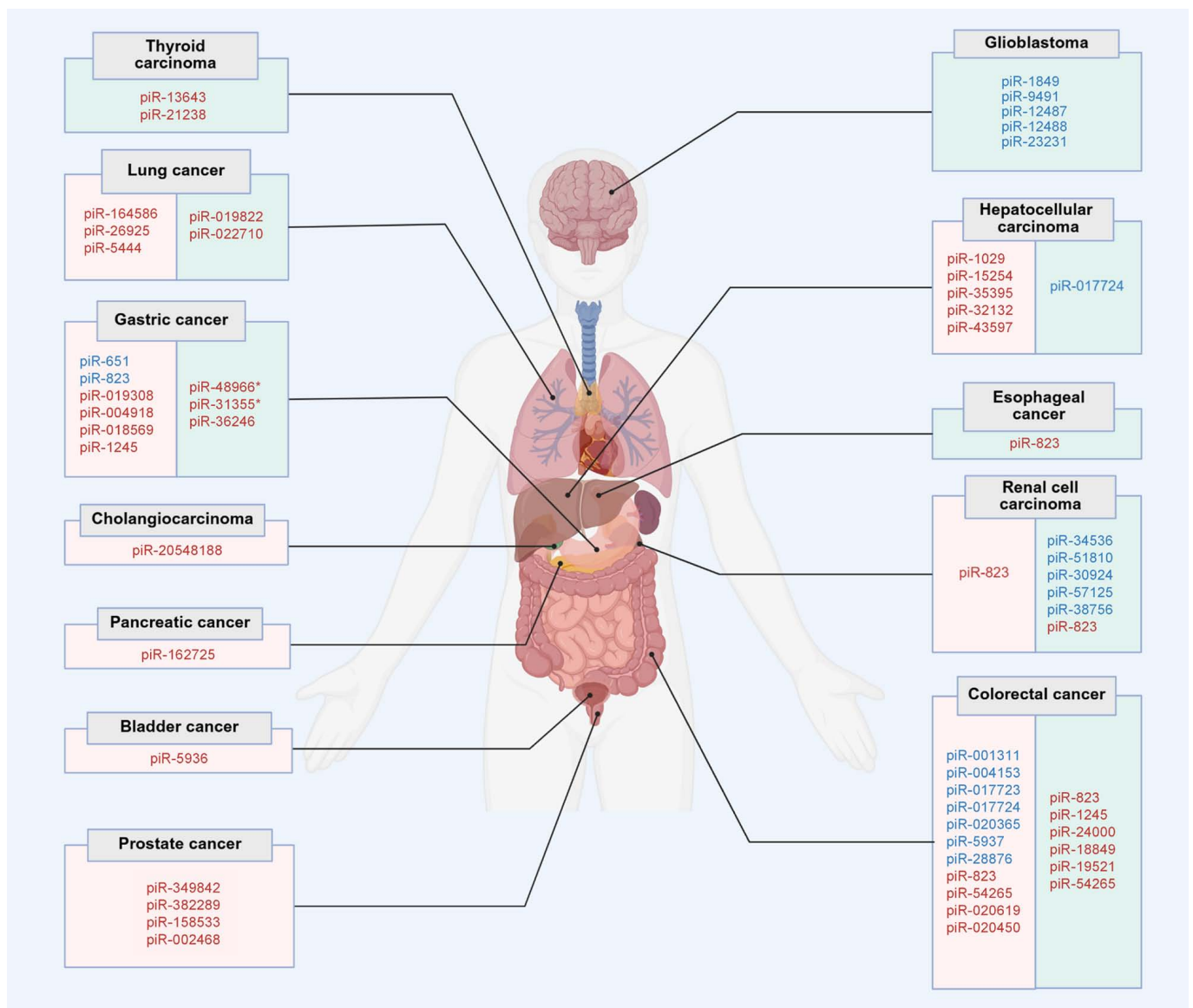


Figure 4. Expression of piRNAs in human cancers. Expression of piRNAs in thyroid carcinoma, lung cancer, gastric cancer, cholangiocarcinoma, pancreatic cancer, bladder cancer, prostate cancer, glioblastoma, hepatocellular carcinoma, oesophageal cancer, renal cell carcinoma and colorectal cancer. Light red box: piRNAs as liquid biopsy biomarkers; light green box: piRNAs as pathological diagnosis biomarkers. PiRNAs in red: Significantly upregulated expression; piRNAs in blue: Significantly downregulated expression. piRNA, PIWI-interacting RNA; PIWI, P-element induced wimpy testis.

tilova-Faltejskova *et al* (148) also found that piR-5937 and piR-28876 distinguish patients with colon cancer from healthy donors with high sensitivity and specificity and outperform conventional markers such as CEA and CA19-9 in stage I disease.

