Online ISSN: 2161-2609
Advancesin Open Access Journals

Published by advancesin@www.cancercellresearch.org

***Cancer cell research, 2024*, Vol. *11*, No.44**

REVIEW ARTICLE

Title: Research Progress of piRNA as an Early Diagnostic Biomarker for Gastrointestinal Tumors

Tsegmed Uranchimeg[1#], Yingcai Wu[2#], Yanling Zheng[3#], Lijuan Wu[2], Qin Hao[4*], Li Feng[5*], Zhenfei Wang[2*]

[1] National Cancer Center of Mongolia, Nam Yan Ju street, Bayanzurkh district, Ulaanbaatar, 13370, Mongolia
[2] The Laboratory for Tumor Molecular Diagnosis, Peking University Cancer Hospital (Inner Mongolia Campus)/Afliated Cancer Hospital of Inner Mongolia Medical University, Hohhot 010020, China
[3] Department of Thoracic Oncology, Peking University Cancer Hospital (Inner Mongolia Campus)/Afliated Cancer Hospital of Inner Mongolia Medical University, Hohhot 010020, China
[4] Department of Abdominal Tumor Surgery, Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010010, China
[5] Department of Abdominal Tumor Surgery, Peking University Cancer Hospital (Inner Mongolia Campus)/Afliated Cancer Hospital of Inner Mongolia Medical University, Hohhot 010020, China

Abstract: Piwi-interacting RNAs (piRNAs) are a distinct class of small non-coding RNAs that have garnered significant attention for their regulatory roles and potential as biomarkers in cancer diagnosis. Recent advancements highlight their aberrant expression in gastrointestinal (GI) tumors, including gastric, colorectal, and esophageal cancers, positioning them as promising candidates for early detection. Technological progress in high-throughput sequencing has enabled the identification of tumor-specific piRNA signatures, such as piR-823 and piR-651, that demonstrate high specificity and sensitivity. Furthermore, circulating piRNAs in bodily fluids present a non-invasive approach to tumor detection. Ongoing studies unravel the piRNA-PIWI protein axis, elucidating its involvement in gene regulation and tumorigenesis. However, challenges such as standardizing detection methods and validating clinical efficacy persist. This review emphasizes the strides made in piRNA research, underscoring their transformative potential in GI tumor diagnostics and paving the way for innovative diagnostic tools.

ARTICLE HISTORY

Received: 15-Dec-2024
Revised: 23-Dec-2024
Accepted: 23-Dec-2024
DOI: *10.54762/ccr2024.44.983-991*

Keywords: Piwi-interacting RNA; Gastrointestinal Tumors; Biomarkers; Early Diagnosis; Non-coding RNA

* Address correspondence to these authors at the Department of Abdominal Tumor Surgery, Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010010, China, Email: Haoqin2015@126.com, Department of Abdominal Tumor Surgery, Peking University Cancer Hospital (Inner Mongolia Campus)/Afliated Cancer Hospital of Inner Mongolia Medical University, Hohhot 010020, China, feng_zhuo1@126.com, and The Laboratory for Tumor Molecular Diagnosis, Peking University Cancer Hospital (Inner Mongolia Campus)/Afliated Cancer Hospital of Inner Mongolia Medical University, Hohhot 010020, China, Email: 20202006@immu.edu.cn.

