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REVISIÓN

piRNAs and PIWI -like proteins in Multiple Myeloma and their future as biomarkers and therapy targets

piARNs y proteínas similares a PIWI en el Mieloma Múltiple y su futuro como biomarcadores y blancos terapéuticos

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Abstract

Multiple Myeloma (MM) is the second most common hematological malignancy and one of the 19 most frequent types of cancer. Its diagnosis is a challenge due to the low rate of disease recognition, and diagnosis delays lead to the characteristic end-organ damage of the disease. New approaches to tackle that diagnosis challenge are required. Emerging evidence shows that Piwi-interacting RNAs (piRNA) promote increased methylation in MM cells. In this analysis, we delve into the latest discoveries surrounding piRNA biogenesis and functions, offering fresh perspectives on the possible uses of piRNAs in detection and diagnosis in MM. piRNA-823 increases in MM cells and positively correlates with the disease stage. Its tumorigenic actions in MM relate to intercellular communication between MM and vein endothelial cells. These findings provide the necessary information to highlight the possible role of piRNA-823 as a biomarker for MM diagnosis.

Keywords: piRNA; multiple myeloma; biomarker; cancer; target.

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Resumen

El Mieloma Múltiple (MM) es la segunda neoplasia hematológica más común y uno de los 19 tipos de cáncer más frecuentes. Su diagnóstico es un desafío debido a la baja tasa de reconocimiento de la enfermedad, y los retrasos en el diagnóstico conducen al daño en los órganos característico de la enfermedad. Se requieren nuevos enfoques para abordar este desafío diagnóstico. La evidencia emergente muestra que los ARN interaccionantes con Piwi (piARN) promueven un aumento de la metilación en las células de MM. En este análisis, profundizamos en los últimos descubrimientos sobre la biogénesis y funciones de los piRNA, ofreciendo nuevas perspectivas sobre los posibles usos de piARNs en la detección y diagnóstico en MM. El piARN-823 aumenta en las células de MM y se correlaciona positivamente con la etapa de la enfermedad. Sus acciones tumorigénicas en el MM están relacionadas con la comunicación intercelular entre las células de MM y las células endoteliales de las venas. Estos hallazgos proporcionan la información necesaria para resaltar el posible papel del piARN-823 como biomarcador para el diagnóstico del MM.

Palabras clave: piARN; mieloma múltiple; biomarcador; cáncer; objetivo.

Introduction

Multiple myeloma (MM) is the second most common hematological malignancy [1,2]. According to the GLOBOCAN report, by 2020, 176 404 new cases of MM were reported, the majority of them (63 439) occurred in Asia, followed by Europe (50 918), North America (35 318), and Latin America and the Caribbean with 15 118 cases; 1 376 in Colombia. In the same year, the number of deaths in Colombia reached 1 305 [3,4].

The disease is characterized by periodic relapses and an insidious progression to treatment resistance[2]. This condition is identified by the unregulated expansion of clonal plasma cells. These cells are highly proliferative B-lymphocytes that have differentiated and can produce various types of immunoglobulins. The overproduction of monoclonal immunoglobulins by the clonal plasma cells results in organ damage, with common clinical symptoms such as anemia, kidney failure, elevated calcium levels, and bone lesions characterized by bone destruction [5,6]. Bone structure impairment represents a significant source of illness in MM, primarily caused by an imbalance in the natural bone remodeling processes involving bone-resorbing osteoclasts and bone-repairing osteoblasts. [7]

The diagnosis of MM is challenging, due to the unspecific symptoms, the low incidence of the disease, and the low rate of disease recognition among the general population and physicians. Those factors contribute to delays in diagnosis. In some Latin American countries, the diagnosis process usually takes approximately two years after the first contact with the patient. In Colombia, according to the Colombian Association of Hematology and Oncology, this estimation is 49 days on average. For these reasons, investigations into a new path to the early identification of MM are necessary. In this article, we review the relation between piRNA and cancer, and the possible role of piRNA-823 as an early biomarker for diagnosis of MM [8].

