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NON-THEMATIC REVIEW

piRNAs: biogenesis and their potential roles in cancer

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Abstract
With the length of about 26–31 nt, PIWI-interacting RNA (piRNA) is a small non-coding RNA (ncRNA) that interacts with PIWI proteins to form the piRNA silencing complex (piRISC). PIWI is a subfamily of Argonaute, and piRNA must bind to PIWI to exert its regulatory role. Current studies indicated that piRNA and PIWI are significantly abnormally expressed in gastric, breast, kidney, colon, and lung cancers, and are involved in the initiation, progression, and metastasis of cancers, which may be the potential diagnostic tools, prognostic markers, and therapeutic targets for cancers. By reviewing piRNA recent studies, this research summarized the mechanism of piRNA generation and the functions of piRNA/PIWI in gastric, breast, kidney, colon, and lung cancers, providing a reference value for further piRNA research.

Keywords piRNAs · PIWI · Cancer metastasis · Biogenesis · piRISC

1 Background

Non-coding RNA (ncRNA) is a group of small RNA molecules with specific functions that can be transcribed but do not encode proteins [1]. piRNA is a kind of ncRNA. With a length of about 26–31 nt, piRNA interacts with members of the PIWI family of proteins specifically expressed in germ cells to form the piRNA silencing complex (piRISC) [2]. The PIWI protein is a subfamily of Argonaute proteins [3]. PIWI protein is the center of piRNA's action pathway, which is required for the biosynthesis and function of piRNA.

piRNA binds specifically to the PIWI protein and performs basic functions in organisms. The PIWI-piRNA pathway is generally considered to be the immune system of the reproductive system, while transposon elements (TEs) are considered to be a threat to the development of germ cells. In many mammalian gene sequences, there is usually 50% or more of TEs. Once these TEs are freed from restrictions, they can be independently copied or decomposed from the original position, and then inserted into another site to interfere with the normal genome sequence, leading to gene mutation [4]. TEs can cause genomic damage and gene mutations, which are closely related to physiological and pathological processes such as infertility and cancer [5, 6]. In recent years, some studies have shown that piRNAs' abnormal expression is associated with the progression of cancer, which may become the diagnostic tools, therapeutic targets, and prognosis biomarkers for cancer [7–9].

However, little is known about piRNA/PIWI. In this study, the formation and characteristics of piRNAs and the research progress on the relationship between piRNA/PIWI and cancer in recent years are reviewed, which will provide references for further exploration of piRNA's related mechanisms.

2 The transcription of piRNAs

The processing of mature piRNAs includes the generation of piRNA precursor (pre-piRNA), the modification of 5′ end and 3′ end, and the methylation of nucleotides, resulting in mature piRNAs [10]. The transcription of piRNAs in animal gonads was

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mainly activated in the clusters of the piRNA genes on heterochromatin. The piRNA cluster of *Drosophila* includes promoter element, RNA polymerase II, and downstream components, whose promoter regions have H3K4me2 dimethylation [11]. This process is mainly accomplished by the newly discovered Moonshiner protein. In heterochromatin, heterochromatin protein Rhino and its related protein Deadlock recruit transcription factors such as Moonshiner protein and RNA polymerase II to form the assembly of the pre-initiation complex (PIC). With the participation of a series of transcription factors, PIC initiates the transcription of piRNA [12]. The RNA polymerase moves along the 3′ to 5′ direction of the heterochromatin DNA template strand, catalyzing the extension of the piRNA strand and eventually producing the pre-piRNA (Fig. 1).

3 The posttranslational modification processing of piRNAs

Drosophila pre-piRNAs transported out of the nucleus through nuclear pores and clustered at the "Dot COM" site of the perinuclear structure [13, 14]. Yb bodies are usually located around the mitochondria [15, 16], and pre-piRNAs bind with Yb bodies of cytoplasm in reproductive plasm (Nuage) [14, 17], which can enhance the assembly capacity of PIWI and piRNAs [15]. The PIWI protein further binds the precursor piRNA to form piRISC by recognizing the 5′-end binding of piRNA. Armitage delivers piRNA intermediates from reproductive material to mitochondrial surface [18, 19] (Fig. 2). Zucchini (Zuc) protein in mitochondria acts as an endonuclease to modify the 5′ end of primary piRNA [20] (Fig. 2). Zuc splits the precursor, releasing a piRISC into the cytoplasm. Papi protein recognizes Trimmer enzyme recruitment at the N end of PIWI protein through its eTudor domain, and thus forms a pruning complex to modify the 3′ end of piRNA [10, 21] (Fig. 2). Processed piRISC is transported into the nucleus through nuclear pores [15], inhibiting the transcription of transposon-related genes and thus inhibiting the production of transposons [22]. piRISC was cut from the binding by forebody, exposing the 5′ end of the forebody and binding to the new PIWI protein. Zuc released the second piRISC by splitting the precursor (Fig. 2). When Yb bodies