# These authors contributed equally to this manuscript.

1. Introduction

Gastrointestinal (GI) tumors, comprising malignancies of the stomach, colorectal region, esophagus, liver, and pancreas, represent one of the most critical challenges in global oncology [1]. According to the most recent statistics from the Global Cancer Observatory (GLOBOCAN 2020) [2], GI cancers account for a significant portion of the global cancer burden, with over 4.8 million new cases diagnosed annually and more than 3.4 million deaths [1] [2] [3]. This makes GI cancers among the leading causes of cancer-related mortality worldwide. Among these, colorectal cancer ranks as the third most frequently diagnosed cancer and the second leading cause of cancer deaths. Gastric cancer, once a dominant cancer globally, continues to affect millions, particularly in high-risk regions such as Eastern Asia and Latin America [4]. Meanwhile, liver and pancreatic cancers, though less prevalent, exhibit dismal survival rates, largely attributed to late-stage diagnoses and the lack of effective systemic therapies. The epidemiology of GI tumors varies greatly by region [3], largely influenced by dietary, genetic, and environmental factors. In high-income countries, colorectal cancer incidence has been steadily increasing due to lifestyle factors such as high-fat diets, physical inactivity, and obesity. Conversely, in low- and middle-income countries, gastric and esophageal cancers are more prevalent,

often linked to Helicobacter pylori infections and chronic esophageal irritation caused by hot beverages or alcohol consumption. Liver cancer, primarily hepatocellular carcinoma (HCC), is significantly more common in regions with high rates of hepatitis B and C virus infections, such as Sub-Saharan Africa and East Asia. Pancreatic cancer, while less common, has seen a rise in incidence globally and is projected to become one of the top three causes of cancer deaths by 2030 due to its aggressive nature and lack of early diagnostic methods [5]. Despite advances in medical imaging, endoscopic techniques, and biomarker discoveries, the prognosis for many GI cancers remains poor. For instance, the 5-year survival rates for pancreatic and liver cancers remain below 10%, underscoring the urgent need for improved early detection methods. Current diagnostic tools such as colonoscopy, endoscopic ultrasonography, and imaging modalities like CT and MRI are limited by accessibility, cost, and invasive nature. Furthermore, serum biomarkers like carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) lack sufficient sensitivity and specificity for reliable early-stage cancer detection. These limitations necessitate the exploration of novel biomarkers that are non-invasive, cost-effective, and highly specific. Over the past decade, non-coding RNAs (ncRNAs) have emerged as key players in cancer biology. Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) have been extensively studied for their roles in gene regulation, with many identified as potential diagnostic and prognostic biomarkers. Recently, a relatively understudied class of small non-coding RNAs, known as Piwi-interacting RNAs (piRNAs) [6], has gained attention due to their unique properties and potential in cancer diagnostics. Originally discovered in the context of germline cells, where they play a critical role in maintaining genomic stability by silencing transposable elements, piRNAs are now being recognized for their aberrant expression and functional significance in various cancer types, including GI tumors. piRNAs are typically 24-32 nucleotides long and form complexes with PIWI proteins, a subfamily of the Argonaute family [7]. This piRNA-PIWI complex is instrumental in epigenetic regulation, post-transcriptional gene silencing, and chromatin remodeling. While these roles are well-characterized in germline cells, increasing evidence suggests that piRNAs also contribute to somatic processes, including oncogenesis. For instance, studies have shown that dysregulated piRNA expression can influence tumor proliferation, metastasis, and resistance to therapy by modulating oncogenic pathways and tumor suppressor genes. In the context of gastrointestinal cancers [8], piRNAs have shown significant promise as both biomarkers and therapeutic targets. Several studies have identified specific piRNAs with aberrant expression patterns in gastric, colorectal, and liver cancers. For instance, piR-823 has been shown to promote gastric cancer progression by influencing tumor microenvironment dynamics [9], while piR-651 has been implicated in the regulation of colorectal cancer cell proliferation. These findings highlight the potential of piRNAs to serve as highly specific biomarkers for early cancer detection [10]. One of the most compelling aspects of piRNAs as diagnostic tools is their stability in body fluids, such as plasma, serum, and urine. This stability, combined with their tumor-specific expression profiles, makes piRNAs ideal candidates for liquid biopsy approaches. Liquid biopsies offer a non-invasive alternative to traditional tissue biopsies, enabling real-time monitoring of tumor dynamics and early detection of malignancies. High-throughput sequencing technologies and advanced bioinformatics tools have further facilitated the identification of piRNA signatures in patient-derived samples, accelerating their translational potential. The mechanistic roles of piRNAs in gastrointestinal cancers are an area of active investigation. Emerging evidence suggests that piRNAs interact with PIWI proteins to regulate a wide range of cellular processes, including DNA damage repair, apoptosis, and immune evasion. For example, piR-823 has been shown to modulate angiogenesis and immune responses in gastric cancer, thereby contributing to tumor growth and metastasis. Similarly, piR-5937 and piR-28876 have been associated with altered epigenetic states in colorectal cancer cells, highlighting their potential role in tumor progression [11]. The involvement of piRNAs in modulating the tumor microenvironment is another exciting avenue of research. By influencing stromal-epithelial interactions and immune cell recruitment, piRNAs can create a microenvironment conducive to tumor growth and resistance to therapy [12]. Understanding these interactions could pave the way for novel therapeutic strategies that target the tumor microenvironment in addition to the tumor itself. Despite their promise, several challenges remain in the clinical translation of piRNA research. One major hurdle is the lack of standardized methods for piRNA detection and quantification. While high-throughput sequencing and PCR-based methods have advanced our understanding of piRNA biology, their clinical application requires robust and reproducible assays. Additionally, the functional roles of many piRNAs remain poorly understood, necessitating further mechanistic studies to elucidate their contributions to tumor biology. Another challenge lies in the validation of piRNAs as clinical biomarkers [13]. Large-scale, multicenter studies are needed to establish the sensitivity, specificity, and predictive value of piRNAs across diverse patient populations. Furthermore, the integration of piRNA-based diagnostics into existing clinical workflows will require collaboration between researchers, clinicians, and industry stakeholders. Given the global burden of gastrointestinal tumors and the urgent need for innovative diagnostic and therapeutic approaches, piRNAs represent a promising frontier in cancer research. This review aims to provide a comprehensive overview of the current progress in piRNA research, focusing on their roles as diagnostic biomarkers and their potential to transform the landscape of gastrointestinal oncology [14]. By summarizing recent findings and highlighting future directions, we hope to inspire further research and facilitate the clinical translation of piRNA-based technologies.