Pathophysiology

MM is one of the malignancies identified within the bone marrow, specifically affecting plasma cells, as a result of genetic or epigenetic aberrations that ultimately alter several impor-

tant cellular processes [9,10]. MM is originated by errors in the maturation of differentiated B cells known as plasma cells. These cells conduct the humoral immune response by the secretion of antibodies. When errors related to their maturation occur, it leads to several disorders that include premalignant conditions (monoclonal gammopathy of undetermined significance and smoldering myeloma) and the malignant condition MM [11].

Epigenetic changes have a role in the pathogenesis of MM; among these alterations, the altered DNA methylation or miRNA deregulation are the most prominent ones [12, 13, 14]. It is worth mentioning that the role of non-coding RNAs in epigenetic regulation is increasing, so it is necessary to recognize that this material is involved in the initiation and promotion of processes such as tumour progression [15, 16]. A distinct class of small ncRNAs, called Piwi-interacting RNAs (piRNAs), are involved in the regulation of de novo DNA methylation [17, 18, 19]. These piRNAs are considered as guide material for the PIWI protein sequences, through which the Piwi-piRNA signaling pathway is described, which is considered key in the silencing mechanism of transposable factors through the DNA methylation process. Which in this case is responsible for protecting the genetic material from the germline cells and of course regulates their expression [17,18,20,21].

New Molecular Pathways: Biosynthesis and Mechanism of Action of piRNA

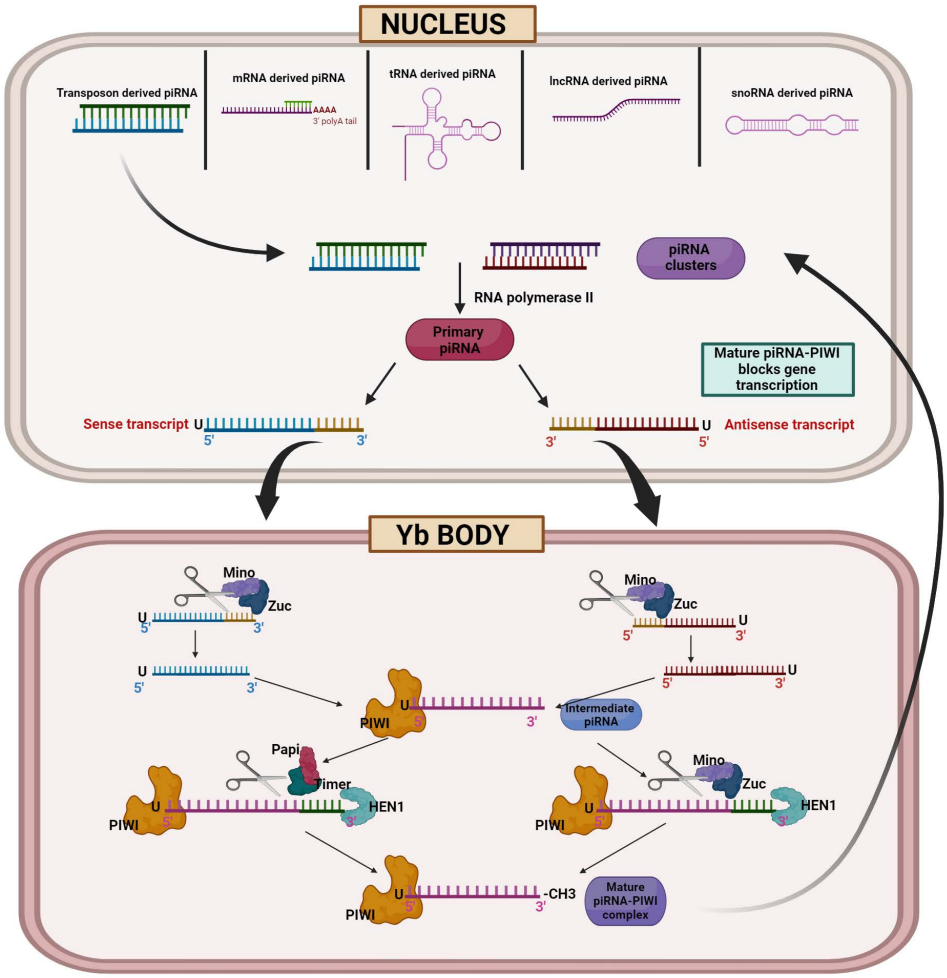
About 98% of the gene's transcriptome is non-coding RNA (ncRNA), and they can be divided into two main categories: housekeeping ncRNA and regulatory ncRNA. The latter are classified by their molecular size in small ncRNAs (less than 200 nucleotides) and large ncRNAs (more than 200 nucleotides). The small ncRNAs are diverse and include the PIWI-interacting RNAs, known as piRNAs [22]. In addition, there are three groups of piRNAs: lncRNA, (produced from the full-length transcript), mRNA (generally derived from 3' untranslated regions - UTRs), and transposon-derived piRNAs (transcribed from two genomic strands, producing both piRNA and antisense piRNA) [15,23]. On the other hand, PIWI Proteins are nuclear proteins initially discovered in Drosophila. They are involved in the silencing of retrotransposons, the control of male germ line motility, and the maintenance and self-renewal of stem cells of the germ line. PIWI-like protein 1 (PIWIL1 or HIWI), PIWIL2 (HILI), PIWIL3 (HIWI3), and PIWIL4 (HIWI2); These are 4 of the PIWI proteins that have been identified in humans in carcinogenic processes. [23].