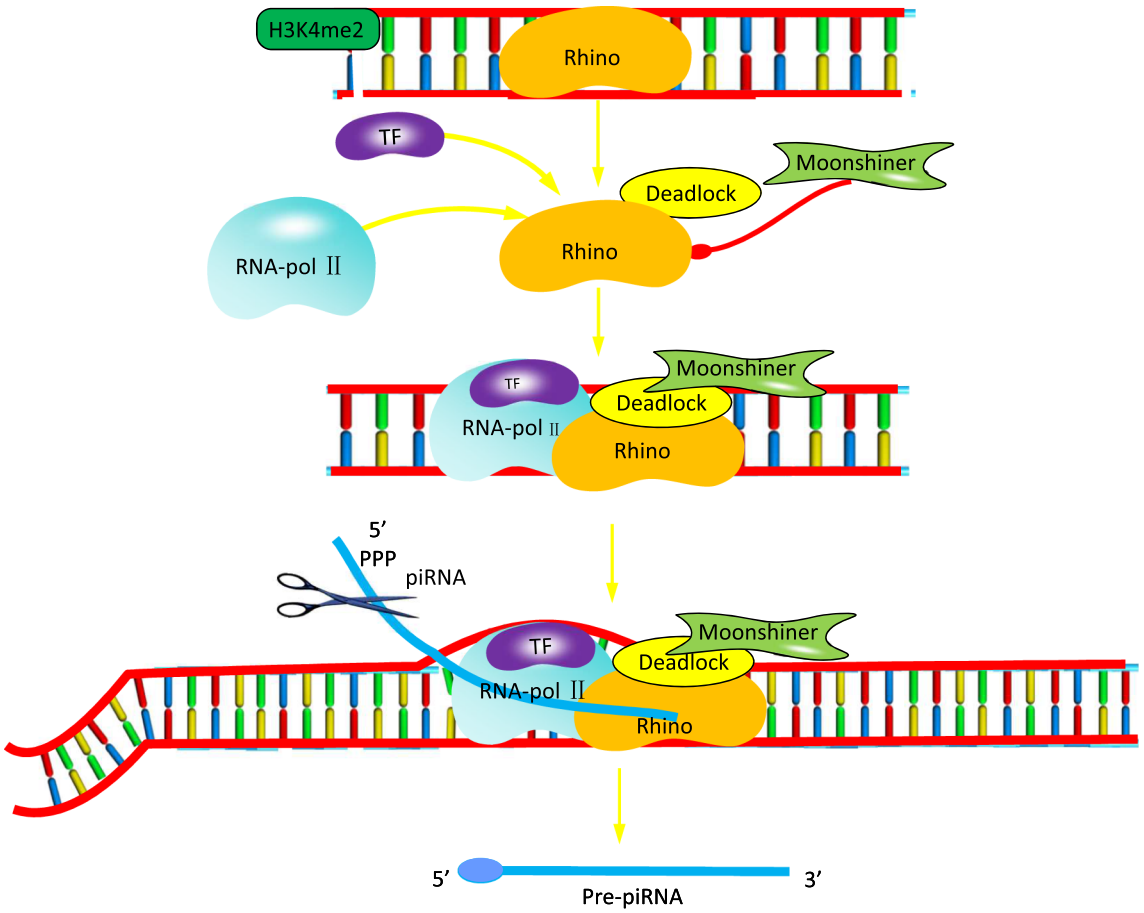

Fig. 1 The transcriptional process that *Drosophila* piRNA gene cluster is transcribed to produce the precursor piRNA. Rhino and Deadlock proteins on the piRNA gene cluster recruit Moonshiner protein, RNA polymerase II, and other transcription factors to form the pre-initiation complex PIC, which initiates piRNA transcription. RNA polymerases move in the 3′ to 5′ direction of the heterochromatin DNA template strand, catalyzing the extension of the piRNA strand, and eventually producing the pre-piRNA

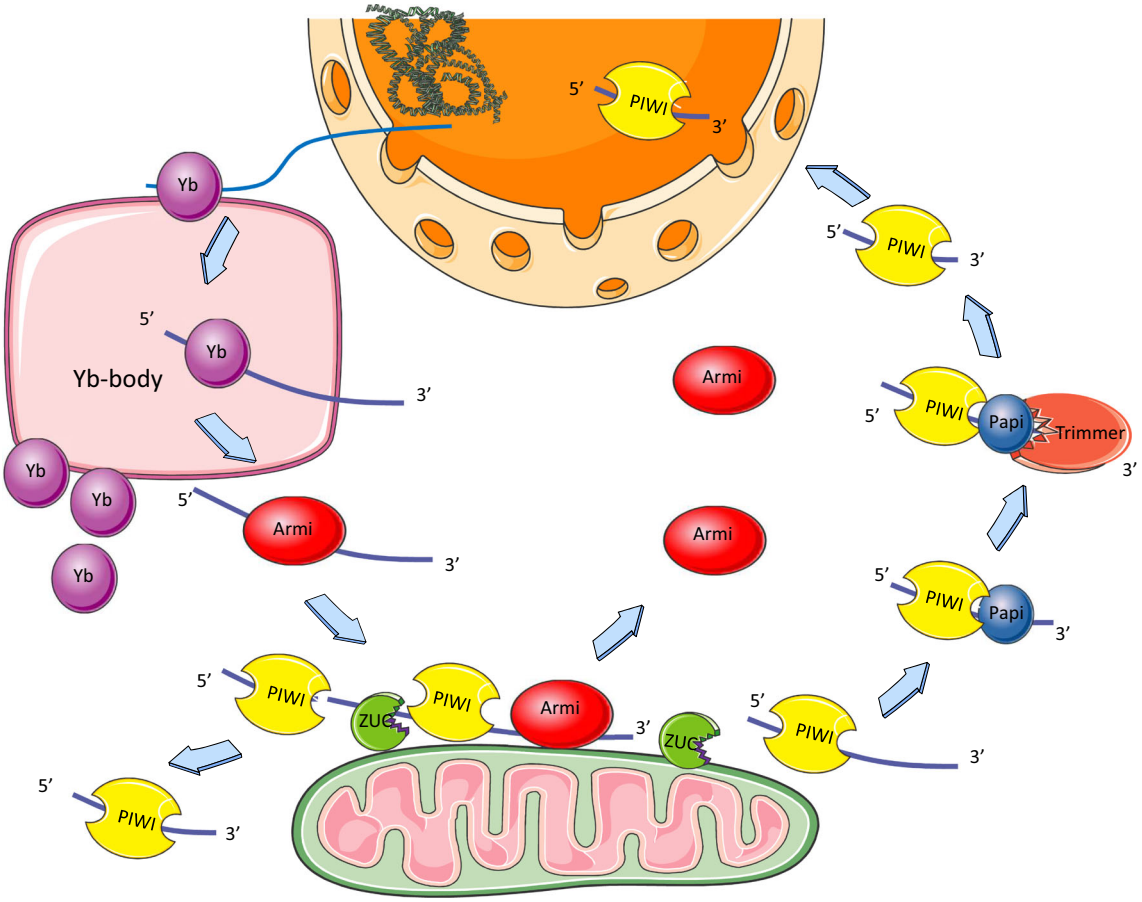

Fig. 2 The post-translational modification processing of piRNAs. Pre-piRNA transports the nucleus through nuclear pores and aggregates in perinuclear structures. Combining with Yb bodies, Armitage delivers piRNA intermediates from reproductive bodies to the mitochondrial surface. The PIWI protein binds to piRNAs. Zucchini (Zuc) protein in mitochondria acts as an endonuclease to modify the 5′ end of primary piRNA. Zuc splits the precursor, releasing a piRISC into the cytoplasm. Papi protein recruits Trimmer enzyme to modify the 3′ end of piRISC. Processed piRISC is transported into the nucleus through nuclear pores, inhibiting transposon-related gene transcription

are lacking, the precursor would choose to combine with Armitage to generate piRNA which is unrelated to silent transposons [23].

4 The link between piRNAs and cancer

Cancer is a major cause of human death. In recent years, through the researches on the role of piRNA and PIWI in cancer, scientists have found shreds of evidence supporting the close links between piRNA/PIWI and various tumors. The abnormal expression of piRNA is associated with a variety of cancers and may play a pro-cancer or anti-cancer role in cancer initiation, progression, and metastasis. piRNA and PIWI's potential clinical significance as diagnostic tools, therapeutic targets, and prognosis biomarkers in cancer was highlighted. Some studies have shown that high piRNA expressions are associated with various cancers, and inhibition of piRNA expression can inhibit the development of cancer [24]. This work reviewed the abnormal expression of piRNAs and PIWI molecules in different tumors to further investigate the close relationship between piRNA and tumors (Table 1).

