World Journal of Biomedical Sciences
ISSN: 2960-0189
DOI: https://doi.org/10.61784/wjbs3016

CURRENT RESEARCH STATUS OF PIWI-PIRNA IN MALIGNANT TUMORS

Mi Meng[1], Bo Yu[2*]
[1]*Department of Thoracic Oncology, The second Affiliated Hospital of Zunyi Medical University, Zunyi, China.*
[2]*Department of Urology, The second Affiliated Hospital of Zunyi Medical University, Zunyi, China.*
Correspondence Author: Bo Yu, Email: docyubo2407@163.com

Abstarct: PIWI-interacting RNA (piRNA) is a type of small non-coding RNA linked to the PIWI branch of the Argonaute family of proteins. PiRNA was initially identified in animal germline cells and is essential for germline development. Recent findings suggest that beyond reproductive functions, aberrant expression of PIWI/piRNA is strongly associated with several human cancers. The PIWI-piRNA complex in the reproductive system preserves genomic integrity by suppressing transposable elements. Recent research has also demonstrated that the PIWI-piRNA complex regulates protein-coding genes, thereby impacting the development and progression of human tumors. With further insights into piRNA biology, it has become clear that in addition to directly cleaving and degrading target RNA akin to the Ago/miRNA family, PIWI proteins/piRNA can regulate human cancers through epigenetic factors, such as DNA methylation and RNA methylation. In this article, we provide an overview of the molecular mechanisms underlying piRNA biogenesis and the latest functional insights into piRNA's role in human cancers, along with recent research advancements.
Keyword: ncRNA (Non-coding RNA); Piwi-interacting RNA (piRNA); Malignant neoplasms; Biological marker

1 INTRODUCTION

In the entire human genome, only about 2% of genes are translated into proteins to exert functions, whereas more than three-quarters of the genome can be transcribed into RNA. Alterations in transcriptional products are typically triggered by functional changes in somatic cells within the cancer genome[1]. Molecules that undergo transcription but are not subsequently translated into proteins are termed non-coding RNA (ncRNA), among which piRNA is one type. Non-coding RNA can be categorized based on nucleotide length into long non-coding RNA (>200 nt) and small non-coding RNA (≤200 nt) [2]. Small non-coding RNA (sncRNA) molecules are key regulators of gene expression at both co-transcriptional and post-transcriptional stages. Non-coding RNA also comprises various subclasses, including micro RNA, PIWI-interacting RNA, small interfering RNA, small nuclear RNA, small nucleolar RNA, small cytoplasmic RNA, transfer RNA, and ribosomal RNA [3]. Research to date indicates that ncRNA holds significant importance in cellular functions [4]. So far, millions of piRNAs have been identified across different species, including over 20,000 in the human genome, making it one of the most numerous but least explored classes of small non-coding RNA [5]. PIWI proteins are members of the PAZ-PIWI domain (PPD) family of RNA-binding proteins, and in humans, there are four types: PIWIL1 (HIWI), PIWIL2 (HILI), PIWIL3 (HIWI3), and PIWIL4 (HIWI2) [6]. The piRNA and PIWI proteins create the piRNA-induced silencing complex (piRISC), which plays a role in regulating target sequences by transcriptional or post-transcriptional degradation, or by controlling the expression of other genes through transposon activity modulation [7].

2 THE STRUCTURE AND FUNCTION OF PIRNA

2.1 Formation of piRNA

In typical organisms, piRNA is derived from longer single-stranded precursors processed via the ping-pong cycle. Initially, PIWI protein and primary piRNA catalyze the cleavage of the precursor, producing a primary precursor piRNA, which further generates a responder precursor piRNA. This responder precursor is then methylated to produce the responder piRNA, which goes on to form new primary piRNA. In the entire ping-pong cycle pathway, the sequence of this new piRNA aligns with the sequence of the initial primary piRNA, completing a new cycle [8-11].

2.2 Pathophysiological Role of Human PIWI-piRNA Complex

Due to the significant role of PIWI-piRNA complexes in gametogenesis and fertility in animals, recent studies have found extensive gene mutations in piRNA pathway genes among patients with human infertility [12]. In addition, dysregulation of PIWI proteins has been frequently observed in other pathological conditions [13]. In animal research, mutations in PIWIL1 in mice have been shown to impair the piRNA-independent functions of MIWI. Considering the crucial role of piRNA in animal fertility, substantial efforts have been made in recent years to screen for mutations in PIWI pathway genes linked to human infertility [14]. A recent report indicated that four unrelated infertile males of Middle Eastern ancestry were found to carry mutations in the PNLDC1 gene [15]. Another study discovered a

