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Review

The Regulation and Role of piRNAs and PIWI Proteins in Cancer

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Abstract: P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs) are regulatory small non-coding RNAs that participate in transposon inactivation, chromatin regulation, and endogenous gene regulation. Numerous genetic and epigenetic factors regulate cell proliferation and tumor metastasis. PIWI proteins and piRNAs have been revealed to function in regulating upstream or downstream of oncogenes or tumor-suppressor genes in cancer tissues. In the present review, we summarize major recent findings in uncovering the regulation and role of PIWI proteins and piRNAs in tumorigenesis and highlight some of the promising applications of specific piRNAs in cancer therapeutics and as cancer biomarkers.

Keywords: piRNA; PIWI; PIWIL; small RNA; non-coding RNA; cancer; cancer stem cell; oncogene; tumor-suppressor gene; non-invasive cancer biomarker

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1. Introduction

Dysregulation of the piRNA pathway may cause epigenetic changes or gene dysregulation, which contributes to diverse diseases such as cancers [1–3]. piRNAs are small non-coding regulatory RNAs similar to microRNAs (miRNAs) and small interfering RNAs (siRNAs). piRNAs are 26–35 nucleotide long, with 2′-O-methylation at their 3′-end [4–6]. piRNAs are bound to PIWI, an Argonaute/PIWI family protein, and form a piRNA-induced silencing complex (piRISC). piRISC is thought to regulate its target gene through four mechanisms: transcriptional gene silencing (TGS) or activation (TGA), and post-transcriptional gene silencing (PTGS) or activation (PTGA) [7].

Both PIWI and piRNAs are highly abundant in germ cells and their initially identified functions include transposon silencing, which ensures genome integrity maintained in germ cells [8–10]. Accumulating evidence, however, supports that the PIWI-piRNA pathway not only serves as a transposon inactivator but also plays a crucial role in regulating endogenous gene expression, just like miRNAs or other mechanisms [11–14]. Numerous recent studies reveal that its role is not limited to the germ cells as it also has non-gonadal somatic roles, including stem cell self-renewal, axon regeneration, and memory formation [4,15–17]. Lately, its relationship with somatic cancers has been extensively studied and revealed unprecedented mechanisms regulating gene expression.

Here, we summarize the aberrant expression of PIWI proteins and piRNAs in various cancer tissues and cell lines and discuss recently disclosed molecular mechanisms of how these PIWI proteins or piRNAs cause tumorigenesis and metastasis. PIWI is known to function through interacting with piRNAs, but recent studies report novel actions of PIWI in a piRNA-independent manner. Although it remains unclear whether the dysregulated piRNA pathway directly contributes to cancer pathogenesis, both PIWI and piRNAs are promising therapeutic targets as well as novel biomarkers for cancer diagnosis and prognosis.

2. PIWI Proteins in Diverse Cancers

In humans, there are four PIWI proteins termed PIWIL (for PIWI-like RNA-mediated gene silencing) proteins: PIWIL1 (also called HIWI1 for Human PIWI), PIWIL2 (HILI for HIWI-like), PIWIL3 (HIWI3), and PIWIL4 (HIWI2) [18,19]. PIWI proteins have long been considered to be germline-specific; they help maintain the stemness of germ cells in sexually reproductive animals [20,21]. PIWI proteins also play a crucial role in stem cell regulation in planarians' adult pluripotent stem cells (neoblasts) and fly intestinal stem cells [22,23]. These findings support the idea that PIWI proteins might maintain the 'stemness' of the cells. Thus, when a PIWI protein is ectopically expressed in the somatic cells, it probably gives a 'stemness' to the somatic cells and can potentially make cancerous cells or even cancer stem cells. However, the functional role of the ectopically expressed PIWI is still perplexing. A higher level of transposon expression, which is likely boosted by aging [24–26], is known to be tightly correlated with human cancers [27,28]. The higher expression of PIWI is likely suggested to be induced to suppress the transposon activity [29]. Then, is it just reactive to the high-level expression of transposon? Given the fact that an ectopic expression of PIWI is linked with more aggressive cancer behaviors and its inhibition reverses such phenotypes, PIWI likely has a pro-cancerous role [2,30].

Mentis and colleagues performed a meta-analysis to reveal the association of a cancer prognosis with the expression of PIWI family proteins by analyzing multiple databases [31]. Although highly variable results were seen across cancer types, higher PIWIL1 and lower PIWIL4 expression levels tend to be associated with a worse prognosis in cancer.

Here, we introduce several interesting recent findings of mainly PIWIL1 regulation in cancer cells (Table A1). In endometrial cancer, estrogen-estrogen receptor alpha (ERα) signaling upregulates the expression of oncogenic PIWIL1, likely via promoting the hypomethylation of the *PIWIL1* promoter [32,33]. Similarly, promoter DNA hypomethylation of *PIWIL1* has been reported to upregulate *PIWIL1* expression in both lung adenocarcinoma cell lines and tissues [34]. The overexpressed PIWIL1 promotes cell proliferation, migration, and invasion, and *vice versa*, suggesting that PIWIL1 could promote the malignant phenotypes in lung cancer. Importantly, its high expression shows a correlation with shortened survival of lung cancer patients.

