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REVISIÓN

piRNAs and PIWI-like proteins in cancer and their future as biomarkers and therapy targets in breast cancer

piARNs y proteínas similares a PIWI en el cáncer y su futuro como biomarcadores y objetivos terapéuticos en el cáncer de mama

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Abstract

Globally, breast cancer (BC) is the most common type of cancer, with 2.296.840 new cases in 2020 with almost 700.000 related deaths. Due to the high burden this represents, it is imperative to expand horizons to be able to find novel biomarkers and therapeutic targets that can be used to improve prognosis, treatment, and survival of breast cancer patients. In recent years, numerous studies have been conducted looking for the association between piRNAs (PIWI – interacting RNAs) and the development, pathogenesis, metastasis, and progression of different types of cancer, including BC. PiRNAs are small molecules (24-31 nucleotides) that interact with the PIWI-protein complex (PIWI-like proteins - HIWI/HILI in humans) performing regulatory functions by inducing transcriptional, post-transcriptional, translational, and post-translational epigenetic changes, which has been observed to contribute to cancer development through modifications in cell proliferation, transposon silencing, genome rearrangement, epigenetic regulation, protein regulation, and stem cell maintenance. In breast cancer, a strong association has been found between the expression of some piRNAs, PIWI-like proteins and tumor development. If a specific piRNA could be associated with a distinct type of cancer, it could then be used as an early biomarker which would allow for a better prognosis. Findings surrounding these molecular mechanisms could also spark interest in studies focusing on the modification of the expression of piRNAs in cancer cells. In this article, we intend to review in a straightforward manner the current information about piRNAs/PIWI-like proteins focu-

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sing on their expression in BC.

Keywords: breast neoplasms; tumor biomarkers; early detection of cancer; therapeutic uses.

Resumen

A nivel global, el cáncer de mama (CM) es el tipo de cáncer más común, con 2.261.419 nuevos casos en 2020 y casi 700.000 muertes relacionadas. Debido a la alta carga que esto representa, es imperativo expandir horizontes para poder encontrar nuevos biomarcadores y objetivos terapéuticos que puedan utilizarse para mejorar el pronóstico, el tratamiento y la supervivencia de las pacientes con cáncer de mama. En los últimos años, se han llevado a cabo numerosos estudios buscando la asociación entre los piARNs (ARNs que interactúan con PIWI) y el desarrollo, la patogénesis, la metástasis y la progresión de diferentes tipos de cáncer, incluido el CM. Los piARNs son moléculas pequeñas (24-31 nucleótidos) que interactúan con el complejo proteico PIWI (proteínas similares a PIWI - HIWI/HILI en humanos) realizando funciones regulatorias al inducir cambios epigenéticos transcripcionales, post-transcripcionales, traduccionales y post-traduccionales, lo que se ha observado que contribuye al desarrollo del cáncer mediante modificaciones en la proliferación celular, silenciamiento de transposones, reordenamiento del genoma, regulación epigenética, regulación de proteínas y mantenimiento de células madre. En el cáncer de mama, se ha encontrado una fuerte asociación entre la expresión de algunos piARNs, proteínas similares a PIWI y el desarrollo tumoral. Si un piARN específico pudiera asociarse con un tipo específico de cáncer, entonces podría utilizarse como un biomarcador temprano que permitiría un mejor pronóstico. Los hallazgos en torno a estos mecanismos moleculares también podrían despertar interés en estudios que se centren en la modificación de la expresión de piARNs en células cancerosas. En este artículo, pretendemos revisar de manera sencilla la información actual sobre los piARNs/proteínas similares a PIWI, centrándonos en su expresión en el CM.

Palabras clave: neoplasias de la mama; biomarcadores de tumor; detección precoz del cáncer; usos terapéuticos.

Background

In 2020, breast cancer (BC) became the neoplasm with the highest incidence around the world, surpassing lung cancer, with 2.296.840 new cases and 666.103 related deaths1. This fact elucidates the importance of developing not only better primary prevention programs, but also improved molecular profiling, such as novel molecular biomarkers that can result in a better prognosis and treatment. This need has resulted in the discovery of several useful molecular biomarkers throughout the years, nevertheless, there is still much to learn about the infinite abnormalities that lead to the development of cancer; among them, piRNAs.