In prostate cancer, piR-349843, piR-382289, piR-158533 and piR-002468 are upregulated in urinary EVs, suggesting diagnostic potential (157). Meanwhile, in non-small cell lung cancer, piR-164586 exhibits stronger diagnostic performance than cytokeratin-19 fragment, may have prognostic value after surgery and correlates with clinical features, including age and TNM stage (149).

Additional studies have identified aberrant piRNA expression in LUAD, with serum exosomal piR-26925 and piR-5444 potentially serving as diagnostic biomarkers (150). In gastric cancer (GC), Ge *et al* (155) proposed serum exosomal piR-019308, piR-004918 and piR-018569 as robust diagnostic

biomarkers, with piR-019308 and piR-004918 also showing potential for metastasis monitoring. Zhou *et al* (156) further reported that elevated piR-1245 in gastric juice is associated with tumour size, TNM stage, poorer OS and poorer PFS; Cox regression confirmed piR-1245 as an independent prognostic risk factor for OS, supporting its value in GC detection and prognosis.

In cholangiocarcinoma and gallbladder cancer, plasma exosomal piR-10506469 is significantly upregulated, whereas piR-10506469 and piR-20548188 decrease after surgical resection, suggesting diagnostic relevance (151). Rui *et al* (35) identified five serum exosomal piRNAs—piR-1029, piR-15254, novel-piR-35395, novel-piR-32132 and novel-piR-43597—as effective biomarkers for HCC diagnosis, including in low-tumour-burden cases. In pancreatic ductal adenocarcinoma, piR-162725 improves the diagnostic sensitivity of CA19-9 when used in combination (34). In bladder cancer,

Table III. Correlation between piRNA expression in cell-free body fluids and tissue samples and clinicopathological features of cancers.

A, Correlation between cell-free piRNA expression in body fluids and clinicopathological features of cancers

Tumor type	piRNAs	Sample type	Expression	Clinicopathological association	Area under the curve	(Refs.)
Colorectal cancer	piR-001311	Serum	Down	-	0.726	(145)
	piR-004153				0.729	
	piR-017723				0.756	
	piR-020365				0.786	
	piR-017724				0.749	
				Overall survival and progression-free survival		
	piR-823	Serum	Up	Tumor differentiation and TNM stage	0.933	(146)
	piR-54265				0.896	
	piR-020619	Serum	Up	-	0.871	(147)
	piR-020450				0.841	
Pancreatic cancer	piR-54265	Serum	Up	TNM stage, progression-free survival and overall survival	-	(23)
	piR-5937				-	
	piR-28876	Serum	Down	TNM stage	0.8060	(148)
	piR-162725				0.8065	
		Serum	Up	-	-	(34)
	piR-164586				-	
		Serum extracellular vesicles	Up	TNM stage	0.624	(149)
	piR-26925				0.624	
		Serum extracellular vesicles	Up	-	0.751	(150)
	piR-5444				0.751	
Cholangio-carcinoma	piR-20548188	Plasma extracellular vesicles	Up	TNM stage	0.713	(151)
					-	
		Serum exosomes	Up	-	0.861	(35)
	piR-1029				0.861	
		Serum exosomes	Up	-	0.885	(152)
	piR-15254				0.885	
	piR-35395	Plasma extracellular vesicles	Up	-	0.967	(153)
	piR-32132				0.967	
	piR-43597	Plasma extracellular vesicles	Up	-	0.885	(154)
	piR-5936				0.942	
Bladder cancer		Serum	Up	-	-	(152)
	piR-823				-	
		Urine	Up	-	0.626	(153)
	piR-823				0.626	
		Peripheral blood	Down	-	0.7433	(154)
	piR-651				0.7433	
		Serum exosomes	Up	T stage and distant metastasis	0.841	(155)
	piR-823				0.841	
	piR-019308	Serum exosomes	Up	Metastasis	0.822	(156)
	piR-004918				0.820	
Renal cell carcinoma		Gastric juice	Up	-	0.754	(156)
	piR-018569				0.754	
		Gastric juice	Up	-	0.732	(156)
	piR-1245				0.732	
		Gastric juice	Up	Tumor size, TNM stage, overall survival and progression-free survival	0.885	(156)
	piR-1245				0.885	
		Gastric juice	Up	-	0.885	(156)
	piR-1245				0.885	
		Gastric juice	Up	-	0.885	(156)
	piR-1245				0.885	
		Gastric juice	Up	-	0.885	(156)

Table III. Continued.