2. piRNA in GI Tumor Diagnosis

2.1 Aberrant expression in tumors

Aberrant expression of Piwi-interacting RNAs has emerged as a critical feature in the molecular landscape of gastrointestinal cancers, including gastric, colorectal, and esophageal cancers [15]. Dysregulated piRNA expression not only marks the transition from normal to malignant states but also plays active roles in promoting tumor progression. For instance, certain oncogenic piRNAs are overexpressed, driving enhanced cell proliferation, evasion of apoptosis, and angiogenesis, which are hallmarks of cancer [16]. Conversely, the suppression of tumor-suppressor piRNAs disrupts regulatory pathways that maintain cellular homeostasis, thereby facilitating unchecked tumor growth. In colorectal cancer, studies have demonstrated that specific piRNAs like piR-651 are upregulated, contributing to the activation of signaling pathways such as Wnt/β-catenin [17], which are pivotal in cancer metastasis and invasion. Similarly, in gastric cancer [17], piR-823 has been shown to reshape the tumor microenvironment by modulating immune responses and supporting vascularization, thus promoting metastatic potential. The unique and often tumor-specific patterns of piRNA deregulation offer insights into the complex molecular networks underlying GI cancers [18]. Moreover, the stable nature of piRNAs in body fluids presents a significant opportunity for non-invasive biomarker development, enabling early detection and real-time monitoring of tumor dynamics. Understanding the intricate mechanisms behind piRNA dysregulation is not only crucial for deciphering the biology of GI tumors but also holds immense potential for the development of targeted therapeutic strategies and personalized medicine approaches [19].

