Provisional chapter

Part 2: Deregulated Expressions of PIWI Proteins and piRNAs as New Candidate Biomarkers and Potential Therapeutic Tools in Cancer

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Abstract

Epigenetic abnormalities are early events in carcinogenesis and associate heterogeneity of DNA methylation, modifications of histones, and deregulation of noncoding RNAs. Aberrant expressions of PIWI proteins and piRNAs were recently observed in numerous subtypes of malignant tumors and were implicated in occurrence of most cancer hallmarks such as cell proliferation, genomic stability, apoptosis inhibition, invasion, and metastatic spread. However, this pathway is a new emerging research field, and further investigations are necessary to elucidate their oncogenic or tumor-suppressing status. Since the aberrant expression of this pathway may induce stemness, analysis of relationship between PIWI proteins, piRNAs, and cancer stem cells may open new avenues in cancer research. The objective of this review is to provide a broad overview of the emerging implication of PIWI proteins and piRNAs in carcinogenesis and their potential clinical interest as diagnostic and prognostic biomarkers and therapeutic tools.

Keywords: piRNA, PIWI proteins, transposable element (TE), transcriptional and post-transcriptional silencing, piRNA cluster, heterochromatin, DNA methylation, ping-pong cycle, Nuage

1. Deregulation of the PIWI-piRNA pathway in cancer

Recent evidences have suggested that activation of the PIWI-piRNA pathway has important implications in carcinogenesis (**Table 1**). PIWI proteins and piRNAs are deregulated in numerous human cancers such as breast cancer, prostate cancer, hepatic cancer, gastric cancer, malignant melanoma, and lung cancer. Experimental analyses have demonstrated that

PIWI proteins	PiRNAs	Cancer type	Expression	Cancer hallmarks	Mechanisms in cancer	References
PIWIL1		Breast cancer	Overexpression	Proliferation and stemness	Cell-cycle regulation	[10]
PIWIL1		Lung cancer	Overexpression	CSC self-renewal	Maintenance of CSCs	[9]
PIWIL1		Ovary cancer	Overexpression	Migration and invasion		[72]
PIWIL1		Gastric cancer	Overexpression	Proliferation	Cell-cycle regulation	[8, 22, 62]
PIWIL1		Colon cancer	Overexpression	Proliferation and stemness	Interactions with OCT4 and SOX2	[17]
PIWIL1		Liver cancer	Overexpression	Proliferation		[12]
PIWIL1		Cervix cancer	Overexpression	Migration and invasion		[5]
PIWIL1		Seminoma	Overexpression	Proliferation		[21]
PIWIL1		Glioblastoma	Overexpression	Proliferation, migration, and invasion	Overexpression of MMP2 and MMP9	[11]
PIWIL1		Sarcomas	Overexpression	Genomic instability	Decreasing of differentiation and inhibition of TSGs (p15, p21, and p27)	[73]
PIWIL2		Colon cancer	Overexpression	Proliferation, migration, and invasion	MMP9 transcriptional activation	[14, 24]
PIWIL2		Breast cancer	Overexpression	Proliferation, apoptosis inhibition, migration, and invasion	Maintenance of CSCs, proliferation via STAT3/cyclin D1 activation, and apoptosis inhibition via STAT3/Bcl-xL activation	[16, 26]
PIWIL2		Liver cancer	Overexpression	Apoptosis inhibition	PIWIL2/STAT3/c-Src activation and repression of p53 transcription	[12, 13]
PIWIL2		Cervix cancer	Overexpression	Apoptosis inhibition	PIWIL2/STAT3/c-Src activation and repression of p53 transcription	[29]
PIWIL2		Ovary cancer	Overexpression	Genomic instability	Chromatin decondensation, cell survival, and DNA repair	[65]
PIWIL4		Colon cancer	Overexpression	Migration and invasion		[74]
PIWIL4		Lung cancer	Overexpression	Proliferation	Increasing of cyclin A and cdk2 expression	[68]

PIWI proteins	PiRNAs	Cancer type	Expression	Cancer hallmarks	Mechanisms in cancer	References
PIWIL4		Cervix cancer	Overexpression	Proliferation, migration, and invasion	Impairing apoptosis via p14ARF/p53 pathway	[34]
	piR-651	Breast, colon, gastric, and lung cancers	Overexpression	Proliferation, invasion, and metastasis	Cyclin D1 and CDK4 overexpression	[28, 43, 69, 70]
	piR-932	Breast cancer	Overexpression	Stemness, EMT, and invasion	TGF-β pathway activation and hypermethylation of the TSG Latexin	[48]
	piR-823	Gastric cancer	Underexpression	Chromatin regulation and angiogenesis	Hypomethylation via decreased DNMT3A/B and decreased pro-angiogenic activity	[5]
	piR-823	Multiple myeloma	Overexpression	DNA methylation and angiogenesis	Hypomethylation via decreased DNMT3A/B and decreased pro-angiogenic activity	[44]
	piR-Hep1	Liver cancer	Overexpression	Migration and invasion	Akt phosphorylation and apoptosis inhibition via STAT3/Bcl-xL pathway	[41, 71]
	piR-017061	Pancreatic cancer	Underexpression			[42]
	piR-L-163	Lung cancer	Underexpression	Proliferation and invasion	Cell-cycle regulation	[38]
	piR-ABC	Bladder cancer	Underexpression	Inhibition of proliferation apoptosis promotion	TNFSF4 regulation	[75]