PIWIL1 is also found to be overexpressed in glioblastoma [35]. PIWIL1 is expressed at higher levels in glioma stem cells compared to non-glioma stem cells and this expression regulates the viability and self-renewal of glioma stem cells. PIWIL1 is localized in the cytoplasm and regulates target messenger RNA (mRNA) stability. In a mice model of glioblastoma, *Piwil1* knockdown reduces tumor growth and promotes animal survival, supporting that PIWIL1 plays a regulatory role in stem cell maintenance. Interestingly, miRNA-154-5p is downregulated in glioblastoma and functions as a tumor suppressor by directly targeting *PIWIL1* mRNA (Figure 1) [36]. Thus, these data present an important axis of the miRNA and PIWI pathway in malignant glioblastoma although it is yet unclear whether PIWIL1 functions with piRNAs in the brain.

Several studies have further focused on the prognostic effects of PIWI subcellular localization. In patients with esophageal squamous cell carcinoma, the cytoplasmic, but not nuclear, PIWIL1 expression is associated with poorer clinical survival, indicating its potential involvement in cancer development [37]. In patients with bladder cancer, a combination of cytoplasmic and absent nuclear expression of PIWIL2 is significantly associated with tumor progression and poorer clinical survival [38]. These results suggest that PIWI family proteins may have a distinct role in the nucleus and cytoplasm. Speculatively, PIWI proteins act as suppressors of the transposon activity in the nucleus, while they regulate endogenous RNA stability or translational efficiency in the cytoplasm. Recent studies have also revealed piRNA-independent roles of PIWIL1, which is likely occurred in the cytoplasm (see below). More research is needed to identify the subcellular localization of PIWI proteins and their target RNAs in diverse types of cancers. Further research should unravel their precise cytoplasmic roles, which would provide useful information for developing diagnostic strategies.

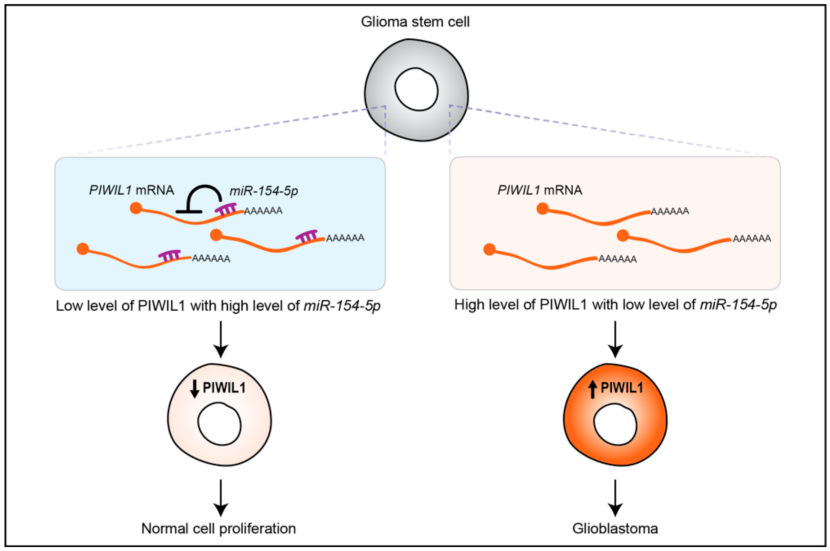

Figure 1. An axis of the miRNA and PIWI pathway in malignant glioblastoma. PIWIL1 is expressed at higher levels in glioma stem cells compared to non-glioma stem cells and localized in the cytoplasm [35]. Downregulated PIWIL1 by miRNA-154-5p (miR-154-5p) inhibits malignant behavior of glioblastoma, while upregulated PIWIL1 in the lack of miR-154-5p leads to malignant glioblastoma [36].

3. Oncogenic piRNAs and Tumor-Suppressor piRNAs

piRNAs are recently obtaining a greater appreciation for their role in carcinogenesis. During cancer development, the expression of certain piRNAs is increased, so these types of piRNAs are considered to be oncogenes. Oncogenic piRNAs contribute to cancer development and progression by increasing cell proliferation and growth, inhibiting apoptosis, and enhancing cell migration and invasion [2,39,40]. In contrast, the expression of certain piRNAs is decreased during tumorigenesis, so these types of piRNAs are considered to be tumor suppressor genes. Tumor suppressor piRNAs upregulate tumor suppressor genes and downregulate oncogenes to inhibit tumor development. There are growing examples of both oncogenic piRNAs and tumor suppressor piRNAs identified in various types of cancers. Here, we summarize a few examples of these piRNAs identified in recent studies (Table A2).

In breast cancer, both oncogenic and tumor-suppressing piRNAs were identified. piR-36026 is found to be an oncogenic piRNA, which is highly expressed in human breast cancer cell line MCF-7 and its downregulation inhibits cancer progression [41,42]. Lee and colleagues developed a double-stranded piR-36026 molecular beacon, which allows visualization of endogenous piR-36026 and simultaneously inactivates the function of piR-36026 [42]. They further identified at least two endogenous targets, *SERPINA1* and *LRAT*, which contain piR-36026 binding sites in the 3′ untranslated region (3′ UTR) and coding region, respectively. These target genes are directly hybridized and downregulated by piR-36206. Importantly, the piR-36026 molecular beacon shows a therapeutic effect in breast cancer, supporting that it may serve as a breast cancer-targeting theragnostic probe.