A, Correlation between cell-free piRNA expression in body fluids and clinicopathological features of cancers

Tumor type	piRNAs	Sample type	Expression	Clinicopathological association	Area under the curve	(Refs.)
Prostate cancer	piR-349843	Urinary extracellular vesicles	Up	-	0.757	(157)
	piR-382289				0.777	
	piR-158533				0.723	
	piR-002468				0.783	

B, Correlation between piRNA expression in tissue samples and clinicopathological features of cancers

Tumor type	piRNAs	Sample type	Expression	Clinicopathological association	AUC	(Refs.)
Colorectal cancer	piR-823	Tissue samples	Up	Overall survival	-	(158)
	piR-1245	Tissue samples	Up	T stage, lymph node and distant metastasis	-	(122)
	piR-24000	Tissue samples	Up	Tumor differentiation, distant metastasis and TNM stage	0.8175	(159)
	piR-18849	Tissue samples	Up	Tumor differentiation and lymph node metastasis	-	(160)
	piR-19521	Tissue samples	Up	Tumor differentiation	-	(23)
	piR-54265			TNM stage, progression-free survival time and overall survival time		
Gastric cancer	piR-48966 ^a	Tissue samples	Up	-	0.91	(161)
	piR-31355 ^a				0.95	
	piR-36246				0.86	
Esophageal cancer	piR-823	Tissue samples	Up	Lymph node metastasis	0.713	(162)
Hepatocellular carcinoma	piR-017724	Tissue samples	Down	Tumor stage, vascular invasion and overall survival	-	(163)
Thyroid carcinoma	piR-13643	Tissue samples	Up	TNM stage	0.813	(164)
	piR-21238				0.820	
Glioblastoma	piR-1849	Tissue samples	Down	-	0.8422	(165)
	piR-9491				0.978	
	piR-12487				0.9283	
	piR-12488				0.9339	
	piR-23231	Tissue samples	Down	Overall survival	-	(165)
Renal cell carcinoma	piR-34536	Tissue samples	Down	Progression-free survival, cancer-specific survival and overall survival	0.815	(166)
	piR-51810				0.829	
	piR-30924	Tissue samples	Down	Tumor stage, tumor grade, recurrence and overall survival	0.61	(167)
	piR-57125				0.87	
	piR-38756				0.71	
	piR-823	Tissue samples	Up	Disease-free survival	0.795	(153)

Table III. Continued.

B, Correlation between piRNA expression in tissue samples and clinicopathological features of cancers

Tumor type	piRNAs	Sample type	Expression	Clinicopathological association	Area under the curve	(Refs.)
Lung cancer	piR-019822 piR-022710	Tissue samples	Up	Disease-free survival and overall survival Disease-free survival	-	(168)

piRNA^a (named as piRNA clusters): piRNAs mainly originate from transposon-enriched genomic regions known as piRNA clusters, typically located in heterochromatic regions near centromeres or telomeres. piRNA, PIWI-interacting RNA; PIWI, P-element induced wimpy testis.

piR-5936 expression increases from controls to high-risk patients and muscle-invasive bladder cancer, indicating association with disease severity (152). Urinary piR-823 has also demonstrated considerable diagnostic potential in RCC (153).

piRNAs also have value as pathological diagnostic markers across multiple cancers (23,122,153,158-168) (Table III). In CRC, piR-1245 overexpression in tumour tissues is associated with adverse clinicopathological features, including poor differentiation and metastasis, and serves as a robust prognostic marker for survival (122). Similarly, elevated piR-24000 expression is associated with aggressive CRC features, including poor differentiation, distant metastasis and advanced stage; receiver operating characteristic curve analysis revealed strong performance in distinguishing patients with CRC from healthy individuals (159). Feng *et al* (158) identified tissue piR-823 expression as an independent prognostic factor for OS in CRC. Meanwhile, high expression of piR-18849 and piR-19521 is associated with poor CRC differentiation, with piR-18849 also linked to lymph node metastasis (160). piR-54265 is also significantly upregulated in CRC tissues relative to non-tumour tissues; higher levels in tumour or serum are significantly associated with poorer survival (23).

