Review article

PIWI-interacting RNAs (PiRNAs) as emerging biomarkers and therapeutic targets in biliary tract cancers: A comprehensive review

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ARTICLE INFO

ABSTRACT

Cancers affecting the biliary tract, such as gallbladder cancer and cholangiocarcinoma, make up a small percentage of adult gastrointestinal malignancies, but their incidence is on the rise. Due to the lack of dependable molecular biomarkers for diagnosis and prognosis, these cancers are often not detected until later stages and have limited treatment options. Piwi-interacting RNAs (piRNAs) are a type of small noncoding RNA that interacts with Piwi proteins and has been linked to various diseases, especially cancer. Manipulation of piRNA expression has the potential to serve as an important biomarker and target for therapy. This review uncovers the relationship between PIWI-interacting RNA (piRNA) and a variety of gastrointestinal cancers, including biliary tract cancer (BTC). It is evident that piRNAs have the ability to impact gene expression and regulate key genes and pathways related to the advancement of digestive cancers. Abnormal expression of piRNAs plays a significant role in the development and progression of digestive-related malignancies. The potential of piRNAs as potential biomarkers for diagnosis and prognosis, as well as therapeutic targets in BTC, is noteworthy. Nevertheless, there are obstacles and limitations that require further exploration to fully comprehend piRNAs' role in BTC and to devise effective diagnostic and therapeutic approaches using piRNAs. In summary, this review underscores the value of piRNAs as valuable biomarkers and promising targets for treating BTC, as we delve into the association between piRNAs and various gastrointestinal cancers, including BTC, and how piRNAs can impact gene expression and control essential pathways for digestive cancer advancement. The present research consists of a thorough evaluation presented in a storytelling style. The databases utilized to locate original sources were PubMed, MEDLINE, and Google Scholar, and the search was conducted using the designated keywords.

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1. Introduction

Biliary tract obstruction is a common clinical problem that can be caused by various factors, including gallstones, cancerous tumors, inflammation of the pancreas, metastatic disease to the liver, and disease of the bile ducts [1]. Extensive research has been conducted on both humans and animals, which consistently demonstrates that blockages in the bile duct can potentially result in cholestasis and severe harm to the liver [2–5]. As an important cause of cholestasis, biliary tract cancers (BTCs) encompass a diverse range of malignant tumors that are typically categorized into distinct subgroups based on their anatomical location. These subgroups include extrahepatic cholangiocarcinoma (eCCA), intrahepatic cholangarcinoma (iCCA), ampulla of Vater cancer (AVC), and gallbladder cancer (GBC) [6–8]. Cholangiocarcinoma, as a term, encompasses two subtypes: eCCA and iCCA. These subtypes, in turn, can be further classified as distal cholangiocarcinoma (dCCA) and perihilar cholangiocarcinoma (pCCA) [9,10]. The anatomical classification of BTC effectively reflects the distinguishing characteristics of BTC subgroups across various aspects [11]. Though it may seem simple, this classification system accurately captures the complexities of BTCs. BTCs, also known as bile duct cancers, are responsible for approximately three percent of all gastrointestinal (GI) cancers. Interestingly, it claims the noteworthy status of being the runner-up among primary liver cancers (PLCs), immediately following hepatocellular carcinoma (HCC) [12,13]. The prevalence of BTCs is indeed increasing in countries across the Western hemisphere, and this may be attributed to various factors including better disease recognition and its association with the rising incidence of iCCA. The connection between BTCs and iCCA underscores the need for thorough research and awareness regarding these conditions [14]. The incidence of eCCA and GBC appears to remain stable, contrasting with the increasing incidence of BTCs. This suggests that there might be distinct factors contributing to the rising occurrence of BTCs compared to other subgroups of BTCs. It's disheartening to hear that the overall prognosis for BTCs remains poor, with a low 5-year survival rate and high rates of recurrence [15,16]. Apart from the mentioned risk factors, an elevated probability of developing BTCs has been associated with primary sclerosing cholangitis (PSC), infections of hepatitis B & C, cirrhosis, and Hepatic Steatosis [17–20]. The symptoms experienced and their nature depend on the location of the primary tumor and any associated metastases. Symptoms can arise from direct compression, such as biliary obstruction, or they may be constitutional or related to underlying conditions like chronic liver disease. Due to their lack of specificity, patients often present with advanced-stage disease. Some patients may not exhibit any symptoms, and the presence malignancy is typically discovered incidentally through abnormal liver function tests or imaging conducted for unrelated purposes [21]. Although surgery is typically considered the primary course of action for cases in the early stages, a substantial portion of individuals diagnosed with biliary BTC are unfortunately identified during advanced stages, rendering surgical intervention impractical [22,23]. Enhancing patient outcomes for individuals diagnosed with biliary tract malignancies [24] is an immediate priority. This necessitates the advancement of early detection strategies, the identification of new biomarkers, and the exploration of innovative therapeutic methods [23,25–27]. At present, the treatment approach for patients in advanced stages of the disease primarily involves palliative chemotherapy [28]. Nevertheless, the landscape of treatment for BTCs, particularly iCCA, is undergoing a significant transformation due to the rapid development of targeted therapies. This transformation is evident in the increasing feasibility of targeting FGFR (fibroblast growth factor receptor) fusions and IDH-1 & −2 (isocitrate dehydrogenase) mutations as viable treatment options [29]. At the early stage, both patients with CCA and GBC experience a deficiency in circulating diagnostic biomarkers. The emergence of extracellular vesicles, particularly exosomes, has presented a promising opportunity for cancer diagnosis due to their convenient and rapid accessibility. Consequently, the discovery of exosomal biomarkers offers a fresh approach to diagnosing CCA and GBC [16]. The involvement of BTCs leads to the disruption of numerous signaling pathways that are essential in the advancement and deterioration of the condition [30]. Within various signaling pathways, certain ones hold greater significance, particularly the mitogen-activated protein kinase (MAPK) pathway. This pathway is known to be excessively activated in various cancers of the digestive system, such as biliary tract cancers (BTCs), pancreatic cancer, and colon cancer [31,32]. Phosphotidylinositol-3 kinase (PI3K)/Akt pathway, which is vital for various biological functions in malignant cells, such as proliferation, senescence and survival [33], is another one. An interruption in the control of this pathway has shown a significant impact on BTCs [34–37]. The JAK/STAT signaling cascade is another pathway that is pertinent to BTCs [38–40] as is the PI3K/AKT/mTOR pathway [41]. All of them of are promising targets for therapy in patients with BTCs.

Many research studies have demonstrated the significant impact of non-coding RNAs on the onset and advancement of digestive system cancers. One particular type, known as PIWI-interacting RNAs (piRNAs), may possess notable functions in either promoting or inhibiting cell death processes [42]. PiRNAs are additionally implicated in the major signaling pathways discussed earlier, including the PI3K/AKT signaling pathway [43] and the STAT signaling pathway [44], which are involved in apoptosis. Abnormal piRNA expression has been documented in human cancers, including the digestive system cancers. These results suggest that the piRNA signaling pathways may have a connection to cancer formation [45]. Growing evidence suggests that particular piRNA molecules have a significant impact on the growth, progression, and response to chemotherapy in different types of digestive cancers. However, further investigation is necessary to fully understand the consequences of an imbalanced PIWI-piRNA pathway in these malignancies. PiRNAs are increasingly being recognized as potentially valuable indicators for detecting and predicting digestive system cancers, as well as potential targets for future therapeutic strategies [46].

2. Literature search strategy

2.1. Description of search methodology

For this comprehensive review, a systematic literature search was conducted to identify relevant studies focusing on the role of PIWI-Interacting RNAs (PiRNAs) as emerging biomarkers and therapeutic targets in biliary tract cancers. The search strategy aimed to

gather a diverse range of research articles, reviews, and clinical studies to provide a comprehensive overview of the topic.

2.2. Databases used

The following databases were utilized to locate original sources.

1. PubMed: A widely used biomedical database providing access to a vast collection of peer-reviewed literature in the field of medicine, biology, and related disciplines.
2. MEDLINE: A subset of PubMed, MEDLINE offers a comprehensive index of biomedical literature, including articles from reputable journals and research publications.
3. Google Scholar: A multidisciplinary database encompassing scholarly articles, theses, books, conference papers, and other scholarly works, providing broad coverage across various fields of study.

2.3. Keywords and inclusion/exclusion criteria

The search was conducted using the following keywords and phrases, tailored to capture relevant literature on PiRNAs and biliary tract cancers:
- "PIWI-interacting RNAs"- "PiRNAs"- "Biliary tract cancers"- "Gallbladder cancer"- Cholangiocarcinoma"- "Biomarkers"- "Therapeutic targets"- "Diagnostic applications"- "Prognostic applications"

2.4. Inclusion criteria

- Studies published in peer-reviewed journals.
- Articles focusing on the role of PiRNAs in biliary tract cancers.
- Research articles, reviews, and clinical studies written in English.
- Studies available in full-text format.

2.5. Exclusion criteria

- Non-English language publications.
- Studies not directly relevant to the role of PiRNAs in biliary tract cancers.
- Conference abstracts, letters, editorials, and commentaries.

The search strategy aimed to encompass a broad range of literature while maintaining relevance to the topic of interest. By utilizing these databases and carefully selecting keywords and criteria, we aimed to ensure a comprehensive and systematic approach to gathering relevant literature for this review.

3. piRNAs

3.1. Origin of piRNAs

PiRNAs, which are a type of non-coding RNAs (ncRNAs), have their origins traced back to the transcription of a significant portion of mammalian genomes [47]. Unlike coding RNAs, ncRNAs lack the ability to encode proteins but possess informational content and exhibit diverse functionalities. These functionalities encompass translation suppression, regulation of chromosome dynamics, RNA editing, mRNA degradation, as well as playing a crucial role in chromosome segregation and maintenance [48,49]. It is intriguing to note that ncRNAs play a role in numerous disease-related biological processes, including cancer.

Based on evidence, ncRNAs are divided to structural ncRNAs (rRNA, tRNA) and regulatory ncRNAs including miRNA, piRNA, siRNA, crasiRNA, TelsRNA and RNA interference (RNAi).

In contrast, ncRNAs can be classified into two distinct classes according to their functional roles. The first category encompasses short ncRNAs, denoted as SncRNAs, which consist of fewer than 200 nucleotides. These SncRNAs possess the capacity to regulate biological processes either at the DNA or RNA levels. The second category comprises long ncRNAs, commonly known as lncRNAs, which are characterized by their length exceeding 200 nucleotides and their inability to undergo translation into proteins. It is worth noting that lncRNAs perform diverse functions within the cellular contex [50]. To reiterate, lncRNAs have a length greater than 200 nucleotides and lack the translational capacity to synthesize proteins. SncRNAs, which encompass a diverse range of non-coding oligonucleotide regulators, play crucial roles in morphological and physiological processes. Gene modulation, a crucial procedure in governing genetic activity, is significantly impacted by their participation at both the levels of transcription and post-transcription, as stated in Ref. [51]. Extensive investigation has been dedicated to the exploration of diverse subtypes of sncRNAs, encompassing small nuclear RNAs, small nucleolar RNAs, and RNAi molecules. RNAi, a notable gene regulatory mechanism prevalent among numerous eukaryotes, includes small interfering RNAs (siRNAs) and piRNAs [52–54]. Additionally, microRNAs (miRNAs) play a significant role in RNA interference, also known as RNA silencing, which has emerged as a prominent pathway for gene regulation in various eukaryotic organisms [55,56].