Conversely, piR-36712 is found to be a tumor-suppressing piRNA in breast cancer. Tan and colleagues analyzed the expression profile of piRNAs in breast cancer using the Cancer Genome Atlas (TCGA) database and examined the expression levels of the top 20 highly expressed piRNAs in tissue samples of breast cancer patients [43]. They found that piR-36712 is significantly downregulated in breast tumors compared with their non-tumor tissues and associated with clinical outcomes. In breast cancer cell lines, the overexpression of piR-36712 suppresses cancer cell proliferation and malignant phenotypes, whereas its knockdown promotes cell proliferation. Furthermore, it has been demonstrated how piR-36712 suppresses breast cancer (Figure 2). piR-36712 interacts with RNAs pro-

duced by *SEPW1P*, a *SEPW1* pseudogene, and destabilizes it. *SEPW1P* helps to increase *SEPW1* RNA stability by acting as a molecular sponge for miRNA-7 and miRNA-324, resulting in the de-repression of SEPW1. Thus, piR-36712 subsequently inhibits SEPW1 expression by reducing *SEPW1P* levels. When piR-36712 is downregulated in breast cancer, SEPW1 is highly expressed, and consequently, P53, P21, and E-cadherin levels are suppressed. Eventually, it promotes cancer cell proliferation, invasion, and migration. Thus, piR-36712 suppresses cancer development by regulating endogenous gene expression. Interestingly, the treatment of piR-36712 analog enables to inhibit tumor growth and shows synergistic anticancer effects with chemotherapeutic agents, paclitaxel and doxorubicin, for breast cancer in mice. These findings support the combinatorial therapeutic strategy of piRNA-targeted therapy and standard chemotherapy, potentially also with immunotherapy [43].

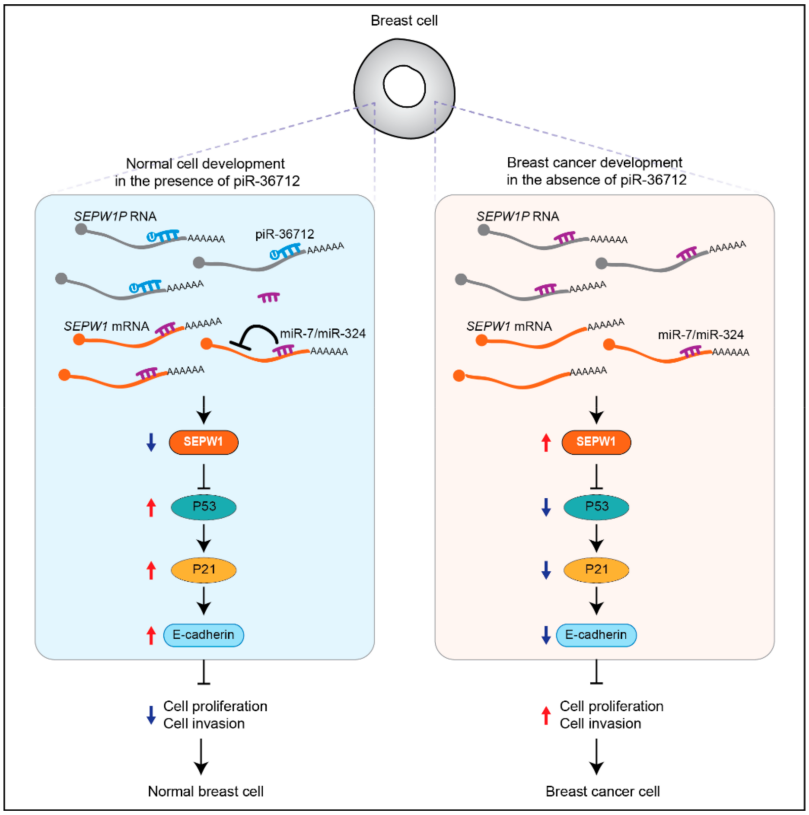

Figure 2. A tumor-suppressing piRNA in breast cancer. Left, piR-36712 binds and destabilizes *SEPW1P* RNA, a *SEPW1* pseudogene, which inhibits SEPW1 expression by reducing *SEPW1P* levels. It increases P53, P21, and E-cadherin levels, and consequently, suppresses cancer development. Right, when piR-36712 is downregulated in breast cells, SEPW1 is highly expressed, and P53, P21, and E-cadherin levels are suppressed, which promotes breast cancer development [43].

