Epigenetic roles of PIWI-interacting RNAs (piRNAs) in cancer metastasis (Review)

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Abstract. P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs) are epigenetic-related short ncRNAs that participate in chromatin regulation, transposon silencing, and modification of specific gene sites. These epigenetic factors or alterations are also involved in the growth of a variety of human cancers, including lung, breast, and colon cancer. Accumulating evidence has revealed that tumor metastasis and invasion involve genetic and epigenetic factors. Cancer metastasis is characterized by epigenetic alterations including DNA methylation and histone modification. Changes in DNA methylation, H3K9me3 heterochromatin and transposable elements have been detected in several cancers. piRNAs may function in gene silencing and gene modification upstream or downstream of oncogenes in cancer cell lines or cancer tissues. In addition to piRNAs, PIWI proteins can be used as biomarkers for prognosis, diagnosis and clinical evaluation and may be factors in cancer metastasis. Here, we elucidated the possible mechanisms by which piRNAs regulate cancer metastasis, including but not restricted to influencing DNA and histone methylation and transposable elements.

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1. Introduction

P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs) belong to a new class of ncRNAs that have been associated with many cancers (1). piRNAs are involved in the gene regulation process in which certain nucleotides bind coding regions in gene promoters (2). piRNAs function in the epigenetic regulation of DNA methylation (3), transposable silencing and chromatin modification (4). PIWI is a type of Argonaute protein that binds to piRNAs and carries out unique functions in somatic and germ cells, and stably expressed piRNAs have also been detected in human blood (5). piRNAs may be used as biomarkers for cancer diagnosis (6). Four PIWI proteins have been discovered in humans: PIWI1, PIWI2, PIWI3, and PIWI4 (7-10). Furthermore, PIWI expression levels are associated with different types of cancers and clinical stages (11-13). Additionally, piRNAs have been linked to proliferation, apoptosis (14), genomic instability (15), invasion, and metastasis (16) in cancer cells. The levels of PIWI and piRNAs were revealed to be significantly altered between tumor tissues and non-tumor tissues. The clinical pathological features of tumors are associated with PIWI and piRNA expression. Therefore, additional studies are needed to understand the role of piRNAs in cancer and their epigenetic mechanisms and to shed light on the potential of piRNAs for the diagnosis and prediction of clinical cancer stages.

Cancer formation involves genetic, epigenetic, and pathological mechanisms (17). Cancer is a complicated disease with distinct genetic, epigenetic and pathological features (18). Despite recent advancements in precision medicine, the assessment of pathological and clinical features remains the primary and most accurate method for diagnosing carcinoma. Preventing tumor metastasis is still a formidable problem in the world. Carcinogenesis involves genetic machineries that are present beginning in early childhood but are altered over time. Abnormal and inappropriate epigenetic alterations can regulate carcinoma development (19). Activation of oncogenes and inactivation of tumor suppressors or other cell factors and pathways cannot fully account for metastatic cancers. Under

certain circumstances, epigenetic mechanisms related to piRNAs may be responsible for the downstream inactivation of genes in tumors (20). Epigenetic modifications alter gene expression rather than changing the DNA sequence (21). These alterations are based on DNA methylation and histone protein modification, and non-coding RNAs (ncRNAs) frequently participate in this process.

2. Epigenetic functions of PIWI and piRNAs

piRNAs play crucial roles in protecting genomic stability by inhibiting transposon activity and maintaining minimum levels of transposons in germ line, mammalian cells and other cell types (22). piRNAs reside in clusters within heterochromatin-euchromatin boundaries and exhibit repeat-rich regions with ancient fragmented transposon copies. Amplification of piRNAs occurs through the 'ping-pang' cycle (23). This cycle is initiated by the emergence of a primary piRNA from piRNA clusters. Primary piRNAs are antisense sequences to expressed transposons and have the ability to cleave their targets, while secondary piRNAs form the AGO3 complex. The AGO3-bound piRNAs interact with transposon targets, which include antisense transposon sequences. This interaction then produces antisense piRNAs, and the 'ping-pang' cycle continues.

PIWI proteins have been reported to be selectively expressed in precancerous stem cells, tumor cell lines and cells of various malignancies (24). PIWI and Aubergine (Aub) proteins accumulate in the pole plasm and transfer maternal piRNAs into germ line cells (25). It was previously believed that PIWI proteins could maintain genomic integrity in animal germ cells by silencing transposons. Without these proteins, piRNAs cannot inactivate transposons within a single generation. In mammals, the PIWI proteins MILI, MIWI2 and intracisternal A-particle (IAP) act as retrotransposons and function as transposon-inhibiting factors via transposon gene silencing (TGS) (26). MILI is active in the cytosol, and MIWI2 is active in the nucleus (27). MILI, also known as PIWIL2, and MIWI2, also known as PIWIL4, suppress transposons in the cytoplasm and nucleus. MIWI is a slicer similar to Argonaute family members, which include PIWI proteins. The function of the slicer MIWI depends on its binding motif, which has a conserved Argonaute domain sequence (27). A high degree of MIWI complementarity is required for piRNA targeting. MIWI-associated piRNAs endonucleolytically cleave their target RNAs. Therefore, silencing of transposons depends on the piRNAs involved. Using next-generation sequencing, we can analyze histone modifications and methylation across entire genomes. Moreover, we can identify ncRNAs that participate in these regulatory processes. This technology provides a link between epigenetic modifications and transcription (28).