Two main pathways have been described in piRNA biogenesis; the primary and the secondary (also called the "Ping-pong cycle"). The primary occurs in somatic and germ cells, and the secondary occurs only in germ cells.

In the nucleus, the primary pathway takes place where RNA polymerase II transcribes piRNA genes from small nucleotide sequences to form a long single-stranded precursor. Subsequently, this precursor is exported from the nucleus, and the transcripts are transported to the cytoplasm via UAP56 to the processing sites: Yb bodies. The process is possible because the RNA precursors carry signals that allow piRNA precursors to be recognized. In Yb bodies, Zucchini (Zuc) and its co-factors produce piRNA intermediates with a 5' uracil to produce short-chain piRNA intermediates with 5' uracil [23]. During this first processing, essential proteins for germline development are required: Vreteno (Vret), Minotaur (Mino), and Gasz. Small non-coding RNAs (piRNAs) bearing the nucleotide base Uracil are mainly selected in PIWI or Aub factors for ovarian somatic cells. However, the Piwi protein is expressed and is part of the primary piRNA pathway. On the other hand,

molecular chaperones such as Hsp83 and Shut result in the maturation and loading of piRNAs. The 3' end formation is carried out either by another cleavage event by Zuc, which results in the formation of phased Piwi-associated piRNAs, or by a putative exonuclease named 'trimmer' and its cofactor Papi resected to their mature size: the piRNA intermediates. Finally, those piRNA intermediates undergo methylation by Hen1 to form mature piRNA-Piwi complexes that can enter the nucleus to exert silencing (shown in Figure. 1) [24,25].