Beyond CRC, tissue-derived piRNAs exhibit prognostic potential in several malignancies. Zuo *et al* (169) reported that piR-000627, piR-005553 and piR-019346 are associated with biochemical recurrence risk in prostate cancer, suggesting potential utility. Similarly, Vinasco-Sandoval *et al* (161) identified piR-48966 clusters (*), piR-49145 and piR-31335* as potential GC biomarkers. Meanwhile, piR-823 exhibits high specificity for oesophageal squamous cell carcinoma detection, with elevated levels associated with an increased risk of lymph node metastasis (162). Wu *et al* (163) reported that piR-017724 is significantly downregulated in HCC and correlates with malignant progression and poor prognosis, supporting its potential as both a biomarker and therapeutic target. In papillary thyroid carcinoma, piR-13643 and piR-21238 are significantly upregulated, correlate with clinical stage and outperform Hector Battifora mesothelial-1 in distinguishing malignant from benign thyroid nodules (164). Furthermore, piR-23231 expression is significantly associated with OS in patients with glioblastoma treated with the Stupp regimen (165).

In ccRCC, piR-34536 and piR-51810 represent prognostic biomarkers, with reduced tissue expression independently predicting shorter PFS, cancer-specific survival and OS (166). Similarly, piR-30924 and piR-57125 are independent prognostic predictors of ccRCC (167). In lung squamous cell carcinoma, upregulation of piR-019822 and piR-022710 is associated with increased recurrence risk and poorer disease-free survival and OS (168).

Overall, these candidate piRNAs may serve as biomarkers in various cancers, but larger independent cohorts are needed before clinical translation.

piRNAs as therapeutic targets in cancer. piRNAs exhibit broad therapeutic potential in cancer. Current strategies primarily include direct delivery of piRNA inhibitors or mimics and nanoparticle-based delivery of piRNA-targeting agents (Fig. 5).

Several studies have evaluated direct piRNA-targeting approaches. In CRC, Mai *et al* (23) identified piR-54265 as a potential therapeutic target and demonstrated that synthetic antagopiR-54265, delivered intratumorally or intravenously, significantly suppressed tumour growth and metastasis in mouse xenograft models. Combining antagopiR-54265 with 5-fluorouracil was more effective than either monotherapy, although potential toxicity requires further investigation. In lung cancer, Xu *et al* (91) reported that inhibiting PM2.5-induced lung cancer-upregulated piRNA (PMLCPIR) promoted apoptosis and enhanced cisplatin sensitivity; intratumoral injection of PMLCPIR antagomir also suppressed lung tumour growth in mice. In LUAD, agopiR-211106 inhibited xenograft progression and enhances cisplatin sensitivity (170), whereas antagopiR-137463 suppressed tumour growth and metastasis in nude mice through the lncRNA LOC100128494 axis and improved anti-PD-1 responses in immunocompetent mouse models (134). Han *et al* (171) proposed selective inhibition of the piR-30473/Wilms tumour 1-associated protein/hexokinase 2 axis as a therapeutic strategy for diffuse large B-cell lymphoma, with antagomir-30473 significantly reducing xenograft growth. In MM, Ma *et al* (172) showed that chemically modified antagomir-4800 inhibits xenograft growth, whereas Yan *et al* (26) reported significant anti-MM activity following treatment with chemically modified piR-823 antagomir (antagomir-823).