2.2 Circulating piRNAs

Circulating piRNAs in plasma or serum represent a groundbreaking advancement in non-invasive cancer diagnostics, particularly for gastrointestinal tumors [20]. These small, stable RNA molecules possess remarkable specificity and sensitivity in detecting tumor-associated molecular alterations, even at early stages of cancer development [11]. Unlike traditional tissue biopsies, which are invasive and often limited to localized analysis, circulating piRNAs provide a systemic snapshot of tumor dynamics, capturing insights into both primary tumors and metastatic lesions. Their extraordinary stability in body fluids, attributed to their association with protective protein complexes, ensures that circulating piRNAs remain intact and detectable, even in challenging clinical conditions [21]. Recent studies have highlighted specific piRNA signatures in gastric and colorectal cancers, showcasing their ability to differentiate malignant from benign conditions with high accuracy. For example, panels of circulating piRNAs have demonstrated promising diagnostic performance in detecting colorectal cancer at its preclinical stages, offering a potential window for early therapeutic intervention. Beyond diagnosis [22], these circulating biomarkers hold immense potential for real-time monitoring of treatment efficacy and disease progression [23]. However, translating these findings into routine clinical practice requires standardized protocols for piRNA isolation, quantification, and interpretation, as well as large-scale validation in diverse patient populations. Despite these challenges, circulating piRNAs have the potential to redefine cancer screening paradigms, providing a minimally invasive yet highly effective tool for improving early detection and patient outcomes in GI oncology.

2.3 Prognostic value

The prognostic value of piRNAs in gastrointestinal cancers is an area of burgeoning interest [22], with evidence suggesting that specific piRNAs serve as reliable indicators of patient outcomes and tumor behavior. Certain piRNAs exhibit expression patterns that correlate closely with critical clinical parameters such as tumor grade, metastatic potential, and survival rates [24]. For instance, elevated levels of oncogenic piRNAs in gastric cancer patients are associated with aggressive phenotypes, including enhanced invasion and resistance to chemotherapy, thereby signaling poorer prognoses. Conversely, tumor-suppressive piRNAs, whose downregulation is linked to disrupted cellular homeostasis [25], often correspond to advanced disease stages and reduced survival. Beyond static prognosis, piRNAs also provide dynamic insights into therapeutic responses, offering potential as biomarkers to predict and monitor the efficacy of treatments. In colorectal cancer, piR-823 has been linked to both tumor proliferation and the modulation of the tumor microenvironment, making it a candidate for evaluating the impact of targeted therapies [25]. Furthermore, the inherent stability of piRNAs and their detectability in liquid biopsies enable longitudinal monitoring of disease progression, providing clinicians with actionable data for personalized treatment adjustments [26]. As research continues to elucidate the functional roles of piRNAs in tumor progression, their integration into clinical workflows holds the promise of refining prognostic models and enhancing precision medicine approaches for gastrointestinal oncology.

3. Current progress in piRNA

3.1 piRNA pathway and mechanisms

The piRNA pathway represents a sophisticated and multi-faceted mechanism that plays pivotal roles in both normal cellular processes and tumorigenesis, particularly in gastrointestinal cancers [16]. Piwi-interacting RNAs, a class of small non-coding RNAs, are primarily known for their interactions with PIWI proteins, a subfamily of Argonaute proteins [27]. This piRNA-PIWI protein complex is crucial for silencing transposable elements, regulating gene expression, and maintaining genomic