Table 1. Role of PIWI and/or piRNA in carcinogenesis. This table shows recent evidences that deregulation (overexpression and/ or underexpression) of the PIWI-piRNA and PIWI proteins pathway in different cancer types has important implications in cancer hallmarks.

PIWI proteins and piRNAs altered levels of expression are associated with major hallmarks of cancer, including genomic chronic proliferation, differentiation, and survival [1]. Emerging roles of PIWI-piRNA-mediated epigenetic alterations in cancer seem related to promotion of a stem-like state of tumor cells through aberrant DNA methylation. Experimental studies in *Drosophila* have confirmed that PIWI proteins and piRNAs functions are upstream modulators of cell cycle progression and proliferation through control of DNA synthesis, maintain of chromatin structure, and assembly of mitotic spindle. In *Drosophila*, maternal alterations of the three PIWI proteins, Piwi, Aub, and Ago3, were associated with chromatin disorganization, asynchronous nuclear division, abnormal nuclear morphology, and cell cycle arrest. Analysis

of this pathway in cancer is still in its infancy, and investigation is currently underway to determine whether ectopic expression of piRNAs and PIWI proteins could have a driver role. Expression status and roles of the PIWI proteins and piRNAs remain poorly understood in cancer, and most of the current data about association of this pathway with carcinogenesis result from clinic-pathological reports. Aberrant expressions of these genes have been associated with various hallmarks of cancer and prognostic/predictive factors [2, 3].

1.1. Deregulation of PIWI proteins in cancer

Based mainly on both *in vivo* and *in vitro* functional studies combined with clinico-pathologic analysis, mounting evidence has identified all four human PIWI proteins as new molecular players in carcinogenesis [4]. Alterations of their expression levels are actually better investigated than piRNAs, with PIWI proteins overexpressed in germline and somatic malignant tumors. Deregulated PIWI proteins observed in somatic malignant tumors can be included in the cancer/testis antigens (CTAs) class and could thus be pertinent targets of immunotherapy [5]. They are linked to most of cancer hallmarks, suggesting an oncogenic role [6]. The four PIWI proteins PIWIL1/HIWI, PIWIL2/HILI, PIWIL3, and PIWIL4/HIWI2 were thus found to be involved in cancer cell proliferation, apoptosis, invasion, and metastasis and could represent diagnostic and prognostic biomarkers.

1.1.1. Proliferation, apoptosis, and stemness

PIWIL1: PIWIL1-induced DNA hypermethylation generates genetic and epigenetic changes promoting carcinogenesis [7]. PIWIL1 was upregulated in seminoma. In gastric cancer, PIWIL1 progressive increased expression was observed from preneoplastic lesions to invasive cancer, whereas inhibition of PIWIL1 suppressed growth of tumor epithelial cells and induced cell-cycle arrest at the G2/M phase. PIWIL1 induced cyclin B1 overexpression and promoted cell cycle progression from G2 to M phase [8]. In sarcomas, PIWIL1 upregulation was associated with repression of tumor differentiation and indefinite proliferation and inversely correlated with TSGs expression, including p15, p21, and p27. Lung cancer stem cells overexpressed PIWIL1 whose knockdown was associated with inhibition of sphere formation ability and colony forming capacity in nude mice [9]. The promoting role of PIWIL1 in cell proliferation was also demonstrated in breast cancer [10]. In glioma, PIWIL1 repression was associated with inhibition of cell proliferation by promoting apoptosis and increasing cell cycle arrest. PIWIL1 induced cell cycle progression through increasing cyclin D1 and cyclin B levels of expression and repressed expression of p21, an inhibitor of cyclin D1 and CDK4, whereas its suppression through siRNAs blocked S phase entry *via* p21 upregulation and cyclin D1 downregulation [11]. In hepatocellular carcinoma, PIWIL1 was underexpressed leading to reduction of proliferation and migration of cancer cells [12].