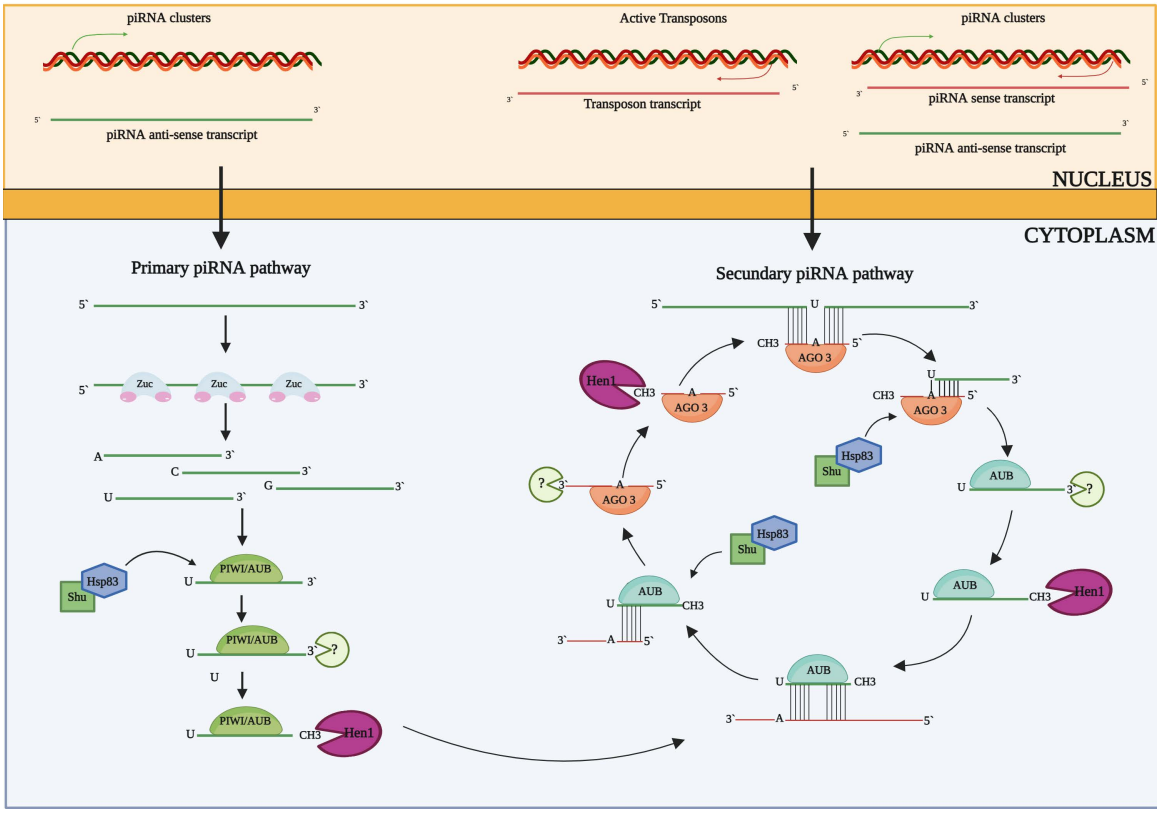
Figure. 1.
piRNA biogenesis pathways: Primary pathway. PiRNA production is initiated in the nucleus with DNA polymerase II, short nucleotide fragments are formed, forming a long single-stranded precursor that subsequently passes into the cytoplasm. There a cut is generated at the 3' end, it is methylated and maturation is generated, forming the piRNA/PIWI protein complex. Adapted to Czech B, Hannon GJ (2016) [12].



After the piRNA/PIWI protein complex is organized, the target gene is directed where, through complementarity, it binds to piRNA and target DNA, a process that occurs in the nucleus. This binding promotes the activation of gene silencing mechanisms and prevents transcription of the target gene. Therefore, piRNAs are transcriptional regulators that act on saltimbanquis genes or so-called transposable elements, also recruiting histone methyltransferases such as H3K9me3. The structure resulting in the establishment of heterochromatin is changed, which due to its disposition does not allow gene transcription, and therefore transcriptional silencing occurs [26].

The ping-pong mechanism: after the primary piRNA is produced, it is transferred from the nucleus to the cytoplasm, where a second amplification takes place. This is possible because Aub proteins bind to mature antisense piRNAs, the binding allows a second amplification cycle so that the piRNAs drive the Aub protein to cleave or separate the transcribed fragments, which represent the transposons and identify the initial piRNA factors generated through initial piRNAs. The generated products are added to the Ago3/AUB complex by other known Shu and Hsp83 allowing piRNA/Ago3 or piRNA/AUB binding, subsequently subjected to 3'-end clipping to mature sense piRNAs and methylation, then Ago3 and its associated mature sense piRNAs can be used as templates to generate new piRNAs based on sequence complementarity. Finally, the piRNA/Ago3 complexes and the resulting new piRNAs can load the Aub protein, via secondary factors such as Shu and Hsp83, which are also cleaved and methylated on the 3' end related to the primary piRNA pathway (shown in Figure. 2) [23].