Cancer cells develop abnormal modifications that relate to their tumor status. These variations are possible due to changes in gene expression developed mostly through epigenetic changes such as hypermethylation of DNA in a specific gene, general hypomethylation of DNA and the dysregulation of small non-coding RNAs2.

Small non-coding RNAs have become important due to their epigenetic regulatory role

in germline cells, stem cells and differentiated cells[2,3,4]. A new subtype of small non-coding RNAs called PIWI-interacting RNAs (piRNAs) has been recently described. These piRNAs interact with the PIWI protein complex, member of the argonaute protein family and known in humans as HIWI/HILI (PIWI-like proteins), playing an essential role in the differentiation and maintenance of germline and stem cells[2,4,5]. These piRNAs were first described in Drosophila germline cells as small non-coding RNAs transcribed from repetitive elements, such as retrotransposons and DNA transposons[4,6], and were found to be essential for genome stability during the genome-wide reprogramming that occurs mainly in primordial germline cells. During this reprogramming, methyl marks are erased, activating genes such as transposons, which can result in genome damage[5,6]. In this scenario, piRNAs inhibit transposable elements avoiding DNA damage that can derive from their activation[6,7].

Later, piRNAs and PIWI-like proteins were described in mammals like mice, and afterwards in humans, initially in gonads and subsequently in somatic tissues. Recent evidence shows the existence of an aberrant expression of PIWI-like proteins in cancer, including BC cells, which relates directly to an abnormal expression of specific piRNAs with distinct homologous or opposite functions [2, 8, 9].

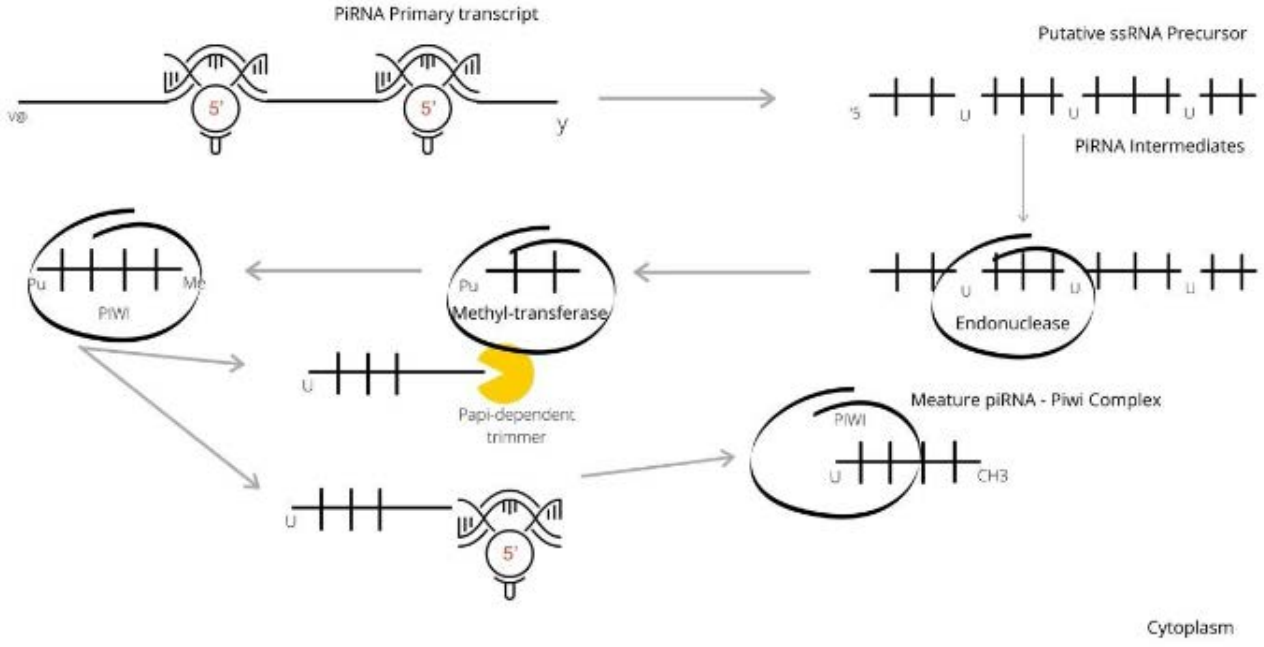
We hereby intend to review what is currently known about piRNAs and cancer, their relation to BC, and their implications in diagnosis, prognosis, and treatment.