Specifically, siRNAs, with a nucleotide length ranging from 20 to 30, assume a crucial function in modulating gene expression pathways and post-translational protein events. These small RNAs, generated either by the enzyme Dicer or through mechanisms not involving Dicer, then associate with the RNA-induced silencing complex (RISC) comprising Argonaute proteins [57]. The targeting of RISCs to specific genes relies on the complementarity between small RNAs and target gene transcripts. This interaction leads to the suppression of gene expression through various mechanisms, such as promoting RNA instability, inhibiting translation, cleaving the transcripts, or inducing heterochromatinization [58,59].

PiRNAs, miRNAs, and siRNAs have several notable distinctions. One key difference is their interaction with different subfamilies of Argonaute proteins. PiRNAs utilize PIWI subfamily proteins to exert their effects, whereas miRNAs and endogenous short interfering RNAs (endo-siRNAs) team up with members of the Argonaute subfamily. Additionally, RNAs generated via a Dicer-independent process bind to proteins from the PIWI subfamily [60,61].

The length of piRNAs ranges from twenty-one to thirty-five nucleotides, and they are generated from single-stranded precursors in a Dicer-independent manner. Conversely, miRNAs and siRNAs, which are twenty to twenty-four nucleotides, originate from stem-loop precursors or double-stranded RNA and undergo processing via Dicer RNase III [62]. Furthermore, across diverse animal taxa, piRNAs display 2′-*O*-methylation at their 3′-terminus, whereas siRNAs in plants and Drosophila are predominantly characterized by 2′-*O*-methylation [63–65]. This variation in 2′-*O*-methylation patterns across different organisms adds to the complexity and diversity of the RNA regulatory mechanisms. Recognized for their impact on cancer biology, piRNAs are a class of small RNA molecules that have gained prominence in recent times. In the case of humans, the identification of over thirty thousand piRNAs has taken place [66]. Primarily, their role lies in directing the machinery responsible for chromatin silencing towards particular DNA sequences within the genome. These targeted sequences encode transposons, thus unveiling the crucial connection between piRNAs and gene regulation [67].

The previous belief that piRNAs primarily functioned in germlines has been overturned, as it is now understood that they have a
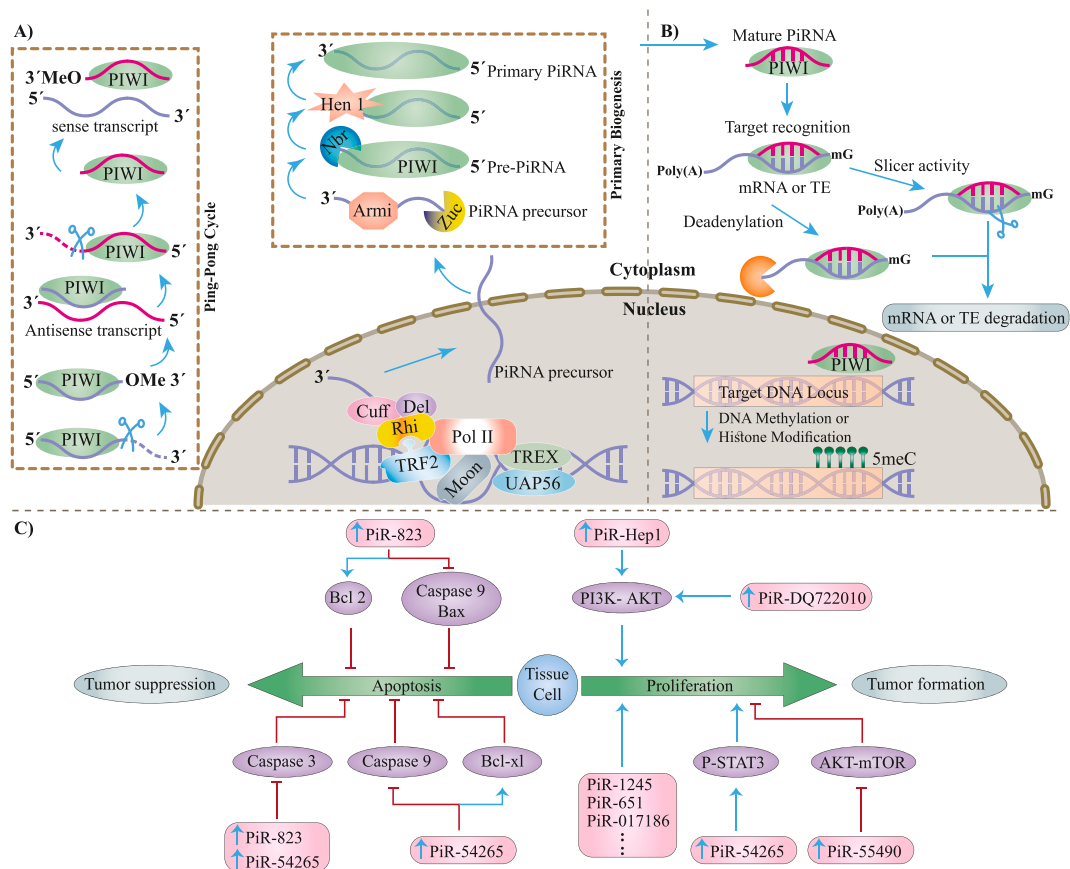

Fig. 1. This figure provides a visual representation of the piRNA biogenesis process (A). It begins with the generation of piRNA precursors from dual-strand clusters by RNA polymerase II and related factors. Upon entering the cytoplasm, the RNA helicase Armitage (Armi) unwinds the complex secondary structures of piRNA precursors. Then, the endonuclease Zucchini (Zuc) converts precursors into pre-piRNAs by adding a 5′ monophosphate group. These pre-piRNAs are loaded onto PIWI proteins, where the 3′–5′ exonuclease Nibbler (Nbr) trims them further. At the same time, the newly formed 3′ terminal ends undergo methylation at the 2′ oxygen with the help of the small RNA 20-*O*-methyltransferase Hen1, signifying the completion of the primary biogenesis process. Additionally, the Ping-Pong cycle involves PIWI proteins associating with sense or antisense piRNAs, leading to the cleavage of corresponding piRNA precursors (A).

crucial role in both somatic tissues and cancer tissues [68]. The scientific community has put forth a hypothesis suggesting that a distinct cluster of piRNAs may possess the capacity to exert control over protein-coding genes via DNA methylation. Should these modulatory targets prove to be pertinent to cancer, it has the potential to significantly influence the progression and manifestation of cancerous conditions [69,70]. Molecules with lengths typically ranging from twenty-six to thirty-one nucleotides are generated from single-stranded precursors through a process independent of the RNase III enzyme. In contrast, siRNAs and microRNAs are shorter, ranging from 21 to 23 nucleotides. The mechanisms governing the functions and formation of piRNAs are largely unexplored, primarily due to the distinct nature of the piRNA pathway compared to mi and endo-siRNA pathways. Additionally, piRNAs are predominantly restricted to reproductive tissues [71,72], further complicating their study. PiRNAs originate from longer transcripts that are synthesized from different sections of the genome, including 3′-UTR regions, introns, and repetitive sequences [59,73]. PiRNAs are truly captivating and play critical roles in a wide range of biological processes. Some of their important functions include silencing transposons (mobile genetic elements), regulating epigenetic modifications, and facilitating germline development [69,74,75]. Furthermore, the diversity of these molecules is quite remarkable, with mammals carrying a large number of different sequences and Drosophila boasting more than 1.5 million unique sequences and Drosophila boasting more than 1.5 million unique sequences [76]. Despite their initial discovery in germline cells and their main role in regulating germline preservation, piRNAs have been detected in somatic tissues, suggesting their involvement in gene expression within those cells as well [77,78]. The transcription of piRNA precursors occurs within piRNA clusters, followed by cytoplasmic modifications and nuclear transport, where they form complexes with PIWI proteins [79]. While unidirectional piRNA clusters are prevalent in somatic cells, dual-stranded clusters are more common in germline cells [80]. 2-OH structures are commonly observed at the 3′ ends of mature primary piRNAs, while their 5′ ends are characterized by the presence of uridine. These distinctive methylation patterns and nucleotide compositions contribute to the unique nature of piRNAs [81]. Notably, piRNAs demonstrate stability in body fluids and are not subject to degradation during circulation [82].

3.2. Biogenesis of piRNAs

The origin of piRNAs can be categorized into 3 classes: transposons, mRNAs, and lncRNAs. While previous studies have primarily focused on the transposon-derived piRNAs [83], recent discoveries indicate that the main source of genomic piRNAs in humans is the genetic material found in protein-coding genes and lncRNA genes, instead of transposons. This discovery suggests that there are likely other functions associated with human piRNAs, extending beyond their established role in transposon silencing. These findings point towards the intriguing possibility that human piRNAs might be involved in diverse cellular processes and play a broader regulatory role in gene expression. Ongoing research aims to unravel the multifaceted functions of piRNAs in the human genome [76,84–87].

The process of piRNA biogenesis is complex, with some variations depending on the cell type, organism, and purpose. However, the fundamental steps of piRNA biogenesis remain conserved [88]. In contrast to the biogenesis processes of siRNA and miRNA, piRNA biogenesis can be categorized into two distinct pathways: primary and secondary amplification, which is commonly referred to as the ping-pong amplification loop. This intricate mechanism ensures the generation and maintenance of a diverse pool of piRNAs, enabling them to carry out their specialized roles in protecting the genome against the detrimental activities of transposons [83].

The maturation process of piRNAs involves multiple sequential steps. These steps include the formation of piRNA precursors, modification of the 5′ and 3′ ends, and the addition of nucleotide methylation. These collective processes ultimately lead to the formation of fully mature piRNAs, which are essential for their functional activities (Fig. 1A) [79].

3.2.1. The primary processing pathway

PiRNA clusters, which are typically found in pericentromeric or subtelomeric regions of the genome, serve as origins for primary piRNAs. These clusters, spanning from several to over 200 kb length, consist of numerous transposon fragments [11,47]. It is worth noting that the majority of piRNA clusters exhibit bidirectional transcription, resulting in the production of piRNAs from both genomic strands [59,89]. The transcription process of piRNA precursors in the nucleus involves multiple proteins. These include RNA polymerase II, Moonshiner (Moon), the Rhino-Deadlock-Cutoff complex (RDC complex), TATA-box binding protein-related factor 2 (TRF2), three prime repair exonuclease (TREX), and 56-kDa U2AF-associated protein (UAP56) [72,90]. At the onset, pi clusters attract RNA polymerase II, in which the promotion of transcription is facilitated by the RDC complex. Moreover, the involvement of the RDC complex and TRF2 in transcription initiation is accompanied by the interaction of Moon. The inhibition of Rops formation is ensured by TREX, while splicing dual-strand clusters are suppressed by UAP56 [78]. Afterward, the nuclear pores play a crucial role in facilitating the transportation of piRNA precursors [91]. Additionally, the RNA helicase Armitage (Armi) is responsible for unraveling the secondary structures [78].