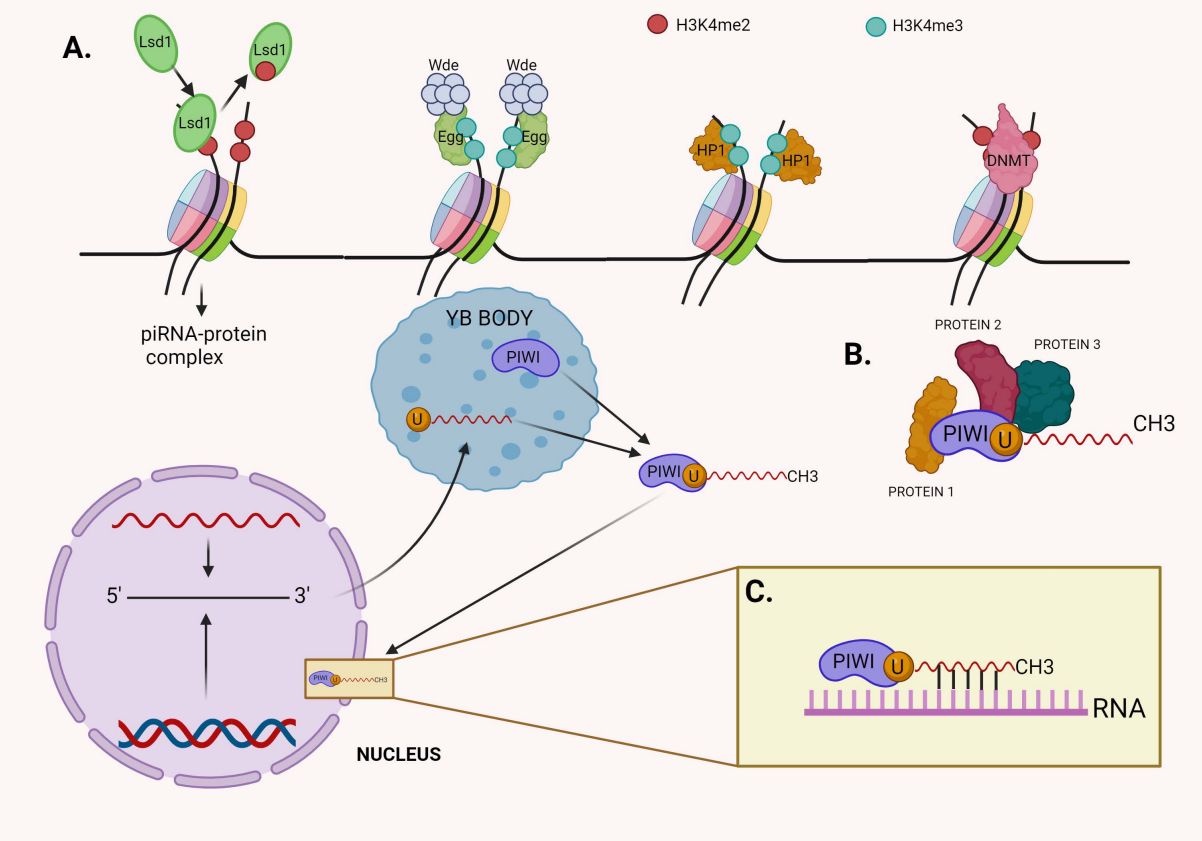
Figure. 2.
piRNA biogenesis pathways: Ping-pong cycle. Secondary route through the ping-pong mechanism. It begins after the production of piRNA in primary synthesis24.



