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REVIEW

Synergistic Immunoregulation: harnessing CircRNAs and PiRNAs to Amplify PD-1/PD-L1 Inhibition Therapy

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Abstract: The utilization of PD-1/PD-L1 inhibitors marks a significant advancement in cancer therapy. However, the efficacy of monotherapy is still disappointing in a substantial subset of patients, necessitating the exploration of combinational strategies. Emerging from the promising results of the KEYNOTE-942 trial, RNA-based therapies, particularly circRNAs and piRNAs, have distinguished themselves as innovative sensitizers to immune checkpoint inhibitors (ICIs). These non-coding RNAs, notable for their stability and specificity, were once underrecognized but are now known for their crucial roles in regulating PD-L1 expression and bolstering anti-cancer immunity. Our manuscript offers a comprehensive analysis of selected circRNAs and piRNAs, elucidating their immunomodulatory effects and mechanisms, thus underscoring their potential as ICIs enhancers. In conjunction with the recent Nobel Prize-awarded advancements in mRNA vaccine technology, our review highlights the transformative implications of these findings for cancer treatment. We also discuss the prospects of circRNAs and piRNAs in future therapeutic applications and research. This study pioneers the synergistic application of circRNAs and piRNAs as novel sensitizers to augment PD-1/PD-L1 inhibition therapy, demonstrating their unique roles in regulating PD-L1 expression and modulating immune responses. Our findings offer a groundbreaking approach for enhancing the efficacy of cancer immunotherapy, opening new avenues for treatment strategies. This abstract aims to encapsulate the essence of our research and the burgeoning role of these non-coding RNAs in enhancing PD-1/PD-L1 inhibition therapy, encouraging further investigation into this promising field.

Keywords: CircRNA, piwi-RNA, immunotherapy, PD-1/PD-L1 blockade, immunotherapy sensitizer, combination therapy

Introduction

According to GLOBOCAN, there were 19.3 million.[1], new cancer cases and 10.0 million cancer-related deaths worldwide in 2020.[2] In 2019, the World Health Organization (WHO) estimated that cancer ranks as the primary or secondary cause of death before the age of 70 in 112 out of 183 countries.[2] As a formidable ailment, the available therapeutic options for cancer remain limited.[3] The advent of immune checkpoint inhibitor therapy, including PD-1 (Programmed Cell Death Protein 1) and PD-L1 (Programmed Death-Ligand 1) inhibitors, has revolutionized cancer treatment and, to some extent, improved clinical outcomes for cancer patients. However, a significant portion of cancer patients still struggle to achieve sustained and optimal clinical benefits.[3–5]

Therefore, the concept of incorporating a sensitizer into PD-1/PD-L1 inhibitor-based therapy has been developed to enhance clinical benefits for cancer patients.[6] Existing or potential sensitizers, such as tyrosine kinase inhibitors, carbonic anhydrase XII inhibitors, histone deacetylase 2 inhibitors, and RNA therapy, have expanded the range of options and possibilities for combating malignancies.[6–9]

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The awarding of the 2023 Nobel Prize for the pioneering application of mRNA vaccines in combating COVID-19 has underscored the potential of gene therapy to modulate the immune response, bringing it widespread recognition. Furthermore, the safety of such therapies has gained acceptance, heightening the imperative to leverage gene therapy in cancer treatment, especially considering its proven ability to regulate immune system functions.[10] Moreover, recent findings have provided initial evidence supporting the practicality of utilizing Immune Checkpoint Inhibitors (ICIs) in conjunction with non-coding RNA (ncRNA) for cancer treatment.[11,12] To elaborate, in the Phase 2 KEYNOTE-942 trial (NCT03897881), the combination of mRNA-4157 (V940) vaccine and pembrolizumab, a PD-1 inhibitor, exhibited a notable reduction in the risk of distant metastasis or mortality by approximately 65%, compared to pembrolizumab used as a standalone adjuvant therapy in (sample size: 257) patients with resected melanoma at a high risk of recurrence.[11,12] The median follow-up period was 23 months for the mRNA-4157 plus pembrolizumab group and 24 months for those receiving pembrolizumab monotherapy. At the 18-month mark, the rate of distant metastasis-free survival (DMFS) stood at 91.8% for the combined therapy, as opposed to 76.8% for the pembrolizumab monotherapy (Hazard Ratio, 0.347; 95% Confidence Interval, 0.145–0.828; P = 0.0063).[11–13] This result represents the first demonstration of efficacy for an investigational mRNA-based cancer treatment in a randomized clinical trial.