3. Epigenetic functions of specific transposable elements

Transcriptional silencing, heterochromatin formation, transgene silencing, HP1α alterations, histone modifications and transposon suppression are all associated with PIWI, piRNAs, the piRNA pathway and specific transposable elements (29).

As mediators of eukaryotic evolution, transposable elements are classified as either retrotransposons or DNA transposons. Notably, retrotransposons can cause genomic variations (30), alter chromatin structures and change the expression of nearby genes by integrating into genomic locations. Retrotransposons include long terminal repeat (LTR) and non-LTR retrotransposons. LTRs are similar to retroviruses in their structure, and non-LTRs are similar to mRNAs (31).

LTRs can encode structural proteins to form virus-like particles (VLPs), which can regulate gene transcription. RNA derived from LTRs can be reverse-transcribed into cDNA and can thereby integrate into the genome (32). RNA polymerase II located at the 5'end of LTRs can transcribe LTRs. These RNA molecules are then packaged into viral particles and use the reverse transcription machinery to generate full-length DNA. The first priming event occurs between the 5'end and the 3'end of LTR, and the second priming event occurs near the 3'end of LTR leading to production of a double-stranded cDNA molecule through an additional strand transfer. Thus, the cDNA is integrated into the host genome. The main difference between retrotransposons and infectious retroviruses is the presence of the envelope (env) gene in the latter, which enables viruses to infect other cells. In contrast, exon open reading frames (ORFs) are found in retrotransposons and retroviral genomes (33).

Non-LTRs are classified as either autonomous non-LTRs, such as LINEs, or non-autonomous LTRs, such as short interspersed nuclear elements (SINEs) (34). Retrotransposition-competent LINE1 includes a 5'-untranslated region (UTR) that is rich in CpG-islands; ORF1, which binds to RNA and ORF2, which encodes proteins such as endonuclease, reverse transcriptase and cysteine-histidine-rich domains. Similar to mammalian RNAs, LINE1 RNA has a poly (A) tail at the 3'-UTR. RNA polymerase II transcribes ORF1 and ORF2 in the cytoplasm (35).

Mutations are generated during the process of evolutionary change. Proofreading polymerases repair damaged DNA sequences and eliminate potential mutations. However, some piRNAs can also mediate the activity of transposable elements (36). HP1, a chromatin-organizing protein, can affect transposon activity by regulating piRNA expression or by directly mediating the expression of transposons. We can therefore conclude that some chromatin-organizing proteins, such as HP1, act upstream or downstream of piRNAs to regulate transposons. Mutations in Aub, PIWI, and Su(var)205 are known to increase the activity of transposable elements in germline cells (37,38) (Fig. 1).

4. Epigenetic functions of specific piRNA pathway proteins

Uncontrolled transposons threaten genomic integrity, and these alterations can be transferred to the next generation. piRNAs bind with their partner PIWI to recognize and silence transposable elements in germ cells. Both cytoplasmic and nuclear PIWI proteins target the genome to mediate transcriptional silencing. In mice, transposon inhibition by piRNAs occurs via DNA methylation at CpG islands in the sequences of transposable elements. During this process, the piRNA pathway mediates and maintains high levels of the repressive H3K9me3 mark in LINE regions in germ cells. Furthermore, piRNAs recognize full-length elements of the actively transposing LINE family (39).