4.1 The potential role of piRNA and PIWI in gastric cancer

Some studies found a close relationship between piRNAs and the malignancy of gastric cancer [25–27]. Through piRNA microarray analysis of gastric cancer tissues and adjacent normal tissues, the researchers found that the expression of piR-651 in gastric cancer tissues was higher than that in the control group [24, 28], transfected piR-651 inhibitor into gastric cancer cells, and gastric cancer cells were blocked by piR-651 inhibitor in G2/M phase [24]. This suggests that piR-651 may be involved in the progression of gastric cancer (Fig. 3(A)). The expression of piR-823 was significantly decreased in gastric cancer tissues, and the transfection of piR-823 simulants in gastric cancer cells showed a significant inhibitory effect on the growth of gastric cancer cells [28, 29]. This indicates that the decreased expression of piR-823 in

Table 1 The function of the abnormal expression of piRNA and PIWIL in different cancer cells

Cancer	Molecules	Expression	Function	Reference
Gastric cancer	piR-651	Up	Diagnostic tool or therapeutic target	Cheng et al. (2011), Cui et al. (2011)
	piR-823	Down	Diagnostic tool or therapeutic target	Cui et al. (2011), Cheng et al. (2012)
	PIWIL1	Up	Prognosis biomarker	Wang et al. (2012), Gao et al. (2018)
Breast cancer	piR-651	Up	Diagnostic tool	Cheng et al. (2012), Oner et al. (2016)
	piR-4987	UP	Prognosis biomarker	Krishnan et al.(2016)
	piR-36,712	Down	Prognosis biomarker or therapeutic target	Tan et al. (2019)
	piR-021285	Up	Prognosis biomarker	Fu et al. (2015)
	piR-932	Up	Therapeutic target	Zhang et al. (2013)
	PIWIL1	Up	Diagnostic tool	Litwin et al. (2018)
	PIWIL2	Up	Therapeutic target	Zhang et al. (2013)
Renal cancer	piR-32051	Up	Prognosis biomarker	Li et al. (2015)
	piR-39894	Up	Prognosis biomarker	Li et al. (2015)
	piR-43607	Up	Prognosis biomarker	Li et al. (2015)
	piR-30924	Up	Prognosis biomarker	Busch et al. (2015)
	piR-38756	Up	Prognosis biomarker	Busch et al. (2015)
	piR-57125	Down	Prognosis biomarker	Busch et al. (2015)
	piR-34536	Down	Prognosis biomarker	Zhao et al. (2019)
	piR-51810	Down	Prognosis biomarker	Zhao et al. (2019)
	PIWIL1	Down	Prognosis biomarker	Stohr et al. (2019)
	PIWIL2	Down	Prognosis biomarker	Stohr et al. (2019)
	PIWIL4	Down	Prognosis biomarker	Stohr et al. (2019)
	piR-823	Down	Diagnostic tool	Iliev et al. (2016)
Colorectal cancer	piR-54265	Up	Therapeutic target	Mai et al. (2018)
	piR-823	Up	Therapeutic target	Yin et al. (2017)
	piR-18849	Up	Prognosis biomarker	Yin et al. (2019)
	piR-19521	Up	Prognosis biomarker	Yin et al. (2019)
	piR-1245	Up	Prognosis biomarker	Mai et al. (2018)
	PIWIL 1	Up	Prognosis biomarker	Sun et al. (2017)
	piR-5937	Up	Diagnostic tool	Vychytilova-Faltejskova et al. (2018)
	piR-28876	Up	Diagnostic tool	Vychytilova-Faltejskova et al. (2018)
Lung cancer	piR-55490	Down	Prognosis biomarker	Peng et al. (2016)
	piR-651	Up	Diagnostic tool	Zhang et al. (2018), Li et al. (2016)
	PIWIL1	Up	Prognosis biomarker	Reeves et al. (2012)

gastric cancer tissues may be associated with the initiation and metastasis of cancer, which means that piR-823 may have an anti-cancer effect in the progression of gastric cancer (Fig. 3(A)). The abnormal expression of piR-651 and piR-823 in normal and cancerous tissues is closely related to the prognosis of gastric cancer, which may be used as the diagnostic tools or therapeutic targets for gastric cancer.

In addition, the expression of PIWIL1 was upregulated in gastric cancer cells, suggesting poor prognosis in patients with gastric cancer [30–32]. In AGP 01 gastric cells with PIWIL1 gene knocked out, a series of oncogenes were found to be decreased in expression, while cancer suppressor genes were increased [32]. This indicates that PIWIL1 may promote the progression of gastric cancer by regulating the expression of pro-cancer and anti-cancer genes [31, 32]. These findings suggested that the high expression of PIWIL1 could be used as an important prognosis marker to predict the prognosis of the patients with gastric cancer (Table 1). At present, there is still a lack of research in this field, and the specific mechanism of piR-651, piR-823, and PIWIL1's involvement in the invasion and metastasis of gastric cancer is still very