homozygous mutation in PNLDC1 that significantly impairs piRNA trimming activity and is causally linked to male infertility in humans [16]. This evidence directly supports the idea that piRNA biogenesis defects contribute to male infertility in humans. Homozygous loss-of-function mutations in TDRD7 have been observed in infertile patients, and TDRD7-knockout mice display similar disease phenotypes, suggesting these mutations are key pathological drivers of infertility [17]. Furthermore, recent genotyping analyses have shown that specific single nucleotide polymorphisms (SNPs) in piRNA pathway genes TDRD1 and SPGF are associated with a significant increase in infertility among patients [18-20]. Despite the identification of extensive gene variations in piRNA pathway genes among infertile men, only a few have been experimentally validated as causing infertility in animal models, underscoring the crucial role of PIWI-piRNA in male infertility. In oocytes, the PIWI-piRNA complex is crucial for the development of golden hamsters [21], and the dysfunction of PIWI-piRNA, involving PIWIL3 and piRNAs, in human oocytes may also contribute to female infertility. These findings indicate that PIWI-piRNA represents a promising direction for future genetic research.

3 PIWI-PIRNA COMPLEX IN TUMORS

The expression of PIWI genes is abnormally induced across various cancers, with PIWI functions related to genomic instability, aneuploidy, cell cycle progression, cell metabolism, stemness, DNA methylation, and gene regulation in cancer cells [5]. Additionally, while numerous studies have associated PIWI proteins with cancer progression, whether they act together with piRNA in cancer cells is still under debate. Numerous small RNAs have been reported across various tumor tissues and cell lines; however, it is still uncertain whether they are genuine piRNAs, as most lack confirmed association with specific PIWI proteins. Li discovered that PIWIL1 acts as an oncogene in pancreatic cancer patients, promoting metastasis even in the absence of piRNA[22]. Shi found that PIWIL1 exhibits piRNA-independent functions that drive the progression of gastric cancer[23]. Another study indicated that PIWIL1 is not associated with piRNA in colorectal cancer cell lines [24], implying an independent role of PIWIL in human cancer cells. Conversely, in skin cancers, piRNA and PIWIL2 interact to form an active complex, collectively involved in cancer development [25]. Further studies are needed to determine whether the role of PIWI proteins in specific cancers depends on piRNAs. Although the role of PIWI proteins and piRNAs in human diseases is compelling, most evidence to date is correlative, such as from genetic association studies. Future genetic research in model organisms may clarify the wider impact of PIWI-piRNA functions beyond the mammalian germline.

3.1 Regulatory Mechanisms of PIWI/piRNA in Human Cancer

3.1.1 PIWI/piRNA influences DNA methylation

DNA methylation (methylation) is an epigenetic modification. DNA methylation can cause abnormalities in restriction enzyme cleavage sites and DNA enzyme-sensitive sites, further leading to chromatin hyper-spiralization and even condensation, losing transcriptional activity. If such gene substitution mutations occur during cell division, with base mismatches not corrected, it may trigger related genetic diseases or cancer. lncRNA can recruit DNMT to increase DNA methylation levels, while recent studies suggest that piRNA can influence hypermethylation in certain human cancers by recruiting DNMT [26]. The interplay between DNA methylation and piRNA significantly influences genome stability and expression, eventually causing abnormal shifts in cellular signaling, which drive disease progression [27]. Many studies have shown that DNA methylation alterations in tumor cells are closely linked to PIWI/piRNA-related diseases. One study demonstrated that overexpression of piR-651 can enhance breast cancer cell proliferation and invasion. This regulatory mechanism involves PIWIL2 recruiting DNMT1 to the promoter region of the tumor suppressor gene PTEN, causing methylation of the PTEN promoter and consequently reducing its expression [27]. Litwin discovered that PIWIL1 is expressed in breast cancer[28], whereas PIWIL2 is not expressed. Additionally, Fu demonstrated that piR-021285 facilitates methylation of the proto-oncogene ARHGAP11A 5'UTR/first exon and suppresses its expression level[29], though the precise regulatory mechanism remains to be studied further. Ding discovered that piRNA-823 overexpression enhances DNMTs expression[30], including DNMT1, DNMT3A, and DNMT3B, triggering breast cancer cell stemness via the Wnt signaling pathway, thus inducing high methylation of the APC gene. Beyond breast cancer, piR-823 is also upregulated in multiple myeloma (MM) and esophageal squamous cell carcinoma (ESCC). In MM, piR-823 overexpression can sustain cellular stemness and enhance tumorigenic potential by activating DNMT3B and elevating DNA methylation levels [31]. Yan showed that piRNA-823 upregulates DNMT3A and DNMT3B at both mRNA and protein levels[32], causing methylation of the tumor suppressor gene p16 (INK4A), thus promoting oncogenic effects in MM. Su demonstrated that piR-823 and DNMT3B are both overexpressed in ESCC and are positively correlated[33]. piR-823 facilitates ESCC progression via DNMT3b-mediated DNA methylation, with PIWI/piRNA-mediated DNA methylation also crucial in other tumors. In prostate cancer, overexpression of piRNA-31470 prompts DNMT1 and DNMT3α to bind to the CpG island of glutathione S-transferase P1 (GSTP1) through PIWIL4. Subsequently, GSTP1 undergoes methylation, which suppresses its transcription. GSTP1 inactivation heightens the susceptibility of normal cells to oxidative stress, raising the risk of prostate cancer [34]. Wu's study demonstrated that the overexpression of LV-has-piR-011186 binds to specific sequences, recruiting DNA and histone methylation proteins to the CDKN2 promoter gene, thus facilitating cell proliferation and inhibiting apoptosis in U937 leukemia cells [35]. In lung cancer, RASSF1C, a principal member of the RASSF1 gene family, enhances PIWIL1 expression and regulates piRNA expression. This research proposes the RASSF1C/PIWIL1/piRNA axis, which