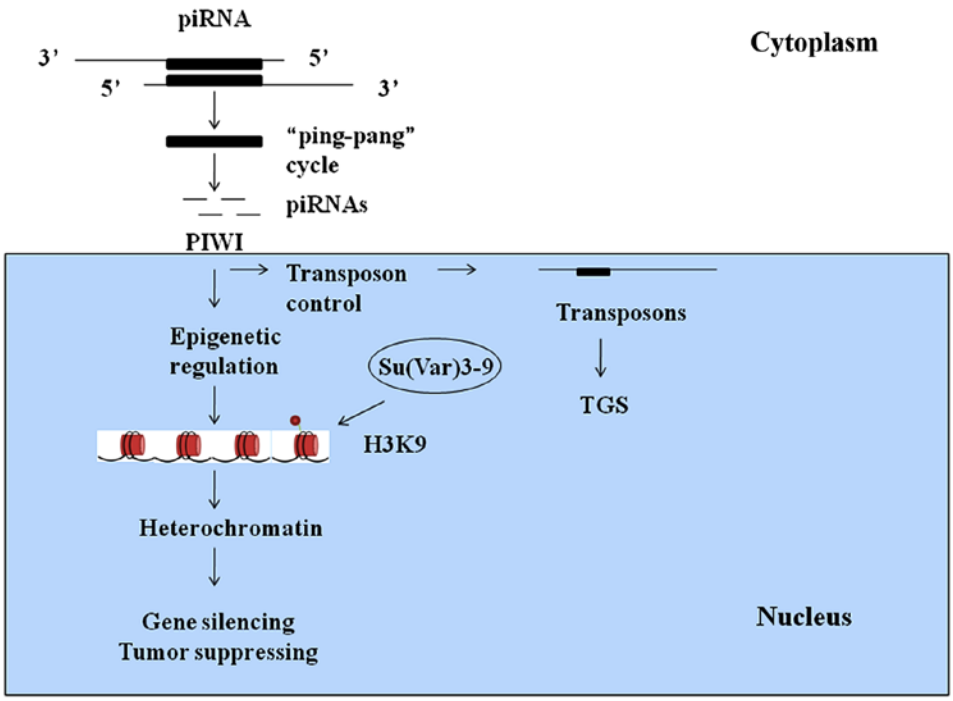

Figure 1. piRNAs bind to PIWI proteins to induce epigenetic regulation and transposon control.

Scientists recently identified two piRNA pathway proteins that are related to transposon silencing (40). The first, CG9754, is a downstream piRNA pathway factor that participates in the nuclear PIWI-piRNA complex involved in transcriptional silencing and heterochromatin formation. CG9754 is the first protein to target RNA or DNA, in heterochromatin and transcriptional silencing (41-43). PIWI is only able to silence downstream proteins if it is bound to piRNAs that are engaged with target genes (44-46). Recruitment of CG9754 directly to DNA or indirectly to RNA results in potent transcriptional silencing. Thus, CG9754 is sufficient to induce transcriptional silencing by binding to RNA. In ovarian somatic cells (OSCs), CG9754 is a downstream protein of PIWI (47-49). When vectors were used to delete CG9754, a decrease in the level of H3K9me3 was observed, and the insertion of transposable elements into transcriptional genomic regions was repressed. CG9754 mediates heterochromatin formation and integrates other cell factors, such as HP1α, which induces transcriptional silencing. SetDB1 is downstream of HP1α and CG9754, an important HMT effector (50-52). EXD1 is the second important piRNA pathway protein. MILI slicing acts as a switch that initiates piRNA processing. Slicing activates different types of primary piRNAs in MILI and MIWI. EXD1 is a component of the PET (PIWI-EXD1-TDRD12) complex, which mediates transcriptional gene silencing (53).

5. piRNAs and PIWI with cancer

piRNAs exhibit a long lifetime in cells, even though they are only 24 to 30 nt long (54), indicating that they are not easily degraded and may exist in cell nuclei and cytoplasm for a longer time than other RNAs (55). piRNAs play an important role in cancer development (56). Real-time PCR and next-generation sequencing analyses have made the relationship between piRNAs and carcinogenesis increasingly clear (11). Compared with non-cancerous tissues, the expression of piR-651, piR-823, piR-4987, piR-20365, piR-20485 and piR-20583 was revealed to be altered in cancer cell lines (12,13).

piRNAs can serve as biomarkers for the prognosis, diagnosis and clinical evaluation of cancer (8). The expression level of piR-823 in gastric cancer tissues was revealed to be lower than that in non-cancerous gastric tissues (57). A transfection-mediated increase in the piR-823 level inhibited the growth of gastric cancer cells. These results were also observed in nude mice. Thus, piR-823 may be a potential marker for cancer diagnosis (58).

The expression levels of PIWIL2, PIWIL4, and piR-823 were associated with the tumor-node-metastasis (TNM) stage of renal cell carcinoma (10). Additionally, increased piR-4987 expression regulated lymph node metastasis in breast cancer.

Here, we present an example to clarify the biomarker characteristics of piRNAs. Through small RNA sequencing and real-time PCR analysis of frozen benign kidney tissues and renal cell carcinoma tissues, 26,991 piRNAs were revealed to be expressed in kidney tissues (59). In the tumor samples, 19 types of piRNAs were found to be deregulated, including 2 types that were upregulated and 17 types that were downregulated. Furthermore, differentiation was much more obvious in the metastatic renal cell carcinoma samples (16). By comparing the localized tumor samples to the metastatic samples, 46 piRNAs were found to be aberrantly expressed, 44 of which were upregulated, while only 2 were downregulated. The increased piR-32051, piR-39894, and piR-43607 are similar in length, highly homologous and derived from the same piRNA cluster on chromosome 17. Increased expression of these piRNAs is found in late-stage tumors, which means they are highly associated with renal cell carcinoma metastasis (60).