piRNAs, PIWI-like proteins and piRNAs biogenesis

piRNAs are a subtype of small non-coding RNA molecules that interact with PIWI-like proteins to form the piRNA/PIWI silencing complex known as piRISC3. They are slightly bigger in size than miRNAs with a length of 24 to 31 nucleotides (nt) and play crucial roles in gene expression regulation[3,10]. PiRNAs have 5' Uridine signature, Adenosine in the tenth position, and a 2'-O-methylation signature at 3' end[2,3,5]. Mature piRNAs bind to PIWI-like proteins to form the piRNA/PIWI complex which is involved in gene expression regulation, such as transposon silencing, spermiogenesis, genome rearrangement, epigenetic regulation, protein regulation and the maintenance of germ stem cells[3,6,10]. Both piRNAs and PIWI-like proteins have been described across many different species. Nevertheless, piRNAs sequences show a surprising number of variations among different species.

PiRNA biosynthesis is controlled by a complex positive regulation mechanism given by homologous piRNAs, thus being a classic control mechanism[3,5]. Biogenesis involves two pathways, primary amplification and secondary amplification or ping pong mechanism. The primary amplification relies on RNA polymerase II to transcribe piRNA clusters consisting of 200 kb genomic loci, single-chain groups contain a series of promoters including Pol II Ser5P and H3K4me2 that promote transcripts through RNA polymerase II, forming a long single stranded piRNA precursor (pre-piRNA). piRNAs require post-translational modifications to become mature piRNAs, maturation takes place with cleavage of the 3' end by riboendonuclease Zucchini[3,5,11,12], methylation following this process results in piRNA/PIWI complex (figure 1). Once this process is finished, and piRNAs are mature, they bind to PIWI-like proteins to form the piRNA/PIWI complex[3,5]. Secondary amplification is regarded as a more primitive mechanism in which sense piRNA/PIWI complexes cleave piRNA cluster transcripts to produce piRNA intermediates with a 5' Uracil that are then loaded into another PIWI protein[3,13]. In this pathway, piRNAs/PIWI complexes are used to generate new RNAs as substrates for new piRNA formation[3,5,11,12,13].

Figure 1.
Simplified piRNA primary Biosynthesis Process 3,5,11,12,13



piRNAs, PIWI-like proteins and cancer

Several studies have found a correlation between piRNA/PIWI and distinct types of cancer. Abnormalities in piRNA expression have been related to both cancer development and cancer protection. Some of them even showing a correlation with diagnosis, invasion, progression, metastasis, and prognosis. Some molecular mechanisms related with piRNA/PIWI complexes are shown in Table 1.

Table 1.
Interaction of the piwi / piRNA complex with the cellular environment

Cellular processes	Action mechanism
piRNA/PIWI mediated transcriptional gene silencing (TGS)	The piRNA/PIWI complex enters the nucleus, and links with the genomic target through a nascent transcription by complementary sequence. Afterwards, it induces TGS when recruiting silencing machinery components[4]. The piRNA/PIWI complex also recruits DNA methyltransferase (DNMT), methylating CpG sites4.
piRNA/PIWI complex mediated postranscriptional gene silencing (PTGS)	The piRNA interaction requires a strict base pairing inside of the 2-11 nt 5´ (five prime) portions of the piRNA and a less strict base pairing inside of 12-21 nt. The piRNA-induced silencing complex (piRISC) mediates the deadenylation and the disintegration of mARN through a similar mechanism of miARN with piRNA guide and CAF1 , impeding their function by complementarity of sequence 3.
Relationship between piRNA/PIWI complex and protein structures	The piRNA/PIWI complex binds directly to some proteins through piRNAs or PIWI PAZ domain. This relationship supplies the interactions of multiple proteins, altering their subcellular location.

piRNAs/PIWI as biomarkers in cancer

PiRNAs, being relatively short in length, can easily cross the cell membrane and enter the systemic circulation, thus being easily detected in peripheral blood. They can also be detected in tissue samples and even some PIWIL proteins can be detected using immunohistochemistry stains[13, 14]. The importance of piRNAs in cancer lies in the possibility of using it as a biomarker due to its upstream action in various signaling pathways highly related to the etiology, progression, and development of distinct types of cancer. Different specific blood biomarker piRNAs have been identified in several tumors[14,15,16,17] (Table 2). However, a detectable serum biomarker piRNA for BC is yet to be characterized.