integrity [28], especially in germline cells. However, accumulating evidence has highlighted its aberrant activation or suppression in somatic cells, including in the context of GI tumors, where these pathways contribute significantly to oncogenesis and tumor progression. In the piRNA pathway, the biogenesis of piRNAs begins with the processing of long single-stranded precursor RNAs transcribed from specific genomic regions called piRNA clusters [29]. These precursors are cleaved into intermediates and subsequently matured into functional piRNAs through the actions of endonucleases, such as Zucchini, and exonucleases. Mature piRNAs, typically 24-32 nucleotides in length, bind to PIWI proteins to form the functional piRNA-PIWI complex [30]. This complex is transported into the nucleus or remains in the cytoplasm to execute its regulatory functions. Its primary roles include transcriptional silencing, epigenetic modification, and post-transcriptional regulation through the targeting of complementary RNA sequences[31]. In GI cancers, such as gastric, colorectal, and liver cancers, the deregulation of piRNA pathways has been implicated in key oncogenic processes, including tumor initiation, proliferation, and metastasis [32]. The piRNA-PIWI complex regulates oncogenes and tumor suppressor genes through a variety of mechanisms. For instance, in colorectal cancer, studies have shown that specific piRNAs promote oncogenic pathways such as Wnt/β-catenin signaling by suppressing antagonistic regulators, thereby facilitating tumor growth and invasion [33]. Conversely, tumor-suppressor piRNAs can downregulate oncogene expression or promote the activity of tumor-suppressive pathways, although their silencing due to epigenetic modifications or other factors often contributes to tumor progression. A critical aspect of the piRNA pathway in GI tumors is its role in epigenetic regulation. piRNAs guide PIWI proteins to specific genomic loci [34], where they recruit chromatin-modifying enzymes to induce DNA methylation or histone modifications. This activity is particularly significant in the silencing of transposable elements, which, when derepressed, can cause genomic instability-a hallmark of cancer. In gastric cancer, aberrant DNA methylation mediated by piRNA pathways has been associated with silencing key tumor suppressor genes, leading to enhanced tumor aggressiveness [35]. Similarly, in hepatocellular carcinoma, the deregulation of piRNAs involved in chromatin remodeling has been linked to altered gene expression profiles that favor tumorigenesis. Beyond epigenetics, the piRNA-PIWI complex exerts post-transcriptional regulation by degrading target mRNAs or preventing their translation [36]. This mechanism often involves sequence-specific interactions between piRNAs and their mRNA targets, akin to the action of microRNAs. In GI tumors, this regulatory axis has been implicated in the fine-tuning of pathways involved in cell cycle progression, apoptosis, and angiogenesis. For example, piR-823 has been shown to enhance angiogenesis in gastric cancer by modulating vascular endothelial growth factor (VEGF) expression, highlighting its role in creating a tumor-permissive microenvironment [37]. The involvement of piRNA pathways in the tumor microenvironment is another critical area of research. GI tumors are characterized by a complex microenvironment comprising immune cells, stromal cells, and extracellular matrix components, all of which influence tumor behavior. piRNAs have been found to modulate immune cell recruitment and activity, as well as stromal-epithelial interactions, thereby impacting immune evasion and metastasis. For instance, piRNAs that regulate cytokine signaling pathways can alter the immune landscape of GI tumors, enabling them to escape immune surveillance [38]. Despite these advances, the precise molecular mechanisms underlying piRNA functions in GI tumors remain incompletely understood. One of the major challenges is identifying the full spectrum of piRNA targets and elucidating their context-specific roles. High-throughput sequencing technologies and advanced bioinformatics tools have significantly expanded our understanding of piRNA pathways, yet the complexity of their interactions with PIWI proteins and other cellular components necessitates further investigation. [39] Functional studies using gene editing technologies such as CRISPR/Cas9 are beginning to shed light on the direct roles of specific piRNAs in GI tumor biology, offering new insights into their therapeutic potential [40]. The therapeutic implications of targeting the piRNA pathway in GI cancers are profound [41]. By restoring the normal function of tumor-suppressive piRNAs or inhibiting oncogenic piRNAs, it may be possible to reverse tumor progression or enhance the efficacy of existing treatments [42]. For instance, small molecules or RNA-based therapeutics designed to modulate piRNA-PIWI interactions could serve as targeted therapies for GI cancers. Additionally, the stability of piRNAs in body fluids positions them as valuable biomarkers for early diagnosis, prognosis, and treatment monitoring, underscoring the translational relevance of piRNA research. The piRNA pathway represents a complex but highly promising target in the study of GI tumors. [15] Its multifaceted roles in regulating oncogenes, tumor suppressors, and the tumor microenvironment underscore its potential as both a biomarker and therapeutic target. Continued exploration of the piRNA-PIWI axis will not only deepen our understanding of GI tumor biology but also pave the way for innovative diagnostic and therapeutic strategies, ultimately improving patient outcomes in gastrointestinal oncology.