In colorectal cancer, several piRNAs are suggested to be oncogenes. piR-54265 induces colorectal cancer in combination with PIWIL2/STAT3/phosphorylated-SRC (p-SRC) complex [44]. PIWIL2/p-SRC/piR-54265 promotes the phosphorylation of STAT3. The phosphorylated STAT3 enters into the nucleus and induces the expression of anti-apoptotic genes and invasion-related genes such as matrix metallopeptidases, leading to tumor proliferation, invasion, and metastasis. Thus, PIWI-piRNA enables the formation of a ribonucleoprotein complex with other proteins and helps protein phosphorylation that leads to colorectal cancer development. piR-54265 is found to be the only piRNA upregulated among the top 20 highly expressed piRNAs in colorectal cancer tissues, and interestingly, serum piR-54265 levels were found to be significantly elevated in periphery blood sam-

ples collected from colorectal cancer patients [44,45]. Thus, serum piR-54265 seems to be a promising biomarker for population screening, early diagnosis, and prognosis of colorectal cancer. Intriguingly, piR-54265 is suggested to be generated from a small nucleolar RNA (snoRNA), SNORD57, based on their sequence homology [46] (See below). A recent study reanalyzed the published data sets and revealed that SNORD57 rather than piR-54265 is expressed in blood samples of patients [46]. Thus, the expression and oncogenic role of piR-54265 should be re-evaluated thoroughly.

piR-1245 is also suggested to be an oncogenic piRNA for colorectal cancer [47]. piR-1245 is overexpressed in colorectal cancer patients and its expression is correlated with advanced and metastatic stages. piR-1245 appears to repress the expression of several tumor suppressor genes involved in cell proliferation and apoptosis. Similarly, piR-24000 and piR-823 are also identified as oncogenic piRNAs in colorectal cancer, which are overexpressed in colorectal cancer patients [48,49]. These results support that these oncogenic piRNAs could serve as diagnostic and prognostic biomarkers as well as potential therapeutic targets for colorectal cancer.

In glioma endothelial cells, PIWIL1 appears to act with piR-33221 (piRNA-DQ593109) and suppress blood-tumor barrier (BTB) permeability via regulating *MEG3*/miR-330-5p/RUNX3 axis [50]. The PIWIL1/piR-33221 piRISC complex decays *MEG3*, a long non-coding RNA. As a result, it eventually upregulates genes involved in tight junction formation, and in turn, BTB permeability is suppressed. Therefore, PIWIL1 suppresses BTB permeability via the formation of piRISC with piR-33221 in glioma endothelial cells. PIWI proteins and piRNAs in the germline are involved in germline development by forming piRISC. This mechanism seems to be reused in somatic cancer cells. Numerous examples of oncogenic piRNAs suggest that piRISC functions in cancer.

In blood cancer, several piRNAs are identified as oncogenes. piR-30473 promotes the progression of diffuse large B-cell lymphoma (DLBCL), the most common lymphoma, by regulating N6-methyladenosine (m6A) posttranscriptional RNA modification [51]. piR-30473 inhibition leads to decreased tumorigenesis of DLBCL by reducing the expression of Wilms' tumor 1-associating protein (WTAP) (Figure 3A). piR-30473 targets *WTAP* 3′ UTR and, in this case, this interaction stabilizes *WTAP* mRNA. As WTAP forms an m6A methyltransferase complex, the aberrantly high expression of WTAP by piR-30473 increases m6A methylase activity [51,52]. Like piR-30473, the high expression of WTAP is associated with a poor prognosis in DLBCL patients. Thus, these results suggest that piR-30473 promotes DLBCL progression by regulating m6A RNA methylation.

piR-823 promotes the progression of multiple myeloma, another type of blood cancer by regulating *de novo* DNA methylation [53]. piR-823 is upregulated in multiple myeloma patients and cell lines and positively correlated with a poor prognosis [53,54]. piR-823 inhibition leads to decrease tumorigenesis of multiple myeloma by reducing DNA methyltransferases, with a consequent re-expression of tumor suppressor genes (Figure 3B). Furthermore, piR-823 leads to enhance 'stemness' of multiple myeloma stem cells at least partially by activation of DNA methyltransferase [24]. Interestingly, piR-823 can be delivered by multiple myeloma-derived extracellular vesicles and modulates the tumor microenvironment [55]. Multiple myeloma-derived extracellular vesicles transport piR-823 to endothelial cells, which leads to promoting the malignant transformation of endothelial cells. Thus, piR-823 directly contributes to myelomagenesis by silencing tumor-suppressor genes via regulating de novo DNA methylation, and encapsulated piR-823 is released to establish a favorable microenvironment that ultimately promotes myelomagenesis.