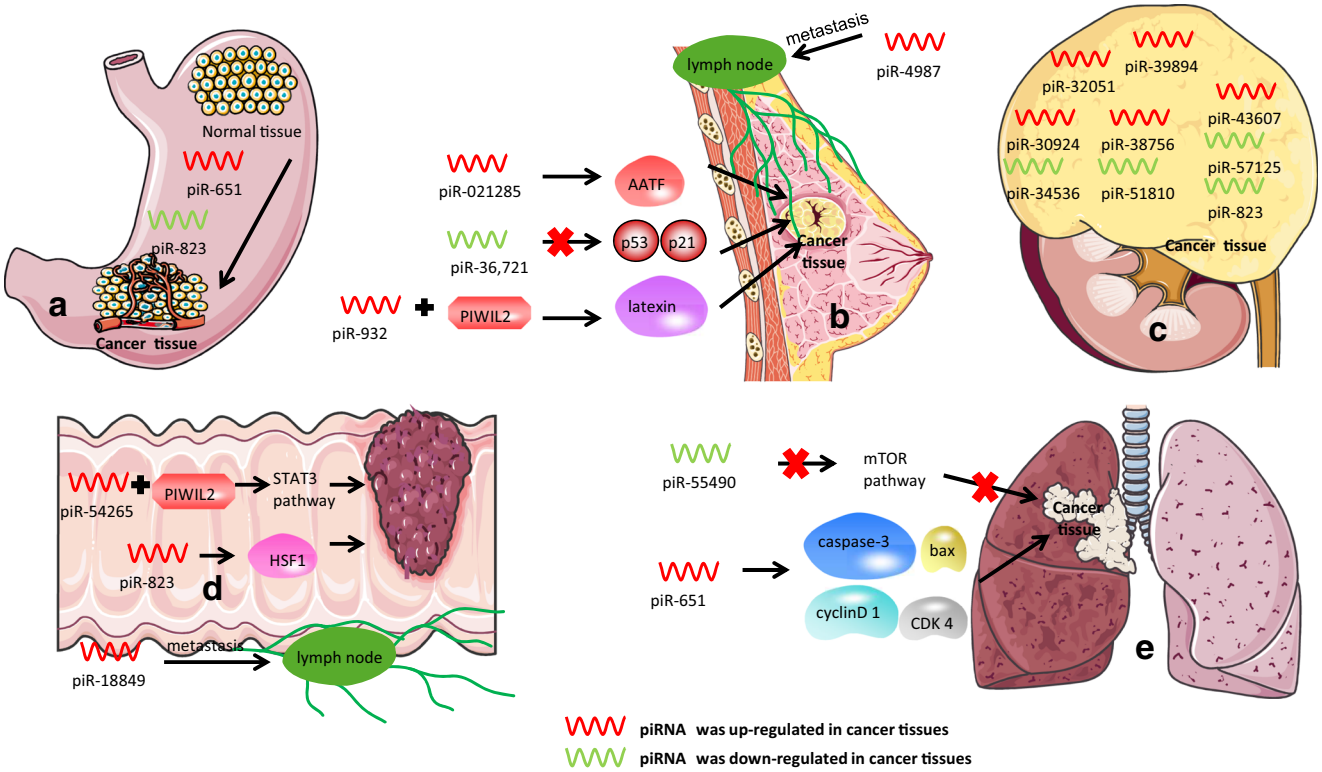

Fig. 3 Abnormal expression of piRNA in tumor tissues and related mechanisms to promote or inhibit cancer. (A) Abnormal expression of piR-651 and piR-823 in gastric cancer. (B) piR-021285 promotes the methylation of AATF and other related oncogenes, which leads to the metastasis of breast cancer. The decreased expression of piR-36721 resulted in decreased expression levels of the tumor suppressor genes p53 and P21, which promoted the progress of cancer cells. piR-932 binds to PIWIL2 to promote the methylation of Latexin, thus promoting the progression of breast cancer. piR-4987 promotes lymph node metastasis of breast cancer. (C) Abnormal expression of different piRNAs in renal cancer. (D) piR-54265 combined with PIWIL2 protein, activated the STAT3 signaling pathway, and promoted the proliferation and metastasis of colorectal cancer. pir-823 acts as a tumor promoter by upregulating the phosphorylation and transcriptional activity of HSF 1. piR-188489 promotes lymph node metastasis in colorectal cancer. (E) piR-55490 inhibits the development of lung cancer by inhibiting the activation of MTOR pathway in lung cancer cells. By changing the expression levels of apoptosis-related proteins caspase-3 and bax, pir-651 induces the expressions of cyclinD 1 and CDK 4, regulates cell proliferation, and thus promotes lung tumor metastasis

unclear. However, the abnormal expression of piRNA/PIWI between gastric cancer tissues and normal tissues shows promising prospects.

4.2 piRNA and PIWI promote or inhibit the metastasis of breast cancer

Breast cancer is a common malignancy in women. Some studies have pointed out that piRNAs play important roles in the initiation of breast cancer [33, 34] and can be used as prognostic markers [34, 35]. The high expression of piR-651 was confirmed in breast cancer cell lines [24, 36]. The high expression of piR-4987 in breast cancer is associated with lymph node metastasis [35] (Fig. 3(B)). The expression of piR-36712 in breast cancer patients is significantly lower than that in normal tissues, and the lower expression of piR-36712 tends to predict worse prognosis of patients with breast cancer. When the expression of piR-36712 was upregulated in cancer cells, it was found that the expression levels of the tumor suppressor gene p53 and P21 were increased, leading to the arrest of the cell cycle in the G0/G1 phase of cancer cells [37]. This means that piR-36712 can play an anti-cancer role in breast cancer, and also suggests that piR-36712 can be used as a prognostic marker or therapeutic target for breast cancer (Fig. 3(B)). The invasion of breast cancer cell lines transfected with piR-021285 was enhanced, and piR-021285 mediated the methylation of AATF, ARHGAP11A, PIP4K2B, THAP 10, and other related oncogenes in breast cancer tissues, which promotes the progression of breast cancer [38]. This means that piR-021285 can be used as a potential regulator of invasive breast cancer (Fig. 3(B)). In addition, piR-932 binds to PIWIL2 to promote the methylation of Latexin in breast cancer tumor cells, and finally promote the progression of breast cancer [39] (Fig. 3(B)). It showed that both of them may become potential targets to block the metastasis of breast cancer. Through molecular studies of frozen fragments of breast cancer tissues and adjacent non-malignant breast tissues, PIWIL1 was detected only in cancer and breast lesions, but not in most normal breast tissues [40]. PIWIL2 expression was higher than that of the control group [39], and PIWIL3

and PIWIL4 genes were associated with prognosis [34]. Researchers also found that PIWIl4 may be involved in the regulation of estrogen target gene expression and migration ability of breast cancer cells in estrogen-receptor-positive breast cancer patients, resulting in hormone therapy and drug resistance of targeted therapy in estrogen-receptor-positive breast cancer patients [41] (Table 1). Therefore, piRNA and PIWI seem to play an important role in the initiation and metastasis of breast cancer, but there is still a lack of comprehensive studies to fully understand the relevant mechanisms of breast cancer.

4.3 piRNA and PIWI predict the prognosis of renal cancer

In the aspect of renal cancer, some studies have shown that piRNA is closely related to the metastasis and prognosis of renal cancer. The upregulation of piR-32051, piR-39894, piR-43607, piR-30924, and piR-38756 in renal clear cell carcinoma (ccRCC) is closely related to the metastasis and low cancer-specific survival rate [42, 43]. In ccRCC, the expressions of piR-57125 were downregulated [43], as were the expressions of piR-34536 and piR-51810 isolated from the mitochondria of cancer cells [44]. This means that all of the above piRNAs may serve as prognostic markers for ccRCC (Fig. 3(C)). However, the role of these piRNAs in the mechanism of renal cancerization is not yet clear.

In renal cell carcinoma (RCC), the expression levels of PIWIL1, PIWIL2, and PIWIL4 gradually decrease with the increase in clinical stage, and the worse prognosis of patients [45]. piR-823 plays a complex role in the pathogenesis of RCC and is positively correlated with poor prognosis (Fig. 3(C)). In urine, piR-823 is upregulated, and preliminary data indicated that urine piR-823 has a perfect diagnostic value in RCC patients [46]. The researchers detected the expression of PIWI-like 1 protein in the two groups of renal cell carcinoma patients by immunohistochemistry, and found that the positive PIWI-like 1 protein revealed the poor prognosis of the patients [47]. It can be seen that kidney cancer is closely related to piRNA/PIWI (Table 1).

4.4 piRNA and PIWI can be the markers of colorectal cancer

The studies indicated that piRNAs have important clinical significance as diagnostic tools and therapeutic targets for colorectal cancer (CRC). The expression of piR-54265 in colorectal cancer cells was significantly higher than that in normal tissues. Through the experiments with mice xenografts, researchers found that piR-54265 combined with PIWIL2 protein to activate the STAT3 signaling pathway and promote the invasion and metastasis of colorectal cancer [48] (Fig. 3(D)). The expression of piR-823 is upregulated in colorectal cancer, and piR-823 plays a role in promoting tumor by upregulating the phosphorylation and transcriptional activity of heat shock transcription factor-1 (HSF-1) (Fig. 3(D)). What's more, inhibiting the expression of piR-823 could block cancer cells at G1 stage [49] (Table 1). These important findings suggest that piR-823 and piR-54265 may serve as therapeutic targets for colorectal cancer.