Significantly increased piR-651 levels have been observed in non-small cell lung carcinoma (NSCLC). In A549 cells, an NSCLC cell line, piR-651 increased cell viability and metastasis. The expression of piR-651 in A549 cells decreased the proportion of cells arrested in the G0/G1 phase, thereby promoting proliferation. Oncogenes and tumor suppressor genes can be detected, and piR-651 was revealed to promote cancer growth via cyclin D1 and CDK4. These results have also been verified in lung cancer tissues from patients (61).

piR-1245 has been revealed to be overexpressed in colorectal cancer, lung, breast, stomach, bladder, kidney and prostate cancer, indicating its significant role in carcinogenesis. Poor differentiation, advanced T stage, lymph node and distant metastasis were revealed to be closely related to higher expression of piR-1245 in colorectal cancer (CRC). piR-1245 also played a crucial role in clinical pathology and revealed poor overall survival (OS) in colorectal cancer patients. piR-1245 directly targeted ATF3, BTG1, DUSP1, FAS, NFKBIA, UPP1, SESN2, TP53INP1 and MDX1 to regulate tumor progression. Thus, it may be a prognostic biomarker in CRC (62).

Similar to miRNAs, which are stable in tissues, piRNAs are relatively stable and can be used to obtain reliable results in quantitative piRNA expression studies (63). piRNAs can be detected in patient plasma (64), suggesting their potential use as biomarkers to predict TNM stage and disease prognosis (65). Furthermore, piRNAs can serve as a switch allowing tumors to proliferate and metastasize (66). Additionally, piRNAs can be indicative of patient outcomes and can be helpful in selecting effective surgical methods, radiotherapy and chemotherapy to prolong patient survival time (67).

PIWI proteins have been found in human cancers, such as breast, lung, gastric, hepatocellular, colon, renal cell carcinoma, endometrial and ovarian cancer. PIWI acts via a distinct pathway to regulate carcinogenesis. PIWIL can affect transcription, causing an increase in Bcl-XL, Stat3 and cyclin D1 expression (68).

Cancer stem cells (CSCs), contain epigenetic alterations and signaling pathways characteristic of stem cells including self-renewal capacity, rapid proliferation and multiline age differentiation. PIWIs may be cancer testis antigens (CATs) and act as oncogenes or constitute markers for CSCs (69). Metastatic cancer cells appear to undergo epithelial-mesenchymal transition (EMT) and CSC-like phenotype (70). PIWIL2 expression was associated with altered expression of EMT markers.

As a member of the PIWI gene family, MILI binds to piRNAs and plays multiple roles in gene silencing (71) and chromatin remodeling (72). Transposon methylation has been observed in tumor cell lines and many types of human cancer (73-75). MILI was revealed to control the activity of LINE1 by methylating its CpG island (76). The hypomethylation of LINE1 increased the risk of cancer development and may be an indicator of cancer grade and lymph node metastasis (77,78). Wang *et al* found that MILI affected melanoma cell metastasis and cancer-related gene expression by regulating LINE1 methylation (79). MILI is expressed in the melanoma cell line B16 but not in the highly metastatic mouse melanoma model B16BL6. Notably, knockdown of MILI in B16 cells activated MAGEA expression and increased cell migration, whereas MILI overexpression in the B16BL6 model inhibited MAGEA expression and decreased cell migration, yielding the opposite results (65,80). Depletion of MILI/MIWI2 in mice led to reduced DNA methylation of LTR-retrotransposon promoter regions (81). Thus, LINE1 methylation by MILI was revealed to controls the expression of cancer-related genes and cell migration, and MILI plays a key role in melanoma metastasis and tumor progression.

6. Epigenetic mechanisms of cancer metastasis related to piRNAs

The function of piRNAs in tumors is related to transposable elements and changes in chromatin structures that are caused by epigenetic alterations, such as DNA hypomethylation (60). For example, in HeLa cells, piRNAs play an important role in inhibiting transposons by interacting with the HILI protein (82). The relationship between piRNAs and epigenetics is elaborated above, and the epigenetic changes account for a large proportion in tumors. Perhaps piRNAs and transposable elements, upstream or downstream of epigenetic alterations in tumors, affect the metastasis ability of tumors. Next, we will illustrate the epigenetic mechanisms of cancer metastasis related to piRNAs.

Cancer is a genetic and epigenetic disease (83). Cancer cells have the ability to invade tissues, enter systemic circulation, and extravasate into surrounding interstitial tissues, resulting in distant capillary retention (84). The metastasis phenotype is associated with epigenetic alterations that are involved in the cancer metastasis process. Metastasis involves cellular invasion, migration and angiogenesis of the primary carcinoma. Detection of genetic or epigenetic abnormalities can be used to identify epigenetic alterations in lesions that are morphologically normal (85). Understanding the epigenetic mechanisms of tumor metastasis related to piRNAs can assist in the identification of new tumor markers and treatments (86).