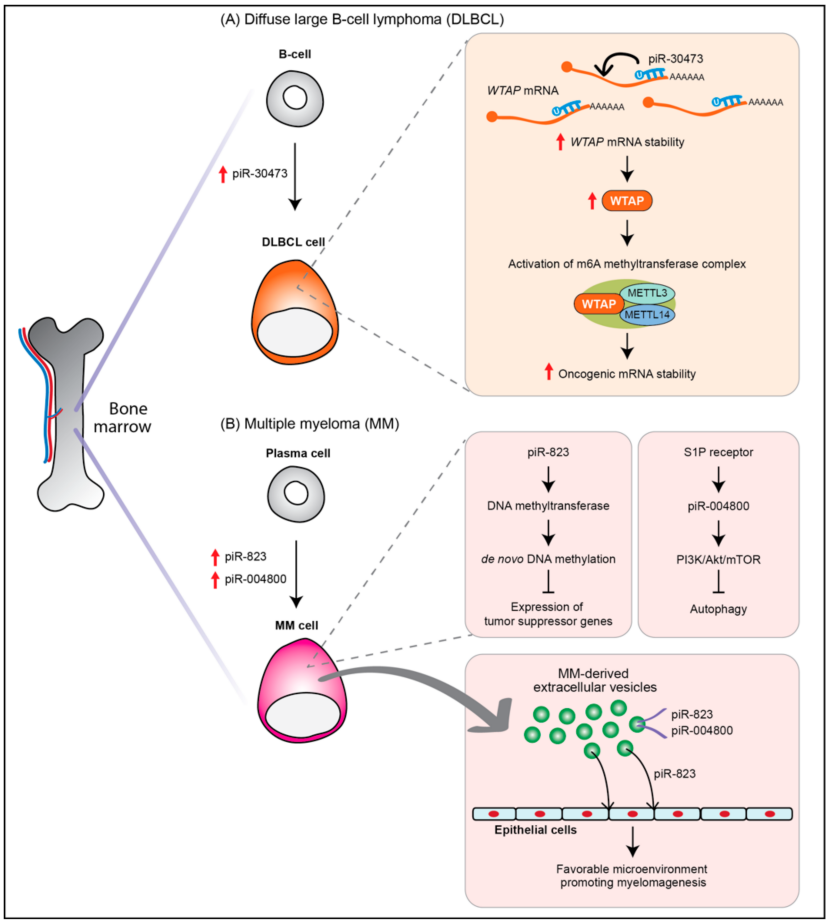

Figure 3. Oncogenic piRNAs in blood cancer. (**A**) piR-30473 promotes the progression of diffuse large B-cell lymphoma (DLBCL) by regulating N6-methyladenosine (m6A) posttranscriptional RNA modification [51,52]. (**B**) piR-823 and piR-004800 promote the progression of multiple myeloma (MM) and are found in MM-derived extracellular vesicles [24,53,54]. The encapsulated piR-823 is released to endothelial cells and leads to establish a favorable microenvironment that ultimately promotes myelomagenesis [55].

In addition to piR-823, piR-004800 is also found in multiple myeloma-derived extracellular vesicles, particularly in exosomes from the patient's bone marrow supernatant as well as cell lines [54]. piR-004800 is positively correlated with a poor prognosis and plays an oncogenic role by regulating the sphingosine-1-phosphate receptor (S1PR) signaling pathway. Downregulation of piR-004800 induces autophagic cell death in multiple myeloma cell lines as well as in xenografts of mice induced by multiple myeloma cells. In addition, the downregulation of piR-004800 decreases the expression of protein kinase B (Akt) and mammalian target of rapamycin (mTOR), thus piR-004800 is suggested to promote phosphoinositide 3-kinase (PI3K)/Akt/mTOR pathway that inhibits autophagy and promotes cell proliferation and survival (Figure 3B). Thus, the presence of piR-004800 in multiple myeloma-derived exosomes might have a role that inhibits autophagic cell death although it remains to be tested.

Autophagy is recognized to have a dual role in cancer, generally, performing a tumor-suppressing role in non-cancerous cells and performing a tumor-promoting role in established tumors [56]. Thus, it suggests that piR-004800 promotes cancer initiation by inhibiting the autophagy performing a tumor-suppressing role in normal cells. The importance of the interplay between autophagy and exosome in cancer has only recently been recognized and has revealed an intricate relationship in the context of cancer initiation, progression, and recurrence [57].

4. The Role of PIWI in Cancer in a piRNA-Independent Manner

As mentioned above, some PIWI family proteins have been often observed to be induced in cancer cells, but other components in the piRNA pathway do not seem to be generally induced [58]. In many cases, the overall expression of piRNAs is not altered and remains low. Although there are still obvious examples of the piRNA-dependent manner of PIWI by forming a piRISC complex, a recent study shows that the aberrant expression of select genes in the piRNA pathway including PIWI does not reactivate piRNA silencing in colon cancer cell lines [58]. These lines of evidence suggest a piRNA-independent manner of PIWI in tumorigenesis. Here, we summarize this new strategy that contributes to tumorigenesis.

Two recent studies suggest the regulation of PIWI in tumorigenesis in a piRNA-independent manner. In pancreatic cancer, piRNA-unloaded PIWIL1 appears to act as an oncoprotein [59]. PIWIL1 is aberrantly induced in multiple types of human cancer tissues and cancer cell lines. Among the numerous cancer cell lines, the human pancreatic ductal adenocarcinoma cell line, BxPC-3, is found to express the highest level of PIWIL1, and this overexpressed PIWIL1 promotes pancreatic tumor growth and metastasis. Intriguingly, PIWIL1 plays this oncogenic role without a partner piRNA in this cell line. Instead, this unloaded PIWIL1 appears to interact with the anaphase-promoting complex/cyclosome (APC/C) E3 complex and acts as a co-activator to target a cell adhesion-related protein, PININ (Figure 4A). As PININ acts as a key regulator in pancreatic ductal adenocarcinomas cell metastasis, priming PININ proteolysis via APC/C-PIWIL1 complex regulates tumorigenesis. Further studies should identify additional substrates of APC/C-PIWIL1 in pancreatic ductal adenocarcinoma cells.