modulates the expression of Gem-interacting protein (GMIP) mRNA in lung cancer via DNA methylation, thus influencing the migration of cancer cells [36].

3.1.2 PIWI/piRNA impacts m6A RNA methylation

Recently, the epigenetic RNA modification m6A has garnered considerable interest. This RNA modification occurs via m6A "writers," "erasers," and "readers," which are proteins responsible for methylating, demethylating, and identifying m6A in RNA, respectively. The primary demethylases for m6A are currently FTO and ALKBH5, highlighting that m6A methylation, similar to DNA methylation and histone modification, is a reversible modification [37]. Specific RNA-binding proteins must recognize m6A before carrying out distinct biological functions. Methylation-recognizing proteins such as heterogeneous nuclear ribonucleoproteins (HNRNP), eukaryotic translation initiation factor 3 (eIF3), and the IGF2 mRNA-binding protein (IGF2BP) family impact mRNA fate in an m6A-dependent manner, affecting RNA transcription, processing, translation, and metabolism. Additional studies suggest that m6A RNA methylation may also affect tumorigenesis [38]. Consequently, researchers are investigating whether piRNA and m6A have a relationship that could collectively impact cancer progression. For instance, the study by Xie demonstrated that piRNA-14633 promotes cervical cancer (CC) progression via the piRNA-14533/METTL14/CYP1B1 axis[39]. In this study, piRNA-14633 was highly expressed in CC cells, raising m6A RNA methylation levels and METTL14 mRNA stability. Additionally, METTL14, regulated by piRNA-14633, can directly target CYP1B1 in CC cells, thereby enhancing CC cell growth, migration, and invasion. Han showed that piRNA-30473 acts in diffuse large B-cell lymphoma (DLBCL) by regulating WTAP, serving as a prognostic marker in DLBCL patients[40]. piRNA-30473 is overexpressed in DLBCL and modulates m6A methylation levels through WTAP and IGF2BP2 expression. This upregulates HK2 expression in cells by increasing HK2 mRNA stability, targeting its 5'UTR. Both studies suggest that piRNA plays a role in m6A modification by controlling m6A methyltransferase expression. Additionally, studies have found that piRNA can modulate global m6A levels by interacting with specific m6A methyltransferases and impacting their activity. For instance, Liu discovered that piRNA-36741 influences osteoblast differentiation through the regulation of BMP2's METTL3-dependent m6A methylation[13]. The CHAPIR (cardiac hypertrophy-associated piRNA)-PIWIL4 complex facilitates pathological hypertrophy and cardiac remodeling by repressing m6A methylation of the PArp10 mRNA transcript, thus regulating NFATC nuclear accumulation[41]. Mechanistically, these two studies concluded that the piRNA-PIWIL4 complex directly interacts with METTL3, modulating METTL3-mediated m6A modification of targeted gene transcripts. While these studies have shown that the piRNA-PIWI protein complex can influence m6A modification by regulating m6A methyltransferase expression or affecting its m6A activity, several critical questions remain unanswered. For instance, piRNAs, as a form of ncRNA, can bind to the 3'UTR or CDS regions of mRNA and recruit DNA methyltransferases via PIWI proteins to control DNA methylation of target genes, akin to miRNA. Whether specific piRNAs regulate only the m6A levels of target genes they associate with, rather than global m6A levels in the cell, warrants further exploration. Furthermore, investigating this issue in depth may reveal the molecular processes through which m6A is specifically and dynamically deposited within the transcriptome. Currently, with only a few studies available, the regulatory mechanisms of piRNA and m6A in human cancers exhibit immense potential and warrant further research to uncover deeper underlying mechanisms.