The modification of primary piRNA's 5′ end occurs within the mitochondria and involves the action of the Zucchini (Zuc) protein, which acts as an endonuclease [92]. Subsequently, the precursors undergo processing by the same endonuclease Zuc, resulting in the formation of pre-piRNAs. These pre-piRNAs are subsequently loaded onto PIWI proteins, including PIWI and Aubergine. They undergo trimming at the 3′ end with the help of Nibbler (Nbr), which functions as a 3′ to 5′ exonuclease [78]. At the same time, the newly formed 3′ terminal ends of pre-piRNAs undergo methylation at the 2′-oxygen position. This methylation process is facilitated by Hen1, which is a small RNA 2′-O-methyltransferase [93]. According to current research findings, the process of 2′-O-methylation is believed to contribute to the enhanced stability of piRNAs [93–95]. This series of events is known as the primary biogenesis of piRNAs, resulting in the production of primary piRNAs [90].

Then piRNAs enter the nucleus. The mechanism of piRNA nuclear entry remains unknown in humans, unlike in Drosophila and mice where PIWI loading is essential for this process [68]. Previous studies have indicated that the HIWI/HIWI2-piR-sno75 complex has the ability to activate the TRAIL gene. This activation is accomplished by enlisting histone methyltransferases KDM6A and MLL3 to

the promoter of the TRAIL gene. This suggests a potential connection between HIWI/HIWI2 and the import of piRNAs into the nucleus through histone modification [96]. Additionally, it has been observed that PIWI proteins detected in the nucleus include HIWI/HIWI2 and HILI, indicating a possible involvement of HILI in this process as well [68,97–99].

3.2.2. The secondary amplification pathway

In the process of secondary amplification, primary piRNAs are activated by AGO3 and Aubergine (Aub) proteins. This activation leads to the production of mature piRNAs [90]. Aub protein specifically attaches to antisense-strand piRNAs and cuts sense piRNA precursors. This cleavage generates the 5′ end of a new piRNA and forms sense piRNAs that associate with Ago3. On the flip side, when it comes to Ago3, it has an affinity for sense-strand piRNAs and carries out the cleavage of antisense piRNA precursors. This cleavage event leads to the creation of antisense piRNAs, which then load onto Aub protein [100,101]. Interestingly, this entire process results in the generation of a new piRNA that possesses the exact same sequence as the initial piRNA that triggered the cycle [90]. In a fairly brief timeframe, this cleavage process repeats itself, giving rise to multiple piRNAs. This abundant production of piRNAs is commonly known as secondary biogenesis or the ping-pong cycle. Subsequently, PIWI proteins bind to these piRNAs and facilitate their transportation back to the nucleus, where their primary role is to silence target genes [90].

Our knowledge about the precise mechanism of piRNA biogenesis is still limited, primarily derived from studies conducted on Drosophila germline cells. However, in mice, a comparable pathway has been identified, albeit with the participation of distinct Piwi proteins. Interestingly, there are indications suggesting that the process in humans might bear resemblance to that observed in Drosophila [76,102].

The core components of piRNA biogenesis in mammals, including humans, are the initiation of a ping-pong cycle and the phased production based on mitochondria. These two processes have been found to be highly conserved [68].

3.3. Functions of piRNAs

SiRNAs, miRNAs, and piRNAs are recognized for their capacity to silence or induce mRNA degradation, thereby regulating gene expression. PiRNAs, predominantly involved in epigenetic silencing within the nucleus, are also capable of participating in transcript silencing in the cytoplasm, resembling the function of miRNAs (Fig. 1B) [67,103].

3.3.1. piRNAs and transposon silencing

The PIWI protein plays a vital role in organisms by specifically binding to piRNA and carrying out essential functions. The PIWI-piRNA pathway is widely recognized as the immune system of the reproductive system, protecting germ cell development from the potential threats posed by transposon elements (TEs). TEs, which often make up 50 % or more of mammalian gene sequences, can cause disruptions in the normal genome sequence and lead to gene mutations when they are released from their constraints. These TEs have the ability to replicate independently or fragment from their original position and integrate elsewhere, thereby inducing genomic damage and gene mutations that are closely linked to physiological and pathological processes such as infertility and cancer [72,104, 105].

At first, the role of piRNAs in safeguarding against transposon movement within germline cells of flies was documented. Remarkably, subsequent investigations have validated this finding across a wide spectrum of organisms, spanning from hydras to humans. The body of evidence supporting this notion has grown significantly [106–108]. Transposons, commonly referred to as jumping genes, display similarities to internal viruses and present a threat to genetic integrity through the act of "duplicating and transferring" their genetic material into the DNA of the host organism. This activity gives rise to a range of repercussions. The introduction of exons disrupts the arrangement of coding sequences, whereas the insertion of introns possesses the capability to modify splicing patterns. Consequently, this could lead to the generation of newly formed fusion proteins that may prove detrimental [109].

In the process of piRNA biogenesis, piRNA clusters are situated within transposon elements, further highlighting the close relationship between piRNAs and TEs. Consequently, it is believed that piRNAs play a role in silencing transposons through epigenetic mechanisms [110].

3.3.2. PIWI-interacting RNAs and the influence on DNA methylation

DNA undergoes a chemical modification known as DNA methylation, where certain bases are selectively altered by the addition of S-Adenosyl-methionine (SAM) by the enzyme DNA methyltransferase (DNMT) [111]. The process of DNA modification, known as DNA methylation, plays a pivotal role in methylating CpG islands located within promoter regions. This, in turn, hinders the initiation of transcription. Intriguingly, Piwi proteins have a vital function in guiding DNMT to interact with transposable elements or specific genes, leading to the repression of these elements or genes [112]. The activation of PIWIL1 in the piRNA-PIWIL1 pathway can result in a global decrease in hypomethylation and specific regional alterations in hypermethylation [113]. This suggests that piRNAs have the potential to induce DNA methylation at non-transposon sites. The influence of piRNAs on DNA methylation is still not fully understood in terms of its molecular mechanism, but their evolutionary importance in this process is highly significant [114]. Furthermore, the interplay between piRNAs and Piwi proteins extends to the machinery responsible for histone methylation, wherein they contribute to the regulation of methylation of specific lysine residues on histones, specifically H3K and H4K. By facilitating the recruitment of histone methyltransferases to target transcripts, piRNAs and Piwi proteins play a crucial role in transcriptional repression [112]. However, the exact relationship between histone modification and piRNAs is currently not fully resolved [90].

3.3.3. PIWI-interacting RNAs and messenger RNA (mRNA)

It is vital to conduct additional research to ascertain whether piRNAs exhibit comparable functionalities to microRNAs. This is crucial since both types of RNA molecules are instrumental in the regulation of gene expression. Therefore, exploring potential parallels between piRNAs and microRNAs is imperative to gain a comprehensive understanding of their roles and mechanisms in gene regulation. Once transcribed, piRNAs can function similarly to microRNAs by inducing mRNA degradation, thereby inhibiting protein synthesis [115,116]. There are two main mechanisms through which mRNA degradation takes place. One involves the slicing of mRNA by PIWI, while the other is a deadenylation-dependent process [117]. Deadenylation refers to the shortening of the poly(A) tail of mRNAs and serves as the main pathway for mRNA decay and the inhibition of translation [78,102].

3.4. Detection of piRNAs

The detection of piRNAs can be achieved through a combination of computational and experimental methods. Commonly, piRNA detection is done using methods such as RNA-sequencing (RNA-seq), northern blotting, *in situ* hybridization, Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) [46], support vector machine (SVM) [118] and machine learning predictors. The last one mentioned, include computational methods that can be trained to predict piRNA-disease associations based on piRNA and disease features. They can be used to identify potential piRNA-disease associations and predict new ones [119].

4. PIWI

PIWI proteins, members of the Argonaute family, are primarily expressed in germline cells, particularly those induced by P-element activity [106]. Their fundamental function lies in the regulation of gene expression, where they accomplish this task by identifying short RNAs that act as guides, directing them towards their designated target genes. In humans, there are four types of PIWI proteins known as PIWIL1, PIWIL2, PIWIL3, and PIWIL4. Each of these proteins plays a role in RNA silencing and gene regulation processes [120]. Specialized proteins possess the capacity to interact with piRNAs. When they form a complex, referred to as piRNA-induced silencing complex (piRISC), these proteins exhibit crucial functions in a wide array of biological processes. These include the modulation of epigenetic mechanisms, suppression of transposable elements, safeguarding the integrity of the genome, facilitating gametogenesis, and actively participating in piRNA generation [59]. Recent research has established a connection between PIWI proteins and several cancer characteristics, such as cell growth, the preservation of genomic stability, the evasion of programmed cell death, cell invasion, and the spread of cancer cells to distant sites [121,122]. The implications indicate that PIWI proteins exhibit promise for their application in cancer diagnosis and prognosis. Furthermore, there is an increasing body of research that showcases noticeable variations in expression patterns between samples obtained from healthy individuals and those affected by tumors [123–125].

5. The role of piRNAs in cancer

Recent research investigations have established a clear association between cancer progression and the dysregulated expression of piRNAs, underscoring their immense potential in diverse oncological applications. These small ncRNAs present opportunities as diagnostic aids, therapeutic targets, and prognostic biomarkers for cancer, as highlighted in Ref. [45].

Abnormal piRNA expression has been detected across multiple cancer types, serving as a hallmark of disrupted piRNA biogenesis. The disruptions in the intricate process of piRNA production and maturation have a significant impact on initiating tumor formation and progression [68].

By aiding in the degradation of transcripts and influencing chromatin formation, piRNAs assume a pivotal role in suppressing transposon mobilization. Besides, these perturbations result in the formation of complexes involving PIWI proteins, which are members of the Argonaute protein family. The intricate roles of piRNAs and PIWI proteins underscore their essentiality in preserving the genomic integrity of germline cells. Their functions contribute significantly to maintaining the stability and proper functioning of reproductive cells.

Research focused on piRNAs has traditionally revolved around their importance in germline cells. However, as research in this field progresses, a growing body of evidence suggests that abnormal patterns of piRNA and PIWI protein expression manifest across diverse cancer types. This realization calls for a reassessment of how piRNAs and PIWI proteins function and their significance beyond germline cells. We now need to reevaluate and explore the various roles and relevance of these molecules in contexts outside of germline cells. This understanding prompts us to reconsider how piRNAs and PIWI proteins contribute and hold importance in different cellular domains beyond germline cells.

Unveiling the intricate interplay between piRNAs, PIWI proteins, and cancer unveils a potential avenue for deeper understanding and novel therapeutic interventions. The expanding landscape of piRNA research holds promise in expanding our knowledge of cancer biology and revolutionizing therapeutic strategies in the battle against this complex disease [83].

Researchers [78] has shown a strong and convincing body of evidence that suggests non-coding RNAs play a crucial role in the advancement of various types of human cancer. These studies have shed light on the role of ncRNAs in driving the development and advancement of various types of cancer in the human body. The findings emphasize the significant contribution of ncRNAs to the intricate processes and mechanisms underlying cancer development in humans. Although piRNAs have received relatively less attention compared to other ncRNAs, their significance lies in their pivotal involvement in governing epigenetic processes and subduing gene activity in retrotransposons post-transcription. This consequential function is achieved through their intricate interaction

with PIWI proteins [62,126]. Our understanding of the functions of PIWI proteins and piRNAs in the context of human cancers is gradually evolving [127].