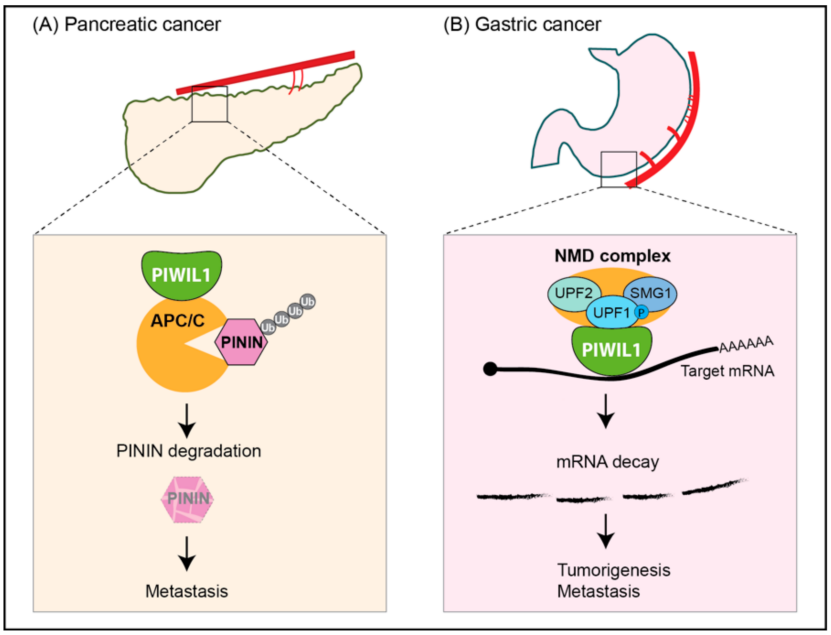

Figure 4. piRNA-independent regulation of PIWI protein. (**A**) In pancreatic cancer, unloaded PIWIL1 interacts with the anaphase-promoting complex/cyclosome (APC/C) E3 complex and acts as a co-activator to target a cell adhesion-related protein, PININ [59]. (**B**) In gastric cancer, unloaded PIWIL1 degrades many target RNAs via the recruitment of UPF1-mediated nonsense-mediated mRNA decay (NMD) components [60].

Another piRNA-independent role of PIWIL1 was revealed in gastric cancer [60]. Similar to the pancreatic ductal adenocarcinoma cell, PIWIL1 is highly expressed in gastric cancer tissues and cell lines and acts as an oncoprotein. Interestingly, abolishing the piRNA-binding activity of PIWIL1 does not affect its oncogenic function and piRNAs are not present in gastric cancer cells, suggesting that PIWIL1 functions in gastric cancer

development in a piRNA-independent manner. PIWIL1 appears to degrade many target RNAs via the recruitment of UPF1-mediated nonsense-mediated mRNA decay (NMD) components (Figure 4B). The mechanism of how PIWIL1 binds target mRNAs without piRNAs should be addressed.

These studies start to reveal an emerging role for PIWI proteins independent of piRNAs, with many open questions remaining. Do piRNA-independent functions of PIWI in partnership occur only under pathological conditions, or do such functions also occur under normal physiological conditions? Is it possible to block the oncogenic functions of PIWIL1 when a sufficient amount of piRNAs is supplied? More examples should help address these remaining questions and further elucidate detailed mechanisms underlying a piRNA-independent regulation of PIWI proteins.

5. Databases of piRNA Expression in Cancers

Several piRNA expression databases have been generated within the last decade and recently some databases include information on cancer-related piRNA expression changes. piRBase (http://regulatoryrna.org/database/piRNA/, accessed on 12 July 2021) is a comprehensive database of piRNA sequences, which was launched in 2014 to assist piRNA functional study [61,62]. In the piRBase release v2.0, eight cancer types (breast, bladder, colorectal, gastric, kidney, liver, myeloma, and pancreas cancer)-related piRNAs were added with piRNA expression changes in cancer tissues or cell lines [61].

piRNA-eQTL (http://njmu-edu.cn:3838/piRNA-eQTL/, accessed on 12 July 2021), by using piRBase as reference data, has been developed, which is an expression quantitation trait locus (eQTL) database between single nucleotide polymorphisms (SNPs) and piRNA expression [63]. By combining piRNA expression and genotype data in 33 cancer types, piRNA-eQTL provides a systematic evaluation of the effects of various cancers on piRNA expression and the potential roles of piRNAs in the development of cancers. In the piRNA-eQTL, there are millions of SNP-piRNA pairs in tumor and normal tissues.

piRPheno (http://www.biomedical-web.com/pirpheno/, accessed on 12 July 2021) is a human disease-associated piRNA database [64]. Through manual curation of publications, piRPheno provides 9057 associations between 474 piRNAs and 204 diseases, including cancers. As piRPheno provides a confidence score and a clinical correlation on each of these associations, it enables easy exploration of the human disease-related piRNAs.

A recent study gives a caution that the current piRNA databases possibly contain erroneous entries, which are supposed to belong to other non-coding RNAs including miRNA, transfer RNA (tRNA), snoRNA, and long non-coding RNA (lncRNA) fragments [46]. The concept of miscellaneous-piRNAs (m-piRNAs) has been introduced to distinguish between 'canonical' piRNAs (90% of the piRNAs directly derived from the piRNA clusters) and other small RNAs circumstantially associated with PIWI proteins. However, the snoRNA-derived piRNAs have also been previously recognized by other researchers and they consider this group of piRNAs are also piRNAs that have just a different origin. Thus, some researchers classify piRNAs into five groups based on origin: transposon-derived, mRNA-derived, lncRNA-derived, tRNA-derived, and snoRNA-derived piRNAs [65]. Although research on piRNA biogenesis mechanisms remains insufficient, the piRNA community should soon have a clear definition of piRNA to avoid further confusion.