Table 2.
piRNAs in different cancer types and their serum detection [15,16,17,18,19].

Cancer type	piRNA	Blood detection
Gastric	piR-651	Low
	piR-823	
Colorectal	piR-5937	Low
	piR-28876	
	piR-54265	High
Renal	piR-823	High
Multiple myeloma	piR-823	High
Classical Hodgkin's lymphoma	piR-651	Low

PIWI proteins, part of the piRNA/PIWI complex, are expressed in situations of stress and constant aggression in the cellular environment. The PIWI protein family (PIWIL1,2,3 and 4) is associated with specific tumor conditions, thus being possible biomarkers in cancer[15, 16, 17]. Studies have been conducted in different types of cancers, finding a close relationship between piRNAs, PIWI proteins and malignancies. Here we elucidate some examples:

Renal cancer

In renal clear cell carcinoma (RCCC), piR-32051, piR-39894, piR-43607, piR-30924, and piR-38756 have been found to be related to poor prognosis, lower survival and even metastasis. PiR-823 is also related to the pathogenesis and prognosis of renal cancer (RC), and its high detection in urine has a very important diagnostic value [3,18]. PIWIL1 can be detected through immunohistochemistry and its high expression has been linked to poor prognosis in RC. Moreover, PIWIL1, PIWIL2, and PIWIL4 decrease their expression with the increase of clinical stage, relating to poor prognosis [3,20,21].

Gastric cancer

In gastric cancer (GC), microarray analysis of malignant tissue and adjacent normal tissue found a decreased expression of piR-823 compared to healthy cells, and when piR-823 expression was stimulated, malignant cells' growth was inhibited. In contrast, an increased expression of piR-651 was found in cancer cells when compared with normal tissue cells, also noticing that when inhibiting piR-651, cancer cells were blocked in G2/M phase. These findings suggest that piR-651 may be involved in GC progression and that piR-823 plays an important role in cancer inhibition, and that its decreased expression in cancer can relate to metastasis[3,22,23]. Furthermore, PIWIL1 has been observed to be upregulated in GC cells, in PIWIL1 knockout cells, several oncogenes were downregulated, and cancer suppressor genes were upregulated. This could signify that PIWIL1 is associated with poor prognosis and plays an important role in cancer development and progression[3, 23].

Colorectal cancer

Probably one of the cancers in which piRNAs have demonstrated to be of great importance. piR-823 plays a contributing role, although, in contrast to gastric cancer, it is upregulated in colorectal cancer (CRC), promoting the activation of Heat shock transcription factor – 1(HSF-1) [3,24]. The inhibition of piR-823 results in cell cycle arrest in G1 in cancer cells (3). Through animal models, it has been shown that the interaction between piR-54265 and PIWIL2 activates STAT3 signaling pathway, resulting in enhanced invasion, progression, and metastasis of CRC cells25. Therefore, both piR-54265 and piR-823 could have prognostic value and could be ultimately used as therapeutic targets for CRC [3,24,25]. Interestingly, serum piR-5937 and piR-28876 show a high sensitivity and specificity for the early diagnosis of early-stage CRC, even better than carcinoembryonic antigen (CEA) and CA19-9 [26]. Other relevant piRNAs and PIWIL proteins are piR-19521, piR-18849, piR-1245 and PIWIL12[7,28,29]. Upregulation of piR-18849 promotes lymph node metastasis, piR-1245 is increased and aids with cancer cell proliferation resulting in a shorter survival, and PIWIL1 is related to poor prognosis as well [27,28,29].

Lung cancer

piR-651 has been found to be one of the key piRNAs involved in tumorigenesis of lung cancer. It is upregulated in non-small cell lung carcinoma (NSCLC), which has been related to both tumor invasion and metastasis. piR-651 has also been observed to have an influence in apoptosis proteins such as Caspase-3 and bax, as well as in the expression of cyclinD1 and CDK4, promoting metastasis 30,31. These findings further confirm piR-651 influence over development and progression of lung cancer (LC) 3,30,31. Furthermore, upregulation of mTOR in LC is known to enhance tumor progression, piR-55490 inhibition has been related to lower LC cell proliferation through the inhibition of mTOR pathway in tumor cells, suggesting that it could have an anti-tumor function [32]. PIWIL proteins, specially PIWIL1 has been shown to be overexpressed in LC cells, promoting invasion, progression, and metastasis of lung adenocarcinoma, also being associated with a shorter overall survival [33].