3.2 Biomarker studies

The identification of reliable biomarkers for the early detection and monitoring of gastrointestinal cancers has been a critical focus of research, and Piwi-interacting RNAs have emerged as promising candidates [38]. Specific piRNAs, such as piR-823 and piR-651, have demonstrated significant potential as diagnostic biomarkers for gastric and colorectal cancers in clinical studies. Their high specificity, stability in biological

fluids, and tumor-associated expression profiles make them attractive tools for non-invasive cancer diagnosis and prognosis. piR-823, a small non-coding RNA, has been extensively studied in gastric cancer. Its aberrant expression levels have been correlated with tumor progression, including increased cell proliferation and invasion [35]. Clinical studies have shown that piR-823 is upregulated in gastric cancer tissues compared to adjacent non-tumorous tissues [15]. This overexpression is also detectable in the plasma of patients, making it a viable biomarker for liquid biopsy applications. The biological mechanisms underlying piR-823's role in cancer involve its influence on the tumor microenvironment, particularly in modulating angiogenesis and immune response. For instance, piR-823 has been reported to regulate vascular endothelial growth factor (VEGF) expression, which plays a critical role in promoting blood vessel formation to support tumor growth. These findings highlight piR-823's dual role as a diagnostic marker and a potential therapeutic target [39]. Similarly, piR-651 has been identified as a biomarker for colorectal cancer. Studies have consistently demonstrated its upregulation in colorectal cancer tissues and its association with key clinical parameters, including tumor stage and metastasis. Notably, piR-651 has shown excellent performance in distinguishing colorectal cancer patients from healthy controls when measured in serum samples, with high sensitivity and specificity. This makes it a promising candidate for early detection, particularly in asymptomatic individuals [15]. Furthermore, piR-651's expression has been linked to the activation of oncogenic pathways, such as Wnt/ β -catenin signaling, underscoring its role in tumor progression and its potential as a therapeutic target. Beyond piR-823 and piR-651, other piRNAs are also being explored for their diagnostic potential in GI cancers. High-throughput sequencing technologies and advanced bioinformatics tools have facilitated the identification of piRNA expression profiles unique to specific cancer types [35]. For example, panels of piRNAs have been developed to enhance diagnostic accuracy. A multi-piRNA signature has been proposed for gastric cancer diagnosis, achieving higher sensitivity and specificity than traditional serum biomarkers like carcinoembryonic antigen (CEA) [43]. These multi-marker approaches leverage the combinatorial power of piRNAs, capturing diverse aspects of tumor biology and reducing false-positive rates. The use of piRNAs as biomarkers is particularly appealing due to their inherent stability. piRNAs are protected from degradation by their association with PIWI proteins and incorporation into extracellular vesicles such as exosomes. This stability ensures that piRNAs remain intact in harsh conditions, such as prolonged storage or exposure to RNases in biological fluids, making them ideal for clinical applications [44]. Additionally, the non-invasive nature of piRNA-based diagnostics, which rely on easily accessible samples like blood or urine, addresses one of the major limitations of traditional biopsy-based methods. However, translating piRNAs into clinical practice involves several challenges. Standardized protocols for piRNA isolation, quantification, and data interpretation are necessary to ensure reproducibility and reliability [45]. While next-generation sequencing (NGS) and quantitative PCR (qPCR) are commonly used for piRNA detection, these techniques require further optimization for routine clinical use. Moreover, large-scale validation studies are essential to confirm the diagnostic utility of piRNAs across diverse populations and clinical settings. Such studies should aim to establish reference ranges for piRNA expression and evaluate their performance relative to existing biomarkers. The translational potential of piRNAs extends beyond diagnosis to monitoring treatment response and predicting outcomes. For instance, dynamic changes in circulating piRNA levels during chemotherapy or targeted therapy could provide real-time insights into treatment efficacy. This would enable personalized adjustments to therapeutic regimens, improving patient outcomes. Furthermore, piRNAs might also serve as prognostic biomarkers, offering valuable information about disease recurrence or metastasis risk [46]. piRNAs, particularly piR-823 and piR-651, represent a promising frontier in the quest for effective diagnostic biomarkers for GI cancers. Their tumor-specific expression, stability, and non-invasive detectability address many of the limitations of current diagnostic methods. As research progresses, the integration of piRNA-based biomarkers into clinical workflows holds the potential to revolutionize cancer diagnosis and management, paving the way for more accurate, early detection and personalized treatment strategies in gastrointestinal oncology.