3.1.3 PIWI/piRNA impacts histone modifications

The nucleosome, as the basic unit of chromosome function, is composed of a histone octamer. Some histones in eukaryotes stabilize the nucleosome, with free amino acid residues at their termini that can undergo covalent modifications, such as acetylation, phosphorylation, ubiquitination, ADP-ribosylation, and glycosylation, with methylation and acetylation being the most significant. Histone acetylation involves a fully reciprocal dynamic equilibrium regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs conduct acetyl group electrons from acetyl-CoA to particular lysine residues at the histone N-terminus. The positive charge of the acetyl group and residues expands the DNA structure and relaxes the nucleosome, promoting DNA-transcription factor integration and initiating transcription of specific genomic regions. Histone methylation usually occurs at lysine and arginine residues, leading to diverse impacts of methylation metabolites on histone functions. These modifying enzymes are classified into two parts: active markers and repressive markers. Active markers, such as H3K4me3, H3 and H4 acetylation, and H3K36me2 and H3K36me3, help form open chromatin transcription channels within the nucleosome's 3D structure. Repressive markers, like the methylation of histone H3 lysine 9 and 27 (H3K9me3 and H3K27me3), induce chromatin condensation. Various piRNAs are capable of recruiting specific histone-modifying enzymes, resulting in diverse effects. A study shows that pi-sno75 upregulates pro-apoptotic protein TRAIL expression, exerting inhibitory effects in breast cancer. Mechanistically, pi-sno75 associates with PIWIL1/4 and induces H3K4 methylation/H3K27 demethylation of TRAIL through recruitment of the MLL3/hCOMPASS complex[42]. Wu showed that CDKN2B-related piRNAs, like hsa-piR-011186 and hsa-piR-014637[35], are overexpressed in leukemia cells U937, where their upregulation inhibits CDKN2B expression, thereby advancing the cell cycle and inducing apoptosis. At the mechanistic level, hsa-piR-011186 and hsa-piR-014637 interact with DNMT1, Suv39H1, and EZH2 proteins to form a complex that modulates DNA and histone H3 methylation levels at the CDKN2B promoter site. This particular piRNA complex expedites the epigenetic modification of the cell cycle, offering new insights into the progression of leukemia. Another study found a strong association between reduced PIWIL2 expression and the downregulation of DNMT1[27], histone H1, HP1, and SUV39H1 in invasive breast cancers (IBCs), which correlates with chromatin accessibility and genome methylation. PIWI/piRNA dysfunction, resulting in DNA demethylation and reactivation of transposable elements, is an epigenetic mechanism underlying changes in genome integrity and immune responses in various tumor cells. Furthermore, in IBC, low PIWIL2 expression correlates closely with an increase in cytotoxic

immune CD8+ cells, supporting the potential of PIWI/piRNA as predictive biomarkers for immunotherapy.

3.1.4 Additional carcinogenic mechanisms of PIWI/piRNA

In germ cells, PIWI proteins, through their slicer enzyme activity, can cleave transposon RNA complementary to piRNA, leading to post-transcriptional silencing of transposon genes and closely linking to the piRNA biogenesis process. However, in cancer, the mechanism differs. Based on current research, PIWI proteins and piRNAs independently regulate mRNA decay. For instance, Liu et al. discovered that PIWIL1 acts as a co-activator of the APC/C complex[22], targeting cell adhesion protein Pinin for proteolytic ubiquitination in the absence of piRNA, thereby promoting pancreatic cancer metastasis. A related study by Lin et al. found that in gastric cancer cells with high PIWIL1 expression[23], piRNA levels were low but still facilitated cancer cell proliferation and transformation. Mechanistically, PIWIL1 might negatively regulate tumor suppressor gene expression via binding proteins, which are key mediators of the mRNA decay mechanism. PIWI proteins can directly associate with specific functional proteins (e.g., ubiquitinases and phosphorylases) to perform epigenetic regulatory roles in tumors independently of piRNA. Naturally, further research is needed to determine whether this phenomenon occurs in normal cells as well. Additionally, various piRNAs can act similarly to miRNAs, regulating post-transcriptional networks in cancer by interacting with RNA to inhibit target functions. In lung cancer cells, piRNAs can control cancer cell growth, apoptosis, metastasis, and invasion [43]. In colon cancer cells, piRNAs control formation, pyroptosis, and cell cycle progression via HSF1, BTG1, and FAS [44]. In head and neck squamous cell carcinoma associated with HPV16/18, piRNA FR140858 deletion may enhance the expression of the minichromosome maintenance complex [45]. In breast cancer, PIWIL1 associates with piR-36712 to form the RISC, targeting the degradation of the SEPW1 pseudogene SEPW1P. This leads to SEPW1 mRNA competing with SEPW1P RNA for microRNA-7 and microRNA-324, downregulating SEPW1 expression and suppressing proliferation, migration, and invasion [46]. In neuroblastoma cells, piRNA-39980 directly targets the JAK3 gene, thereby promoting cell proliferation, enhancing metastasis, and inhibiting cellular senescence [47]. In bladder cancer, piRNA DQ594040 expression is reduced, where it suppresses cell proliferation and induces apoptosis by targeting TNFSF4 [48]. These findings on PIWI's functions and mechanisms in tumors establish a robust foundation for its widespread application in the field of precise tumor diagnosis.