Epigenetic evaluations involve examination of DNA methylation profiles, certain RNA expression profiles (87) and histone modification profiles (88). Epigenetic alterations do not involve changes in DNA sequence (89). DNA methylation and histone modification are the major types of epigenetic alteration (90). In normal tissues, DNA methylation can prevent X chromosome activation and gene mutations (91), and histone modifications can dynamically regulate gene activity.

Here, mechanisms related to epigenetic alterations are briefly summarized. First, DNA methylation not only affects the expression of individual genes but also affects DNA domains by interacting with nucleosomes, thus altering DNA packaging. DNA methylation is inevitable and occurs on the cytosine of CpG dinucleotides. In mammalian cells, methylation is conferred by four main DNA methyltransferases (DNMTs): DNMT1 (92), DNMT3A (93), DNMT3B (94,95) and DNMT3L (96). DNMT1 adds methyl groups to hemi-methylated CpG sites, DNMT3A and DNMT3B methylate novel CpG sites, and DNMT3L interacts with DNMT3A and 3B to facilitate methylation of retrotransposons. DNA demethylation and remethylation comprise a balanced process that is disrupted in cancer cells (97). Tumor progression and metastasis occur due to changes in DNA methylation. Studies investigating DNA methylation in promoter regions have been

fruitful, leading to the discovery of adenomatous polyposis coli (APC), retinoic acid receptor β-2 and H-cadherin (98), which are also associated with tumor progression (99). In primary testicular tumors, scientists detected a gain of 5'end promoter CpG island methylation of the PIWIL1, PIWIL2, PIWIL4 and TDRD1 genes in association with transcriptional silencing. The DNMT3L/PIWIL2/TDRD1 complex is responsible for the loss of DNA methylation at LINE1 and IA transposons (100). The extent of DNA methylation in tumor tissues is lower than that in normal tissues, and the degree of DNA hypomethylation increases with the progression of malignancy. DNA hypomethylation is conducive to mitotic recombination, leading to chromosomal deletions and translocations, which promote chromosomal rearrangements (101). DNA methylation in malignant cells can reactivate genomic DNA repeat elements, such as long interspersed element 1 (LINE1) (77) and Alu (102). These demethylated transposons can be transcribed or transposed to other genomic regions and disrupt the genome (103). Transposable elements, which are abundant in the human genome, are highly mutagenic due to their ability to target protein-coding genes for insertion, resulting in chromosome breakage and promoting illegitimate genome rearrangement.

Another mechanism related to cancer is histone modification. There are two types of nuclear chromatin, namely, heterochromatin and euchromatin (104). In normal tissues, heterochromatin is stable during various cell cycle phases and silences during transcription (105), and the genes in euchromatin are actively transcribed. As part of an interplay with DNA methylation, facultative heterochromatin, which is associated with allelic exclusion, genomic imprinting, X chromosome stabilization (106), immunoglobulin (Igh/Igk) and T-cell receptor-α and -β (107) gene loci, is vital for normal cell lineage development and cell differentiation via somatic methylation and inactivation of germline-specific genes (108).

Histone 3 methylation and heterochromatin. Initiation, propagation and maintenance of heterochromatin are largely controlled by trimethylation of lysine 9 on histone H3 (H3K9me3) and other synergistic epigenetic modifications (109). Chromosomal regions that are abundant in repetitive DNA help H3K9me3 stabilize constitutive heterochromatin, facultative heterochromatin and intermediate or transient heterochromatin, the 3 heterochromatin subtypes. By preventing abnormal chromosome segregation, recombination and DNA replication, H3K9me3 regulates constitutive heterochromatin to stabilize genomic integrity (110).

Histone H3 determines the formation of different chromatin structures. Methylation of the N-terminal lysine of histone H3 is vital for well-documented histone modifications. H3K9me3, H3K36me3, and possibly H3K79me3 facilitate the opening of the chromatin configuration to form euchromatin, which is also associated with serine 10 phosphorylation and lysine 9 acetylation of histone H3 for active transcription of genes. H3K9me3 and H3K27me3 mainly function in the initiation, propagation and maintenance of highly compact heterochromatin to silence gene expression (111).

Histone proteins expose DNA euchromatin to facilitate the binding of transcription factors. Methylation and acetylation are the two major mechanisms by which histone function is regulated (112). Methyltransferases and demethylases modify the lysines of histone H3 to form mono-, di-, or tri-methylated lysines, which contribute to chromatin structure and gene transcription (113). Histone methyltransferases (HMTs) regulate histone proteins by transferring methyl groups from S-adenosylmethionine to lysine or arginine residues in histones. Histone acetylation is regulated by histone acetyl-transferases (HATs) and deacetylases (HDACs) (114). Not surprisingly, there are other histone modifications. The balance of these modifications and their effects on histone structure ultimately coordinate DNA exposure. Histone methylation is a key event in gene transcription, and it is plausible to speculate that this type of modification can regulate DNA replication, recombination, and damage repair (115).