3.3 Technological advances in piRNA

The rapid development of high-throughput sequencing technologies and advanced bioinformatics tools has revolutionized the identification and characterization of piRNA signatures in gastrointestinal tumors, marking a pivotal advancement in cancer research [47]. High-throughput sequencing, such as RNA-Seq, allows for comprehensive profiling of small RNA populations [48], including piRNAs, across various sample types, such as tumor tissues, plasma, and exosomes. This technology has provided unprecedented insights into the expression patterns and functional roles of piRNAs in GI cancers [49]. For example, sequencing-based studies have identified distinct piRNA expression profiles that differentiate gastric and colorectal cancer tissues from adjacent non-tumorous tissues, revealing their potential as diagnostic biomarkers. Furthermore, sequencing has enabled the discovery of novel piRNAs that were previously unannotated, expanding the catalog of small RNAs associated with oncogenesis. Bioinformatics tools have played an essential role in translating sequencing data into biologically meaningful insights. Computational pipelines [31], such as piRNA annotation tools, target prediction algorithms, and

pathway analysis software, have facilitated the identification of piRNA-target interactions and their associated pathways. In GI cancers, bioinformatics-driven studies have uncovered key piRNA signatures involved in tumor progression, such as piRNAs targeting Wnt/β-catenin and VEGF pathways [29]. Additionally, machine learning approaches integrated with bioinformatics pipelines have been employed to develop predictive models based on piRNA expression patterns. These models show high accuracy in classifying cancer types, stages, and even treatment responses, underscoring their diagnostic and prognostic potential. The integration of multi-omics data with piRNA research has further enhanced the understanding of their roles in tumorigenesis. By combining piRNA sequencing data with genomic, transcriptomic, and proteomic datasets, researchers have elucidated the regulatory networks governed by piRNAs. For instance, studies in colorectal cancer have linked piRNA expression with DNA methylation and histone modification profiles, highlighting their role in epigenetic regulation [50]. Furthermore, single-cell RNA sequencing (scRNA-Seq) has begun to unravel the heterogeneity of piRNA expression at the cellular level, revealing their distinct roles in tumor and stromal cells within the tumor microenvironment. Technological advances have also improved the detection of circulating piRNAs in body fluids, a critical step for non-invasive diagnostics. Small RNA sequencing platforms have demonstrated the ability to detect low-abundance piRNAs in plasma and serum with high sensitivity, paving the way for liquid biopsy applications [51]. However, these technologies are not without challenges. High-throughput sequencing generates vast amounts of data, requiring significant computational resources and robust analytical pipelines to manage, process, and interpret the results accurately. Moreover, standardization in sample preparation, data analysis, and reporting is necessary to ensure reproducibility and clinical applicability [24]. Technological advances in high-throughput sequencing and bioinformatics tools have dramatically accelerated piRNA research in GI tumors, unveiling their diagnostic, prognostic, and therapeutic potential [52]. These innovations not only expand the scope of piRNA-related discoveries but also bring the field closer to translating these findings into clinical applications, offering new hope for improved cancer detection and treatment.