3.2 Tissue Distribution of piRNA

In addition to being aberrantly expressed in cancer cells, PIWI proteins and piRNAs are found in other somatic tissues. In the nervous system, despite the low abundance of piRNA in the sea hare's nervous system, it is characterized by a 1U preference and 3' end 2'-O-methylation [49]. Notably, neuronal PIWI proteins and piRNAs are crucial for neuron development, encompassing functions such as learning and memory. A recent study showed that pathogenic tau protein lowers PIWI and piRNA levels in fruit fly brains, accelerating neuronal death in neurodegenerative tauopathy through TE suppression [50]. Indeed, mutations in piRNA pathway genes are highly correlated with neurodegenerative disorders in the human nervous system. For instance, a whole-exome sequencing study of 2,500 families with a child diagnosed with autism spectrum disorder (ASD) but without ASD in relatives suggested an association between PIWIL2 and PIWIL4 gene variants and autism spectrum disorder [51]. Moreover, transcriptomic analysis of the human brain reveals differential expression of a subset of piRNAs in Alzheimer's disease patients, with most of these piRNAs associated with Alzheimer's risk single nucleotide polymorphisms [52]. Recent studies have verified that PIWI-piRNA is present in both tissue cells and body fluids, including blood. In 2013, Huang et al. detected piRNA in plasma exosomes via RNA sequencing of RNA in plasma exosomes[53]. Yuan et al. found that a significant number of piRNAs are stably present in plasma extracellular vesicles by sequencing RNA from patient plasma extracellular vesicles[54]. Bahn and Hong et al. likewise found piRNA in human saliva and semen by conducting RNA sequencing on these fluids[55, 56]. El-Mogy et al. utilized next-generation sequencing to study RNA levels in various body fluids (whole blood, plasma, serum, saliva, cell-free saliva, urine, and cell-free plasma)[57], confirming the presence of piRNA in a range of human body fluids.

3.3 Future Perspectives for Targeted Therapy Involving piRNA/PIWI Proteins

Tumor treatment still faces challenges from inefficient and invasive diagnostic tools and adverse effects of therapy (such as low recovery rates, limited universality, and frequent follow-up), but targeted therapies and related diagnostic methods have emerged. Targeted therapy aims to interrupt specific pathways and functional proteins during tumorigenesis, especially mutated molecules with aberrant expression. Multiple piRNAs and PIWI proteins have been identified as either downregulated or upregulated in tumor germ cells and other cancer tissues, facilitating cancer cell formation or inhibiting cancer cell growth. Studies have shown a strong correlation between piRNA and PIWI protein expression with high malignancy in pathological grading and clinical metastasis. In tumors, piRNA/PIWI complexes can modulate oncogenic effects via epigenetic pathways. The epigenetic mechanisms of piRNA/PIWI proteins facilitate or inhibit cell proliferation, migration, and metastasis at the genetic level. In the future, modifying the expression or stability of molecules related to m6A RNA, histone, and DNA methylation in cancer-specific pathways might be achievable by upregulating or downregulating piRNA/PIWI complex expression. Naturally, if more epigenetic pathways regulated by the piRNA/PIWI complex are identified in a specific cancer, the likelihood of developing targeted drugs will substantially rise. The use of miRNA in tumor-targeted therapy faces three challenges: specificity, deliverability, and tolerance, where piRNA demonstrates significant improvements. First, regarding cell specificity, many piRNAs are expressed in both germ and cancer cells, suggesting high cell specificity. Moreover, numerous

piRNAs complementarily match target gene bases and modulate target gene expression at the genetic or post-transcriptional level, demonstrating strong gene specificity. Nonetheless, piRNA and miRNA face similar challenges, like off-target effects and immunogenicity triggered by sequence similarity or excessive induction, often exceeding anticipated endogenous levels. Second, regarding deliverability, piRNA is more stable in the bloodstream than miRNA due to its 3' end 2'-O-methylation structure, giving piRNA better deliverability. Third, in terms of tolerance or side effects, piRNA and PIWI proteins have fewer side effects due to their cell-specific expression. Therefore, due to these advantages, PIWI/piRNA holds substantial potential for future applications.