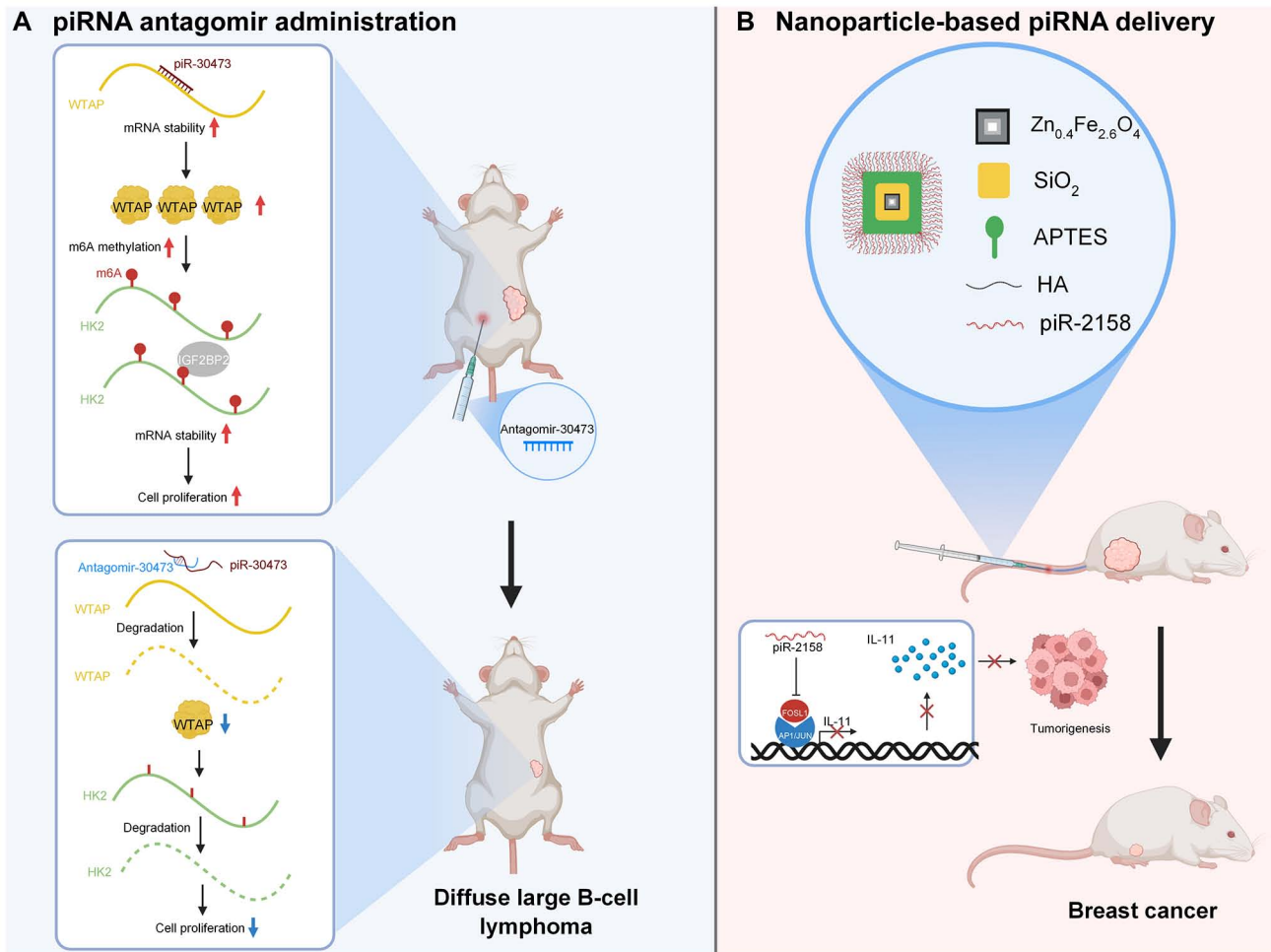


Figure 5. PiRNA-targeted intervention and delivery strategies. (A) PiRNA antagomir administration. In a DLBCL mouse model, administration of piR-30473 antagomir suppresses the piR-30473/WTAP/HK2 signalling axis, attenuating tumour growth. (B) Nanoparticle-based piRNA delivery. HA-coated magnetic nanoparticles loaded with piR-2158 were used to treat breast cancer-bearing mice. This strategy effectively inhibits tumorigenesis by suppressing IL-11 expression. piRNA, PIWI-interacting RNA; PIWI, P-element induced wimpy testis; DLBCL, diffuse large B-cell lymphoma; WTAP, Wilms tumour 1-associated protein; HK2, hexokinase 2; HA, hyaluronic acid; IL-11, interleukin-11; IGF2BP2, insulin-like growth factor 2 mRNA-binding protein 2; APTES, 3-aminopropyltriethoxysilane; FOSL1, transcription factors FOS like 1; AP1, activator protein-1.

Other candidate targets include piR-57125 in metastatic ccRCC (173), piR-001773 and piR-017184 in prostate cancer (174) and sEV-derived piR-30937 in PNENs, where knockdown of piR-30937, combined with anti-CD276 treatment, inhibited tumour growth and metastasis by enhancing T-cell-mediated immunity (132). In TNBC, Wu *et al* (175) found that a chemically modified piR-YBX1 mimics suppressed tumour proliferation and metastasis in xenograft models, further supporting the therapeutic potential of piRNA modulation.