Epigenetic mechanisms play a crucial role in governing the expression of genes in both normal and diseased situations. Among the various types of small ncRNAs, piRNAs have emerged as potential participants in the epigenetic network, playing a role in both transcriptional and post-transcriptional regulation. Previous research has suggested that piRNAs and PIWI proteins could have notable effects on the onset, prognosis, and therapeutic approaches for cancer. On the other hand, the precise clinical relevance of these molecules remains uncertain. This article presents a concise overview of piRNA biogenesis and their potential functions within the epigenetic network, which could be implicated in cancer. Moreover, there is an exploration into potential strategies for harnessing the potential of PIWI proteins and piRNAs as prognostic and diagnostic biomarkers, along with their use in cancer therapies [128].

During the initiation, progression, and metastasis of cancer, piRNAs play a dual role, either promoting or inhibiting cancer development [129]. These small RNAs not only influence the proliferation, programmed cell death, and invasive properties of tumor cells but also exert control over the process of cancer cell spread to distant sites [130].

Numerous studies have presented growing evidence indicating the presence of PIWI proteins, including HIWI, PIWIL2-like proteins, and PIWIL2, in different types of tumor cells, in both mice and humans [48,131]. Moreover, the presence of piRNAs has been detected in these cells as well [48]. Anomalously expressed piRNAs have been identified in human cancers. The results indicate a potential link between the piRNA pathway and the development of cancer. However, the specific functional roles of individual piRNAs in human cancer remain poorly understood. To achieve a comprehensive understanding of the impact of piRNAs on cancer, additional research is necessary. However, these findings emphasize the importance of unraveling the specific contribution of the piRNA pathway to tumor development and open up new possibilities for tumor treatment [45].

Furthermore, the presence of piRNAs in individuals with cancer has shown a significant correlation with clinical outcomes, emphasizing their pivotal contribution to the advancement of the disease. Through the suppression of transposable elements and the modulation of gene expression after transcription, piRNAs play a crucial role in safeguarding the integrity of the genome. Recent data suggests that piRNAs, akin to miRNAs and other small ncRNAs, assume both oncogenic and tumor suppressive roles in the development of cancer [72].

The tissue-specific expression patterns of piRNAs across different human tissues indicate their diverse roles in regulating important signaling pathways. Their ability to modulate gene activity at both the transcriptional and post-transcriptional levels highlight their multi-faceted functionality [132]. Excitingly, these piRNAs hold immense potential as diagnostic or prognostic biomarkers, as well as appealing targets for therapeutic interventions in cancer [133,134].

It has been postulated by researchers that the unique expression patterns and abundance of piRNAs in cancer may signify their active participation in regulatory mechanisms associated with the disease [72].

Researchers have proposed that the dynamic levels of circulating piRNAs, which can undergo changes, hold promise as highly sensitive and specific cancer biomarkers in contrast to circulating miRNAs [135]. The potential of piRNAs as cancer biomarkers in body fluids remains largely unexplored in existing studies [136]. The specific roles and mechanisms of piRNAs in cancer are still not comprehensively understood [132].

PiRNAs also contribute to carcinogenesis by participating in DNA methylation, deposition of histone markers, degradation of mRNA, and modification of proteins. This contribution is partly attributed to the dysregulation of piRNAs induced by oncogenic stress [68].

Tumor-suppressor piRNAs play a crucial role in defending against cancer development. PiRNAs demonstrate a diverse array of protective properties. They have the ability to stifle cell proliferation, facilitate apoptosis induction, impede the formation of colonies, diminish migration and invasion capacities, and halt cell cycle progression [137]. Moreover, experiments involving xenografts have revealed that overexpression of tumor-suppressor piRNAs often leads to slower tumor growth and decreased metastasis [138–141]. In contrast, oncogenic piRNAs have been identified as detrimental contributors to the advancement of cancer. They promote cell proliferation, restrict apoptosis, support the establishment of colonies and angiogenesis, and amplify migration and invasion capabilities [44,142–144]. Studies involving xenografts have demonstrated that heightened levels of oncogenic piRNAs correlate with accelerated tumor growth, enhanced angiogenesis, and intensified metastasis (Fig. 1C) [44,143].

5.1. Antiapoptotic/proproliferative piRNAs

Numerous scientific studies have shed light on the significance of piRNAs in the advancement of cancer, specifically their proproliferative and anti-apoptotic properties. An exemplification of this phenomenon is the documented influence of hsa_piR_011186 on cell behavior. By modulating the methylation of DNA and histone H3 within the CDKN2B promoter area, this particular piRNA has been observed to exert a twofold effect: suppressing apoptosis and promoting proliferation. Consequently, the expression of the CDKN2B gene, regulated by this promoter region, is altered, thus contributing to cellular behavior changes implicated in cancer progression [145]. Another research conducted by Zhang and colleagues showcased the profound impacts of piR-651 inhibition in non-small cell lung cancer. Their findings elucidated that curbing the activity of piR-651 resulted in a notable decline in cell proliferation rate alongside a noteworthy elevation in apoptosis levels (166). In addition to the effects on cell proliferation and apoptosis, Zhang et al. also observed alterations in the expression of proteins linked to apoptosis in this context. It suggests that piR-651 may play a crucial role in modulating the molecular mechanisms involved in programmed cell death [146]. Additionally, the piRNA-54265 was found to be highly expressed in colorectal adenocarcinoma, and functional experiments revealed its binding to the essential PIWIL2 protein. The activation of STAT3 signaling, which promotes cellular proliferation, is attributed to the complex formed by PIWIL2, STAT3, and phosphorylated-SRC (*p*-SRC). This complex, known as PIWIL2/STAT3/p-SRC, plays a significant role in initiating the

signaling pathway that leads to increased cell growth [44].

PiRNA-823, a well-known piRNA, has been extensively studied for its role in regulating cell growth across various tumor cell types. Researchers have found that piRNA-823 exhibits upregulation in cell lines of multiple myeloma (MM). By exerting control over de novo DNA methylation and angiogenesis, the silence of piRNA-823 has demonstrated its ability to impact the expression of proteins associated with apoptosis. This reveals a connection between piRNA-823 and the regulation of cellular programmed death as well as the formation of new blood vessels. In vitro experiments provided evidence that the suppression of piRNA-823 led to a decrease in the secretion of vascular endothelial growth factor (VEGF) and a subsequent inhibition of angiogenesis. This was evident from the reduced expression of CD31 and CD34, indicating a significant impact on angiogenic markers. These findings not only support the role of piRNA-823 in inhibiting apoptosis but also indicate its potential in enhancing proangiogenic activity, thus promoting vascular endothelial angiogenesis [147].

A recent study has revealed that the transportation of extracellular vesicles carrying piRNA-823 has a notable impact on tumor progression through its influence on endothelial cells. This research highlights the significant role of piRNA-823 in the development of tumors. When piRNA-823 is overexpressed, a considerable enhancement in the growth of EAhy 926 cells is observed. This boost is accompanied by heightened levels of IL-6, ICAM-1, and VEGF, implying their active participation in the process. Additionally, there is evidence suggesting that apoptosis, which refers to programmed cell death, is dampened under these conditions. These findings highlight the potential impact of piRNA-823 on various cellular activities, including proliferation, modulation of cytokine expression, cell adhesion, and inhibition of programmed cell death. Conversely, diminished expression of RNA-823 led to contrary outcomes (164). In a study on colorectal cancer cells, Jie Yin and colleagues discovered that inhibiting piR-823 hampered cell proliferation and induced apoptosis by augmenting the transcriptional activity of HSF1 [142].

The study conducted by Tang and colleagues found that there was a significant rise in piR-823 levels within activated hepatic stellate cells (HSCs). The elevated levels of piR-823 played a crucial role in fueling the growth of hepatic stellate cells (HSCs), as well as the synthesis of alpha-smooth muscle actin (a-SMA) and COL1α1. Conversely, suppressing the activity of piR-823 resulted in a decline in HSC function. Furthermore, the cooperation between piR-823 and eukaryotic initiation factor 3B (EIF3B) led to an increased expression of transforming growth factor β1 (TGF-β1) [148].

5.2. Antiproliferative/proapoptotic piRNAs

Different types of cancers may experience a reduction in the expression of a specific group of piRNAs, which have antiproliferative and proapoptotic properties. According to Chu et al., an investigation on bladder cancer revealed that the excessive expression of R-60152 hampers cell growth while promoting apoptosis [137]. In a similar vein, Jacobs et al. demonstrated that piR-8041 reduces the proliferation of glioma cell lines, induces apoptosis, and obstructs cell survival pathways, with both *in vivo* and *in vitro* experiments supporting these findings [141]. Das et al. conducted a study where they identified a noteworthy phenomenon: the overexpression of piR-39980 was found to substantially decrease cell proliferation and induce apoptosis in HT1080 fibrosarcoma cells. This effect is achieved by targeting the 3′-untranslated region (3′UTR) of a specific gene called ribonucleotide reductase subunit M2 (RRM2) [149]. Lastly, Tan et al. made a noteworthy discovery indicating lower levels of piRNA-36712 in breast cancer cells compared to healthy breast tissues. It's interesting that the depletion of piRNA-36712 contributes to increased cancer cell proliferation. This effect is believed to occur due to its interaction with the SEPW1 pseudogene SEPW1P RNA [139].

6. piRNAs and BTCs signaling pathways

The primary cause of the observed malignant characteristics in BTCs is the dysfunctional operation of cellular signaling networks that typically control cell growth, survival, and differentiation. Various cell-surface receptors, such as EGFR (epidermal growth factor receptor), VEGFR (vascular endothelial growth factor receptor), HER2 (human epidermal growth factor receptor 2), and MET (hepatocyte growth factor receptor), play a significant role in influencing these behaviors. All of these receptors are part of the tyrosine kinase receptor family and exhibit increased expression in various cancer types. They play a vital role in cell differentiation by activating specific signaling pathways. The functional aspects of BTCs are greatly impacted by intracellular signaling cascades, particularly the RAS-RAF-MEK and PI3K-AKT-mTOR pathways. These cascades make substantial contributions to the overall behavior of the disease. Additionally, specific genetic abnormalities have been identified for each subtype of BTCs, and epigenetic mechanisms also play a role in disrupting these pathways. Non-coding RNAs, including piRNAs, have emerged as important players in driving epigenetic changes within cells.

PiRNAs have been implicated in potentially promoting tumor formation by enhancing the phosphorylation and transcriptional activity of various signaling proteins, including heat shock transcription factor 1 (HSF1), Akt, and signal transducer and activator of transcription 3 (STAT3) [44,112,142,150,151]. In hepatocellular carcinoma (HCC), the excessive expression of piR-Hep1 enhances cell viability, motility, and invasiveness. This effect is achieved by initiating AKT phosphorylation, which subsequently amplifies the PI3K/AKT signaling pathway [151]. Conversely, piR-54265 interacts with the PIWI domain of PIWIL2, facilitating the assembly of the PIWIL2/STAT3/phosphorylated-SRC (*p*-SRC) complex. Consequently, this complex stimulates the phosphorylation and activation of STAT3 by *p*-SRC, thereby promoting malignancy in colorectal cancer (CRC) cells [44].