4 CONCLUSIONS AND FUTURE DIRECTIONS

In recent years, the piRNA field has achieved substantial advancements, offering deeper insights into piRNA cluster transcription, biogenesis, and multiple aspects of piRNA function. Certain cellular processes have been identified as key to piRNA biology, one of which is splicing regulation, seemingly vital for the biogenesis of categorized piRNA precursor transcripts. Variations in nuclear export factors are also involved in piRNA biology by identifying specific unspliced piRNA precursor transcripts and facilitating transposable element (TE) silencing. An intriguing link has also been uncovered between translation and piRNA biogenesis, where the ribosome directs mRNA processing into piRNA. This ribosomal function is conserved and participates in the biogenesis of other small RNA classes. Despite these advances, several questions remain unresolved, such as whether piRNA genuinely participates in homologous TE suppression, how active TEs are transformed into piRNA loci, and which pathways collaborate with piRNA in TE silencing, all of which are critical issues to be addressed. Relevant studies indicate a connection between piRNA biological function and cellular biology. For example, some piRNA biogenesis takes place on the outer mitochondrial membrane, though the link to mitochondria remains unclear. Moreover, the intricate relationship between piRNA biology and ribonucleoprotein condensates is not well understood.

COMPETINGINTERESTS

The author(s) declare financial support was received for the research, authorship, and/or publication of this article.

FUNDING

This study was supported by the Guizhou Provincial Health Commission (2024GZWJKJXM0704).

REFERENCES

[1] PCAWG Transcriptome Core Group, Calabrese C, Davidson NR, et al. Genomic basis for RNA alterations in cancer. Nature, 2020, 578(7793): 129-136.
[2] Cech T R, Steitz J A. The noncoding RNA revolution-trashing old rules to forge new ones. Cell, 2014, 157(1): 77-94.
[3] Shi J, Zhou T, Chen Q. Exploring the expanding universe of small RNAs. Nat Cell Biol, 2022, 24(4): 415-423.
[4] Slack F J, Chinnaiyan A M. The Role of Non-coding RNAs in Oncology. Cell, 2019, 179(5): 1033-1055.
[5] Liu Y, Dou M, Song X, et al. The emerging role of the piRNA /piwi complex in cancer. Mol Cancer, 2019, 18(1): 123.
[6] He J, Chen M, Xu J, et al. Identification and characterization of Piwi-interacting RNAs in human placentas of preeclampsia. Sci Rep, 2021, 11(1): 15766.
[7] Iwasaki Y W, Siomi M C, Siomi Het, et al. PIWI-Interacting RNA: Its Biogenesis and Functions. Annu Rev Biochem, 2015, 84: 405-33.
[8] Saxe J P, Chen M, Zhao H, et al. Tdrkh is essential for spermatogenesis and participates in primary piRNA biogenesis in the germline. EMBO J, 2013, 32(13): 1869-1885.
[9] Anzelon T A, Chowdhury S, Hughes S M, et al. Structural basis for piRNA targeting. Nature, 2021, 597(7875): 285-289.
[10] Tang W, Tu S, Lee H C, et al. The RNase PARN-1 trims piRNA 3'ends to promote transcriptome surveillance in C. elegans. Cell, 2016, 164(5): 974-984.
[11] Izumi N, Shoji K, Sakaguchi Y, et al. Identification and functional analysis of the pre-piRNA 3' trimmer in silkworms. Cell, 2016, 164(5): 962-973.
[12] Mann J M, Wei C, Chen C, et al. How genetic defects in piRNA trimming contribute to male infertility. Andrology, 2023, 11(5): 911-917.
[13] Liu J, Chen M, Ma L, et al. piRNA-36741 regulates BMP2-mediated osteoblast differentiation via METTL3 controlled m6A modification. Aging (Albany NY), 2021, 13(19): 23361-23375.
[14] Wang X, Gou LT, Liu M F. Noncanonical functions of PIWIL1/piRNAs in animal male germ cells and human diseases†. Biol Reprod, 2022, 107(1): 101-108.
[15] Nagirnaja L, Mørup N, Nielsen J E, et al. Defective piRNA Processing, and Azoospermia. N Engl J Med, 2021, 385(8): 707-719.
[16] Gou L T, Kang J Y, Dai P, et al. Ubiquitination-Deficient Mutations in Human Piwi Cause Male Infertility by Impairing Histone-to-Protamine Exchange during Spermiogenesis. Cell, 2017, 169(6): 1090-1104.e13.