The upregulation of piR-18849 and piR-19521 is positively correlated with the malignancy of colon cancer tissues, and the increased expression of piR-18849 will promote lymph node metastasis of colon cancer [50] (Fig. 3(D)). piR-1245 is highly expressed in colorectal cancer cells. piR-1245 promotes the proliferation of tumor cells and significantly shortens the overall survival time of colorectal cancer patients by regulating the expression of tumor suppressor genes [51]. In addition, high expression of PIWIL1 indicates poor prognosis in CRC patients [52]. These suggest that piR-18849, piR-19521, piR-1245, and PIWIL1 can be the important molecular markers for the prognosis of CRC patients. Through the analysis of piRNA expression profile in serum samples of patients and healthy people, it was found that the sensitivity and specificity of piR-5937 and piR-28876 for detection of stage I CRC patients were higher than the current biomarkers of CEA and CA19-9 [53] (Table 1). This suggests that piR-5937 and piR-28876 may play an important role in the early diagnosis of rectal cancer and have important clinical significance.

The early diagnosis of colorectal cancer is of vital significance for the survival of colorectal cancer patients. Although the overall molecular mechanism of colorectal cancer has not been clarified, piRNA and PIWI are of great significance for the early diagnosis of colorectal cancer. It is hoped that piRNA and PIWI's relevant studies can be paid attention to and have the opportunity to be applied in clinical practice as soon as possible for the benefit of patients.

4.5 The mechanisms that involve piRNA and PIWI in lung cancer

The pathogenesis of lung cancer is still unknown. The scientists found a link between the abnormal expression of piRNAs in lung cancer and the growth of lung cancer cells. The mammalian or mechanical target of rapamycin (mTOR) is a serine/threonine kinase. When the mTOR pathway was overactivated, it promotes the progression of tumors [54]. Peng et al. found that the expression of piR-55490 was decreased in lung cancer tissues compared with normal lung tissues, and the inhibition of piR-55490 could promote the proliferation of lung cancer cells by inhibiting the activation of mTOR pathway in lung cancer cells. This suggests that piR-55490 may have an anti-cancer effect in the initiation of lung cancer (Fig. 3(E)) [55]. Yao et al. found that piR-651 is overexpressed in non-small cell lung cancer (NSCLC) cell lines [56]. Through a wound healing test, it was found that the relative migration distance of lung cancer cells transfected

with piR-651 inhibitor was smaller, and it was inferred that piR-651 promoted tumor invasion and metastasis [56, 57]. After transfection of piR-651 inhibitor into lung cancer cells, it was found that piR-651 affected the initiation and progression of lung cancer by changing the expression levels of apoptosis-related proteins caspase-3 and bax [56, 57]. Studies have also shown that piR-651 regulates cell proliferation by inducing the expression of cyclinD 1 and CDK 4, thus promoting tumor metastasis [58] (Fig. 3(E)). Thus, piR-651 is involved in the initiation, invasion, and metastasis of non-small cell lung cancer, and can be a potential cancer diagnostic tool.

RASSF1C is an important gene that promotes the growth of lung cancer cells [59]. Multiple studies have found that excessive expression of RASSF1C will affect the expression of PIWI1 gene and may affect the progression of lung cancer [60, 61]. It has also been found that PIWIL1 overexpression promotes the initiation, invasion, and metastasis of lung adenocarcinoma cells, and is associated with a shorter survival period in lung cancer patients [62]. This suggests that PIWIL1 may be a therapeutic target of lung cancer (Table 1).

5 Conclusions and perspectives

The understanding of the pathways in piRNAs is increasing, but many mysteries remain. Many of the molecules and mechanisms involved in the formation of piRNAs are still unknown. What substances mediate the transport of pre-piRNA out of nuclear pores? The mechanism that involves molecules in the transport of pre-piRNA to Yb bodies and recognizes each other has not been discovered. What other molecules are involved in the transport of Yb bodies and Armitage regulating piRNA mitochondria spatially and temporally? The mechanisms that allow mature piRISC to enter the nucleus to act as silent transposons are not well understood. In this paper, the progress of piRNA research in recent years is reviewed, and relatively complete mechanisms of piRNA pathways are described in detail, which provides further development for subsequent studies. Since piRNA was discovered in 2006, the close connection between piRNA pathways and the physiological and pathological state of the body is still being explored. Once thought of as a dud, piRNA achievements in scientific research have grown.

The link between piRNAs/PIWI and cancer has focused on diagnostic tools, prognosis biomarker, and therapeutic targets. An increasing number of studies confirmed the abnormal expressions of piRNAs/PIWI in cancer and normal tissues. At present, research on cancer treatment mainly focuses on immunotherapy. But piRNA's/PIWI's extraordinary appeal may lead to new breakthroughs and new peaks in cancer treatment. However, there is still a lack of researches on the relationship between piRNAs/PIWI and cancer. Currently, most studies focus on the abnormal expression of piRNAs/PIWI in tumors. The mechanisms by which abnormal piRNA/PIWI expression mediates tumorigenesis remain unclear. Be that as it may, it is hoped that more studies will reveal the specific link between piRNAs and cancer, leading to new challenges and breakthroughs.

Abbreviations ccRCC, clear cell renal cell carcinoma; CRC, colorectal cancer; ncRNA, non-coding RNA; NSCLC, non-small cell lung cancer; PIC, pre-initiation complex; PICS, piRNA biogenesis and chromosome segregation; piRNA, PIWI-interacting RNA; piRISC, piRNA silencing complex; pre-piRNA, piRNA precursor; RCC, renal cell carcinoma; TEs, transposon elements; Zuc, Zucchini; HSF-1, heat shock transcription factor-1; MTOR, mammalian or mechanical target of rapamycin

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Compliance with ethical standards

Consent for publication All authors consent for publication.

Conflict of interest The authors state that there is no conflict of interest.

References

1. Ferreira, H. J., & Esteller, M. (2018). Non-coding RNAs, epigenetics, and cancer: tying it all together. *Cancer Metastasis Reviews, 37*(1), 55–73. https://doi.org/10.1007/s10555-017-9715-8.
2. Xiao, Y., & Ke, A. (2016). PIWI takes a giant step. *Cell, 167*(2), 310–312. https://doi.org/10.1016/j.cell.2016.09.043.
3. Rodriguez-Leal, D., Castillo-Cobian, A., Rodriguez-Arevalo, I., & Vielle-Calzada, J. P. (2016). A primary sequence analysis of the ARGONAUTE protein family in plants. *Frontiers in Plant Science, 7*, 1347. https://doi.org/10.3389/fpls.2016.01347.
4. Russell, S. J., & LaMarre, J. (2018). Transposons and the PIWI pathway: genome defense in gametes and embryos. *Reproduction, 156*(4), R111–r124. https://doi.org/10.1530/rep-18-0218.
5. Theron, E., Maupetit-Mehouas, S., Pouchin, P., Baudet, L., Brasset, E., & Vaury, C. (2018). The interplay between the Argonaute proteins Piwi and Aub within Drosophila germarium is critical for oogenesis, piRNA biogenesis and TE silencing. *Nucleic Acids Research, 46*(19), 10052–10065. https://doi.org/10.1093/nar/gky695.
6. Weng, W., Li, H., & Goel, A. (2019). Piwi-interacting RNAs (piRNAs) and cancer: emerging biological concepts and potential clinical implications. *Biochimica Et Biophysica Acta. Reviews on Cancer, 1871*(1), 160–169. https://doi.org/10.1016/j.bbcan.2018.12.005.
7. Chalbatani, G. M., Dana, H., Memari, F., Gharagozlou, E., Ashjaei, S., Kheirandish, P., Marmari, V., Mahmoudzadeh, H., Mozayani, F., Maleki, A. R., Sadeghian, E., Nia, E. Z., Miri, S. R., Nia, N. Z., Rezaeian, O., Eskandary, A., Razavi, N., Shirkhoda, M., & Rouzbahani, F. N. (2019). Biological function and molecular

