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Review Article

PiRNA Biogenesis and Their Role in Human Cancers and Other Diseases: A Narrative Review

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Abstract

PIWI-interacting RNAs (piRNAs) with the length of approximately 26-30 nucleotides are a distinct class of small non-coding RNAs that mainly expressed in the animal gonads. Other small RNAs originate from double stranded precursors but piRNAs derive from long single-stranded primary transcripts, which expressed from distinct genomic regions. piRNAs are involved in silencing of mobile elements named transposons and their main role is germline maintenance. Recent studies have opened new insights on biological and clinical significance of piRNAs in various diseases. Abnormal expression of piRNAs is a remarkable feature in many diseases especially human cancers, which emphasize on their important biological role in disease progression. Furthermore, they can be served as biomarkers and therapeutic targets for tumor diagnostics and treatment. In this review, we explained piRNAs characteristics, biogenesis process and functions, discuss new findings about involvement of these elements in various disease and their potential to be used as diagnostic biomarkers.

Keywords: Piwi interacting RNAs; Biomarkers; Small noncoding RNAs

Introduction

Piwi-interacting RNAs (piRNAs) are a class of small non-coding RNAs with the length of 26–30 nucleotides which express in animal gonads and originates from intergenic repetitive elements in the genome called piRNA clusters (1) and they suppress transposons in order to preserve the integrity of the genome (2). To precise regulation of development and homeostasis of living organisms, control of gene expression by RNA silencing is carry out through a specific manner (3,4). Small non-coding RNAs and Argunaute proteins are the main factors of RNA silencing, which form RNA induced silencing complexes (RISCs) that play a central role in posttranscriptional gene regulation (5).

The loss of piRNAs functions results in transposons' lack of control, and leads to genome damage and deficiency in gonadal development and fertility (6,7). Therefore, transposon silencing mediated by piRNAs is obligated in the animals which go through sexual reproduction (8,9). piRNAs can guard the genome against invasive non-self DNA elements. Therefore, piRNA pathway considered as a small RNA-base genome immune system (2). piRNAs were first recognized through the researches on the Stellate locus in Drosophila. This locus is composed of repeated copies of a gene, which encodes a casein kinase II β-subunit homologue. The biological function of Drosophila Stellate protein is un-

known, yet mutations in Stellate suppressor result in overexpression of Stellate protein during spermatogenesis. This result in formation of Stellate crystals which leads to decreased fertility (10). Most piRNA sequences are antisense to transposon RNAs and can suppress them (11). In mammals, a great number of germ line piRNAs are derived from transposon elements, but most of them (80%) are derived from about 140 loci, corresponding to long non-coding RNAs, not recognized as transposon elements (12,13).

In human and mice piRNA clusters exist in almost similar locations, indicating a conserved biological function in germline development; yet, they are different in terms of nucleotide sequences. These clusters are strand- specific, implying that only one strand transcribes the piRNA precursors (14). piRNA clusters span around 100,000 bases and mainly consist of different transposable elements and their remains, and are enriched in transposon sequences (11). Mutations (especially loss of function mutations) in piRNAs and their cofactors (PIWI proteins) causes transposon disregulation and leads them to randomly insert copies of themselves or move within the genome (15). In this review we tend to point out the main characteristics of piRNA and their function and their role in clinical approaches.

piRNA clusters

piRNAs are classified based on processing factors, the basis of their origins, and Argonaute-binding partners (16). Unique characteristics of clusters such as their contents and lengths and the number of clusters have emerged through this study. For example, Drosophila genome contains 142 piRNA clusters and human genome contains 186 piRNA clusters (17). Normally, piRNA clusters length might range from a few kilo bases to hundred sand they carry fragments of transposons, yet the amount of transposon contents and types varies in different animals (18). In *Drosophila*, piRNA clusters locate near the telomere and centromere regions, mainly between heterochromatin and euchromatin boundaries (1). In mammals, piRNA clusters disperse within chromosomes comparatively randomly, but are syntenically preserved in higher animals (18).

Based on the orientation of transcription in each locus, piRNA clusters classified into three groups: uni-strand, dual-strand, and bidirectional piRNA clusters. Uni-strand clusters only transcribed in one direction. Means that RNA polymerase only chooses one strand of the DNA as its template. In dual- strand clusters both of the strands of a DNA region undergo transcription process. The produced RNA strands are complementary so they can make a double strand RNA molecule (19,20). In bidirectional clusters, transcription occurs in both strands as well but the two newly synthesized RNA strands cannot hybrid because transcription occurs in different directions (21).

piRNA biogenesis and maturation

There are two main pathways for piRNA biogenesis including: the primary processing pathway and the ping-pong cycle which amplifies secondary piRNAs. Primary piRNAs tend to have a uridine (U) at their 5' position (1U bias), while secondary piRNAs have a 10nt residue (10A bias) which is complementary with the 5' ends of primary piRNAs (22,23).