Multiple Myeloma

Abnormalities in DNA methylation contributes to the development of multiple myeloma (MM), piR-823 has been related to de-novo DNA methyltransferases (DNMT3A and DNMT3B) mainly in CD138+ myeloma cells, that when upregulated, results in methylation and consequent inactivation of tumor suppressor genes such as p16 [34]. piR-823 inhibition results in an important decrease of vascular endothelial growth factor which ultimately results in the decrease of angiogenesis (34,35). These findings could possibly end up in the development of piR-823 target therapy. Very recent evidence relates the upregulation of piR-004800 through sphingosine-1-phosphate receptor (SP1R) to higher stages of multiple myeloma, and when piR-004800 was inhibited, it resulted in apoptosis and autophagic cell death, accompanied by cell proliferation inhibition [36]. This could mean that piR-004800 plays a very important role in multiple myeloma progression, which could have a great importance in the future as a novel therapeutic target.

Classical Hodgkin's lymphoma

Recent evidence has shown piR-651 as an independent prognostic factor in Classical Ho-

dgkin's lymphoma (CHL), patients with decreased levels of piR-651 had worse outcomes and less treatment response. piR-651 levels in serum were also lower in patients with CHL, showing a progressive increase with treatment and remission [19]. It has also been shown that, PIWIL1 and PIWIL2 were overexpressed in CHL, suggesting an increase in activity of the piRNA/PIWI pathway in these cancer cells, additionally, it was shown that the inhibition of piR-651 results in an increased expression of gene ABCC5, which codes for an efflux pump of several chemotherapy drugs such as doxurrubicin, ultimately conferring chemoresistance[37]. Additionally, piR-20365 and piR-20582 were found to be overexpressed in CHL compared to controls[38].

piRNAs/PIWI and breast cancer: what is known and future applications

Several studies have linked specific types of piRNA with different cellular actions in BC tumor cells. These actions range from increasing cell proliferation to inhibiting proliferation [38, 39]. Some examples are shown in Table 3.

It is relevant to elucidate the most important associations between piRNAs, PIWIL proteins and breast cancer that have been recently identified. Both piR-651 and piR-4987 have been shown to be overexpressed in BC cells, the latter being related to lymph node metastasis[3,4,9,39,40]. PiR-36712 has been shown to be downregulated in BC cells compared to normal tissue cells, having a correlation with worse prognosis. When piR-36712 was upregulated, tumor suppressor genes such as p21 and p53 were upregulated, blocking cell cycle in G0/G1 phase. Studies show that piR-021285 is a possible modulator of BC invasiveness, this is possible through methylation mechanisms of BC related genes, thus resulting in a higher or lower progression of cancer through epigenetic changes induces by piRNAs [41,42]. PIWIL1 was found almost exclusively in BC tissue when compared to normal tissue, this finding could be of relevance, nevertheless more studies are needed [43]. PIWIL2 expression was found to be higher in BC than in controls, and interestingly, piR-932 and its interaction with PIWIL2 was found to enhance BC through Latexin methylation, promoting BC development, which could mean that both piR-932 and PIWIL2 could be used as biomarkers and target therapy in the future, with piR-932 being up to date only found in BC cells [3,44]. PIWIL3 and PIWIL4 were related to prognosis, being the latter also related to the regulation of the expression of estrogen receptors and BC cells invasiveness in BC with positive estrogen receptors expression [44].

The abnormal expression of these several piRNAs (piR-4987, piR-021285, piR-021285, piR-823, piR-932, piR-36712, piR-016658, piR-016975) could eventually be used as both therapeutic targets and biomarkers in BC, nevertheless, a better understanding of their regulation pathways is needed. There are many other piRNAs that have been found to be involved in BC, showing a downregulation in DQ596311, DQ596670, DQ598252, DQ598183, and DQ597341 and an overexpression of DQ570994, DQ597960, DQ598677 when compared with normal tissues. It was also noted that DQ570994 was related to chemotherapy response, as well as that the overexpression of DQ571955 associated with an estrogen receptor positive BC was related to decreased disease-free time after radiotherapy, which could imply that DQ571955 could be used as a predictor of treatment response in this type of estrogen receptor positive BC [45,46,47]. Another piRNA related to estrogen receptor positive luminal BC was piR-823, which was demonstrated

to alter DNA methylation and active Wnt signaling pathway [46,48].