mechanism of piRNA in cancer. *Pract Lab Med, 13*, e00113. https://doi.org/10.1016/j.plabm.2018.e00113.
8. Trzybulska, D., Vergadi, E., & Tsatsanis, C. (2018). miRNA and other non-coding RNAs as promising diagnostic markers. *Ejifcc, 29*(3), 221–226.
9. Krishnan, P., & Damaraju, S. (2018). The challenges and opportunities in the clinical application of noncoding RNAs: the road map for miRNAs and piRNAs in cancer diagnostics and prognostics. *Int J Genomics, 2018*, 5848046. https://doi.org/10.1155/2018/5848046.
10. Zhang, Y., Liu, W., Li, R., Gu, J., Wu, P., Peng, C., et al. (2018). Structural insights into the sequence-specific recognition of Piwi by Drosophila Papi. *Proceedings of the National Academy of Sciences of the United States of America, 115*(13), 3374–3379. https://doi.org/10.1073/pnas.1717116115.
11. Goriaux, C., Desset, S., Renaud, Y., Vaury, C., & Brasset, E. (2014). Transcriptional properties and splicing of the flamenco piRNA cluster. *EMBO Reports, 15*(4), 411–418. https://doi.org/10.1002/embr.201337898.
12. Andersen, P. R., Tirian, L., Vunjak, M., & Brennecke, J. (2017). A heterochromatin-dependent transcription machinery drives piRNA expression. *Nature, 549*(7670), 54–59. https://doi.org/10.1038/nature23482.
13. Dennis, C., Zanni, V., Brasset, E., Eymery, A., Zhang, L., Mteirek, R., Jensen, S., Rong, Y. S., & Vaury, C. (2013). "Dot COM", a nuclear transit center for the primary piRNA pathway in Drosophila. *PLoS One, 8*(9), e72752. https://doi.org/10.1371/journal.pone.0072752.
14. Dennis, C., Brasset, E., & Vaury, C. (2019). flam piRNA precursors channel from the nucleus to the cytoplasm in a temporally regulated manner along Drosophila oogenesis. *Mob DNA, 10*, 28. https://doi.org/10.1186/s13100-019-0170-7.
15. Murota, Y., Ishizu, H., Nakagawa, S., Iwasaki, Y. W., Shibata, S., Kamatani, M. K., Saito, K., Okano, H., Siomi, H., & Siomi, M. C. (2014). Yb integrates piRNA intermediates and processing factors into perinuclear bodies to enhance piRISC assembly. *Cell Reports, 8*(1), 103–113. https://doi.org/10.1016/j.celrep.2014.05.043.
16. Szakmary, A., Reedy, M., Qi, H., & Lin, H. (2009). The Yb protein defines a novel organelle and regulates male germline stem cell self-renewal in Drosophila melanogaster. *The Journal of Cell Biology, 185*(4), 613–627. https://doi.org/10.1083/jcb.200903034.
17. Pandey, R. R., Homolka, D., Chen, K. M., Sachidanandam, R., Fauvarque, M. O., & Pillai, R. S. (2017). Recruitment of Armitage and Yb to a transcript triggers its phased processing into primary piRNAs in Drosophila ovaries. *PLoS Genetics, 13*(8), e1006956. https://doi.org/10.1371/journal.pgen.1006956.
18. Ge, D. T., Wang, W., Tipping, C., Gainetdinov, I., Weng, Z., & Zamore, P. D. (2019). The RNA-binding ATPase, Armitage, couples piRNA amplification in Nuage to phased piRNA production on mitochondria. *Molecular Cell, 74*(5), 982–995 e986. https://doi.org/10.1016/j.molcel.2019.04.006.
19. Munafo, M., Manelli, V., Falconio, F. A., Sawle, A., Kneuss, E., Eastwood, E. L., et al. (2019). Daedalus and Gasz recruit Armitage to mitochondria, bringing piRNA precursors to the biogenesis machinery. *Genes & Development, 33*(13–14), 844–856. https://doi.org/10.1101/gad.325662.119.
20. Ipsaro, J. J., Haase, A. D., Knott, S. R., Joshua-Tor, L., & Hannon, G. J. (2012). The structural biochemistry of Zucchini implicates it as a nuclease in piRNA biogenesis. *Nature, 491*(7423), 279–283. https://doi.org/10.1038/nature11502.
21. Izumi, N., Shoji, K., Sakaguchi, Y., Honda, S., Kirino, Y., Suzuki, T., Katsuma, S., & Tomari, Y. (2016). Identification and functional analysis of the pre-piRNA 3′ trimmer in silkworms. *Cell, 164*(5), 962–973. https://doi.org/10.1016/j.cell.2016.01.008.
22. Hirakata, S., Ishizu, H., Fujita, A., Tomoe, Y., & Siomi, M. C. (2019). Requirements for multivalent Yb body assembly in transposon silencing in Drosophila. *EMBO Rep, 20*(7), e47708. https://doi.org/10.15252/embr.201947708.
23. Ishizu, H., Kinoshita, T., Hirakata, S., Komatsuzaki, C., & Siomi, M. C. (2019). Distinct and collaborative functions of Yb and Armitage in transposon-targeting piRNA biogenesis. *Cell Rep, 27*(6), 1822-1835.e1828. https://doi.org/10.1016/j.celrep.2019.04.029.
24. Cheng, J., Guo, J. M., Xiao, B. X., Miao, Y., Jiang, Z., Zhou, H., et al. (2011). piRNA, the new non-coding RNA, is aberrantly expressed in human cancer cells. *Clinica Chimica Acta, 412*(17–18), 1621–1625. https://doi.org/10.1016/j.cca.2011.05.015.
25. Lin, X., Xia, Y., Hu, D., Mao, Q., Yu, Z., Zhang, H., et al. (2019). Transcriptomewide piRNA profiling in human gastric cancer. *Oncology Reports*. https://doi.org/10.3892/or.2019.7073.
26. Martinez, V. D., Enfield, K. S. S., Rowbotham, D. A., & Lam, W. L. (2016). An atlas of gastric PIWI-interacting RNA transcriptomes and their utility for identifying signatures of gastric cancer recurrence. *Gastric Cancer, 19*(2), 660–665. https://doi.org/10.1007/s10120-015-0487-y.
27. Lin, X., Xia, Y., Hu, D., Mao, Q., Yu, Z., Zhang, H., Li, C., Chen, G., Liu, F., Zhu, W., Shi, Y., Zhang, H., Zheng, J., Sun, T., Xu, J., Chao, H. H., Zheng, X., & Luo, X. (2019). Transcriptomewide piRNA profiling in human gastric cancer. *Oncology Reports, 41*(5), 3089–3099. https://doi.org/10.3892/or.2019.7073.
28. Cui, L., Lou, Y., Zhang, X., Zhou, H., Deng, H., Song, H., Yu, X., Xiao, B., Wang, W., & Guo, J. (2011). Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using piRNAs as markers. *Clinical Biochemistry, 44*(13), 1050–1057. https://doi.org/10.1016/j.clinbiochem.2011.06.004.
29. Cheng, J., Deng, H., Xiao, B., Zhou, H., Zhou, F., Shen, Z., & Guo, J. (2012). piR-823, a novel non-coding small RNA, demonstrates in vitro and in vivo tumor suppressive activity in human gastric cancer cells. *Cancer Letters, 315*(1), 12–17. https://doi.org/10.1016/j.canlet.2011.10.004.
30. Wang, Y., Liu, Y., Shen, X., Zhang, X., Chen, X., Yang, C., & Gao, H. (2012). The PIWI protein acts as a predictive marker for human gastric cancer. *International Journal of Clinical and Experimental Pathology, 5*(4), 315–325.
31. Gao, C. L., Sun, R., Li, D. H., & Gong, F. (2018). PIWI-like protein 1 upregulation promotes gastric cancer invasion and metastasis. *Onco Targets Ther, 11*, 8783–8789. https://doi.org/10.2147/OTT.S186827.
32. Araujo, T., Khayat, A., Quintana, L., Calcagno, D., Mourao, R., Modesto, A., et al. (2018). Piwi like RNA-mediated gene silencing 1 gene as a possible major player in gastric cancer. *World Journal of Gastroenterology, 24*(47), 5338–5350. https://doi.org/10.3748/wjg.v24.i47.5338.
33. Hashim, A., Rizzo, F., Marchese, G., Ravo, M., Tarallo, R., Nassa, G., et al. (2014). RNA sequencing identifies specific PIWI-interacting small non-coding RNA expression patterns in breast cancer. *Oncotarget, 5*(20), 9901–9910. https://doi.org/10.18632/oncotarget.2476.
34. Krishnan, P., Ghosh, S., Graham, K., Mackey, J. R., Kovalchuk, O., & Damaraju, S. (2016). Piwi-interacting RNAs and PIWI genes as novel prognostic markers for breast cancer. *Oncotarget, 7*(25), 37944–37956. https://doi.org/10.18632/oncotarget.9272.
35. Huang, G., Hu, H., Xue, X., Shen, S., Gao, E., Guo, G., Shen, X., & Zhang, X. (2013). Altered expression of piRNAs and their relation with clinicopathologic features of breast cancer. *Clinical & Translational Oncology, 15*(7), 563–568. https://doi.org/10.1007/s12094-012-0966-0.
36. Oner, C., Turgut Cosan, D., & Colak, E. (2016). Estrogen and androgen hormone levels modulate the expression of PIWI interacting RNA in prostate and breast cancer. *PLoS One, 11*(7), e0159044. https://doi.org/10.1371/journal.pone.0159044.