PIWIL2: PIWIL2-induced Stat3/Bcl-XL pathway activation promoted oncogenesis [13]. In breast cancer, PIWIL2 played an anti-apoptotic role and increased cancer stem cells proliferation by promoting the STAT3/Bcl-xL pathway [14]. PIWIL2 activated the STAT3/cyclin D1 pathway. The splice isoform PL2L60 of PIWIL2 was overexpressed in tumors and was implicated in G0/1 to S phase transition and cancer cells proliferation through

activation of NF-κB and upregulation of STAT3 and Bcl-2 [15, 16]. In this way, PIWIL2 can directly bind to STAT3 protein *via* its PAZ domain and edify a PIWIL2/STAT3/c-Src triple protein-protein complex. Then, c-Src-induced phosphorylated STAT3 is translocated to the nucleus and binds to p53 promoter, inhibiting its transcription and reducing apoptosis in cancer cells. PIWIL2 silencing could reduce tumor proliferation and colony formation but increase apoptosis *in vitro*. PIWIL2 inactivation in colon cancer cells reduced proliferation and colony formation [14]. A significant positive correlation at mRNA level was observed between PIWIL1 and OCT4, as well as PIWIL2 and SOX2 in colon cancer cells [17]. In gastric cancer, PIWIL2 upregulated the expression of CDK2, CDK4, cyclin A, and c-Myc-dependent cyclin D1. In lung carcinoma, PIWIL2 enhanced CDK2 and cyclin A expression and initiated mitotic phase [22]. In hepatocellular and cervical carcinoma, PIWIL2 promoted c-Myc expression through interaction of NME2 kinase to c-Myc promoter region [41]. PIWIL1 and PIWIL2 modulated microtubules activity to increase malignant cell proliferation and invasion. PIWIL1 upregulated Stathmin1 expression, a pivotal cytosolic phosphoprotein implicated in edification of the mitotic spindle and segregation of chromosomes [43]. Direct interaction between Stathmin1 and PIWIL1 prevented its inhibition by CaMKII and its degradation by E3 ubiquitin ligase RLIM. PIWIL2 promoted interaction of the tubulin folding cofactor B (TBCB) with HSP90 and repressed binding of TBCB to the E3 ubiquitin ligase adaptor protein gigaxonin, resulting in tubulin polymerization and microtubule edification during cell division [31].

PIWIL4: PIWIL4 also plays an oncogenic role in cervical cancer by inhibiting apoptosis through H3K9 methylation at the *p14-ARF* locus [18]. In glioma, PIWIL4 upregulated cyclin D1 expression and repressed the inhibitor of cyclin D1/CDK4 p16. PIWIL4 also promoted cyclin D1 expression through activation of STAT3, Bcl2, and Bcl-xL, and its expression was modulated by miR384 [46]. In cervical cancer, PIWIL4 promoted survival of cancer cells by inhibiting expression of p14-ARF and p53 and prevented apoptosis by inactivating the p14-ARF/p53 axis. Furthermore, PIWIL4 downregulated PTEN expression and activity at the transcriptional level, through DNMT1-induced hypermethylation of the *PTEN* gene promoter. PTEN inhibits numerous survival factors such as STAT3 and Akt/PI3K and through dephosphorylation induced by its phosphatase activity [1].

Furthermore, overexpression of PIWI proteins contributes to carcinogenesis by decreasing differentiation and promoting cancer stemness. PIWIL1 and PIWIL2 are implicated in maintenance and proliferation of lung and breast cancer stem cells, respectively [15].

1.1.2. Genomic integrity

Cancer cells overexpressing PIWI proteins often have genomic alterations partially resulting from reactivation of TEs. In sarcomas, PIWIL1, and p15, p21 and p27 expression levels are inversely correlated [7]. PIWI proteins maintain genome integrity in tumor cells by using epigenetic mechanisms. PIWIL1-induced carcinomas were characterized by global DNA hypermethylation at nonpromoter CpG regions. In mouse embryonic fibroblasts, PIWIL2 was implicated in DNA repair by promoting histone acetylation, chromatin relaxation, and DNA damage response [1].

1.1.3. EMT, invasion, and metastasis

Reciprocal interactions between PIWI proteins and transcription factors involved in EMT have been identified [19]. In colon carcinomas, PIWI proteins and piRNA 34736 activate genes implicated in EMT [20].

The four PIWI proteins identified in humans are variably overexpressed in numerous cancers and implicated in invasion and metastatic spread. The first example of PIWI protein deregulated in carcinogenesis was that of PIWIL1 overexpression in seminomas [21]. Since then, increased levels of PIWIL1 have been detected in lung, breast, gastrointestinal tract, pancreas, liver, cervical, ovarian, and endometrial carcinomas. In most of these malignant tumors, PIWIL1 overexpression is associated with large tumor size, high histologic grade, advanced stage, and poorer prognosis [22–24]. Compared with PIWIL1 and PIWIL2, PIWIL3 and PIWIL4 have been studied in few cancers.