Mechanisms of action of piRNAs

piRNA intervenes in gene expression at the transcriptional and post-transcriptional levels. At the transcriptional level, they can silence transposons, regulate DNA methylation, and modify histones. For example, the silencing of killer immunoglobulin-like receptors (KIRs), where the piRNA-PIWI complex can bind to the promoter region and favors the recruitment of methylation factors allowing transcriptional silencing of KIRs. This silencing alters the action of NK cells, as KIRs are part of the group of receptors that permit them to act against malignant cells in the cycle of immunosurveillance [27]. At the post-transcriptional level, piRNA regulates the alternative splicing, the endonucleolytic cleavage of RNA, and the interaction with RNAs. They also control the mRNA stability; piRNAs target sequences of transposons have been found in the 3'-UTR or 5'-UTR of mRNAs, resulting in the deadenylation or degradation of the mRNA (shown in Figure. 3) [23,28].

Figure. 3.
Mechanisms of action of pirRNA. (A) Silencing of transposons. At the TGS (transcription gene silencing) level, Lsd1 (lysine-specific demethylase 1) suppresses the activation of the H3K4me2 (histone 3 lysine 4 dimethylation) marks from the promoter regions, the Egg and Wde H3K9me3 (histone 3 lysine 9 trimethylation) on target DNA, HP1 (heterochromatin protein 1) leads to the formation of heterochromatin, the methylate gene CpG sites of DNMT (DNA methyltransferase). (B) piRNAs/piwi-protein complex interaction, this alters the subcellular localization of the proteins and generates the interaction of multiple proteins. (C) At the PTGS (post-transcriptional gene silencing) level, the piRNAs/piwi complex binds to the target RNAs and prevents their function by complementary sequence 22.