37. Tan, L., Mai, D., Zhang, B., Jiang, X., Zhang, J., Bai, R., Ye, Y., Li, M., Pan, L., Su, J., Zheng, Y., Liu, Z., Zuo, Z., Zhao, Q., Li, X., Huang, X., Yang, J., Tan, W., Zheng, J., & Lin, D. (2019). PIWI-interacting RNA-36712 restrains breast cancer progression and chemoresistance by interaction with SEPW1 pseudogene SEPW1P RNA. *Molecular Cancer, 18*(1), 1–15. https://doi.org/10.1186/s12943-019-0940-3.
38. Fu, A., Jacobs, D. I., Hoffman, A. E., Zheng, T., & Zhu, Y. (2015). PIWI-interacting RNA 021285 is involved in breast tumorigenesis possibly by remodeling the cancer epigenome. *Carcinogenesis, 36*(10), 1094–1102. https://doi.org/10.1093/carcin/bgv105.
39. Zhang, H., Ren, Y., Xu, H., Pang, D., Duan, C., & Liu, C. (2013). The expression of stem cell protein Piwil2 and piR-932 in breast cancer. *Surgical Oncology, 22*(4), 217–223. https://doi.org/10.1016/j.suronc.2013.07.001.
40. Litwin, M., Szczepanska-Buda, A., Michalowska, D., Grzegrzolka, J., Piotrowska, A., Gomulkiewicz, A., et al. (2018). Aberrant expression of PIWIL1 and PIWIL2 and their clinical significance in ductal breast carcinoma. *Anticancer Res, 38*(4), 2021–2030. https://doi.org/10.21873/anticanres.12441.
41. Heng, Z. S. L., Lee, J. Y., Subhramanyam, C. S., Wang, C., Thanga, L. Z., & Hu, Q. (2018). The role of 17betaestradiolinduced upregulation of Piwilike 4 in modulating gene expression and motility in breast cancer cells. *Oncology Reports, 40*(5), 2525–2535. https://doi.org/10.3892/or.2018.6676.
42. Li, Y., Wu, X., Gao, H., Jin, J. M., Li, A. X., Kim, Y. S., Pal, S. K., Nelson, R. A., Lau, C. M., Guo, C., Mu, B., Wang, J., Wang, F., Wang, J., Zhao, Y., Chen, W., Rossi, J. J., Weiss, L. M., & Wu, H. (2015). Piwi-interacting RNAs (piRNAs) are dysregulated in renal cell carcinoma and associated with tumor metastasis and cancer-specific survival. *Molecular Medicine, 21*, 381–388. https://doi.org/10.2119/molmed.2014.00203.
43. Busch, J., Ralla, B., Jung, M., Wotschofsky, Z., Trujillo-Arribas, E., Schwabe, P., Kilic, E., Fendler, A., & Jung, K. (2015). Piwi-interacting RNAs as novel prognostic markers in clear cell renal cell carcinomas. *Journal of Experimental & Clinical Cancer Research, 34*, 61–11. https://doi.org/10.1186/s13046-015-0180-3.
44. Zhao, C., Tolkach, Y., Schmidt, D., Toma, M., Muders, M. H., Kristiansen, G., Müller, S. C., & Ellinger, J. (2019). Mitochondrial PIWI-interacting RNAs are novel biomarkers for clear cell renal cell carcinoma. *World Journal of Urology, 37*(8), 1639–1647. https://doi.org/10.1007/s00345-018-2575-1.
45. Iliev, R., Stanik, M., Fedorko, M., Poprach, A., Vychytilova-Faltejskova, P., Slaba, K., Svoboda, M., Fabian, P., Pacik, D., Dolezel, J., & Slaby, O. (2016). Decreased expression levels of PIWIL1, PIWIL2, and PIWIL4 are associated with worse survival in renal cell carcinoma patients. *Onco Targets Ther, 9*, 217–222. https://doi.org/10.2147/OTT.S91295.
46. Iliev, R., Fedorko, M., Machackova, T., Mlcochova, H., Svoboda, M., Pacik, D., et al. (2016). Expression levels of PIWI-interacting RNA, piR-823, are deregulated in tumor tissue, blood serum and urine of patients with renal cell carcinoma. *Anticancer Res, 36*(12), 6419–6423. https://doi.org/10.21873/anticanres.11239.
47. Stohr, C. G., Steffens, S., Polifka, I., Jung, R., Kahlmeyer, A., Ivanyi, P., et al. (2019). Piwi-like 1 protein expression is a prognostic factor for renal cell carcinoma patients. *Scientific Reports, 9*(1), 1741. https://doi.org/10.1038/s41598-018-38254-3.
48. Mai, D., Ding, P., Tan, L., Zhang, J., Pan, Z., Bai, R., Li, C., Li, M., Zhou, Y., Tan, W., Zhou, Z., Li, Y., Zhou, A., Ye, Y., Pan, L., Zheng, Y., Su, J., Zuo, Z., Liu, Z., Zhao, Q., Li, X., Huang, X., Li, W., Wu, S., Jia, W., Zou, S., Wu, C., Xu, R. H., Zheng, J., & Lin, D. (2018). PIWI-interacting RNA-54265 is oncogenic and a potential therapeutic target in colorectal adenocarcinoma. *Theranostics, 8*(19), 5213–5230. https://doi.org/10.7150/thno.28001.