Additionally, the similar study design was applied in a previous clinical trial (KEYNOT-603), which has also already displayed acceptable safety profile along with observed clinical responses in combination of mRNA-4157 with pembrolizumab in patients with various types of cancer (including melanoma, bladder cancer, colorectal cancer (CRC), non-small-cell lung cancer (NSCLC), SCLC, and head and neck squamous cell carcinoma (HNSCC)).[14–16] The trial yielded encouraging findings, demonstrating an overall response rate (ORR) of 33% and a disease control rate (DCR) of 67%. Importantly, the combination therapy exhibited favorable tolerability, as no new safety signals were detected.[14–16] These promising results prompt the expansion of developing non-coding RNAs as a sensitizer for ICIs-based therapy.

Circular RNAs (circRNAs) and piwi-interacting RNAs (piRNAs) are classes of non-coding RNAs that have garnered interest due to their stability, specificity, and regulatory functions in gene expression. Unlike their more transient counterparts, these RNAs offer a unique advantage in therapeutic applications due to their resistance to degradation and their ability to fine-tune gene expression at multiple levels. The theoretical foundation for their use alongside immunotherapy, such as PD-1/PD-L1 inhibitors, is based on empirical evidence highlighting their roles in immune modulation and their potential to overcome resistance mechanisms that limit the effectiveness of current cancer treatments. The interplay between circRNAs, piRNAs, and the immune system is multifaceted. CircRNAs have been shown to act as molecular sponges, sequestering microRNAs and thus preventing them from suppressing target mRNA translation. This action can enhance the immune response against tumors by upregulating the expression of genes involved in antigen presentation and T cell activation. Similarly, piRNAs, which were initially recognized for their role in silencing transposable elements and maintaining genome integrity, have been found to influence the expression of genes crucial for immune surveillance and tumor suppression.

Moreover, recent advancements in RNA biology have illuminated the roles of circRNAs and piRNAs in regulating PD-L1 expression—a key ligand in the PD-1/PD-L1 immune checkpoint pathway. By modulating PD-L1 levels, these RNAs can potentially enhance the efficacy of immunotherapies that target this pathway, offering a novel means to bolster anti-tumor immunity. The ability of circRNAs and piRNAs to interact with the immune system at various levels, from gene expression to post-translational modifications, opens up new avenues for research and therapy development. Combining circRNAs and piRNAs with immunotherapy capitalizes on the synergistic potential to modulate the tumor microenvironment, enhance the recognition and destruction of cancer cells by the immune system, and overcome the limitations of current treatment modalities. This approach is inspired by both the empirical evidence of their roles in cancer and theoretical advancements in our understanding of RNA biology. As research progresses, this strategy holds the promise of developing more personalized, effective, and durable treatments for cancer patients.

CircRNAs and piRNAs, as special subtypes of non-coding RNA known for their immune modulating capabilities and enhanced stability,[17,18] could potentially be effective in enhancing PD-1/PD-L1 inhibitor therapy in cancer treatment. These non-coding RNAs may offer new opportunities for therapeutic exploration, either used alone or in combination. Thus, we here explore potential RNA candidates and their applications in cancer treatment, focusing on their immune regulatory functions, particularly their impact on PD-L1 expression. This highlights the significant potential of such therapies in oncology.