Biogenesis of primary piRNA

The primary pathway engages in germline and surrounding somatic cells in Drosophila, while the ping pong cycle only engages in germline cells. In ovarian somatic cells of Drosophila, during the primary pathway, among all the PIWI subfamily members, just Piwi is expressed. piRNA clusters such as *flamenco* (*flam*) undergo unidirectional transcription and single stranded transcripts are produced (1). The endonuclease Zuc/MitoPLD plays an important role in piRNA biogenesis (24,25). Zuc was initially considered as an enzyme which forms 5' ends of piRNAs, but recently this enzyme can also produce a subset of piRNA 3' ends (directly or indirectly), emphasizing its vital role in the pathway (26). In addition to Zuc, there are a great number of factors required for piRNA biogenesis. These factors are organized into tissue specific processing sites

which called Yb bodies and nuage in Drosophila follicle cells and animal germ cells respectively (27).

The ping-pong cycle

The ping-pong amplification loop is a Post-Transcriptional Gene Silencing (PTGS) mechanism. Ping-pong cycle initiates in the cytoplast with aub-piRISCs together with AGO3, produce secondary piRNAs. The ping-pong cycle occurs at the nuage. Many factors involved in piRNA biogenesis and PIWI proteins, bring together in this structure (28). Aub and AGO3 behave in a complementary manner and cut antisense and sense transcripts of transposons through their slicer activities. The generation of piRNA in a feed-forward mechanism leads to consumption of transposon transcripts and led to silencing of transposons (1,22). Slicer activity causes the cleavage of target RNA in a position between 10 and 11 nucleotides starting from the 5' end of the small RNA (29). Therefore, the 5' end of the secondary piRNA is determined by bilateral cleavage of transposon transcripts. The process of formation of 3' ends is still unknown (30).

In mouse testis, MILI's primary piRNAs are mainly "sense" to the transcripts of the transposons and are unable to target these transcripts. On the other hand, MIWI2 which exists in the nuclear is associated with antisense piRNAs thus it can cause transposon silencing. There is a link between MILI and MIWI2 and DNA methylation of the loci of gene of interest in the genome (23). Yet the molecular mechanism of this process is unclear.

Mechanism and function (regulation of the genome)

Numerous genes are involved in regulation of transposon elements:

Silencing of transposons

Mutations in piRNAs results in overexpression of transposons. Thus, piRNA-PIWI complexes control transposon activity directly. Piwi is localized to the nucleus and binds to HP1a. This factor also involves in assembly of heterochromatin in soma (31,32). *Spn-E* (encodes a necessary helicase for producing piRNA) mutations, lead to lower binding of HP1a to a telomere specific transposon called TART (33). Based on the evidence, piRNA-PIWI complex manage heterochromatin assembly, which leads to transcriptional silencing. According to this supposition, mutations in piRNA leads to the reduction of DNA methylation in mouse testis. Yet, piRNAs also exist in polysome fractions (34). Moreover, Mili, which is a mouse Piwi protein, is associated with translation association factors and might have a role in positive regulation of translation (35). piRNAs also have a role in control of translation.

Gene expression

Only a few sets of piRNAs map to repeated sequences and transposon elements and a great number of piRNAs are mapped to unannotated regions of the genome (36). In Drosophila, some piRNAs derive from 3' end of mRNAs (37). These findings imply to the role of piRNAs in regulation of gene expression. Most of the Drosophila piRNAs are associated with 3' end of a transcription factor, named *traffic jam (tj)*. *Tj* piRNAs in cultured somatic cells showed immune precipitation with Piwi protein, but in ovaries of *zucchini* mutants the level of *tj* piRNAs were reduced. *tj* mutations leads to reduce the level of Piwi proteins in somatic follicle cells. *tj* locus regulates expression of Piwi and piRNAs that derived from this locus. Mutations in piwi and *tj* cause comparable oogenesis deficiencies and result in *FasIII* overexpression, a basic factor for oogenesis (38). Two genes in fly testes, *vasa* and *stellate* are also targets of piRNA pathway (39). *Vasa* gene encodes DEAD box protein, which is particular for germline, and have a role in piRNA production (40). Maternally deposited mRNAs are destroyed during early embryogenesis as transcription is activated. Resulting in a maternal to zygote developmental control. This developmental pathway may involve piRNA pathway (41). Mutations in genes involve in piRNA pathway such as *aub*, *ago3*, *rhi* and *armi* have no significant effect on oogenesis genes (42). Therefore, regula-

tion of gene expression in piRNA might be limited to developmental stages or particular tissues.