6.1. Ras–Raf–MEK–ERK pathway

MAPK signaling pathway plays a critical role in eukaryotic cells by forming a complex and interconnected web for transmitting

signals. It serves as a key mechanism through which cells interpret and respond to external stimuli, brought about by various molecules. MAPK, a widely present serine/threonine protein kinase, assumes a crucial function in this process [152].

In the context of BTCs, a notable percentage of cases (around 20–40 %) indicate the triggering of the RAS/RAF/MEK/ERK pathway. This activation is facilitated by genetic mutations in essential genes like KRAS, NRAS, BRAF, and others [153,154]. The sequence of proteins constituting this pathway enables effective communication between the cell surface and the nucleus, regulating key cellular processes like proliferation, apoptosis, and metabolism [155].

The Ras-Raf-MEK-ERK pathway, a pivotal signaling pathway involved in the development of BTCs carcinogenesis, frequently manifests mutations specifically in KRAS, which acts as an initial component of the pathway [156–158]. Notably, variations in the frequency of KRAS mutations are observed among different anatomical subtypes of BTCs across various geographical regions, as highlighted by diverse research studies [159].

Furthermore, these mutations are associated with poorer overall survival in various analyses [160–163]. In addition to KRAS, other components of this pathway have also been investigated [164].

PIWIL4 expression is widely observed in breast cancer (BC) tissues and different cell lines derived from Triple-negative breast cancer (TNBC). It initiates essential signaling pathways, such as TGF-β, MAPK/ERK, and FGF, which play a pivotal role in cancer progression. These pathways hold significant significance in the development of cancer. This activation ultimately enhances cell survival, division, and migration in TNBC [165].

These piRNAs primarily target cellular pathways such as the MAPK and calcification signaling pathways [166].

6.2. PIK3–AKT–mTOR pathway

The dysregulation of the PI3K/AKT/mTOR pathway in human BTCs has been extensively studied in both laboratory and organism settings. Evaluations have revealed several notable findings. These include the presence of activating mutations in the PIK3CA and PIK3R1 genes, responsible for encoding the PI3K p110a and p85a subunits, respectively. Additionally, various alterations involve somatic mutations in AKT, inactivation or deletion of the tumor suppressor PTEN, and abnormal overexpression or phosphorylation of PI3K, AKT, mTOR, and downstream targets such as p70SK6, p-4E-BP1, and eIF4E. Furthermore, modifications in this pathway can occur through heightened activity of membrane receptors and their ligands, such as growth factors, inflammatory cytokines, pro-angiogenic molecules, and mral-derived peptides [167–170].

The up-regulation of piRHep1 in HCC has been found to stimulate the proliferation and invasion of liver cells. One potential mechanism for this effect is the binding of piRHep1 with PIWIL2, which subsequently results in increased levels of phosphorylated AKT in the PI3K/AKT signaling pathway. The PI3K/AKT signaling pathway holds significant importance in HCC and is widely recognized as an oncogenic pathway [171].

Recent studies have shown that modifying miRNA/piRNA can lead to changes in the Akt pathway in both normal physiological processes and pathological conditions [172]. Interestingly, HIWI has exhibited significant overexpression in the context of testicular gastric cancer and germ cell tumors, while HILI expression has been observed to rise in breast cancer, gastrointestinal stromal tumors, colon cancer, renal cell carcinoma, and endometrial cancer [173]. Tumor cells commonly express PIWIL2, which hinders apoptosis by activating the Stat3/Bcl-XL pathway [174]; interestingly, one of the downstream target genes of this pathway is Akt. This suggests that PIWI and potentially piRNA play a vital role in modifying the Akt pathway within the realm of cancer [172]. Extensive piRNA expression triggers an increase in cell viability by activating and phosphorylating Akt [151]. As a whole, these findings provide valuable insights into the potential integration of PIWI-piRNA in Akt-dependent cell survival during the progression of cancer [172].

The activation of the PI3K-Akt-mTOR signaling pathway is commonly observed in prostate cancer (PC), aiding in the proliferation of tumors through the facilitation of various cellular mechanisms [175]. An intriguing revelation was made by Shorning et al., who identified several plausible binding sites for novel_pir349843, novel_pir382289, novel_pir158533, and hsa_pir_002468 on the mRNA targets of crucial molecules involved in the PTEN-PI3K-Akt-mTOR signaling pathway. This discovery implies the potential involvement of these piRNAs in the advancement of PC [176].

Han et al. conducted a separate investigation [43] wherein they illustrated that the reduced expression of piRNA-DQ722010 triggered the activation of the PI3K/AKT signaling pathway. This activation led to prostate hyperplasia by augmenting the expression of PIK3R3. While the role of piRNAs in modulating cell proliferation and apoptosis through the PI3K/AKT/mTOR pathway has been established, the specific mechanisms by which piRNAs contribute to illnesses are still not fully understood [177].

In a separate investigation conducted by Peng et al. [138], it was discovered that certain piRNAs could suppress cell proliferation and promote apoptosis by inhibiting the AKT/mTOR pathway. That's fascinating! In lung carcinoma specimens and cell lines, researchers observed a distinct absence of piR-55490 expression. However, when piR-55490 was reintroduced, it led to a decrease in the proliferation rates of lung cancer cells. Further investigation revealed that piR-55490 impeded the activation of the AKT/mTOR pathway by binding to the 3′-UTR of mTOR messenger RNA and promoting its degradation. This mechanism is similar to that of miRNA. By binding to the 3′-UTR of mTOR, piR-55490 effectively reduced the expression of mTOR and its target genes, including HIF-1, PGC-1α, and PPARγ. As a result, this reduction in expression played a role in inhibiting the proliferation of lung cancer cells and tumors [138]. It's worth noting that the Akt/mTOR signaling pathway is of significant importance in cancer biology [178].

6.3. Isocitrate dehydrogenase (IDH) signaling pathway

IDH, or Isocitrate dehydrogenase, plays a critical role in cellular respiration as an important metabolic enzyme. Its presence is necessary for the proper functioning of the Krebs cycle. Within the realm of IDH, specifically IDH1 and IDH2, these two subtypes have a

notable function in facilitating the NADP + -dependent oxidative decarboxylation process. This process involves the conversion of isocitrate into α-ketoglutarate (α-KG) and CO2 [179]. These IDH mutations are considered gain-of-function mutations, disrupting the normal catalytic activity of IDH1/2. Consequently, there is an elevated conversion of α-KG to D-2-hydroxyglutarate (D-2HG), an oncometabolite that promotes tumor growth and metastasis through various pathways, including DNA methylation and activation of VEGFR [180,181].

The discovery of mutations in IDH1 and IDH2 is indeed a noteworthy breakthrough in translational research on cholangiocarcinoma. It opens up new avenues for understanding and potentially treating this disease [182]. In cases of iCCA, an estimated percentage of around fifteen to twenty exhibit these mutations [183]. It's interesting to note that the presence of IDH1 and IDH2 mutations is less common in cases of eCCA and GBC. This suggests that different molecular mechanisms may be involved in the development of these types of cancers [184,185].

The frequency of IDH1 mutations is comparatively higher, with the majority of occurring variants located at the arginine 132 residue. Notably, IDH1-R132C is the most prevalent mutation, accounting for 44 % of cases, followed by IDH1-R132G, which constitutes 14 % of cases [183]. As mentioned earlier, these mutations result in elevated levels of 2-hydroxyglutarate (2-HG), an oncometabolite that can serve as a substitute biomarker for IDH-mutant iCCA. This biomarker can be detected in both tissue and blood samples [185]. Elevated levels of 2-HG are linked to higher DNA CpG methylation and altered histone methylation, which subsequently hinder the cellular differentiation of iCCA cells. Additionally, IDH mutations disturb hypoxia signaling, collagen processing, and facilitate epithelial-mesenchymal transition (EMT) by elevating ZEB1 expression and reducing miR-200 levels. Furthermore, IDH1/2 mutations often interact with TK and MAPK-dependent signaling pathways. Notably, iCCA cells frequently display raised levels of total ERK1/2, phospho-ERK1/2, and the downstream target, phospho-CREB [186]. IDH1 and IDH2 mutations occur independently of NRAS/KRAS and FGFR mutations, and they can potentially co-occur with BAP1 mutations [183]. Although the IDH pathway plays a crucial role in biliary tract cancer, no studies have explored the potential correlation between piRNAs and this pathway.

6.4. Wingless-related integration (Wnt) pathway

If the Wnt signaling cascade, which is an intricate intracellular pathway, experiences a breakdown, it can trigger the expression of various genes such as c-myc, c-jun, VEGF, and cyclin D [187,188]. In the investigation carried out on human IHC and CCA cell lines, it was observed that the expression of Wnt and its constituents showed an upsurge. When this pathway was obstructed, it led to heightened apoptosis and the halt of cell cycle progression [189]. In instances of CCA linked to ovarian cancer (OV), a mutated form of a component in the pathway called ubiquitin E3 ligase ring finger 43 (RNF43) was identified in 9.3 % of cases. This mutation exhibited an unfavorable trend in terms of survival (HR 7.775; P < 0.001), indicating a potentially detrimental effect on patient outcomes [190]. However, apart from a preclinical study that examined the effects of Dickkopf-1 (DKK1), an inhibitor of the Wnt pathway, on various tumor cell lines, including BTCs, there are currently no ongoing trials targeting this pathway specifically in BTCs [164,191].

There is growing evidence suggesting that epigenetic mechanisms play a role in regulating the components of the Wnt signaling pathway, actively contributing to the onset and progression of various cancer types [192]. In addition, piRNAs have been recognized as influential agents in gene expression regulation, exerting their effects through mechanisms like mRNA degradation and DNA methylation. As a result, they might have a substantial impact on modulating the activity of factors associated with signaling pathways linked to cancer.

For instance, Shorning et al. revealed that novel_pir349843 and novel_pir382289 exhibit the ability to interact with the mRNAs of crucial genes in the Wnt/B-catenin signaling pathway [176]. However, further investigation is required to gather sufficient evidence on this matter.

7. Important detected piRNAs in BTCs

BTCs are derived from the epithelial cells that form the lining of the biliary tract. In patients with this condition, the expression of piRNAs in GBC can exhibit either significant upregulation or downregulation [16]. Limited studies have investigated the presence of plasma exosomal piRNAs in GBC [193]. In an important study, the primary aim was to detect the distinct piRNA characteristics found in the blood exosomes of individuals who are diagnosed with CCA and GBC, alongside a control group of healthy individuals. The researchers aimed to identify piRNAs that exhibited significant differences in expression levels between healthy individuals and patient groups with CCA and GBC. Among the CCA and GBC cases, ten piRNAs were upregulated, including piR-2660989, piR-20548188, piR-10506469, piR-18044111, piR-23209, and piR-10822895. Notably, piR-17603885 and piR-23209 displayed potential as diagnostic biomarkers for both CCA and GBC. Conversely, piRNAs such as piR-17802142, piR-4262304, piR-9052713, piR-12355115, piR-14022777, and piR-5114107 were downregulated in the CCA and GBC group. A significant observation was made regarding the decreased levels of piR-12355115 in both CCA and GBC cases, indicating its potential as a shared biomarker for these diseases [16]. To validate these findings, selected piRNAs were further assessed using a separate cohort comprising healthy individuals, CCA patients, and GBC patients. The results confirmed the RNA-seq data, showing an increased expression of piR-10506469 within the plasma exosomes of CCA and GBC patients. Additionally, piR-14090389 and piR-20548188 exhibited significant upregulation specifically in the exosomes of CCA patients. Interestingly, the levels of piR-14090389 escalated with the severity of GBC.