6. piRNAs as Non-Invasive Biomarkers for Cancer

piRNAs have been suggested to be promising tissue-based biomarkers for cancer diagnosis and prognosis as their expression changes are associated with cancer development and progression. piRNAs are also suggested to be non-invasive biomarkers since they are present in human body fluids such as blood, saliva, gastric juice, and urine (Figure 5) [66–72]. Fortunately, piRNAs are present in human serum and plasma samples and remain stable even after repetitive freeze-thaw cycles or long-term incubation at room temperature [73]. Such stability might be owing to the 2′-O-methyl modification in piRNAs

by likely protecting them from 3′ uridylation and truncation, which are considered to influence small RNA stability [74,75]. Another, but not mutually exclusive possibility, is that piRNAs in the blood are encapsulated into the extracellular vesicles like microvesicles and exosomes, so they can be protected from ribonuclease digestions. We have mentioned above that piR-823 and piR-004800 are found in multiple myeloma-derived extracellular vesicles and such encapsulated piR-823 is released to establish a favorable tumor microenvironment [24,54,55].

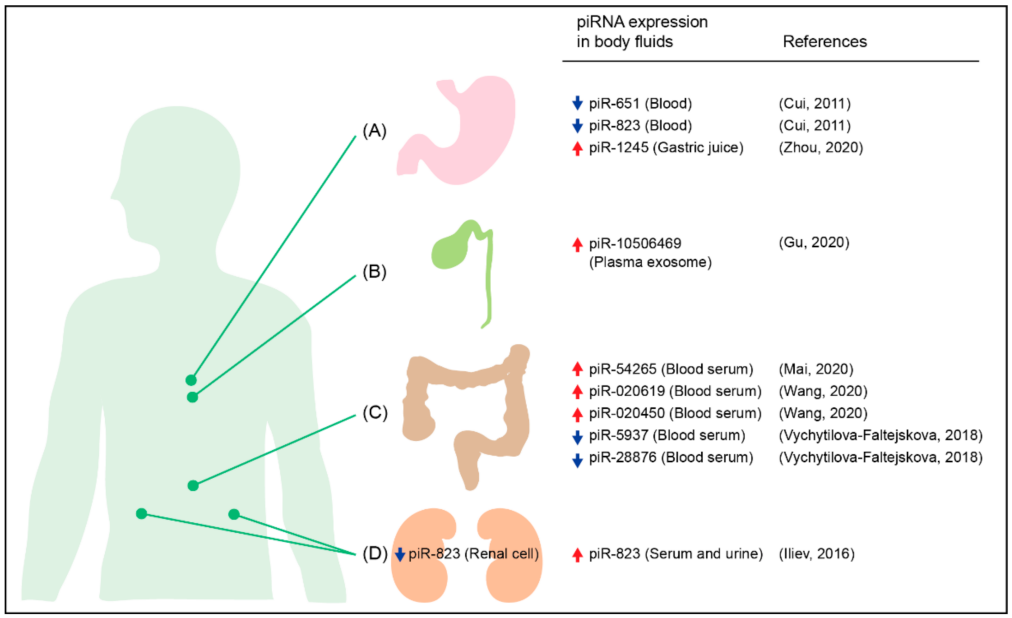

Figure 5. piRNAs as potential non-invasive biomarkers in cancer. (**A**) Gastric cancer. (**B**) Biliary tract cancer. (**C**) Colorectal cancer. (**D**) Renal cancer.

Importantly, exosomal piRNAs in the serum have been quantified. Exosomes are nano-sized (30–120 nm) small membrane-bound extracellular vesicles released from cells, carrying biological large molecules, including DNA, mRNA, and various non-coding RNAs [76]. A growing body of evidence shows that exosomes regulate tumor progression by delivering special components to neighboring cells [77]. Since exosome production is continuous, the analysis of exosomes released from cancer cells could capture the dynamic complexity of cancer [78]. For example, piR-10506469 was found to be significantly increased in plasma exosomes of biliary tract cancer patients and decreased in patients who underwent surgeries [68]. As numerous other piRNAs are also found to be upregulated in the exosome of biliary tract cancer patients, it seems promising to develop more blood-based diagnostic and prognostic biomarkers for biliary tract cancer. Novel piRNA biomarkers should be further identified for cancer detection at an early stage or tracking of tumor progression over time and validated in independent studies with larger sample sets and long-term clinical datasets.