3.4 AI and piRNA in gastrointestinal tumors

Artificial intelligence (AI) has emerged as a transformative tool in the analysis of piRNA data, accelerating discoveries in gastrointestinal tumor research [15]. The integration of AI with high-throughput sequencing data has enabled the identification of complex piRNA expression patterns, revealing their associations with tumor development, progression, and treatment responses [53]. Machine learning algorithms, such as support vector machines (SVMs) and random forests, have been employed to classify GI tumors based on piRNA expression profiles with high accuracy [54]. These models not only differentiate tumor tissues from normal ones but also predict disease stages and metastatic potential. Deep learning approaches, including neural networks [55], have further advanced the analysis by uncovering intricate relationships between piRNA pathways and oncogenic signaling, such as Wnt/β-catenin and VEGF, which are critical in GI cancers. For instance, AI-powered algorithms have successfully identified piRNA panels like piR-823 and piR-651 as diagnostic biomarkers for gastric and colorectal cancers, offering higher sensitivity and specificity than traditional biomarkers [56]. Moreover, AI facilitates the functional annotation of piRNAs by predicting their targets and regulatory roles. Natural language processing (NLP) and bioinformatics tools powered by AI have streamlined the integration of multi-omics datasets, enabling researchers to link piRNA expression with transcriptomic, epigenomic, and proteomic changes in GI tumors. This holistic approach provides a systems-level understanding of tumor biology, revealing novel therapeutic targets. In addition to diagnostics, AI has enhanced the discovery of circulating piRNAs in liquid biopsy samples, aiding the development of non-invasive methods for early cancer detection and monitoring [57]. By analyzing large-scale datasets, AI models identify subtle changes in piRNA levels in plasma or serum, offering real-time insights into tumor dynamics and treatment efficacy [58]. However, challenges remain in fully leveraging AI for piRNA research. The lack of standardized datasets and the complexity of piRNA pathways necessitate the development of more robust and interpretable AI models [59]. Despite these hurdles, the synergy between AI and piRNA research holds immense promise for revolutionizing the understanding and management of GI tumors. By providing unprecedented analytical power and precision, AI-driven piRNA studies are poised to advance early diagnosis, personalized therapies, and improved outcomes for patients with GI cancers.

4. Conclusion

In summary, the emerging role of Piwi-interacting RNAs (piRNAs) in the early diagnosis of gastrointestinal (GI) tumors offers a promising avenue for non-invasive and precise cancer detection. The identification of tumor-specific piRNA signatures, along with their presence in circulating bodily fluids, paves the way for more accessible and reliable diagnostic tools. While significant strides have been made in understanding the piRNA-PIWI protein axis and its involvement in tumorigenesis, challenges remain in standardizing detection methods and ensuring clinical validation. Nevertheless, ongoing research holds immense potential for translating piRNA-based diagnostics into practical applications, ultimately improving early detection, treatment outcomes, and patient survival rates in GI cancers. Continued interdisciplinary efforts will be essential to overcome existing hurdles and harness the full potential of piRNAs as biomarkers for cancer diagnosis.

FUNDING

This study was financially supported by the National Natural Science Foundation of China (82460865), the Inner Mongolia Autonomous Region science and technology planning project (2023KJHZ0023), the Scientific and Technological Innovative Research Project for Inner Mongolia Universities (NMGIRT2327), the Health Science and Technology Plan Project of Inner Mongolia Autonomous Region (202201363, 202202222, 202201256), the Project of Inner Mongolia Medical University (YKD2023QN010, YKD2022ZD004, YKD2022TD003, YKD2023TD010, YKD2021LH032, YKD2021LH006), the High level clinical specialty development technology project for public hospitals in Inner Mongolia Autonomous Region(2023SGGZ074, 2023SGGZ074-06), and the Science and Technology Program of the Joint Fund of Scientific Research for the Public Hospitals of Inner Mongolia Academy of Medical (2023GLLH0126).

CONFLICT OF INTEREST

There is conflict of interest.

ACKNOWLEDGEMENTS

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