PIWIL1 upregulation was implicated in carcinogenesis by promoting expression of CSC transcription factors OCT4, NANOG, and BMI1 with increased self-renewal properties and resistance to chemotherapies [25]. In breast cancer, PIWIL1 modified cell cycle progression through regulating levels of TGF-β receptors, CDK4, CDK6, and CDK8 [26]. During gastric carcinogenesis, PIWIL1 levels of expression were gradually increased in normal tissues, atrophic gastritis, intestinal metaplasia, and invasive carcinoma [25]. In colon cancer, PIWIL1 overexpression induced global DNA methylation and proliferation. In hepatocellular carcinomas, PIWIL1 overexpression promoted invasion and metastatic spread, whereas PIWIL1 inactivation decreased metastatic evolution [27]. In glioblastomas, PIWIL1 inactivation reduced migration by inactivating expression of MMP2 and MMP9 [1]. Conversely, PIWIL1 had an inhibiting effect on invasion in ovarian cancer [28].

PIWIL2 upregulation was identified in various stages of cervical low-grade and high-grade squamous, intraepithelial lesions, and invasive cervical carcinomas and had the potential to be used as a complementary biomarker for p16 [29]. In colon cancer, PIWIL2 overexpression was significantly correlated with more aggressive clinical and pathological parameters, including lymph node and distant metastasis and poor prognosis [30]. PIWIL2 overexpression induced invasion through activation of MMP9, whereas PIWIL2 inhibition decreased migration via E-cadherin upregulation and TWIST, vimentin, and N-cadherin downregulation [30]. Experimental PIWIL2 knockdown was associated with inhibition of colon cancer cells invasion through MMP9 downregulation. In breast cancer, PIWIL2 upregulation in cancer stem cells was correlated with large tumor size, high histological grade, estrogen receptor expression, proliferation marker Ki67, advanced tumor stage, and lymph node metastasis [26]. In prostate cancer, PIWIL2 overexpression was associated with deregulation of EMT factors. In breast cancer, PIWIL2 and piRNA 932 overexpression promoted EMT in CD44+/CD24− CSCs [31].

PIWIL4 was upregulated in breast and renal cell cancers, as compared with normal tissue [32, 33]. PIWIL4 can also induce cervical cancer cell invasion through inhibiting p14/ARF and p53 expression [34].

1.2. Deregulation of piRNAs in cancer

Compared to PIWI proteins, few literatures are available about piRNAs in carcinogenesis. Despite numerous piRNAs are generated in the human genome, only a small number is consistently expressed in normal and tumor tissues [1]. With the previously established evidence on PIWI proteins deregulation in cancer, the hypothesis that piRNAs are also aberrantly expressed in various cancers is very likely because piRNAs are pivotal part of the pi-RISC effector complexes that allow recognition of TEs. Deregulation of piRNAs that target mRNA transcripts containing TE-derived sequences could induce alterations of TSGs and oncogenes [15]. Deregulation of both PIWI proteins and molecules implicated in biogenesis of the PIWI-piRNA are also in favor of a driver role for piRNAs in cancer. Recent evidence suggests that piRNAs control transcriptional and post-transcriptional genes regulation in cancer cells through epigenetic mechanisms associating global DNA hypomethylation, gene-specific DNA hypermethylation, and histones hypoacetylation, which result in oncogenes activation, TSG repression, genomic silencing, and induction of a stem-like state [35, 36]. Furthermore, piRNAs drive carcinogenesis by using nonepigenetic mechanisms such as cell cycle deregulation, proliferation, and invasion [37, 38]. Differential expressions of few piRNAs were reported between malignant tumors and normal tissues. Transcriptomic analysis of 6260 human piRNAs from 11 types of tumors and normal tissues has revealed that among the 20,831 known piRNAs, only 522 piRNAs were expressed in tumor tissues in a cancer-type specific manner, whereas 273 piRNAs were expressed in normal tissues [39].