Boosting PD-1/PD-L1 Inhibitor Efficiency Through PD-L1 Regulation

PD-1 is a protein that exists on the surface of T-cells, a type of immune cell. PD-L1 is its ligand, often expressed on the surface of cancer cells. When PD-L1 binds to PD-1, it sends a signal to the T-cell to reduce its activity, effectively "turning off" the immune response. This mechanism is a normal part of immune regulation, but cancer cells exploit it to protect themselves from immune attack.[19] PD-1/PD-L1 inhibitors disrupt this interaction. They are antibodies that bind either to PD-1 or PD-L1, preventing the two from interacting. By blocking this pathway, these drugs lift the "brakes" on the immune system, allowing T-cells to recognize and attack cancer cells. With the PD-1/PD-L1 interaction blocked, T-cells can more effectively identify and destroy cancer cells.[20] This strengthens the body's natural immune response against the tumor.[21] However, cancer patients treated with PD-1/PD-L1 inhibitors have shown different therapeutic responses, most of them remain unresponsive, and the expression of PD-L1 is the main factor affecting the efficacy. Clinical trial KEYNOTE-189 showed that the efficacy of PD-1/PD-L1 inhibitors is poor when the level of PD-L1 expression is less than 1%.[22] Tumors with low PD-L1 expression may show limited responsiveness to PD-1/PD-L1 inhibitors due to insufficient PD-L1 molecules for engaging immune checkpoint inhibitors.[23] In the clinical setting, both the PD-L1 tumor proportion score (TPS) and the PD-L1 combined positive score (CPS) have emerged as robust indicators of the potential benefits of PD-1/PD-L1 inhibitors therapy.[24] Higher TPS or CPS scores are indicative of more favorable outcomes with PD-1/PD-L1 inhibitors therapy. Consequently, elevating PD-L1 expression levels could render cancer cells more conspicuous to the immune system, thereby enhancing the effectiveness of PD-1/PD-L1 inhibitors in activating the immune response.[25,26]

Research also showed that promoting PD-L1 expression can augment the efficacy of PD-1/PD-L1 inhibitors.[27] For instance, some chemo agents, such as paclitaxel, etoposide, and 5-fluorouracil, were proved to elevate the expression of PD-L1,[28] and the efficacy of immunotherapy could be enhanced by combining with chemotherapy.[29] Besides, chidamide, a class I histone deacetylase (HDAC) inhibitor, has been proven to increase PD-L1 expression. When combined with the anti-PD-1 antibody toripalimab, chidamide showed more efficacious than toripalimab alone in treating metastatic sarcoma patients.[30] Besides, increasing PD-L1 expression by using peripheral serotonin can enhance the efficacy of anti-PD-1 therapy in mice with colorectal cancer (CRC) or pancreatic cancer.[31] Additionally, upregulating PD-L1 expression with Pin1 inhibitors (ATO, ATRA, Sulfopin) has shown a synergistic antitumor effect in combination with anti-PD-1 antibody and gemcitabine therapy for pancreatic cancer treatment in a preclinical study.[32] Consequently, the up-regulation of PD-L1 expression holds the potential to render tumors more responsive to PD-1/PD-L1 checkpoint inhibitors, with circRNAs therapy representing a promising avenue for bolstering immune activation by increasing PD-L1 expression.

Conversely, when PD-L1 expression is inhibited on cancer cells, it means there are fewer inhibitory signals for T cells, which can lead to some benefits, such as enhancing T cell activity, improving the response to PD-1/PD-L1 inhibitors, increasing sensitivity to immunotherapy.[33] For instance, an in vivo study demonstrated that reducing PD-L1 expression through the use of an mTOR inhibitor (rapamycin) can enhance the effectiveness of PD-1/PD-L1 inhibition therapy for NSCLC treatment.[34] Furthermore, the addition of curcumin, a compound known to suppress PD-L1 expression, could enhance the therapeutic effects of anti-PD-1 therapy for HCC both in vitro and in vivo. This enhancement is attributed to the activation of lymphocytes and inhibition of immune evasion mechanisms.[35]

The relationship between PD-L1 expression and the therapeutic effect of PD-1/PD-L1 inhibitors is complex and can vary depending on the specific tumor microenvironment, the patient's immune response, and other factors. Therefore, it is crucial to appropriately choose proper piRNAs and/or circRNAs to regulate the PD-L1 expression (upregulation or downregulation) based on different clinical contexts.[36,37] Undoubtedly, regulating PD-L1 expression as needed to enhance the efficacy of PD-1/PD-L1 inhibitors is a field in urgent need of breakthroughs.