H3K9me3 and transcriptional repressors. H3K9me3 recruits transcriptional repressors such as repressor element 1 silencing transcription factor (REST) and CoREST, which contain histone deacetylases (HDACs) (116,117), H3K4me3 demethylases LSD1 (118) and Rbp4 (119), to actively transcribe gene loci, leading to gene blocking and suppression of gene transcription (120). Recruitment of DNMTs as well as additional histone methylases is responsible for localized chromatin condensation when the tethering of HP1α (heterochromatin protein) and HP1ß to H3K9me2 or H3K9me3 triggers gene silencing (121). Thus, H3K9me3 acts as a natural brake to prevent unnecessary over-transcription of actively expressed genes. Attenuation of H3K9me3 by either over-transcription of demethylases or deficiency of H3K9 methyltransferases will therefore lead to sustained expression of the genes involved in either cell cycle transition or proliferation (122).

H3K9me3 and methyltransferases in cancer. Changes in chromatin structure that are caused by epigenetic alterations can contribute to cancer development. In experimental studies using mice lacking SUV39, a methyltransferase that acts on H3K9, enhanced genomic instability and incidence of B-cell lymphoma were observed (123,124). In addition, polymorphisms of SUV39 can increase lung cancer risk due to piRNA instability and decreased levels of H3K9me3. H3K9 methyltransferases include G9a for mono- and di-methylation and SUV39h1/h2 for di- and tri-methylation of H3K9. Similarly, low levels of RIZI (125), another methyltransferase of H3K9, are frequently observed in lung cancer, breast cancer, hepatocellular carcinoma, colon cancer, neuroblastoma, and melanoma (126). Methyltransferases, such as SUV39 and RZZI act as tumor suppressors, while some demethylases may have oncogenic activity. The low expression of these methyltransferases in tumor cells may be the result of increased cell proliferation, apoptosis resistance and poor differentiation (127). Global regulation of H3K9me3 has been observed in several human cancers, including colorectal, ovarian, and lung cancer, all of which are characterized by deficiency or elevated activity of H3K9 methyltransferases or changes in the expression of H3K9 demethylases (128,129).

Other epigenetic alterations in cancer. Epigenetic changes associated with piRNA expression, affect genes associated with malignant phenotypes (VE-cadherin, VEGF-C, PAX8,

Table I. Summary of epigenetic-related molecular biomarkers.

	Expression	Cancer	Function	Technology	(Refs.)
DNMT1	Low	Colon cancer (HCT116)	DNMT1-associated lncRNAs contributes to aberrant DNA methylation and gene expression during colon tumorigenesis	RIP-seq	(92)
DNMT3A	High	Lung cancer	Regulated by miR-708-5p	Bisulfate sequencing	(93)
DNMT3B	Overexpression	Pancreatic cancer Endometrial cancer (Ishikawa cell lines)	Oncogene	MSP, RT-PCR, and western blotting	(94,95)
DNMT3L	Overexpression	Embryonic stem cell (ESC)	Differentiation delays	MSP, RT-PCR, and western blotting	(96)
LINE1	Higher global methylation levels	Bladder cancer	Increases bladder cancer risk	Pre-diagnostic blood DNA	(77)
Alu (with LINE1)	Hypomethylated	Epithelial ovarian cancer	Direct cancer association	LINE1 and Alu bisulfite pyrosequencing	(102)
H3K9me3 H3K27me3	High	Prostate cancer	Facilitates PCa progression	Epidrug 5-Aza as a model	(111)
HDAC inhibitors (HDACis)	Cancer chemotherapeutic agents	Melanoma cell line SK-Mel-5	Upregulation of activin A	RT-PCR and dual luciferase assay	(116)
HDACs	Histone deacetylase inhibitors (HDACi)	Breast cancer with exemestane and tamoxifen, and in renal cell carcinoma	Anticancer therapeutics	RT-PCR	(117)
LSD1	High	Hepatocellular carcinoma Oral cancer	CSC self-renewal and tumorigenicity in HCC Notch signaling activated	RT-PCR	(118)
Rbp4	High	Colorectal cancer Colon cancer	Diagnosis serum biomarkers	ELISA method	(119)
SUV39	High	Cancer cell lines	HDAC/Suv39/G9a pathway	Analysis of MICA/B surface expression by FACS	(99)
RIZ1	Hypermethylated and downregulated	Hepatocellular carcinoma	HBx repressed RIZ1 expression via DNMT1	CHIP	(125)
	Low	Endometrial cancer (EC)	Potential therapeutic targets	Immunohistochemical analysis	(126)
	Low	SiHa cervical cancer cells	Stronger cell inhibition than paclitaxel alone	RT-PCR	(127)
UPA	High	Lymphangioleiomyomatosis (LAM)	Attenuates LAM progression and poten tially other TSC-related disorders	RT-PCR	(132)
TIMP3	High	Cervical cancer	Proliferation, migration, and invasion in cervical cancer	RT-PCR	(135)
	High	Ovarian cancer	Diagnostic and prognostic biomarker	RT-PCR	(136)

Keratin 7, CD13, laminin, urokinase, α3-integrin subunit and c-met) (8).