49. Yin, J., Jiang, X. Y., Qi, W., Ji, C. G., Xie, X. L., Zhang, D. X., Cui, Z. J., Wang, C. K., Bai, Y., Wang, J., & Jiang, H. Q. (2017). piR-823 contributes to colorectal tumorigenesis by enhancing the transcriptional activity of HSF1. *Cancer Science, 108*(9), 1746–1756. https://doi.org/10.1111/cas.13300.
50. Yin, J., Qi, W., Ji, C. G., Zhang, D. X., Xie, X. L., Ding, Q., Jiang, X. Y., Han, J., & Jiang, H. Q. (2019). Small RNA sequencing revealed aberrant piRNA expression profiles in colorectal cancer. *Oncology Reports, 42*(1), 263–272. https://doi.org/10.3892/or.2019.7158.
51. Weng, W., Liu, N., Toiyama, Y., Kusunoki, M., Nagasaka, T., Fujiwara, T., Wei, Q., Qin, H., Lin, H., Ma, Y., & Goel, A. (2018). Novel evidence for a PIWI-interacting RNA (piRNA) as an oncogenic mediator of disease progression, and a potential prognostic biomarker in colorectal cancer. *Molecular Cancer, 17*(1), 16. https://doi.org/10.1186/s12943-018-0767-3.
52. Sun, R., Gao, C. L., Li, D. H., Li, B. J., & Ding, Y. H. (2017). Expression status of PIWIL1 as a prognostic marker of colorectal cancer. *Disease Markers, 2017*, 1204937. https://doi.org/10.1155/2017/1204937.
53. Vychytilova-Faltejskova, P., Stitkovcova, K., Radova, L., Sachlova, M., Kosarova, Z., Slaba, K., et al. (2018). Circulating PIWI-interacting RNAs piR-5937 and piR-28876 are promising diagnostic biomarkers of colon cancer. *Cancer Epidemiology, Biomarkers & Prevention, 27*(9), 1019–1028. https://doi.org/10.1158/1055-9965.EPI-18-0318.
54. Tian, T., Li, X., & Zhang, J. (2019). mTOR signaling in cancer and mTOR inhibitors in solid tumor targeting therapy. *International Journal of Molecular Sciences, 20*(3). https://doi.org/10.3390/ijms20030755.
55. Peng, L., Song, L., Liu, C., Lv, X., Li, X., Jie, J., Zhao, D., & Li, D. (2016). piR-55490 inhibits the growth of lung carcinoma by suppressing mTOR signaling. *Tumour Biology, 37*(2), 2749–2756. https://doi.org/10.1007/s13277-015-4056-0.
56. Yao, J., Wang, Y. W., Fang, B. B., Zhang, S. J., & Cheng, B. L. (2016). piR-651 and its function in 95-D lung cancer cells. *Biomed Rep, 4*(5), 546–550. https://doi.org/10.3892/br.2016.628.
57. Zhang, S. J., Yao, J., Shen, B. Z., Li, G. B., Kong, S. S., Bi, D. D., Pan, S. H., & Cheng, B. L. (2018). Role of piwi-interacting RNA-651 in the carcinogenesis of non-small cell lung cancer. *Oncology Letters, 15*(1), 940–946. https://doi.org/10.3892/ol.2017.7406.
58. Li, D., Luo, Y., Gao, Y., Yang, Y., Wang, Y., Xu, Y., Tan, S., Zhang, Y., Duan, J., & Yang, Y. (2016). piR-651 promotes tumor formation in non-small cell lung carcinoma through the upregulation of cyclin D1 and CDK4. *International Journal of Molecular Medicine, 38*(3), 927–936. https://doi.org/10.3892/ijmm.2016.2671.
59. Reeves, M. E., Firek, M., Jliedi, A., & Amaar, Y. G. (2017). Identification and characterization of RASSF1C piRNA target genes in lung cancer cells. *Oncotarget, 8*(21), 34268–34282. https://doi.org/10.18632/oncotarget.15965.
60. Reeves, M. E., Baldwin, M. L., Aragon, R., Baldwin, S., Chen, S. T., Li, X., Mohan, S., & Amaar, Y. G. (2012). RASSF1C modulates the expression of a stem cell renewal gene, PIWIL1. *BMC Research Notes, 5*, 239. https://doi.org/10.1186/1756-0500-5-239.
61. Reeves, M. E., Firek, M., Chen, S. T., & Amaar, Y. G. (2014). Evidence that RASSF1C stimulation of lung cancer cell proliferation depends on IGFBP-5 and PIWIL1 expression levels. *PLoS One, 9*(7), e101679. https://doi.org/10.1371/journal.pone.0101679.
62. Xie, K., Zhang, K., Kong, J., Wang, C., Gu, Y., Liang, C., Jiang, T., Qin, N., Liu, J., Guo, X., Huo, R., Liu, M., Ma, H., Dai, J., & Hu, Z. (2018). Cancer-testis gene PIWIL1 promotes cell proliferation, migration, and invasion in lung adenocarcinoma. *Cancer Medicine, 7*(1), 157–166. https://doi.org/10.1002/cam4.1248.