Potential Advantages of Applying circRNAs or piRNAs in PD-1/PD-L1 Inhibition Therapy

The journey of RNA-based therapies from concept to clinical reality is a remarkable tale of scientific progress.[38,39] Initially, RNA was understood primarily as a messenger, carrying instructions from DNA to the cell's protein-making machinery.[39] However, the discovery of RNA's multifaceted roles—including regulation, interference, and modulation of gene expression—marked a turning point in our understanding of its potential.[39,40] The field has achieved a significant

milestone with the development of mRNA vaccine technology, a triumph of decades-long research recently spotlighted by the Nobel Prize in Medicine awarded to Katalin Karikó and Drew Weissman.[41] Their groundbreaking work underpinned the rapid development of mRNA-based COVID-19 vaccines, showcasing the power of RNA technology not just in disease prevention but also as a promising tool for treating a wide array of conditions, including cancer. This recognition not only celebrates a pivotal advance in RNA research but also signals the dawn of a new era.[41] In this era, RNA-based therapies are emerging as key players in personalized medicine, promising to revolutionize treatment approaches for a variety of diseases. Exploring the roles of circRNAs and piRNAs, the research is anchored in a landscape of innovation, aiming to harness RNA's therapeutic potential to modulate the immune system in the battle against cancer.

CircRNAs

As previously mentioned, the utilization of PD-1/PD-L1 inhibitors has marked a significant advancement in the realm of cancer treatment. Nevertheless, their therapeutic impact remains constrained when used as standalone treatments. Consequently, there has been a concerted effort to investigate combination therapies aimed at augmenting the response rates of PD-1/PD-L1 inhibitors.[6–9]

As a key point of PD-1/PD-L1 inhibition therapy, the level of PD-L1 expression has been found to influence therapeutic outcomes.[42–46] For instance, the evidence indicates that tumors exhibiting higher levels of PD-L1 expression tend to exhibit a more favorable response to PD-1/PD-L1 inhibition therapy. This underscores the significance of PD-L1 expression as a predictive biomarker for the effectiveness of PD-1/PD-L1 inhibitors.[42–46] Consequently, in certain scenarios, the identification of tumors with low PD-L1 expression and subsequent efforts to elevate its expression may potentially broaden the population of patients who can benefit from PD-1/PD-L1 inhibitor treatment.[33,47] Furthermore, bolstering the immune system's positive regulation can expedite the therapeutic impact of PD-1/PD-L1 inhibitors.[48,49] Therefore, strategies aimed at enhancing PD-L1 expression and modulating the tumor microenvironment are being explored as effective means to enhance the efficacy of PD-1/PD-L1 inhibitors.[33]

Circular RNA (circRNA) is classified as a type of non-coding RNA, which holds pivotal biological roles, setting itself apart from the more commonly known linear RNA structures. They can be found in various organisms and tissues, often existing in substantial quantities that rival or even surpass those of their linear RNA counterparts.[50] Unlike linear RNAs, such as messenger RNAs (mRNAs) and long non-coding RNAs (lncRNAs), which follow a straightforward linear chain with distinct start and end points, circRNAs boast an exclusive circular, covalently closed structure.[51] Briefly, circRNAs form a closed loop structure, devoid of the typical 5' cap or 3' poly-A tail found in linear RNAs.[52] This circularity arises through a process called back-splicing, wherein a downstream splice site joins with an upstream splice site within the same RNA molecule.[52] Their closed-loop configuration renders them less susceptible to degradation by exonucleases, enzymes that typically target linear RNAs by accessing their exposed ends. Thus, circRNAs possess higher stability compared to their linear counterparts.[50–52] Recent evidence has gradually uncovered the multifaceted roles of circRNAs, such as regulating gene expression through interactions with microRNAs (miRNAs) and proteins, sequestrating RNA-binding proteins, and serving as templates for translation.[17,53] Moreover, the circular architecture of circRNAs underpins their unique stability and functionality. They are increasingly recognized as crucial contributors to the regulation of gene expression, with significant roles in various diseases, including cancer and neurological disorders.[17,54,55] More importantly, accumulating evidence has gradually unrevealed that certain circRNAs can not only regulate the expression of immune checkpoint molecules (including PD-L1) but also modulate the anti-cancer immunity, indicating their promising roles in PD-1/PD-L1 inhibition therapy as a novel sensitizer.