[17] Tan Y Q, Tu C, Meng L, et al. Loss-of-function mutations in TDRD7 lead to a rare novel syndrome combining congenital cataract and nonobstructive azoospermia in humans. Genet Med, 2019, 21(5): 1209-1217.
[18] Guan Y, Keeney S, Jain D, et al. Yama, a mutant allele of Mov10l1, disrupts retrotransposon silencing and piRNA biogenesis. PLoS Genet, 2021, 17(2): e1009265.
[19] Qureshi S, Hardy J J, Pombar C, et al. Genomic study of TEX15 variants:prevalence and allelic heterogeneity in men with spermatogenic failure. Front Genet, 2023, 14: 1134849.
[20] Babakhanzadeh E, Khodadadian A, Rostami S, et al. Testicular expression of TDRD1, TDRD5, TDRD9 and TDRD12 in azoospermia. BMC Med Genet, 2020, 21(1): 33.
[21] Roovers E F, Rosenkranz D, Mahdipour M, et al. Piwi proteins and piRNAs in mammalian oocytes and early embryos. Cell Rep, 2015, 10(12): 2069-2082.
[22] Li F, Yuan P, Rao M, et al. piRNA-independent function of PIWIL1 as a co-activator for anaphase promoting complex/cyclosome to drive pancreatic cancer metastasis. Nat Cell Biol, 2020, 22(4): 425-438.
[23] Shi S, Yang Z Z, Liu S, et al. PIWIL1 promotes gastric cancer via a piRNA-independent mechanism, 2020, 117(36): 22390-22401.
[24] Liu J, Zhang S, Cheng B. Epigenetic roles of PIWI-interacting RNAs (piRNAs)in cancer metastasis (Review). Oncol Rep, 2018, 40(5): 2423-2434.
[25] Pammer J, Rossiter H, Bilban M, et al. PIWIL-2 and piRNAs are regularly expressed in epithelia of the skin and their expression is related to differentiation. Arch Dermatol Res, 2020, 312(10): 705-714.
[26] Mann J R, Mattiske D M. RNA interference in mammalian DNA methylation. Biochem Cell Biol, 2012, 90(1): 70-77.
[27] Liu T, Wang J, Sun L, et al. Piwi-interacting RNA-651 promotes cell proliferation and migration and inhibits apoptosis in breast cancer by facilitating DNMT1-mediated PTEN promoter methylation. Cell Cycle, 2021, 20(16): 1603-1616.
[28] Litwin M, Szczepańska-Buda A, Michałowska D, et al. Aberrant Expression of PIWIL1 and PIWIL2 and Their Clinical Significance in Ductal Breast Carcinoma. Anticancer Res, 2018, 38(4): 2021-2030.
[29] Fu A, Jacobs D I, Hoffman A E, et al. PIWI-interacting RNA 021285 is involved in breast tumorigenesis possibly by remodeling the cancer epigenome. Carcinogenesis, 2015, 36(10): 1094-1102.
[30] Ding X, Li Y, Lü J, et al. piRNA-823 Is Involved in Cancer Stem Cell Regulation Through Altering DNA Methylation in Association with Luminal Breast Cancer. Front Cell Dev Biol, 2021, 9: 641052.
[31] Li B, Hong J, Hong M, et al. piRNA-823 delivered bymultiple myeloma-derived extracellular vesicles promoted tumorigenesis throughre-educating endothelial cells in the tumor environment. Oncogene, 2019, 38(26): 5227-5238.
[32] Yan H, Wu Q L, Sun C Y, et al. piRNA-823 contributes to tumorigenesis by regulating de novoDNA methylation and angiogenesis in multiple myeloma. Leukemia, 2015, 29(1): 196-206.
[33] Su J F, Zhao F, Gao Z W, et al. piR-823 demonstrates tumor oncogenic activity in esophageal squamous cell carcinoma through DNA methylation induction via DNA methyltransferase 3B. Pathol Res Pract, 2020, 216(4): 152848.
[34] Nakayama M, Gonzalgo M L, Yegnasubramanian S, et al. GSTP1 CpG island hypermethylation as a molecular biomarker for prostate cancer. J Cell Biochem, 2004, 91(3): 540-552.
[35] Wu D, Fu H, Zhou H, et al. Effects of Novel ncRNA Molecules, p15-piRNAs, on the Methylation of DNA and Histone H3 of the CDKN2B Promoter Region in U937 Cells. J Cell Biochem, 2015, 116(12): 2744-2754.
[36] Amaar Y G, Reeves M E. The impact of the RASSF1C and PIWIL1 on DNA methylation: the identification of GMIP as a tumor suppressor. Oncotarget, 2020, 11(45): 4082-4092.
[37] Sun T, Wu R, Ming L. The role of m6A RNA methylation in cancer. Biomed Pharmacother, 2019, 112: 108613.
[38] Yadav P, Subbarayalu P, Medina D, et al. M6A RNA Methylation Regulates HistoneUbiquitination to Support Cancer Growth and Progression. Cancer Res, 2022, 82(10): 1872-1889.
[39] Xie Q, Li Z, Luo X, et al. piRNA-14633 promotes cervical cancer cell malignancy in a METTL14-dependent m6A RNA methylation manner. J Transl Med, 2022, 20(1): 51.
[40] Han H, Fan G, Song S, et al. piRNA-30473 contributes to tumorigenesis and poor prognosis by regulating m6A RNA methylation in DLBCL. Blood, 2021, 137(12): 1603-1614.
[41] Gao X Q, Zhang Y H, Liu F, et al. The piRNA CHAPIR regulates cardiac hypertrophy by controlling METTL3-dependent N6-methyladenosine methylation of Parp10 mRNA. Nat Cell Biol, 2020, 22(11): 1319-1331.
[42] He X, Chen X, Zhang X, et al. An Lnc RNA (GAS5)/SnoRNA-derived piRNA induces activation of TRAIL gene by site-specifically recruitingMLL/COMPASS-like complexes. Nucleic Acids Res, 2015, 43(7): 3712-3725.
[43] Mei Y, Wang Y, Kumari P, et al. A piRNA-like small RNA interacts with and modulates p-ERM proteins in human somatic cells. Nat Commun, 2015, 6: 7316.
[44] Yin Jie, Jiang Xiao-Yu, Qi Wei, et al. piR-823 contributes to colorectal tumorigenesis by enhancing the transcriptional activity of HSF1. Cancer science, 2017, 108(9):1746-1756.
[45] Krishnan, Aswini R, Qu, Yuanhao, et al. Computational methods reveal novel functionalities of PIWI-interacting RNAs in human papillomavirus-induced head and neck squamous cell carcinoma. Oncotarget, 2017, 9(4): 4614-4624.
[46] Qian L, Xie H, Zhang L, et al. Piwi-Interacting RNAs: A New Class of Regulator in Human Breast Cancer. Front Oncol, 2021, 11: 695077.