The first examples of piRNAs deregulated in cancer were piR-651, piR-823, and piR-932. PiR-651 was aberrantly overexpressed in numerous tumors, such as breast, gastric, colon, and lung cancers, compared to normal tissues. By using small RNA sequencing techniques in breast cancer, over 100 deregulated piRNAs were identified in tumors compared to normal tissues, including piR-34736, piR-36249, piR-35407, piR-36318, piR-34377, piR-36743, piR-36026, and piR-31106. Among them, piR-36743, piR-36026, and piR-31106 were overexpressed, whereas piR-34736, piR-36249, piR-35407, piR-36318, and piR-34377 were underexpressed [33]. Furthermore, estrogen deficiency and the estrogen receptor ERβ controlled metastatic spread of breast cancer cells by directly modulating piRNA expression. Eight piRNAs were significantly differentially expressed between breast tumors and normal tissue, with overexpression of piR-4987, piR-20365, piR-20485, and piR-20582 correlated with lymph node metastasis [33]. In lung cancer, 555 piRNAs were differentially expressed between lung adenocarcinomas and squamous cell carcinomas and normal bronchial tissue. The most frequently underexpressed piRNA in lung cancer was piR-L-163, localized in intron 10 of the *LAMC2* gene. PiR-651 promoted cyclin D1 and CDK4 overexpression, resulting in G1 phase entry [38]. In gastric cancer, piR-823 underexpression and piR-651 overexpression were initially identified by using piRNA microarray and PCR compared to matched nonmalignant tissues. PiR-651 overexpression induced transition from G2 to M phase [40]. A total of 156 piRNAs, including piR-32105, piR-58099, and piR-59056, were significantly differentially expressed by using small RNA sequencing from a series of 320 carcinomas and 38 nonmalignant tissues [1]. In liver cancer, piR-Hep1 was overexpressed compared to normal liver tissues. PiR-Hep1 levels of expression positively correlated with

PIWIL2, suggesting that piR-Hep1 may interact with PIWIL2 to induce carcinogenesis [41]. In pancreatic cancer, piR-017061, localized within HBII-296A snoRNA, was shown to be underexpressed compared to normal pancreatic tissues [42]. In leukemias, piR-823 inhibited p16 expression through activation of DNMT3A and DNMT3B, resulting in upregulation of cyclin D1 and CDK4. PiR_011186A downregulated p15 expression and thus promoted cyclin D1 and CDK4 upregulation through edification of a molecular complex combining DNMT1, EZH2, and Suv39H1 [44]. In kidney cancer, study of 24 tumors revealed that 19 piRNAs were differentially expressed between clear cell renal cell carcinomas and benign tissues. Furthermore, 46 piRNAs were differentially expressed between primary and metastatic carcinomas. Among piRNAs deregulated in renal cell metastatic carcinomas, three overexpressed piRNAs (piR-32051, piR-39894, and piR-43607) were localized on the same piRNA cluster on chromosome 17 [43]. In multiple myeloma, piR-823 was significantly underexpressed in tumors, compared to normal tissues [44]. Furthermore, numerous PiRNAs, including piR-Hep1, piR-651, piR-823, piR-932, piR-L-163, piR-4987, piR-20365, piR-20485, piR-20582 and piR-ABC, were associated with hallmarks of cancer and could be pertinent diagnostic and prognostic biomarkers [40, 41, 45].

1.2.1. Proliferation, survival, and apoptosis

In bladder cancer, piRNAs microarray study identified 106 overexpressed piRNAs and 91 underexpressed piRNAs, including piRNA DQ594040. PiRABC repressed cancer cells proliferation and increased cell apoptosis through TNFSF4 protein overexpression. In multiple myeloma, PiR-823 was upregulated and implicated in proliferation, apoptosis, cell-cycle regulation, and angiogenesis related to DNMT3A and p16-INK4A repression and associated with advanced clinical stage [44]. PiRNA-823 repression promoted deregulation of cell cycle regulators and apoptosis-related proteins and inhibited pro-angiogenic activity [44]. In glioblastoma, piR-598 promoted cancer cells survival and proliferation [46]. In lung carcinoma, piR-55490 underexpression was associated with increased proliferation through reduction of piRNA-induced 3'UTR mTOR mRNA binding and degradation. Furthermore, inhibition of piR-L-163 enhanced DNA synthesis and promoted tumor cells survival and proliferation [38, 47].

1.2.2. Invasion and metastatic spread

In breast cancer, piR-4987 upregulation was significantly associated with lymph node metastasis and PIWIL2-piR-932 complex enhanced EMT through Latexin methylation of its promoter region [31, 48, 49]. In clear cell renal cell carcinoma, 19 piRNAs were differentially expressed between tumor and normal tissues and 46 piRNAs were associated with metastasis and poor survival [43]. PiRNA microarray analysis revealed 235 piRNAs upregulated and 369 piRNAs downregulated in malignant tissue from 106 patient samples. RT-qPCR analysis confirmed that piR-57125 was downregulated in metastatic tumors, whereas piR-30924 and piR-38756 were upregulated [50]. In gastric cancer, downregulated blood levels of piR-823 were correlated with stage, lymph node, and distant metastasis, suggesting that piRNAs may be pertinent blood biomarkers [51]. Furthermore, PiR-651 inhibition promoted growth suppression and cell cycle arrest at the G2/M stage [40]. In hepatocellular carcinoma (HCC), deep sequencing analysis identified oncogenic piR-Hep1 upregulation associated with PIWIL2 overexpression. Inhibition of piR-Hep1 repressed Akt phosphorylation, motility, and