A significant insight was observed when comparing the expression levels of specific piRNAs in blood samples collected before and one week after surgery. For instance, the plasma levels of piR-20548188 and piR-10506469 notably decreased in CCA and GBC cases one week after surgery compared to pre-surgical levels. This finding highlights the potential of these piRNAs as circulating diagnostic

markers for distinguishing GBC and CCA cases from healthy individuals, as well as monitoring post-treatment progress in gallbladder disease patients. In contrast, the levels of piR-14090389 and piR-4333713 were notably diminished in the blood samples collected from CCA and GBC patients one week after undergoing surgery, when compared to the samples obtained prior to the surgical procedure. This suggests that these two piRNAs may serve as indicators of disease progression from a healthy state to CCA, and subsequently to GBC, making them potential biomarkers for diagnosing gallbladder neoplastic and pre-neoplastic diseases [16]. Therefore, these piRNAs exhibit promise as potential biomarkers for CCA and GBC (Fig. 2) [78].

8. piRNA and other cancers in the digestive system

The disruption of piRNAs has emerged as a significant factor in the facilitation or inhibition of human cancer development and progression. These effects are achieved through diverse mechanisms, including DNA methylation, transcriptional suppression, mRNA degradation, and translational regulation. Digestive cancers are responsible for a significant number of cancer-related deaths globally. Out of all the different types of cancer, those affecting the digestive system hold a prominent position in terms of their contribution to mortality rates. PiRNAs have been found to possess the ability to control the expression of crucial genes and pathways that are involved in the progression of digestive cancers. Consequently, they have emerged as promising candidates for diagnostic and therapeutic biomarkers [78].

The scope of digestive system cancers encompasses various types, such as esophageal cancer, HCC, colorectal cancer (CRC), gastric cancer (GC), pancreatic cancer, and BTCs. The increasing amount of evidence strongly indicates a connection between piRNAs, PIWI proteins, and cancers that impact the digestive system (Fig. 3) (Table 1) [78].

8.1. Hepatocellular carcinoma and the role of PIWI-interacting RNAs

HCC, a liver tumor with an alarmingly high global prevalence and known for its devastating impact [194], exhibits a distinguishable developmental progression that encompasses various stages. These stages include cirrhotic nodules (CNs), characterized by liver fibrosis; high-grade dysplastic nodules (HGDNs), and low-grade dysplastic nodules (LGDNs) presenting varying degrees of abnormal cell growth; early hepatocellular carcinoma (eHCC), indicating the emergence of malignant features; and progressed HCC (pHCC), signifying advanced disease progression [195]. Specific piRNAs are distinctive for each stage, delineating their unique characteristics. It is notable that particular sets of piRNAs show characteristic patterns throughout the different stages. Alterations in RNA expression profiles enable differentiation between HCC tissue and liver cirrhosis. For instance, CNs exclusively express piR_LLi_24894, while hsa-piR-020498 & piR-LLi-30552 are predominantly found in DNs, eHCCs, and pHCCs. Additionally, HCC specifically accumulates hsa-piR-013306. In early stages of hepatocellular carcinoma (HCC), the reduced expression of piRNAs has a profound impact on various cellular functions. This includes the activity of genes associated with multiple cellular processes such as the tumor necrosis factor (TNF) receptor, the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, the WNT/b-catenin signaling pathway, the growth arrest and DNA damage-inducible protein 45 (GADD45), the adenosine-monophosphate (AMP)-activated protein kinase (AMPK), the high mobility group box 1 (HMGB1), and the PTEN. These genes play crucial roles in orchestrating cellular events such as telomerase activity, DNA methylation, cell cycle progression, protein ubiquitination, and apoptosis [195].

In the case of dysplastic nodules with a significant degree of cell abnormality and advancing stages of liver disease, the researchers
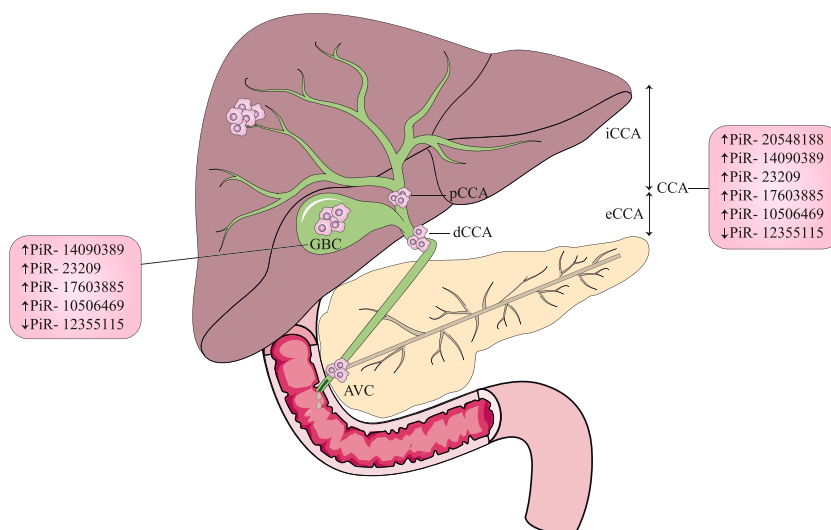

Fig. 2. Blood exosomes as promising biomarkers in the diagnosis of BTCs. Abbreviations: dCCA, distal cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; BTC, biliary tract cancer; GBC, gallbladder cancer; eCCA, extrahepatic cholangiocarcinoma; AVC, ampulla of vater cancer; pCCA, perihilar cholangiocarcinoma.
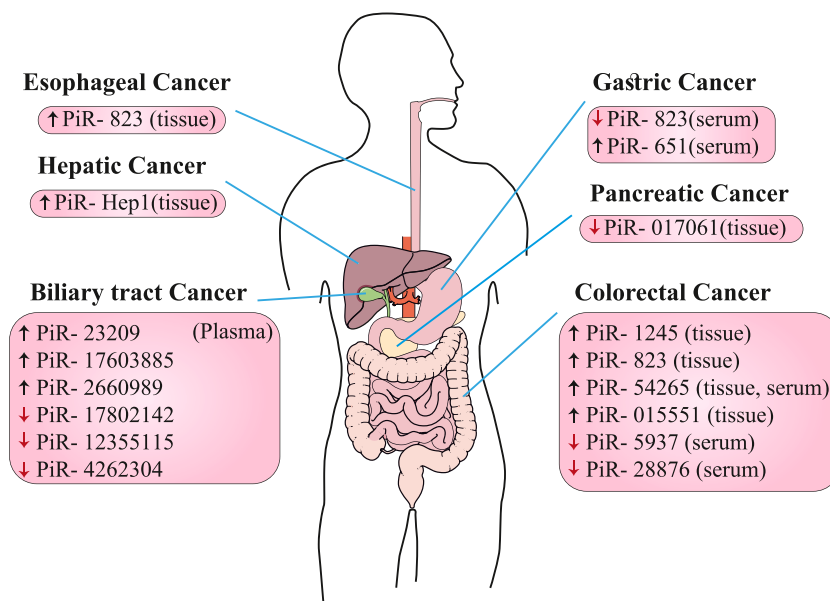
Fig. 3. Expression of piRNAs in digestive system cancers. Up or down regulation has been shown.

observed a substantial increase in the levels of piR-020498. Similarly, in HCC that had progressed to an advanced state, the expression of piR-020498 was remarkably heightened. Additionally, piR-013306 showed unique overexpression specifically in HCC. These findings provide insight into the role of piRNAs in the advancement of HCC and demonstrate distinct expression patterns at different stages. Moreover, the presence of piRNA molecules in all HCC samples reaffirms their importance in liver cancer development [195]. However, the exact mechanisms by which piRNAs work in HCC are still not fully understood [78]. Earlier studies have suggested a strong correlation between certain piRNAs, including piR-Hep1 and piR-823, and the occurrence as well as progression of HCC. HCC cells show a significant twelvefold increase in the expression of piR-Hep1 compared to normal cells. Interestingly, suppressing piR-Hep1 has been found to impede the proliferative, migratory, and invasive capabilities of HCC cells [151].

When HSCs become activated, there is a noticeable increase in the expression of piR-823. This heightened piR-823 activity plays a crucial role in driving HSC proliferation and facilitating the synthesis of α-SMA, COL1a1, and other components of the extracellular matrix. As a result, these processes contribute significantly to the development of cirrhosis. The activation of HSCs during liver fibrogenesis is made possible by the interaction between piR-823 and eukaryotic initiation factor 3B (EIF3B), which subsequently leads to an elevation in the levels of TGF-β1 [148].

8.2. Colorectal cancer and the role of PIWI-interacting RNAs

CRC is a prevalent concern in public health that regrettably holds the unenviable rank of being the third leading cause of cancer-related fatalities globally. Annually, there are over 1.85 million reported cases and approximately 850,000 fatalities [196].

In the context of CRC, the overexpression of piR-823 has been strongly associated with increased cell proliferation. Researchers have witnessed the recruitment of HSF1, a transcription factor known for its role in triggering the production of heat shock proteins through phosphorylation and transcriptional activity, by this particular piRNA. The recruitment ability of piR-823 has a noteworthy impact on the development of colon tumors. Interestingly, patients with CRC who exhibit elevated levels of piR-823 tend to have a significantly poorer prognosis compared to those with lower expression levels, as reported (104). Furthermore, according to Ref. [197], increased levels of piR-823 have been associated with unfavorable treatment responses in individuals diagnosed with stage II and III CRC.

Moreover, piR-54265 levels were found to be increased in CRC tissues as compared to non-tumor tissues. Its expression showed a negative correlation with the survival rate of CRC patients. This specific piRNA engages with PIWIL2 and together they form the PIWIL2/STAT3/phosphorylated-SRC complex. As a result, it promotes CRC metastasis and reduces sensitivity to chemotherapy [44].

Another study revealed a significant decrease in piR-54265 levels among CRC patients following surgical treatment, followed by an increase upon tumor recurrence. piR-54265 exhibited remarkable specificity in the serum of CRC patients, indicating its potential as a promising biomarker [198].

Likewise, piR-1245 demonstrated overexpression in CRC tissues and played an instrumental role in fine-tuning the survival of CRC cells through the modulation of tumor suppressor gene expression. Patients exhibiting high levels of piR-1245 experienced markedly reduced survival durations [144].

According to Ref. [199], the presence of piR-017724 in serum showed a significant positive correlation with both overall survival and progression-free survival rates in CRC patients. This suggests that piR-017724 has the potential to be an independent prognostic

Table 1

Analysis of piRNAs as Diagnostic Tools for Gastrointestinal Cancers.