[47] Roy J, Das B, Jain N, et al. PIWI-interacting RNA 39980 promotes tumor progression and reduces drug sensitivity in neuroblastoma cells. J Cell Physiol, 2020, 235(3): 2286-2299.
[48] Chu H, Hui G, Yuan L, et al. Identification of novel piRNAs in bladder cancer. Cancer Lett. 2015, 356(2 Pt B): 561-567.
[49] Rajan K S, Ramasamy S. Retrotransposons and piRNA: the missing link in central nervous system. Neurochem Int, 2014, 77: 94-102.
[50] Tindell S J, Rouchka E C, Arkov A L. Glial granules contain germline proteins in the Drosophila brain, which regulate brain transcriptome. Commun Biol, 2020, 3(1): 699.
[51] Salloum-Asfar S, Elsayed A K, Elhag S F, et al. Circulating Non-Coding RNAs as a Signature of Autism Spectrum Disorder Symptomatology. Int J Mol Sci, 2021, 22(12): 6549.
[52] Zhang T, Wong G. Dysregulation of Human Somatic piRNA Expression in Parkinson's Disease Subtypes and Stages. Int J Mol Sci, 2022, 23(5): 2469.
[53] Huang X, Yuan T, Tschannen M, et al. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC Genomics, 2013, 14: 319.
[54] Yuan T, Huang X, Woodcock M, et al. Plasma extracellular RNA profiles in healthy and cancer patients. Sci Rep, 2016, 6: 19413.
[55] Bahn J H, Zhang Q, Li F, et al. The landscape of microRNA, Piwi-interacting RNA, and circular RNA in human saliva. Clin Chem, 2015, 61(1): 221-230.
[56] Hong Y, Wang C, Fu Z, et al. Systematic characterization of seminal plasma piRNAs as molecular biomarkers for male infertility. Sci Rep, 2016, 6: 24229.
[57] El-Mogy M, Lam B, Haj-Ahmad T A, et al. Diversity and signature of small RNA in different bodily fluids using next generation sequencing. BMC Genomics, 2018, 19(1): 408.