piRNAs and diseases

Recently, the study of regulatory RNAs has been taken into great consideration. The wide range of research on biogenesis and function of these RNAs has indicated that they have a key role in almost all biological pathways. Deregulation of these molecules such as miRNAs and piRNAs has been observed in several disorders. In this review, we mention the most important classes on piRNA presented in cancer and other disease, with a focus on their role as regulatory RNAs and their potential as biomarkers.

piRNAs have an important role in development and maintenance of germ line by silencing the transposons and protect the integrity of the genome. Now we know that these molecules are also responsible in some processes in somatic tissue as well as gene regulation. The clinical significance of piRNAs as biomarkers for diagnosis and prognosis has also into interest in recent years. Our knowledge about the importance of piRNAs in cancer is due to defining their role in germ cell cancers (43). Although here are a smaller number of piRNAs in somatic cells compared to that of germlines, yet they have a significant contribution to tumorigenesis. In cancer, piRNAs are deregulated compared to normal tissue (just like PIWI). piRNAs show different patterns of expression in different tissue types, which suggested that piRNA expression, is tissue specific (17). In addition, like PIWI, piRNAs'up- or down-regulation can lead to cell machinery imbalances, which might cause increasing cell proliferation (44), invasion and metastasis (45), and decreasing apoptosis (46). Unregulated piRNA expression has been reported in human cancers such as colorectal cancer, lung cancer, breast cancer, bladder cancer, and gastric cancer (47).

Overall, 6260 piRNA transcriptome of human were studied from normal and tumor tissues (48). Eleven organs examined and out of 20831 only 273 piRNAs express in somatic normal tissues and 522 of them expressed in corresponding tumor tissues. piRNAs have a role in cancer cell apoptosis, proliferation, metastasis and invasion and we might consider them as potential prognostic and diagnostic biomarkers during cancer development (49).

In gastric cancer, piR-823 downregulates. No association was observed between its levels of expression and clinicopathological characteristics (50). On the other hand, in a xenograft model, piR-823 mimics (in a dose dependent manner) significantly inhibited gastric cancer cells growth in vitro and the growth of tumor in vivo. Based on these results, piR-823 can be considered as a potential therapeutic target in gastric cancer. piR-651 is an oncogene which is overexpressed in gastric cancer leads to a positive correlation with TNM (tumor node metastasis) stage. Moreover, an inhibitor of piR-651 in G2/M phase might inhibit cell growth. This shows that piRNAs have a key role in tumorigenesis (44). In addition, the amount of both piR-823 and piR-651 in circulating tumor cells were lesser compared to normal controls (51). Furthermore, piR-823 and piR-651 are more sensitive biomarkers than the others such as CA19-9 and CEA for gastric cancer. These molecules are more stable due to their short length and have higher levels in blood samples and they can be isolated and detected in fluid samples very easily (52). Moreover, piR-823 had an oncogenic effect in multiple myeloma (53) and it is overexpressed in colorectal cancer (54), which points out to heterogeneous behavior of piRNAs in different types of cancer. A three piRNA signature (FR290353, FR064000, and either FR387750 or FR157678) in gastric cancer was found to be significantly correlated with survival relapse (48).

RT-PCR shows that four piRNAs including piR-20365, piR-20582, piR20485 and piR-4987 are upregulated in breast cancer (55). On study suggested that piR-932 /PIWIL2 complex might have a positive correlation with epithelial-mesenchymal transition in breast cancer. These data suggested that piR-932 and PIWIL2 might be considered as potential targets for stopping metastasis in breast cancer (56). piR-021285 plays a role in methylation of genes involved in breast cancer (57).

piRABC is downregulated in bladder cancer. In vitro investigations were carried out on human bladder cancer cell lines (59). piRABC overexpression might prevents cell proliferation and colony formation. In addition, the level of PIWIL1/HIWI protein in adjacent tissue were higher than cancerous tissue. This suggests that overexpression of HIWI might lead to reduce bladder cancer risk. The HIWI-piRABC complex may complement to *TNFSF4* 3'UTR and alter bladder cancer development (58).

The pattern of piRNA expression in normal bronchial epithelial cells and lung cancer cells are different (59). In addition, they have demonstrated that piR-L-163 directly binds to ERM and cause ERM phosphorylation. Therefore it have a key role in protein activation (60,61). During cisplatin-based chemotherapy piR-L-138 was upregulated both in vivo and in vitro, suggesting it could be considered as a potential target in patients with lung squamous cell carcinoma for overcoming chemoresistance (62). Compared to their corresponding adjacent normal tissue, piR-Hep1 is upregulated in almost half of the HCC tumors (46.6%). knocking down this piRNA could prevent cell invasion, viability and motility (58).

piRNA/PIWI complex might have a crucial role in the risk of colorectal cancer (63). piRNA-823 might be involved in colorectal carcinogenesis. Silencing this piRNA lead to cell apoptosis and suppress cell viability. They considered piRNA-823 as a potential therapeutic target for colorectal cancer (47). One of piR-598 variants (rs147061479) blocks the tumor suppressive function of piR-598 and thus promoting cell proliferation, which eventually leads to glioma (64). piRNAs can also active gene expression by inducing H3K4 methylation/H3K27demethylation (65).