invasion [41]. In pancreatic ductal adenocarcinoma, piR-017061 located within HBII-296A snoRNA was found to be downregulated in tumors compared to normal tissues [42]. In lung cancer, PiR-651 overexpression promoted survival, invasion, and metastatic progression through cyclin D1 and CDK4 overexpression [70]. In colon cancer, PiR-823 upregulation was correlated with lymph node and distant metastasis, whereas in gastric cancer, piR-823 overexpression suppressed tumor cell growth [1, 69].

1.3. Mechanisms of the PIWI-piRNA pathway deregulation in cancer

Few studies have identified molecular mechanisms implicating PIWI proteins and piRNAs reactivation in carcinogenesis [52, 53]. The first hypothesis concerning deregulation of the PIWI-piRNA pathway in cancer results from its control of TEs propagation. In normal germline stem cells, piRNAs cooperate with abundant PIWI proteins to regulate TEs inactivation *via* DNA methylation. During embryonic development, these cells containing high levels of PIWI proteins develop into various somatic tissues in which PIWI proteins are not normally expressed. In cancer, recent studies have demonstrated that methylation of TEs was decreased, whereas TEs transcripts and proteins were upregulated and correlated with higher metastasis frequency [54, 55]. Prolonged exposure to detrimental environmental factors may promote TEs mobilization with disruption of various TSGs *via* TEs

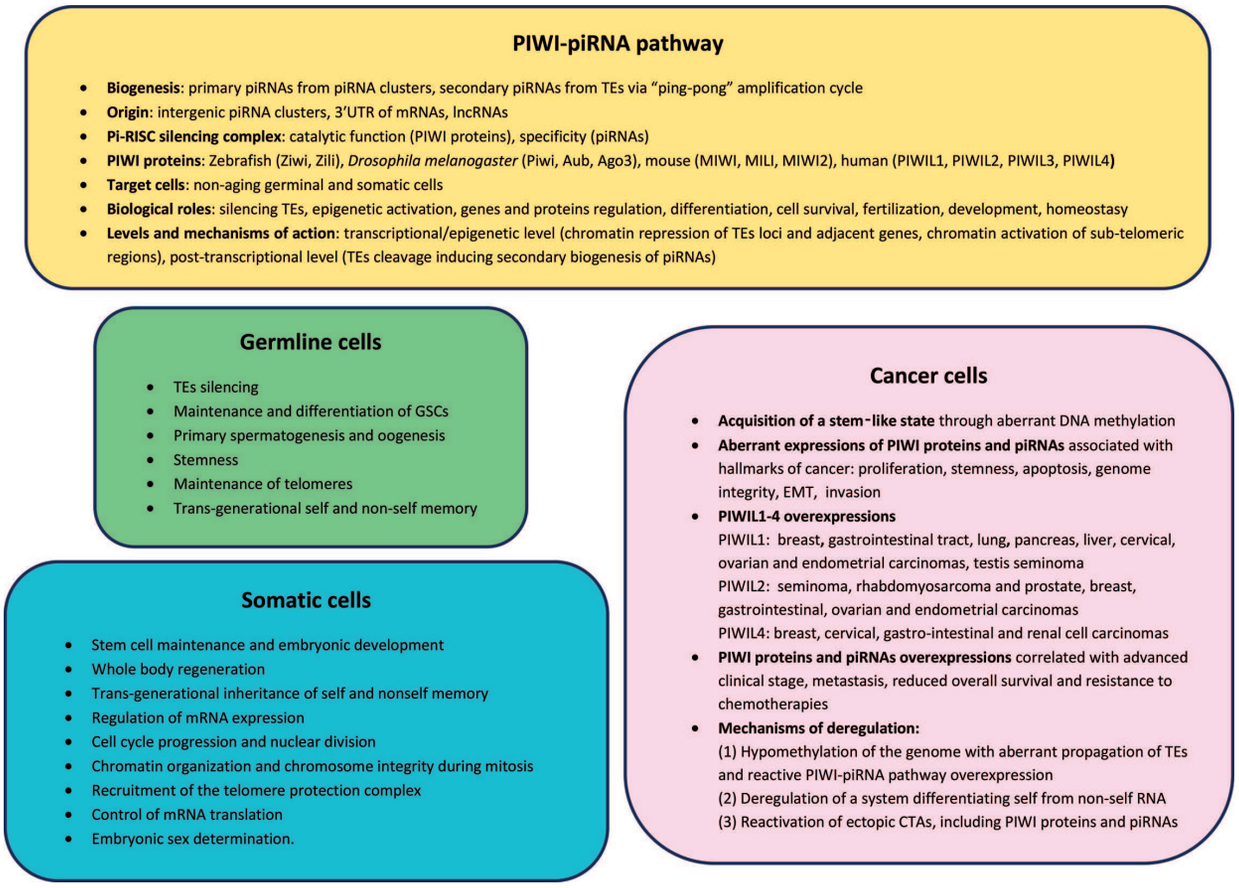

Figure 1. The PIWI-piRNA pathway in germline and somatic cells, and mechanisms of the PIWI-piRNA pathway deregulation in cancer cells.