As for triple negative BC, one of the most aggressive yet most responsive to chemotherapy, it has been found that it shows mainly a downregulation in piR-23662, piR-30293, piR-26527, piR-26528, and piR-26526, as well an upregulation of piR-23672, piR-21131, piR-32745, and piR-1282 45,49. Another recently identified prognostic factor for triple negative BC is Ferritin heavy chain (FTH1), being associated with chemosensitivity and progression45,50; piR-FTH1 with sequence complementarity to mRNA-FTH1 can downregulate fth1 post-transcriptionally through HILI/HIWI2 mechanisms [45, 50].

Table 3.
Examples of piRNAs expressed in BC, function, and expressiveness of each one.

piRNA	Function	Expression level in tumors
piR-36712	It suppressed the proliferation, invasion, and cell migration when combined with SEPW1P RNA (41,42).	Low
piR-021285	It inhibited the cell proliferation and invasion by ARHGAP11A methylation (45,50).	Low
piR-932	It caused EMT through the promotion of CpG island methylation of the Latexin promoter region (3,44). Until now, this piRNA has not been related to other cancer types (REF "a new class of regulator").	High
piR-DQ598677	Pi-RISC formation with the purpose of degrading specific genes such as miRNAs (45,46).	Low

Methods for detection of piRNAs and PIWIL proteins, overview

There are different approaches to piRNA detection depending on the aim of the study. Different objectives may include:

- Assessing the presence of piRNAs
- Determining the exact number of copies present
- Quantifying expressiveness per cell
- Identifying interactions with cellular components and protein structures (for example, piRNAs/PIWIL interactions)

Most of these methods are involved with immunological tests since a high level of specificity is needed for their detection. An overview of the different tests are shown in table 4.

Table 4.
PiRNA testing and detection methods.

Methods	Objective
High-throughput sequencing (HTS), New generation sequencing (NGS).	Evaluates new and known piRNAs.
RT-qPCR	Determines the exact number of piRNA copies per cell and their relative expression.
Southern Blot	Determines the exact number of piRNA copies.
RNA immunoprecipitation (RIP)	Identifies Interaction piRNA/Proteins.
Luciferase reaction	Identifies Interaction piRNA- target RNA.
Microarrays	Identifies DNA methylation DNA through piRNA.

Final considerations

The struggle of being able to identify new biomarkers and treatment targets in cancer is a constant necessity for medical science. Today, thanks to the rapid growth of new technologies, we have been able to identify novel molecular interactions that have very promising prospects for the future. Breast cancer is nowadays the leading cause of cancer, this results in a growing need for better diagnostic, prognostic, and treatment options for patients. PiRNAs have recently emerged as novel prospects to serve this purpose, aiding not only in our understanding of disease development, but also in our development of new approaches to the disease. If the expression of BC-related piRNAs could be identified in serum and evaluated prematurely as screening, it could result in the improvement of disease development and prognosis, allowing to even apply a target-therapy without further invasive techniques that could lead to early treatment and increased survival. We have hereby elucidated two general mechanisms of the currently known piRNAs in BC, one mechanism works by increasing cell growth, invasiveness, proliferation and thus cancer progression, and the other mechanism works by decreasing progression through the inhibition of these cellular processes.

There is still much to understand about the expression of these piRNAs and their resulting effect on cancer development and progression, nevertheless, we are closer to being able to identify every step in these complex pathways that could allow us to develop target therapies and less invasive diagnostic tools for not only breast cancer, but also many other cancers that today have a great impact in patients. Implementation of molecular modifications in search of improving, stopping or even preventing the pathophysiological development of cancer seems closer every day. We hope that by increasing our knowledge on piRNAs and their molecular mechanisms, we can in a very close future be able to improve our current diagnosis techniques, provide early treatment and allow a better prognosis for not only breast cancer, but also all cancer types.

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Contributions

Contributors played a substantial role in conception, design, acquisition, analysis, interpretation, writing, and critical review of the manuscript. All authors approved the final content and accept responsibility for its accuracy and integrity.

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