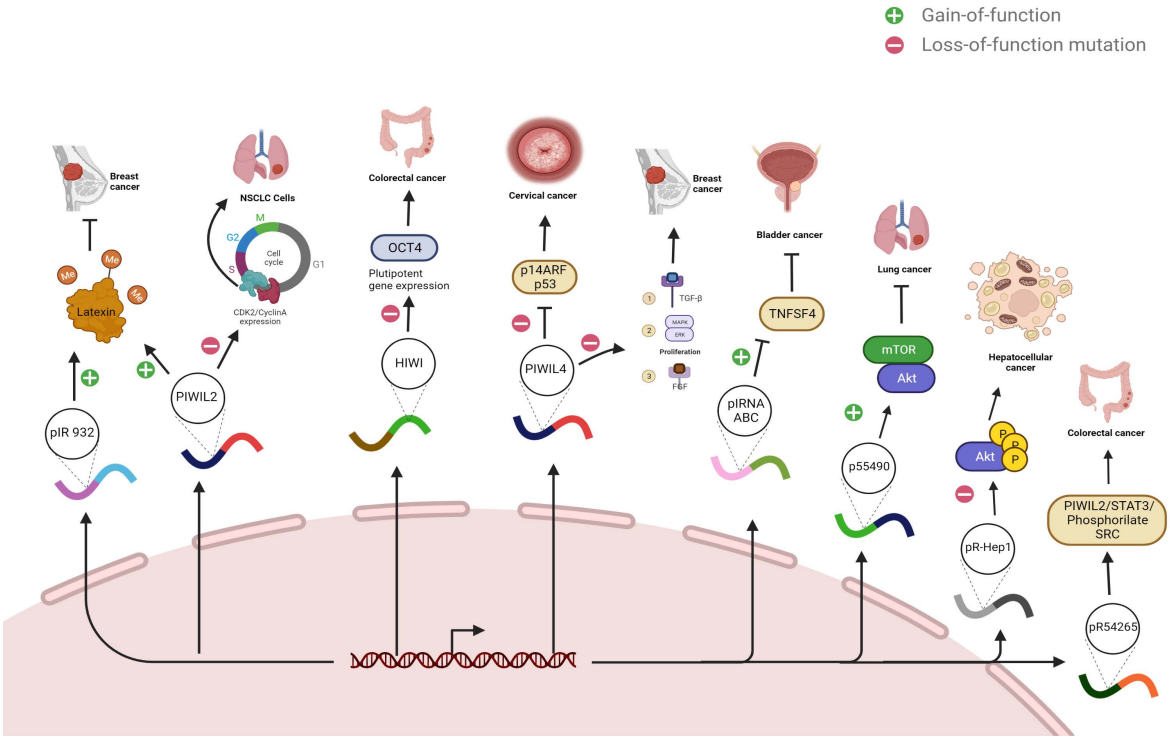


PiRNA and PIWI relationship with cancer

Based on the results of recent studies, piRNAs could be considered potential tumor markers. The data suggest a relation between piRNAs and different types of cancer, such as stomach, esophageal squamous cell carcinoma, colorectal cancer, and breast cancer. In these cases, the piRNAs-PIWI complex favors cancer initiation and progression by increasing or inhibiting the activity of genes involved in cell proliferation, death, and differentiation [21, 29, 30].

In addition, PIWI proteins mediate the repression of transposable elements during meiosis through the binding generated by non-coding RNA (piRNA) and PIWI proteins. This factor binding allows the methylation process, and thus the inhibition of transposons, to generate silencing, triggering dysregulation in different cellular processes [27,31]. PIWI proteins play a role in cancer invasion, migration, proliferation, division, and survival. For example, in breast and lung cancer, the expression of PIWIL2 is increased, and factors such as age, size, stage, and the histology of the tumor are related to PIWIL2 activity. In breast cancer, this protein downregulates the expression of latexin (a tumor suppression protein) due to the methylation of the CpG islands (regulators of gene expression) [32], and PIWIL4 upregulates the level of cell proliferation due to its interaction with the signaling pathways of the growth factors TGF-β and FGF. In cervical cancer, PIWIL4 acts on the p53 gene, one of the most important tumor suppressor genes[33] Similar mechanisms have been described for bladder and hepatocellular cancer. (shown in Figure 4) Finally, in colorectal cancer, the PIWI protein HIWI allows cancer cells to increase their capacity for pluripotency of stem cell properties [34].

Figure 4.
piRNA and hepatocellular cancer. Graphic description of the mechanisms of action of piRNA/PIWI in different carcinogenic conditions



Role of piRNA-823 with MM

New evidence has shown that piRNAs promote increased methylation in MM cells. This methylation silences tumor suppressor genes, which have the function of encoding proteins, regulating cell cycle progression, cell signaling, and apoptosis. Some piRNAs, such as piRNA-823, appear to be increasing in MM patients and are positively correlated with the disease stage. According to H Yan et al., silencing the expression of piRNA-823 in MM cells promotes apoptosis and cell cycle perturbations. This intervention inhibits the proliferation of MM cells in vitro and in vivo. A possible biological explanation is based on the control of angiogenesis. In MM, bone marrow angiogenesis is a hallmark of progression; MM cells and bone marrow ECs (vein endothelial cells) secrete different types of cytokines, which result in the growth of autocrine and paracrine loops between the MM cells and the bone marrow environment. As is known, VEGF (vascular endothelial growth factor) is one of the main proangiogenic cytokines and is responsible for the induction of neoangiogenesis in patients with MM. Evidence supports that the piRNA-823 inhibitor reduced VEGF secretion from MM cells, thereby reducing MM cell-induced proangiogenic activity in EC [35].

The tumorigenic actions of piRNA-823 in MM are related to intercellular communication between MM and ECs cells. Beibei Li et al., determined the relative levels of piRNA-823 in EVs (extracellular vesicles) in 36 MM patients and healthy controls using qRT-PCR. EVs are lipid bilayer spheres that mobilize small proteins and RNAs. EVs reach target cells and regulate biological functions. These are vital for cross-signaling between cancer and surrounding cells in the tumor microenvironment, additionally playing a significant role in cancer development and progression. The investigation found that transfection with piRNA-823 mimetic or treatment with MM-derived EVs significantly promoted ECs proliferation, tube formation, and invasion by enhancing the expression of VEGF, IL-6, and ICAM-1, and it also attenuated apoptosis. In contrast, transfection with the piRNA-823 inhibitor or EVs treatment of piRNA-823 inhibitor-transfected MM cells had opposite effects 1.