integration [56]. A widespread hypomethylation of the genome during progression could induce both aberrant reactivation and propagation of TEs with reactive ectopic overexpression of the PIWI-piRNA pathway. High levels of piRNAs and PIWI proteins observed in somatic cancers could promote abnormal extensive DNA methylation, resulting in silencing of TSGs and acquisition of an aberrant stem-like state [57, 58]. The hypothesis that piRNAs guide PIWIL1 and PIWIL2 to transcriptionally silence TSGs is consolidated by analysis of their orthologs in mice, in which MILI and MIWI2 mutants fail to establish DNA methylation of TEs [59]. Furthermore, by suppressing expression of particular TEs, aberrant reactivation of the PIWI-piRNA axis in cancer may induce genomic and chromosomal instability. Thus, this pathway could be implicated in cancer not only in repression of TEs but also in inactivation of various mRNAs not exposed to germline cells. The second hypothesis is the participation of this pathway in a putative biological system that can differentiate self from nonself RNA [60]. The third hypothesis is the ectopic and coordinated reactivation and overexpression in cancer of cancer/testis antigens (CTA) normally restricted to the germline and including molecules of the PIWI-piRNA axis. As observed in normal gametogenesis, CTAs promote immortalization, implantation, and migration that could correspond in carcinogenesis to transformation, invasion, and metastasis, respectively (**Figure 1**). In this ectopic context, reactivation of the PIWI-piRNA pathway could confer properties of proliferative germline stem cells to cancer cells [61–68].

2. PIWIs and piRNAs as potential biomarkers and therapeutic tools

2.1. PIWI proteins as biomarkers

PIWIL1 overexpression was correlated with poor 5-year survival in malignant glioma, hepatocellular, gastric cancer, colon cancer, pancreatic ductal adenocarcinoma, and ovarian cancer [1]. In hepatocarcinoma, PIWIL1 high levels of expression were correlated with larger tumor size, intrahepatic metastasis, overall survival, and recurrence-free survival [41]. PIWIL2 upregulation was observed in breast cancer, colon cancer, gastrointestinal stromal tumors, renal cell carcinoma, and endometrial carcinoma. In colorectal cancer, PIWIL2 overexpression was associated with aggressive clinico-pathological characteristics and poorer prognostic [30]. In esophageal cancer, PIWIL2 was significantly correlated to high histological grade, advanced clinical stage, and poorer clinical outcomes. Furthermore, PIWIL2 upregulation was identified in a cancer cell subpopulation expressing OCT4 and NANOG stemness factors, suggesting a role in stem-cell maintenance and self-renewal. In breast cancer, PIWIL2 was mainly located in cancer stem cells and showed different expression patterns, with slight nuclear localization in preneoplastic lesions and cytoplasmic/nucleus topography in invasive and metastatic cancers. Its upregulation was correlated with poorer survival [33]. PIWIL2 can promote invasion and metastasis among various cancers by increasing c-Myc expression through binding of NM/NM23 nucleoside diphosphate kinase 2 (NME2) to G4-motif region within c-Myc. In hepatocellular carcinoma, nuclear co-expression of PIWIL2 and PIWIL4 had a worse prognostic phenotype [71]. In ovarian cancer, PIWIL2 overexpression was associated with cisplatin resistance and poorer prognostic [28]. PIWIL3 and PIWIL4 overexpression were associated with

worse prognosis in breast cancer. In gastric cancer, PIWIL1-4 upregulation was significantly associated with TNM stage histological grade and lymph node metastasis [15, 40, 62].