In addition to enhancing immunity, circRNAs may also potentially possess more advantages, compared to miRNAs (Figure 1). For instance, stability: circRNAs boast exceptional stability due to their closed-loop structure, making them highly resistant to degradation by RNases. This robust durability ensures that circRNAs can persist within the tumor microenvironment for prolonged periods, potentially magnifying their therapeutic influence; precision targeting: circRNAs can be precisely engineered to target specific genes or pathways associated with immune checkpoint regulation, including PD-L1.[56,57] This precision enables them to finely tune the immune response, in contrast to miRNAs, which might have broader effects; multifaceted gene regulation: circRNAs act as efficient sponges for miRNAs or RNA-binding proteins, enabling them to

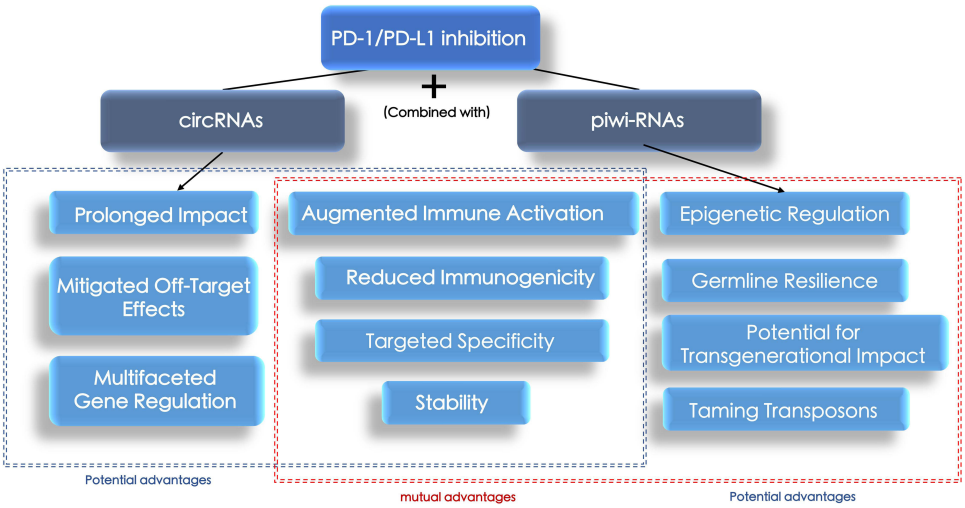

Figure 1 The potential advantages and mutual advantages of adding circRNAs or piwi-RNAs in ICIs-based therapy for treating cancers.

concurrently regulate multiple target genes.[58] This multifaceted regulatory capacity can exert a more substantial impact on immune checkpoint pathways compared to miRNAs, which usually target individual mRNAs;[50] mitigated off-target effects: circRNAs can be designed to have fewer off-target effects due to their specific mode of action involving binding to complementary sequences.[59] In contrast, miRNAs may inadvertently interact with non-target mRNAs, potentially causing off-target effects; reduced immunogenicity: circRNAs typically exhibit lower immunogenicity compared to other RNA molecules, making them less likely to provoke an immune response themselves.[60,61] This characteristic reduces the risk of adverse reactions, making them more suitable for safe therapeutic applications;[60] prolonged impact: circRNAs exert sustained effects on immune checkpoint regulation due to their extended half-life in cells. This protracted action may result in a more enduring response to ICI therapy compared to microRNAs.[62]