The persistence of cancer stem cells (CSCs) in MM is responsible for treatment failure and relapses. Several pathways attempt to explain the development and maintenance of CSC. One hypothesis relies on the immune actions of myeloid-derived suppressor cells (MDSCs). Specifically, the granulocytic MDSCs (G-MDSCs) which mediate immune suppression by inhibiting T cell proliferation, promoting T cell apoptosis, and reducing cytokine secretion by effector T cells. Lisha Ai et al., reported in vitro evidence that MDSCs could induce piRNA-823 expression and, in turn, promote MM cell-like properties through the upregulation of DNA methyltransferases (DNMTs). In this study, the expression of DNMT1, DNMT3A, and DNMT3B was quantified in MM cells, which were cocultured with G-MDSC and identified by qRT-PCR and Western blotting. G-MDSC cocultivation induced DNMT3B expression in RPMI8226 and NCI-H929 cells. Once these cell lines were detected, they were transfected with antagomir-823 with the aim of inhibiting the expression of piRNA-823. This was upregulated by reverting G-MDSC from DNMT3B in MM cell lines. Silencing of piRNA-823 in MM cell lines partially decreased the G-MDSC-induced increase in global DNA methylation. In conclusion, it was shown that piRNA-823 partially controls the stemness of cancer through the activation of DNMT3B and that tumor-associated G-MDSCs promote the properties of MM cancer stem cells in vitro [1].

Methodology to detect piRNA in Multiple Myeloma

The detection of piRNA as a biomarker in cancer, particularly in MM, has emerged as a promising technique to better understand the progression and oncogenic involvement of this disease. Various sampling and analytical techniques, together with the application of advanced tools such as SVM+LSTM software, have allowed investigating the presence and function of piRNAs in different biological samples, shedding light on their potential as indicators of tumor progression and therapeutic response 36.

The techniques used for the detection of piRNAs in MM involve obtaining serum and plasma samples from peripheral blood samples, bone marrow tissue samples from patients with MM and healthy patients, thoracic and lumbar tissue samples infiltrated with CD138+ MM cells, even bone marrow plasma can be used or mononuclear cells can be isolated using Ficoll-Hypaque. Subsequently, RNA isolation can be done by qRT-PCR in different stages of the disease to find out if its levels are correlated with the stage or tumor progression [36,37].

The study of cell lines such as RPMI8226, ARH-77 and U266 has provided a deeper understanding of how specific piRNAs, such as piRNA-823, can regulate the cell cycle and affect the proangiogenic activity of MM in vitro. Detailed analysis of piRNAs in these samples has revealed potential biomarkers that could be critical in the early detection and prognosis of MM. Implementing the use of advanced software, such as SVM+LSTM, has enabled piRNA prediction and correlation with MM oncology [38,39].

Various complementary techniques, such as Western blot, proliferation and apoptosis assays, as well as DNA methylation analysis, in vitro and in vivo assays, along with detailed statistical analyses, have been employed to reveal the mechanism of action of piRNA in the cell cycle and its impact on MM progression [40].

Conclusion

Different evidence shows that there is a limited number of piRNAs present in somatic tissue, several of those already described have been evidenced in the development of different types of cancer by performing functions during proliferation, apoptosis, metastasis, and the capacity of invasion of malignant cells; which makes them potential diagnostic biomarkers.

Overall, the use of piRNAs as biomarkers in the diagnosis and understanding of MM has opened up new possibilities in the early detection of this disease. Sophisticated analysis techniques, combined with the knowledge gained about the function of specific piRNAs, could pave the way for more personalized therapies as a therapeutic target that could aid in the effective management of MM, thus improving the quality of life of affected patients and generating a new innovation in science.

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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Conflict of Interest

Authors declare no commercial or personal relationships influencing the research.

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