Detection of some forms of methylation in tumors can be diagnostic of cancer. 5-Hydroxymethylcytosine (5hmC) in cfDNA, which is found in blood originating from different tissues, is the basis of noninvasive prenatal diagnostic tests, organ transplant rejection diagnostics, and cancer detection. 5hmC is useful in gene regulation and cancer pathogenesis and can be used diagnostically to identify cancer types and track tumor stage. Li *et al* observed a progressive global loss of 5hmC in cfDNA in lung cancer, whereas disease-specific changes in the cell-free hydroxymethylome have been observed in hepatocellular carcinoma and pancreatic cancer (130).

In breast and prostate cancers (131), protease urokinase-type plasminogen activator [UPA (132)] was revealed to promote invasion and was associated with poor prognosis (133). Hypomethylation of the UPA promoter activates tumor genes and thus worsens patient outcomes. Carcinogenesis occurs prior to cancer metastases (134), and hypermethylation of tissue inhibitor of metalloproteinase-3 [TIMP3 (135)] was revealed to promote vascular growth and activate angiogenesis (136).

DNMT3Ab plays crucial role in directing EMT-associated metastasis in gastric cancer (GC). Increased DNMT3A expression was revealed to be closely related to a poor survival rate in GC, breast, lung and liver cancer. Furthermore, TNM stage and lymph node metastasis of GC cells were more closely associated with DNMT3A than with DNMT1 and DNMT3B. Increased expression of DNMT3Ab was demonstrated to promote GC cell migration and invasion as well as EMT progression. DNMT3Ab mediated the E-cadherin gene via DNA hypermethylation and histone modifications of H3K9me2 and H3K27me3. DNMT3Ab effectively regulated the expression of E-cadherin via DNA hypermethylation and histone modifications of H3K9me2 and H3K27me3. DNMT3Ab in cooperation with H3K9me2 and H3K27me3 contributed to the transcriptional regulation of E-cadherin in a Snail-dependent manner and multiple metastasis-associated genes and oncogenic signaling pathways are regulated by DNMT3Ab overexpression. Thus, it was revealed that DNMT3Ab acts as a crucial regulator of metastasis-related genes in GC (137) (Table I).

Gliomas with histone H3 lysine27-to-methionine mutations primarily occur in the central nervous system of young children, which means that there is a link between genetics and cellular context in tumorigenesis. Through single-cell RNA sequencing of 3321 cells from six primary H3K27M-glioma and matched models, Filbin *et al* found that H3K27M-gliomas contained cells that resembled oligodendrocyte precursor cells (OPC-like cells). OPC-like cells tend to exhibit higher proliferation and a greater tumor-propagating potential and some of these cells display PDGFRA signaling (138).

7. Epigenetics of ncRNAs in cancer

EMT and angiogenesis are regulated by miRNAs (139,140). miRNAs function in cell differentiation, proliferation, apoptosis and serve as tumor suppressors and tumor promoters. Additionally, miRNAs can control other genes in certain protein pathways. If the expression of certain miRNAs can be inhibited, cancer growth or cancer metastasis may be suppressed. For example, transfection of breast cancer cells with vectors inhibiting miRNA-155 was revealed to reduce the level of CXCR4. The transfected cells exhibited lower migration and invasion rates *in vitro* and resulted in fewer lung metastases *in vivo* than control cells (141).

miRNAs, short interfering RNAs (siRNAs) and piRNAs are involved in the regulation of mRNA transcripts, chromatin-mediated gene silencing, and DNA rearrangement. miRNAs accelerate de-adenylation of the poly(A) tail and downregulate the expression of some pathways, thereby downregulating the expression of hundreds of target genes. siRNAs can also control transposons. RDR2-dependent siRNAs, which are endo-siRNAs, silence transposons, retroelements and DNA methylation (142). siRNAs bind to a nascent RNA being transcribed at their target site, resulting in RNA-induced transcriptional silencing (RITS) and formation of the RNA-directed RNA polymerase complex (RDRC) at the site of intended heterochromatin formation, which in turn results in TGS. TGS occurs in the nucleus. During this process, siRNAs guide miRNAs to modify chromatin, which also influences the cell cycle (143). miR-127 and miR-136 are released near 2 CpG islands in the Rtl1 transcript and thus regulate RISC-mediated cleavage of the maternal transcript, resulting in late-fetal or neonatal lethality (144).

miRNAs are the best studied ncRNAs (145). miRNAs play a critical role in regulating the maintenance and behavior of stem cells during self-renewal and differentiation. miR-290 serves as a transcriptional repressor of DNMTs (146). DNMTs can epigenetically silence OCT4, a transcription factor in ES cells, which can renew and differentiate into other cell types (147).