Hence, specific circular RNAs (circRNAs) capable of controlling the expression of immune checkpoint molecules, thereby influencing anti-cancer immunity, hold the promise of delivering more precise and targeted therapeutic outcomes with reduced side effects compared to conventional RNA therapies,[63–65] This underscores their potential as novel enhancers for immune checkpoint inhibitors (ICIs)-based therapies. Consequently, we have compiled a summary of these potential candidates (Tables 1 and 2) to foster a comprehensive discussion on their significance, a fact that should not be overlooked. This further underscores the pressing necessity for additional research in this domain.

PiRNAs

As a type of non-coding RNAs, piRNAs have also been found to play a role in immune modulation and response to cancer.[87] It has been suggested that piRNAs can potentially influence immune cell differentiation, response to inflammation, and even affect tumor immunogenicity.[88,89] Moreover, compared to miRNAs and siRNAs, piRNAs may also potentially possess more advantages (Figure 1), for instance:

a. Reduced Immunogenicity: PiRNAs possess a potential advantage in terms of reduced immunogenicity, akin to circRNAs, when compared to other RNA molecules.[90] This property holds promise for mitigating the risk of provoking adverse immune reactions during therapeutic applications.
b. Targeted Specificity: PiRNAs possess a higher degree of specificity for their target sequences.[91] Pinpoint accuracy enables allows for more focused regulation of gene expression, potentially leading to a more effective modulation of immune checkpoint pathways compared to microRNAs.[92]
c. Epigenetic Regulation: PiRNAs actively participate in epigenetic regulation, a mechanism capable of inducing enduring alterations in gene expression patterns.[93] This epigenetic influence may confer a sustained and resilient response to ICI therapy, surpassing the capabilities of microRNAs.

Table 1 Studies of Selected circRNAs That Can Regulate the Expression of PD-L1

CircRNA	Targets	Tumor Type	Experimental Setting	Mechanisms	References
circIGF2BP3	miR-328-3p, miR-3173-5p, PKP3, PD-L1	Non-small cell lung cancer	In vitro, In vivo Human sample	MicroRNA sponge	[66]
circBART2.2	PD-L1, RIG-I, IRF3, NF-κB	Nasopharyngeal carcinoma	In vitro, In vivo	Protein complex stabilization	[67]
circ-CPA4	let-7 miRNA, PD-L1	Non-small cell lung cancer	In vitro, In vivo Human sample	MicroRNA sponge	[68]
circTMTC3	miR-142-5p, PD-L1	Melanoma	Database	MicroRNA sponge	[69]
circFAM117B	miR-142-5p, PD-L1	Melanoma	Database	MicroRNA sponge	[69]
circ-VIM	miR-124, PD-L1	Esophageal cancer	Database, Human sample, In vitro, In vivo	MicroRNA sponge	[70]
circMYO1C	PD-L1	Pancreatic ductal adenocarcinoma	Human sample, In vitro, In vivo	Promote m6A modification	[52]
Circ_0010235	miR-636, PD-L1	Non-small cell lung cancer	Human sample, In vitro, In vivo	MicroRNA sponge	[71]
circPRDM4	HIF-1α, PD-L1	Hepatocellular carcinoma	In vitro, In vivo	Interact with RBP	[72]
Hsa_circ_0136666	miR-497, PD-L1	Colorectal cancer	In vitro, In vivo	MicroRNA sponge	[73]
Circ-METTL15	miR-1299, PD-L1	Non-small cell lung cancer	Human sample, In vitro, In vivo	MicroRNA sponge	[74]
circ-KRT6C	miR-485-3p, PD-L1	Colorectal Cancer	Human sample, In vitro, In vivo	MicroRNA sponge	[75]
CircCHST15	miR-155-5p, miR-194-5p, PD-L1	Lung Cancer	Human sample, In vitro, In vivo	MicroRNA sponge	[76]
CircFOXK2	miR-485-5p, PD-L1	Non-small cell lung cancer	Human sample, Database, In vitro, In vivo	MicroRNA sponge	[77]
Circ_0058058	miR-557, PD-L1	Pancreatic Cancer	Human sample, Database, In vitro, In vivo	MicroRNA sponge	[78]
Circ-HSP90A	miR-424-5p, STAT3, PD-L1	Non-small cell lung cancer	In vitro, In vivo	MicroRNA sponge	[79]
hsa_circ_0068252	miR-1304-5p, PD-L1	Non-small cell lung cancer	Human sample, Database, In vitro	MicroRNA sponge	[80]
circ_001678	miR-326, ZEB1, PD-1, PD-L1	Non-small cell lung cancer	Database, Human sample, In vitro, In vivo	MicroRNA sponge	[81]
hsa_circ_0001598	miR-1184, PD-L1	Breast cancer	Human sample, In vitro, In vivo	MicroRNA sponge	[82]
CircKRT1	miR-495-3p, PD-L1	Oral squamous cell carcinoma	Human sample, In vitro, In vivo	MicroRNA sponge	[83]
Hsa_circ_0003528	miR-511-3p, PD-L1	Non-small cell lung cancer	Human sample, In vitro, In vivo	MicroRNA sponge	[84]
Hsa_circ_0046523	MiR-148a-3p, PD-L1	Pancreatic Cancer	Human sample, In vitro, In vivo	MicroRNA sponge	[85]
circ_0001005	miR-200a-3p, PD-L1	Bladder cancer	In vitro, In vivo	MicroRNA sponge	[86]