Nanotechnology-based systems offer a promising approach for delivering nucleic acid therapeutics to cancer cells (176). Nanoparticle carriers have expanded opportunities for piRNA-based therapy. Ding *et al* (121) found that magnetic nanoparticles carrying anti-piR-823 significantly inhibit tumour growth after tail-vein injection in MCF-7 xenograft-bearing mice. Zhao *et al* (129) reported that piR-2158-loaded nanoparticles suppress breast tumour growth and angiogenesis *in vivo*. In RCC, Wang *et al* (177) developed cancer cell membrane-coated biomimetic nanoparticles to deliver piR-28489 (piR-RCC) plasmids into an orthotopic

mouse model, achieving marked tumour suppression. For HCC, Wei *et al* (178) engineered HCC cell membrane-coated poly lactic-co-glycolic acid (PLGA) nanoparticles co-encapsulating sorafenib and a piR-016975 inhibitor (in-016975/Sora@PLGA-CM). These nanocomposites suppress HCC progression and overcome sorafenib resistance in subcutaneous and orthotopic models by inducing ferroptosis through the piR-016975/Maspin/GPX4 axis (178).

Aptamer-based delivery platforms also demonstrate potential for anticancer therapeutics. Jia *et al* (179) designed aptamer-functionalized DNA tetrahedral nanoprobes for piR-36026 imaging and regulation in breast cancer. These self-assembled probes use intrinsic aptamers for target-cell recognition and exhibit high permeability, low cytotoxicity and resistance to enzymatic degradation, enabling high-fidelity imaging in MCF-7 cells. Their embedded regulatory sequence also modulates intracellular piR-36026 through complementary hybridisation, inducing apoptosis. Together, aptamer-based platforms may support more precise recognition and functional control of piRNAs in cancer therapy.

Collectively, these observations support the therapeutic potential of piRNA-targeted interventions across multiple cancers. Importantly, delivery strategies are evolving from direct administration towards more precise systems based on nanocarriers and aptamers.

5. Conclusions and perspectives

Advances propelled by the Human Genome Project, together with rapid progress in high-throughput technologies, have characterised numerous piRNAs with previously undefined functions. Evidence suggests that piRNAs contribute to tumour initiation and progression by regulating key molecules and downstream signalling pathways through epigenetic, transcriptional and post-transcriptional mechanisms. These features make piRNAs promising candidates for cancer biomarkers and therapeutic targets. Furthermore, compared with conventional molecular biomarkers, piRNAs may offer greater stability, specificity and sensitivity. Their abundance and stability in bodily fluids further support their use in pathological diagnosis, liquid biopsy, treatment monitoring and prognosis assessment. Clarifying their clinical roles and mechanisms in cancer may help advance early diagnosis and targeted therapy.

Recent technological advances have strengthened the potential of piRNAs in cancer diagnosis and targeted therapy.

- i) Development of piRNA-related detection kits. Detection kits based on piRNAs could support tumour pathology and liquid biopsy. Compared with conventional protein biomarkers, certain piRNAs demonstrate high specificity and sensitivity in distinct tumour histotypes, positioning them as strong diagnostic candidates (142,145,148). Current detection methods include reverse transcription-quantitative PCR, high-throughput sequencing, northern blotting and FISH. Paired with platforms such as digital PCR and microfluidic chips, these methods can assess selected piRNAs in tumour tissues or body fluids.
- ii) Development of piRNA-related anticancer therapeutics. Tumour-associated piRNAs are involved in malignant proliferation, invasion, metastasis and metabolic reprogramming, and modulation of selected piRNAs can suppress tumour growth and metastasis in experimental models. Advances in molecular pharmacology, biomaterials and nanotechnology may improve delivery of piRNA agomirs or antagomirs. Ultimately, piRNA-targeted therapies could be combined with chemotherapy, radiotherapy or immunotherapy to improve precision and efficacy.
- iii) Combined application of high-throughput sequencing technology. Since its introduction in 2009, single-cell transcriptome sequencing has become a powerful tool for characterising transcript heterogeneity across cells, cell types and tissues (180). In piRNA research, Yang *et al* (181) applied single-cell Cas9-assisted single-cell small RNA sequencing to identify an oocyte-specific piRNA family with unique features, creating useful resources for studying small RNAs in primate oocytes. Further mining of single-cell datasets may reveal clinically relevant tumour-associated piRNAs.
- iv) Artificial intelligence (AI) can assist in piRNA detection and analysis. AI can establish accurate prognostic models by integrating differential piRNA expression

profiles, clinicopathological features and patient transcriptomic or proteomic data to provide personalized treatment recommendations. Using machine learning algorithms, AI can also identify piRNA molecules associated with tumor biological characteristics from large-scale piRNA sequencing data, further improving diagnostic accuracy and providing strong technical support for precision and individualized medicine.