In mice, the miR-290-295 miRNA cluster was revealed to act as a transcriptional repressor of the DNMTs, Dnmt3a and Dnmt3b, resulting in the appearance of long telomeres and increased telomere recombination. The expression of this cluster remained high in undifferentiated ES cells, but decreased after ES cell differentiation. This example indicates a direct or indirect function of miRNAs in regulating genes involved in self-renewal or differentiation by affecting methylation.

8. Discussion

piRNA expression detected in both patient lymph nodes and serum samples is related to tumor treatment failure. piRNAs can act as tumor promoters or cancer suppressors and can participate in other carcinoma cell activities. piRNAs and PIWI can serve as biomarkers for the prognosis, diagnosis and clinical evaluation of cancer, and can be helpful in selecting effective surgical methods, radiotherapy and chemotherapy to prolong patient survival time. piRNA can serve as a switch, allowing tumors to proliferate and metastasize.

In mammals, PIWI proteins function as transposon-inhibiting factors through TGS. PIWI acts via a distinct pathway to regulate carcinogenesis by affecting transcription and the expression of other carcinoma-related genes that reduce apoptosis and increase cell proliferation and transformation. Transcriptional silencing, heterochromatin formation, transgene silencing, HP1α alteration, histone modifications and transposon suppression are all associated

with PIWI, piRNAs and the piRNA pathway. Transposable elements are classified as either retrotransposons or DNA transposons. Retrotransposons can be further divided into LTR and non-LTR groups. Non-LTRs are divided into the autonomous non-LTRs LINEs and non-autonomous LTRs SINEs. Chromatin-organizing proteins such as HP1, Aub and Su(var)205, act upstream or downstream of piRNAs to regulate transposons. Hypomethylation of LINE1 increases the risk of cancer development and may be an indicator of cancer grade and lymph node metastasis. LINE1 methylation by MILI was revealed to control the expression of cancer-related genes and cell migration, and MILI played a key role in melanoma metastasis and tumor progression. In the sequences of transposable elements, the piRNA pathway mediates and maintains high levels of the repressive H3K9me3 mark in LINE1 regions in germ cells. There are two identified pathway proteins that are related to transposon silencing: CG9754 and EXD1.

Perhaps piRNAs, PIWI, transposable elements and piRNA pathways, upstream or downstream of the epigenetic alterations in tumors, affect the metastasis ability of tumors. In addition, epigenetic alteration of piRNAs, cancer stem cells, CpG island methylation and EMT all participate in cancer metastasis.

Epigenetic alterations associated with cancer metastasis involve DNA methylation, histone modification and certain RNA expression profiles. First, DNA methylation affects the expression of individual genes and DNA domains. The degree of DNA hypomethylation increases as tumors progress and metastasize. DNA methylation and histone modification can activate genomic DNA repeat elements, such as LINE1 and Alu, which can be transcribed or transposed to other genomic regions and disrupt the genome, resulting in chromosome breakage and illegitimate genome rearrangement.

H3K9me3 and other synergistic epigenetic modifications control heterochromatin, but in tumors, that balance has been disrupted. Therefore, with the ability to stabilize genomic integrity by preventing abnormal chromosome segregation, recombination and DNA replication, H3K9me3 and H3K27me3 mainly function in the initiation, propagation and maintenance of highly compact heterochromatin to silence gene expression. Methylation and acetylation are the two major mechanisms that regulate histone function. HMTs regulate histone proteins. Histone acetylation is regulated by HATs and HDACs. Several HMTs exhibit tumor suppressor functions, and some demethylases exhibit oncogenic activity. H3K9me3 recruits transcriptional repressors such as REST and CoREST. The low expression of methyltransferases such as SUV39 and RZZI in tumor cells may be the result of increased cell proliferation and apoptosis resistance and poor differentiation. Global regulation of H3K9me3 has been observed in several human cancers, including colorectal, ovarian and lung cancer, all of which are characterized by deficiency or elevated activity of H3K9 methyltransferases or altered expression of H3K9 demethylases. 5hmC can be used to identify cancer type and track tumor stage. DNMT3Ab cooperated with H3K9me2 and H3K27me3 played a crucial role in directing EMT-associated metastasis in gastric cancer.

Understanding the epigenetic mechanisms of tumor metastasis related to piRNAs can assist in the identification of new tumor markers and treatments. piRNAs can be indicative of patient outcomes and can be helpful in selecting effective surgical methods, radiotherapy and chemotherapy to prolong patient survival time. piRNAs can be used as biomarkers to predict TNM stage and disease prognosis. Since preventing tumor metastasis is still a formidable problem, studies on the potential of piRNAs and epigenetic alternations for diagnosis and prediction of clinical cancer stages are greatly needed.

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Availability of data and materials

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Author's contributions

BC, SZ and JL conceived and designed the study. JL wrote the manuscript. SZ prepared the figure and the table. BC wrote the ncRNA part, reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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