Comparison of genome-wide profiling of piRNAs in Alzheimer disease and normal tissues of brain tissues discovered more than 9000 piRNAs. Among these 103 piRNAs expressed differentially. piRNAs could be considered as potential biomarkers for Alzheimer disease diagnosis (66). piRNAs may act as novel gene expression regulators and stimulate metabolic phenotypes. Henaoui et al. showed that piRNAs regulates pancreatic beta cells gene expression in rat (67).

Both piRNAs and miRNAs affect AKT pathway in heart disease (68). AKT is a survival factor, which plays an important role in increasing the proliferation of myocardial stem progenitor cells and cardiomyocytes (69). There is a modified piRNA Expression in an animal model of cardiac hypertrophy and a specific piRNA recognized in patients' myocardial infarction. piRNAs might play role in activating retrotransposons in several cardiac pathologies. They provide a comprehensive list of piRNAs, expressed in cardiac primitive cells (CPC). This was the first report of piRNA signatures in cardiac progenitors (70).

piRNAs are involved in regenerative medicine (71). After cardiovascular dysfunction, such as ischemia injury, myogenic and cardiac progenitors are in charge for regeneration and tissue repair. The role of piRNA transcripts and stem cells in cardiospheres are in great interest for researchers. Recent studies on piRNA profiling of cardiac progenitors recognized a great number of piRNAs, mostly located in genomic clusters (71). Chuang et al. identified 180 piRNA transcripts involved in myometrium and leiomyoma. Among these, 24 piRNAs including piR-1311, piR-16677, piR-20365, and piR-4153, showed different patterns of expression. The expression of these piRNAs showed a notable decline in leiomyoma compared with myometrium (72).

Knocking down of Caz leads to increasing levels of *flam* pre-piRNA. Therefore, there wsa a binding between Caz and *flam* pre-piRNA (73). FUS binds to new synthesized RNA strand and brings RNA pol II to a halt. Which leads to transcription suppression (74). Caz might be involved in suppression of *flam* pre-piRNA transcription. Caz and Rhino are genetically interacted and it is assumed that FUS is a potential mediator for splicing and transcription (75).

piRNAs as Biomarkers

As mentioned before, abnormal expression of piRNAs and their impact in cancer patients suggests that piRNAs can be considered as valuable

diagnostic biomarkers or potential drug targets (76, 77). In gastric cancer, piR-651 and piR-823 levels were considerably lower in patients with gastric cancer compared to normal tissues, indicating that these two piRNAs can be considered as important biomarkers for gastric cancer detection (51). Several investigations have revealed important piRNA signatures in clear cell renal cancer, used as a prognostic biomarker. In one study 3 piRNAs including piR-30,924, piR-57,125, and piR-38,756 found to have close correlation with overall survival and tumor recurrence (78). Another study identified 46 piRNAs involved in metastasis (79).

In breast cancer, deep sequencing identified piRNA expression profiles from tumor tissues and healthy controls. Eight piRNAs including piR-34,736, piR-36,249, piR-35,407, piR-36,318, piR-34,377, piR-36,743, piR-36,026, and piR-31,106 were selected and proved used as novel prognostic biomarkers. Moreover, Zhang, et al, proposed piR-932 as a newfound piRNA associated with stemness. Following investigations confirmed that piR-932 overexpression through Latein gene demethylation causes induction of an EMT phenotype (80). In colorectal cancer piR-1245 were determined as a potential prognostic biomarker. Moreover, piR-1245 have oncogenic functions and causes tumor progression (81). There are more studies describing the potential of piRNAs to be considered as prognostic biomarkers. However, there are many challenges to transfer these findings to clinics. In diffuse large B-cell lymphoma (DLBCL), piRNA-30473 overexpression leads to aggressive phenotype and lack of this piRNA causes cell cycle arrest and decline proliferation (82).

Conclusion

piRNAs has been discovered and studying their role in biological processes involved in genetic, germline and epigenetic has dragged too much attention. They seem to play important roles in human disease, especially human cancers, and provide sustainable potential for future studies on various disease including cancer. piRNAs together with other regulatory elements provide a complex interaction. Understanding these networks will open new insights toward biological and medical aspects and managing several diseases including cancer.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Conflict of interest

The authors declare that there is no conflict of interests.

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