Diagnostic Capacity	piRNA	Expression	Mechanism	Model	Cell line	Potential clinical utility	Ref
Hepatocellular carcinoma	piR-Hep1	Up	Enhances the survival, mobility, and infiltrative abilities of cells.	Human, *in vitro*	HKCI-4, HKCI-8	Prognostic marker	[151]
	piR-1029, piR-15254	Up	–	Human	–	Diagnostic marker	[242]
	piR-651	Up	–	*In vitro*	HepG2	Diagnostic marker	[207]
Colorectal cancer Gastric cancer	piR-1245	Up	Augments cellular proliferation, movement, and infiltration while preventing programmed cell death through the interaction with specific mRNA molecules located within nuclear exosomes.	Human, *in vitro*	HCT116, SW480	Prognostic marker	[144]
	piR-823	Up	Encourages cellular multiplication and inhibits programmed cell death through the activation of HSF1 phosphorylation.	Human, *in vitro*	HCT116, DLD-1, and FHC	Therapeutic target	[142]
	piR-54265	Up	By controlling STAT3 phosphorylation, it fosters cellular growth and spreading, impedes programmed cell death, and induces resistance to chemotherapy.	Human, *in vitro*, *in vivo*	HCT116 and LoVo, and HEK293T	Diagnostic and prognostic marker/ Therapeutic target	[44]
	piR-015551	Up	Contributes to the regulation of colorectal cancer progression.	Human	–	Risk assessment	[243]
	piR-5937	Down	–	Human	–	Diagnostic marker	[202]
	piR-28876	Down	–	Human	–	Diagnostic marker	[202]
	piRNA-18	Down	Impairs cell growth, movement, and infiltration	Human, *in vitro*, *in vivo*	SW480 and LOVO	Diagnostic marker	[244]
	piR-017724	Down	–	Human	–	Prognostic marker	[245]
	piR-18849, piR-19521	Up	Promote metastasis	Human	–	Prognostic marker	[200]
	piR-020619, piR-020450	Up	–	Human	–	Diagnostic marker	[246]
	piR-24000	Up	Promotes metastasis	Human	–	Diagnostic marker/ Therapeutic target	[201]
	piR-823	Down	Slows down the growth of cells and lowers the methylation of genes that promote tumor formation.	Human, *in vitro*, *in vivo*	GES-1, MGC-803 and SGC-7901	Diagnostic marker/ Therapeutic target	[208, 247]
	piR-651	Up	Promotes the growth of cells and correlates with the TNM staging system.	Human, *in vitro*	GES-1	Diagnostic marker	[207]
	piR-1245	Up	Shows a correlation with both tumor size and TNM stages.	Human	–	Diagnostic and prognostic marker	[234]
	piR-48966, piR-49145, piR-31335	Up	–	Human	–	Prognostic marker	[248]
	piR-019308, piR-004918, piR-018569	Up	Increase metastasis	Human	–	prognostic and Diagnostic marker	[133]
Pancreatic adenocarcinoma	piR-017061	Down	By suppressing the EFNA5 mRNA, it acts as a barrier to hinder the growth of pancreatic cancer cells	Human, *in vivo*, *in vitro*	MiaPaCa, PANC-1, BxPC-3	Diagnostic marker/ Therapeutic target	[210, 211]
	piR-162725	Up	–	Human, *in vitro*	PANC 04.03, PL45, BxPC-3, hTERT-HPNE, RWP1, PANC-1	Diagnostic and prognostic marker	[249]
Esophageal cancer	piR-823	Up	Facilitates aberrant DNA methylation through DNMT3B	Human	–	Diagnostic and prognostic marker	[217]

factor in CRC.

The heightened expression of piR-18849 and piR-19521 demonstrates a positive correlation with the malignant nature of colon cancer tissues. Additionally, patients diagnosed with CRC exhibit a notable correlation between the extent of tumor differentiation, lymph node metastasis, and the increased expression of piR-18849 [200].

piR-24000 seems to have a notable connection with the invasive phenotype of CRC. It's specifically associated with characteristics such as poor tumor differentiation, distant organ metastases, and an advanced stage of the disease. This suggests that piR-24000 could potentially serve as a useful biomarker for identifying invasive CRC cases. And it's great to know that ROC analysis has confirmed its effectiveness in differentiating CRC patients from healthy individuals [201].

Moreover, a decrease in expression levels of piR-001311, piR-5937, piR-017723, piR-004153, piR-017724, piR-58099, piR-28876, piR-020365, piR-59056, and piR-32105 has been observed in CRC patients [199,202,203].

Through the analysis of piRNA expression profiles in serum samples collected from both patients and individuals without the condition, it was revealed that piR-5937 and piR-28876 demonstrate superior levels of sensitivity and specificity in identifying stage I CRC patients compared to the existing biomarkers CEA and CA19-9 [202].

Given the observed variations in clinical presentations, treatment responses, prognoses, and other attributes among different primary sites of CRC such as rectal cancer, right colon cancer, and left colon cancer, it becomes imperative to delve deeper into the potential divergence of piRNA expressions in these distinct locations and their potential associations with prognosis [90].

8.3. Gastric cancer and the role of PIWI-interacting RNA

Despite not featuring in the top ten malignancies in the United States, GC continues to occupy a prominent position as a primary contributor to cancer-related fatalities worldwide [204].

Extensive studies exploring piRNA profiles have revealed the abundance of these small regulatory RNAs within the gastric region of humans [205]. When examining the transcripts of gastric tissues without any pathological conditions alongside GC samples, researchers uncovered that almost half of the piRNAs showed an upregulation specifically in GC samples [206]. This suggests that these upregulated piRNAs could potentially play a significant role in the development and progression of gastric cancer.

Within the realm of GC, piR-651 demonstrates a markedly higher presence in tumor tissues compared to non-cancerous tissues. The inhibitory effects of suppressing piR-651 expression on GC cell growth have been substantiated [207]. It seems that in contrast to the upregulation of some piRNAs in GC samples, both GC cell lines and GC tissues actually demonstrate decreased levels of piR-823. The overexpression of piR-823 has been shown to possess suppressive properties on GC cell growth, as evidenced by experiments conducted on nude mice [208].

Additionally, GC tissues have exhibited increased levels of piR-59056, piR-58099, and piR-32105 [135].

Further research is crucial to unravel the potential link between piRNAs and immune evasion mechanisms in gastric cancer. Moreover, it is crucial to determine if certain piRNAs, akin to piR-823, detectable in peripheral blood, hold promise as biomarkers to predict immunotherapy outcomes and aid in GC diagnosis [90].

8.4. Pancreatic adenocarcinoma and the role of PIWI-interacting RNA

Pancreatic adenocarcinoma stands as a prominent contributor to cancer-related mortality worldwide, and its global impact has more than doubled over the past 25 years [209].

In the context of pancreatic cancer tissues, there is a noteworthy decrease in piR-017061 expression when compared to its expression in normal tissues. This decline is significant, indicated by a 2.3-fold change [210]. By collaborating with PIWIL1, piR-017061 contributes to hindering the progression and proliferation of pancreatic cancer cells through its involvement in facilitating the degradation of EFNA5 mRNA [211]. Similarly, piR-317 demonstrates a reduced expression level in pancreatic cancer tissues, indicating its tumor-suppressive properties. In contrast, piR-1945036 exhibits a significant increase in expression within pancreatic cancer tissues. Remarkably, individuals demonstrating lower levels of piR-1945036 expression display extended survival periods, implying a potential oncogenic function for piR-1945036 [212,213].

A study was conducted to examine exosomes extracted from the serum of healthy individuals, patients with pancreatic cancer, and individuals with intraductal papillary mucosal neoplasms. The researchers employed next-generation sequencing to extract and analyze total RNAs from these exosomes. This approach allowed for a comprehensive analysis of the RNA content within the exosomes, providing valuable insights into the potential diagnostic or prognostic markers related to these conditions. The sequencing results unveiled the elevated expression of specific piRNAs, namely hsa-piR-53108, hsa-piR-56621, hsa-piR-52959, hsa-piR-54479, and hsa-piR-30690, in pancreatic cancer patients when compared to the healthy individuals, indicating their potential oncogenic effects [213,

Table 2
PIWI proteins characteristics in gastrointestinal cancers.

Diagnostic Capacity	PIWI	Expression	Model	Cell line	Effect/clinical utility	Ref
Hepatocellular carcinoma	PIWIL1/HIWI	Up	Human, *in vitro*	MHCC97L, MHCC97H, HepG2, and L02	Promote cancer cell proliferation and migration	[227]
Colorectal cancer	PIWIL1/HIWI	Up	Human, *in vitro*	Caco-2 and HT-29	Promote cancer cell proliferation	[250]
	PIWIL2/HILI	Up	Human	–	Diagnostic and prognostic marker	[225]
	PIWIL3/HIWI3	Up	–	–	Prognostic marker	[251]
	PIWIL4/HIWI2	Up	–	–	Prognostic marker	[251]
Gastric cancer	PIWIL1/HIWI	Up	Human, *in vitro*	RF-48, AGS, NCI-SNU-1, NCI-SNU-5, NCI-SNU-16, and NCI–N87	Diagnostic and prognostic marker	[247]
	PIWIL3/HIWI3	Up	Human, *in vitro*	AGS, N87, SGC7901, MKN45 and BGC823	Increase proliferation, migration and invasion	[252]
Esophageal cancer	PIWIL1/HIWI	Up	Human, *in vitro*	KYSE70, KYSE140 and KYSE45	Prognostic marker	[98]

214]. Conversely, several other piRNAs, such as hsa-piR-46410, hsa-piR-42185, hsa-hsa-piR-43043, hsa-piR-58897, and piR-54888, were found to be downregulated in the pancreatic cancer patients, suggesting their potential as tumor-suppressive factors [213, 214]. Furthermore, there were notable discrepancies in the levels of exosome-derived hsa-piR-016658 and hsa-piR-001311 between pancreatic cancer patients and the control group [215]. Nevertheless, additional investigations are necessary to gain a comprehensive understanding of the functions performed by these exosome-based piRNAs [213].

8.5. Esophageal cancer and the role of PIWI-interacting RNA

Globally, esophageal cancer holds the sixth position among the primary factors contributing to cancer-related fatalities [216].

In esophageal cancer tissues, there was a noticeable increase in the presence of piR-823, which demonstrated a direct association with the likelihood of lymph node metastasis. By employing ROC curve analysis, it was determined that piR-823 serves as a beneficial biomarker to differentiate esophageal cancer from normal controls. Moreover, a positive correlation was discovered between the expressions of DNMT3B and piRNA-823, implying that piRNA-823 could potentially play a cancer-promoting role in esophageal cancer by triggering abnormal DNA-methylation through DNMT3B [217].

Additionally, heightened cytoplasmic levels of PIWIL1 protein in esophageal cancer cells align with increased histological grades, progressed tumor stages, and unfavorable survival rates [98]. To enhance our comprehension of the precise mechanisms involving piR-823 in esophageal adenocarcinomas, it is essential to conduct extensive future investigations [78].