Despite growing interest in piRNAs as cancer biomarkers and therapeutic targets, important barriers remain.

- i) piRNA nomenclature has not yet been fully standardized. Given that identification often uses high-throughput sequencing approaches similar to those used for other small RNAs, including miRNAs, criteria vary across laboratories. Inconsistent naming and classification can assign multiple names to the same piRNA, making literature review, data comparison and clinical translation difficult. Hence, unified databases, naming rules and identification standards are essential.
- ii) Lack of well-established piRNA research systems. piRNAs are essential in the reproductive system, particularly for maintaining germ cell integrity. Disrupting the expression of certain piRNAs or PIWI-related genes can cause abnormal development, fertilisation failure, embryonic defects or lethality in mice. These constraints make piRNA-related knockout models difficult to generate and limit functional studies in cancer.
- iii) Scarce elucidation of the biological functions of piRNAs. Although numerous tumour-associated piRNAs have been reported, systematic evidence for their mechanisms and roles in tumour development remains limited. Currently, piRNAs are classified into three categories (Table SI): a) PIWI-bound and functionally validated piRNAs, for example, piR-54265 (23,182), piR-31115 (130,183,184) and piR-137463 (134); b) partially validated piRNA-like RNAs, for example, piR-651 (120,185), piR-823 (100,186,187), piR-36026 (179,188), piR-MW557525 (124,189); and c) database-annotated small-RNA candidates lacking sufficient experimental validation, for example, piR-7806 and piR-9871 (113). Furthermore, although numerous studies suggest that piRNAs regulate targets at epigenetic, transcriptional and post-transcriptional levels, standardised methods for defining the functions of individual piRNAs or piRNA clusters are lacking. Somatic small RNA annotations should also be interpreted cautiously. Differential expression analyses and computational target predictions indicate only putative associations. Stronger evidence for a causal PIWI-piRNA mechanism requires evidence of PIWI binding, maturation signatures, direct cleavage or methylation of targets, genetic epistasis, rescue experiments, *in vivo* validation and independent replication. Given that PIWI proteins may function independently of piRNAs (86), and because tRNA-derived and other abundant somatic small RNAs may contribute (190), altered PIWI expression alone does not prove that a candidate small RNA and a PIWI protein operate in the same functional pathway.
- iv) Time required to translate basic piRNA research into clinical trials. piRNA specificity and sensitivity may vary by tumour type, and patient-level variability may limit near-term clinical use. For liquid biopsy and other non-invasive applications, detection sensitivity and stability

must be optimised for reliable early diagnosis and prognosis assessment. Evidence for piRNAs as therapeutic targets is also limited; although inhibitors and related interventions have been tested in tumour models, most findings remain preclinical and lack human validation. Broad clinical and mechanistic evidence is required before piRNAs can be widely integrated into clinical applications.

The current understanding of human piRNAs captures only a fraction of their potential biological and clinical importance. Numerous piRNAs with unknown functions require further exploration and progress will depend on improved technologies, experimental models, annotation systems and analytic frameworks. As high-throughput sequencing, AI, biomaterials and related technologies continue to advance, piRNAs are likely to become increasingly important in human disease research, particularly in cancer diagnosis, prognosis and targeted therapy.

Acknowledgements

Not applicable.

Funding

This study was supported by the National Natural Science Foundation of China (grant no. 82373062), the Hunan Provincial Natural Science Foundation of China (grant nos. 2023JJ30929 and 2025JJ80499) and the Central South University Innovation-Driven Research Programme (grant nos. 2023CXQD075 and 2023CXQD077).

Availability of data and materials

Not applicable.

Authors' contributions

XQY, CLL, TTZ, SQS, XYH and ZYO wrote the main manuscript. XQY, CLL and TTZ prepared the figures and tables. CLO and>NNL designed, organized and reviewed this article. Data authentication is not applicable. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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