2.2. PiRNAS as biomarkers

PiRNAs are increasingly investigated as diagnostic and prognostic biomarkers [69]. PiRNAs are small RNAs of only 24–32 nt in length which can pass through cell membrane. Like miRNAs, piRNAs are not degraded in circulation and possess the capability to resist incubation and storage conditions used in laboratories. They are thus detectable in patient samples like blood plasma and serum, saliva, sputum, and urine. Furthermore, piRNAs possess higher sensitivity and specificity compared to an existing lncRNA and miRNA-based biomarkers. They are highly expressed in germinal tissues, but also although at lower levels in various somatic normal tissues, saliva, and plasma-derived exosomes. Furthermore, piRNAs present higher specificity and sensitivity, when compared to miRNAs. In breast cancer, 8 piRNAs were identified as independent prognostic markers and associated with overall survival [63]. Moreover, piR-4987 upregulation in peripheral blood was correlated with lymph node metastasis [48]. In gastric cancer, piR-651 high level of expression in peripheral blood was associated with poor differentiation, advanced TNM stage, and metastasis. A three-piRNA signature, including piR-59056, piR-54878, and piR-62701, could separate patients by risk of recurrence [5]. PiR-651 was also upregulated in other cancers, including lung, colon, and breast tumors. In clear cell renal cell carcinoma, piR-30924 and piR-57125 were shown to be independent prognostic predictors in nonmetastatic patients. In colon cancer, piR59056, piR-54878, and piR-62701 were associated with recurrence-free survival. Few studies have investigated on interactions between piRNAs and PIWI proteins in cancer [70, 72–75]. Actually, it remains unclear whether PIWI proteins independently possess cytoplasmic pro-oncogenic properties that promote cancer cell proliferation, invasion, and metastasis or PIWI proteins located in the nucleus epigenetically modulate numerous biological processes by edifying ribonucleoproteic complexes with piRNAs.

2.3. PIWI proteins and piRNAs as therapeutic tools

Recent studies have revealed the potential for piRNAs as therapeutic tools that target PIWI proteins and oncoproteins [71]. Mounting evidence has revealed that upregulation of PIWI proteins was negatively correlated with patient survival, and that downregulation of PIWI proteins could reduce the number of G2/M phase cells and enhance expression of p53 protein, thus inhibiting proliferation and promoting apoptosis. In addition, PIWI proteins could increase resistance to chemotherapy drugs such as cisplatin, and their downregulation could increase sensitivity of cancer cells to cisplatin. In this way, synthetic piRNAs targeting PIWI genes are potential pertinent tools in transcriptional silencing during cancer progression, and PIWI antibodies targeting PIWI proteins constitute another approach to antagonize cancer cells proliferation at post-transcriptional level. Synthetic piRNAs could also block synthesis of cancer-related proteins by binding to mRNAs. Compared to miRNAs, piRNAs do not require enzymes processing and have better specificity to targets. In a mouse model, artificial piRNAs could be obtained

through expression of sense and antisense transcripts, resulting in epigenetic silencing of target genes [1, 72]. Furthermore, piRNAs could be considered as tools to regulate expression levels of TSGs. A lncRNA (*GAS5*)/SnoRNA-derived piRNA enhanced activation of *TRAIL* gene by site-specifically recruiting MLL/COMPASS-like complexes with induction of H3K4 methylation and H3K27 demethylation, resulting in transcriptional activation of *TRAIL* and inhibition of tumor growth [64].

3. Conclusion and perspectives

PiRNAs and PIWI proteins were first recognized more than a decade ago and are coming into attention with development of high-throughput sequencing technologies and bioinformatics methods. This pathway is considered as a conserved immune-like surveillance process to suppress propagation of TEs in germline cells and various types of somatic mostly nonaging cells. Furthermore, the PIWI-piRNA pathway seems to be implicated in maintain of the genome organization, epigenetic modifications of genes expression, and identification of self and non-self genes that are trans-generationally inherited. Moreover, this axis could be implicated in dual DNA/RNA-level regulation of genes expression. Nevertheless, there is still lack of complete understanding of the functions and interactions of piRNAs and PIWI proteins. Therefore, the complicated biogenesis and functions of piRNAs need further elucidation to improve our understanding of the implication of these molecules in cancer. Since discovery of the unexpected role of this pathway in seminoma, aberrant levels of expression of these molecules have been observed across numerous malignant tumors, though further research is needed to elucidate their oncogenic or tumor-suppressing status. Growing evidence suggests that the PIWI-piRNA pathway modulates occurrence of most of cancer hallmarks. PIWI proteins and piRNAs could be pertinent diagnostic/prognostic biomarkers in cancer and therapeutic tools in targeted therapies. However, the potential driver role of a deregulated PIWI-piRNA pathway in cancer needs to be further evaluated. Furthermore, it remains unclear whether PIWI proteins regulate cancer cell proliferation, apoptosis, metastasis, and invasion in the cytoplasm independently or PIWI proteins perform epigenetic control of homeostasis by taken to the nucleus with piRNAs. Most importantly, since the aberrant expression of this pathway may induce stemness, analysis of relationship between PIWI proteins, piRNAs, and cancer stem cells may open new avenues in future investigations.

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Disclosure/conflict of interest

The authors declare that they have no competing interests.

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