9. Digestive system cancers and the role of PIWI proteins

PIWI proteins show a predominant presence in cancerous tissues, as well as in stem cells and germ cells within most adults [218]. Unlike common treatments that may have an impact on non-cancerous cells, the utilization of these proteins as targets for cancer therapy holds tremendous potential [219]. Certain PIWI proteins have shown a correlation with particular cancer variants, exerting potential effects on extended survival rates and the aggressiveness of tumors (Table 2) [220].

9.1. The influence of PIWI in gastric cancer

PIWIL1 stands out as a noteworthy standalone prognostic indicator in GC. Furthermore, the presence of both PIWIL2 and PIWIL1 is indicative of a decrease in overall survival rates in cancerous tissues. To assess the expression of PIWI proteins, immunohistochemistry analysis was performed on samples from 182 patients, consisting of both normal and gastric cancer tissues. The findings revealed a substantial elevation in the levels of PIWIL1–4 expressions in tumor tissues compared to their normal counterparts [220].

9.2. PIWI's impact on colorectal cancer

When the HIWI gene was suppressed, researchers observed a noticeable decline in the strength of sphere formation and colony cells. This led to an impaired tumor growth of SSCloAldebr cells in nude mice. The findings suggest that the HIWI gene plays a critical role in the growth and progression of these cells, and suppressing its activity could be a potential therapeutic strategy in treating certain types of tumors. Further investigations in this area could provide valuable insights into the underlying mechanisms and help develop targeted therapies for cancer treatment [221].

Researchers have noticed considerably elevated levels of PIWIL2 expression in CRC tissue that has experienced lymph node metastasis, when compared to the normal mucosa of the colon. As a result, a direct correlation was found between increased levels of PIWIL2 expression and heightened pathological and clinical features, indicating a more aggressive nature of the condition. To validate this association, the invasion and migration abilities of colon cancer cells were hindered upon blocking PIWIL2, resulting in a reduction in the transcriptional activation of MMP9. MMP9 is known for its involvement in promoting cancer cell invasion and migration [222, 223].

In patients with colon adenocarcinoma who did not have lymph node metastasis, the presence of HIWI has been associated with an unfavorable diagnosis and prognosis. On the other hand, the expression of HILI mRNA was observed to be lower CRC tissues compared to non-cancerous samples. This suggests a reciprocal regulatory relationship between HILI and HIWI in colorectal cancer. Moreover, the expression of PIWIL2 has been found to be associated with CRC, as well as several indicators indicating unfavorable outcomes and clinicopathological characteristics [224–226].

9.3. PIWI's significance in hepatocellular cancer

The down-regulation of Hiwi in HCC resulted in decreased proliferation and cell migration, pointing to the potential oncogenic involvement of Hiwi in HCC [227]. In HCC, it has been discovered that the combined expression of Piwil2 and Piwil4 within the nucleus has a stronger impact on prognostic outcomes. Relying on a single marker, either Piwil2 or Piwil4 alone, is insufficient for an accurate prognosis assessment [228].

10. piRNAs as cancer biomarkers

Liquid biopsy, a commonly utilized technique for early cancer detection and monitoring tumor progression over time, has shown

potential in the analysis of piRNAs as biomarkers (47). In 2011, the promising role of piRNAs in liquid biopsy was discovered [208].

Detecting cancer at an early stage and initiating prompt treatment greatly benefits the prognosis. Considering that piRNAs have a predominant role in regulating various networks and pathways, their importance in early cancer diagnosis and treatment is substantial. Peripheral blood has been examined for tumor-associated miRNAs, a well-studied class of small non-coding RNAs, which serve as biomarkers for cancer diagnosis [229]. Interestingly, when examining RNA sequencing data, researchers have discovered more than just miRNAs in human blood [230,231]. They have also detected other forms of non-coding RNAs, such as stable piRNAs. These piRNAs, which share a similar length with miRNAs, possess a remarkable ability to move across the cell membrane and circulate freely in bodily fluids. Furthermore, they exhibit exceptional resilience to degradation by ribonucleases, ensuring their stability [232].

Patients who exhibit elevated levels of onco-piRNAs or decreased levels of tumor-suppressive piRNAs frequently encounter unfavorable outcomes, manifesting as reduced progression-free survival (PFS) or overall survival (OS). These findings have been documented in multiple studies [44,139,144,233]. The significance of piRNAs extends beyond their association with cancer stemness, drug resistance, and tumor immunology. By utilizing liquid biopsy to analyze piRNAs, there is a potential to not only diagnose cancer but also predict its prognoses. This means that studying piRNAs in liquid biopsies could provide valuable insights into the development and progression of cancer, allowing for more accurate prognostic predictions. The combination of piRNA targeting with other therapeutic strategies has the potential to revolutionize the realm of cancer care [68].

10.1. piRNAs: new potential biomarkers and therapeutic agents for biliary tract cancers

The utilization of the expression patterns of piRNAs has emerged as a promising avenue for utilizing tissue-specific indicators that aid in the diagnosis and prognosis of various cancers. Understanding the alterations in piRNA expression levels has been directly correlated to the emergence and advancement of cancerous conditions. What adds to the excitement is that piRNAs exhibit detectable traces in bodily fluids such as saliva, blood, urine, and gastric juice, thereby presenting themselves as potential biomarkers that can be accessed non-invasively [16,202,234–236]. Astonishingly, piRNAs demonstrate remarkable stability, persevering through rigorous freeze-thaw cycles and prolonged incubation at room temperature when examined in human plasma and serum samples [82]. The durability of piRNAs can be credited to the presence of the 20-*O*-methyl modification, which serves as a shield against potential degradation factors like thirty uridylation and truncation, both of which are known to impact the stability of small RNAs [237]. Alternatively, piRNAs present in the blood may be encapsulated within extracellular vesicles, such as microvesicles and exosomes, offering resistance against degradation by ribonucleases [238]. It is worth noting that exosomal piRNAs have been quantified in serum. Exosomes, tiny extracellular vesicles enclosed by membranes and measuring 30–120 nm in diameter, are secreted by cells and transport diverse biomolecules such as DNA, mRNA, and ncRNAs. They have a significant impact on the advancement of tumors by enabling intercellular communication [239,240]. Consequently, exploring the exosomes released from cancer cells presents an opportunity to comprehend the intricate nature of cancer as these vesicles are consistently generated [241]. Previous studies have highlighted the significant presence of piRNAs among RNAs isolated from exosomes [215]. Interestingly, multiple exosomal piRNAs exhibit differential expression patterns in plasma samples from patients with CCA and GBC, suggesting their potential as diagnostic biomarkers. Some of these piRNAs undergo dramatic changes in expression, up to 10,000-fold. Given the urgent need for clinical circulating biomarkers for CCA and GBC, our research has identified several promising candidates [16]. The elevated expression of multiple piRNAs in exosomes obtained from individuals with BTCs holds promising prospects for the development of prognostic and diagnostic biomarkers through blood-based testing. To ensure the effectiveness of these innovative piRNA biomarkers, it is crucial to conduct additional research for early detection of cancer at its initial stages and long-term monitoring of tumor advancement. The validation process for piRNAs requires independent studies with larger sample sizes and comprehensive clinical datasets to establish their reliability [238]. This ensures that the findings related to piRNAs and their potential applications in cancer diagnosis and prognosis are robust and applicable across diverse patient populations. It's important to gather extensive data and conduct rigorous research to confirm the effectiveness and reliability of piRNAs as biomarkers.

11. Conclusion

The significance of non-coding RNAs, such as miRNAs and siRNAs, in cancer has been extensively studied. Lately, there has been a marked increase in interest concerning the investigation of piRNAs as potential biomarkers in cancer diagnosis and prognostication. The aim is to identify their potential role in predicting and evaluating the course and consequences of the disease. It appears that piRNAs participate in epigenetic modifications, such as DNA methylation, chromatin remodeling, and mRNA degradation, thereby influencing gene expression regulation.

The unusual manifestation of piRNAs has been identified in different cancer types, hinting at their plausible involvement in the advancement and escalation of cancer. These unique RNAs are thought to have a role in controlling gene expression at various levels, including translation and post-translation. This adds to their importance as potential biomarkers for cancer diagnosis and prognosis. Their presence and abnormalities in cancer cells could provide valuable insights into the disease and aid in developing targeted therapies. Although the dysregulated expression of piRNAs has been linked to diverse cancer types, our comprehension of their precise functions and underlying mechanisms in each particular cancer remains a work in progress. Comprehensive research and investigation are necessary to unravel the intricate relationship between piRNAs and different types of cancer. These studies can shed light on the potential diagnostic and therapeutic applications of piRNAs as biomarkers.

Currently, early detection methods for gastrointestinal cancers are imperative, given the poor prognosis associated with these malignancies. Challenges such as late-stage diagnosis, metastasis, and drug resistance underscore the urgent need for more accurate

biomarkers to aid in the diagnosis and treatment of gastrointestinal cancers. Recent research has brought to light the significance of certain piRNAs in regulating the development, progression, and response to medications in gastrointestinal cancers. It is vital to conduct extensive studies to fully grasp the mechanistic functions of piRNAs in these types of cancer in order to employ them effectively as diagnostic tools, prognostic indicators, and potential therapeutic targets. Understanding the intricate roles of piRNAs in gastrointestinal cancers requires comprehensive investigation.

Overall, this assessment emphasizes the potential of piRNAs as valuable markers and promising targets for treating cancers in the digestive system, including BTCs. The disruption of piRNA expression has been proven to affect gene expression and regulate crucial genes and pathways related to cancer advancement. Due to the lack of reliable diagnostic and prognostic markers for BTCs, it is necessary to identify the disease at later stages, which limits treatment options to mainly palliative care. This analysis presents a distinct viewpoint on the role of piRNAs in cancer detection and treatment. Their potential as markers for early detection and prognosis of BTCs, as well as their potential as targets for intervention, is significant. Furthermore, the potential of combining piRNA-driven markers with other diagnostic and therapeutic approaches is emphasized. More research is needed to fully comprehend the role of piRNAs in BTCs and to develop effective strategies for diagnosis and treatment. These studies have the capability to significantly improve the management and treatment of BTCs and other gastrointestinal cancers. The unique perspective and optimistic future presented in this review demonstrate the potential of piRNAs as valuable tools in combating digestive system cancers.

Funding source

Data availability

All data cited in this review article can be found in the referenced sources and publications.

Furthermore, piRNAs serve diverse functions (B). They play a crucial role in post-transcriptional gene regulation (PTGS) of transposable elements (TE) and mRNA by engaging with piRNA-induced silencing complexes (piRISC). Through various mechanisms like deadenylation or endonuclease activity, they help regulate the expression levels of target RNA. This intricate control ensures precise modulation of gene expression. In addition to their post-transcriptional regulatory role, piRNAs also contribute to transcriptional gene silencing. They facilitate processes such as DNA methylation and histone modifications, which ultimately lead to the repression of TE loci. By participating in these epigenetic modifications, piRNAs help maintain the genomic integrity and stability by suppressing the activity of transposable elements (B).

Notably, piRNAs exert influence on critical cellular processes, including cell proliferation, apoptosis, and the induction or suppression of cancer (C). Their intricate regulation and interactions contribute to the maintenance of cellular homeostasis and the delicate balance of these biological phenomena (C).

CRediT authorship contribution statement

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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