7. Concluding Remarks

Our understanding of PIWI and piRNA biology is rapidly growing. The pathological influence of aberrantly expressed PIWI family proteins in various cancers has been reported, which has triggered extensive research in the field of PIWI-piRNA biology. Many studies have focused on the identification of differentially expressed PIWIs or piRNAs in cancer tissues compared to adjacent non-cancerous normal tissues. In general, PIWIL1 proteins are highly expressed in many tested cancer tissues, which contributes to cancer development in many cases. Although most other components in piRNA biogenesis are still barely detectable in somatic cancer cells [58], at least some piRNAs are reported to be highly expressed in certain types of somatic cancers. Many studies show that these highly

upregulated piRNAs in cancer tissues indeed contribute to cancer development. How is it possible that piRNAs are generated in the absence of piRNA biogenesis components? These mature piRNAs might be produced in other tissues and transferred into somatic tissues. Additionally, some RNAs currently classified as piRNAs might be noncanonical piRNAs, which are mistakenly included in the piRNA databases [46]. If canonical piRNAs are indeed generated in somatic cancer cells in the absence of known piRNA biogenesis components, there should be an alternative mechanism for piRNA biogenesis in somatic cells. In the absence of canonical piRNAs, PIWI could perhaps bind other types of RNAs, which seems to occur at least in cancerous tissues [60,79]. However, a functional readout of these interactions remains largely unknown. Depending on the protein partners of PIWI, these distinct complexes seem to either stabilize or destabilize their target RNAs.

Despite the accumulating information, it is still insufficient to entirely discriminate between a 'passenger (or hitchhiker)' role for the aberrant expression of PIWI and piRNAs in cancer from a 'driver' role in the cancer pathogenesis [80]. In whichever cases, PIWI and piRNAs are promising candidates for cancer biomarkers due to their specificity to cancers. Like circulating miRNAs, circulating piRNAs in body fluids are emerging as a new class of non-invasive biomarkers for cancer due to their high stability [81,82]. The combination of circulating miRNAs and piRNAs could enhance its specificity and sensitivity to detect cancers and provide new opportunities to develop a specific panel for early diagnosis and prognosis. Therefore, a challenge for future research is to elucidate all the context-dependent mechanisms of PIWI regulation in diverse cancers, which should advance our understanding of PIWI-piRNA biology. Ultimately, this advance will help develop not only clinically relevant and promising biomarkers but also therapeutic targets for treatment.

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Appendix A

Table A1. Expression and role of PIWIL1 in cancer.

Cancer	Expression in Cancer; Mechanism	Sample Tested	Role; Mechanism	Ref.
Endometrial cancer	Up; Estrogen-ERα signaling and hypomethylation of *PIWIL1* promoter	Patients, Ishikawa, RL95–2 cell lines	Oncogenic; Inducing epithelial-mesenchymal transition	[32,33]
Lung adenocarcinoma	Up; Hypomethylation of *PIWIL1* promoter	Patients, A549, H1299 cell lines	Oncogenic	[34]
Glioblastoma	Up; Downregulation of miR-154-5p that targets *PIWIL1* mRNA	Patients, glioma stem cell lines (4121, 3832, 387, 3359) & glioblastoma cell lines (U251, U87, A172, LN229, SNB19, LN308)	Oncogenic	[36]

Table A1. *Cont.*

Cancer	Expression in Cancer; Mechanism	Sample Tested	Role; Mechanism	Ref.
Esophageal squamous cell carcinoma	Up (cytoplasmic); Unknown	Patients, Kyse140 cell line	Oncogenic	[37]
Pancreatic ductal adenocarcinomas	Up; Unknown	Patients, BxPC-3, AsPC-1 cell lines	Oncogenic; piRNA-independent function; acting with APC/C to degrade PININ	[59]
Gastric cancer	Up; Unknown	Patients, AGS, HGC-27, N87, SNU-1/5/16 cell lines	Oncogenic; piRNA-independent function; acting with NMD components to likely degrade tumor suppressors mRNAs	[60]

Table A2. Oncogenic piRNA and tumor-suppressor piRNA.

Cancer	piRNA	Expression in Cancer	Samples Tested	Role	Mechanism	Ref.
Breast cancer	piR-36026 (DQ597960)	Up	Patients, MCF7 cell line	Oncogenic	Targeting *SERINA1* and *LRAT* mRNA	[41,42]
	piR-36712	Down	Patients	Tumor suppressing	Increasing P53 expression by targeting *SEPW1P* RNA	[43]
Colorectal cancer	piR-54265	Up	Patients (tissue & blood), HCT116, LoVo, SW480, SW620, HT-29, DLD-1 cell lines	Oncogenic	Activating STAT3 signaling by interacting with PIWIL2	[44–46]
	piR-1245	Up	Patients, HCT116, SW480 cell lines	Oncogenic	Likely regulating a panel of tumor suppressor genes mRNA	[47]
	piR-823	Up	Patients, HCT15/116, DLD-1 cell lines	Oncogenic	Enhancing the transcriptional activity of HSF1 by promoting its phosphorylation	[49]
	piR-24000	Up	Patients	Oncogenic	Unknown	[48]
Glioma	piR-33221 (DQ593109)	Up	Glioma endothelial cells	Oncogenic	Acting with PIWIL1 to suppress BTB permeability via regulating *MEG3*/miR-330-5p/RUNX3 axis	[50]
Diffuse large B-cell lymphoma	piR-30473	Up	Patients	Oncogenic	Stabilizing *WTAP* mRNA via m6A RNA methylation	[51,52]
Multiple myeloma	piR-823	Up	Patients, Plasma cell, RPMI8226, ARH-77, U266, KM3 cell lines	Oncogenic	Inducing *de novo* DNA methylation	[53]
	piR-004800	Up	Patients (exosomes from bone marrow supernatant), RPMI8226, U266 cell lines	Oncogenic	Regulation of PI3K/Akt/mTOR pathway	[54]

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