Table 2 Basic Information of Selected circRNAs

Gene Name	Species	Location	Genomic Length	Spliced Sequence Length	RNA-Binding Protein Sites Matching to circRNAs (Binding Sites)	RNA-Binding Protein Sites Matching Flanking Regions of circRNA
CircPRDM4	Human	chr12:108,136,973–108,140,201	3228 bp	355 bp	AGO2(1); EIF4A3(3); FMRP(7); FXR2(1); HuR(1); IGF2BP1(1); IGF2BP2(2); IGF2BP3(3)	DGCR8; EIF4A3; HuR; IGF2BP1; IGF2BP3; PTB
Circ-KRT6C	Human	chr12:52,863,194–52,865,516	2322 bp	704 bp	EIF4A3(2)	EIF4A3
CircKRT1	Human	chr12:53,068,519–53,069,224	705 bp	705 bp	NA	AGO(1); Lin28b(1)
CircMYO1C	Human	chr17:1,382,724–1,382,999	275 bp	186 bp	FIF4A3(1)	AGO2; EIF4A3
CircFOXK2	Human	chr17:80,521,229–80,545,148	23,919 bp	1367 bp	AGO(14); EIF4A3(16); EWSR1(2); FMRP(17); FUS(1); HuR(15); IGF2BP1(9); IGF2BP2(11); IGF2BP3(9);	AGO2; EIF4A3; FUS; PTB
CircIGF2BP3	Human	chr7:23,353,140–23,401,360	48,220 bp	1242 bp	AGO(6); EIF4A3(19); HuR(3); IGF2BP1(1); IGF2BP3(13); LIN28A(1)	AGO; EIF4A3; HuR; HNRNPC; IGF2NP3; TDP43
Circ-CPA4	Human	chr7:129,948,146–129,964,020	15,874 bp	2068 bp	AGO(18); EIF4A3(14); DGCR8(1); HNRNPC (1); PTB(9); TDP43(1); TIAL1(1); U2AF65(1)	EIF4A3
CircCHST15	Human	chr19:23,542,382–23,543,054	672 bp	672 bp	NA	NA
Circ-METTL15	Human	chr11:28,250,417–28,255,132	4715 bp	4715 bp	AGO2(1); EIF4A3(7); FUS(1); PTB(3)	AGO2; EIF4A3; PTB; TDP43

Abbreviation: NA, not applicable.

d. Germline Resilience: PiRNAs are celebrated for their stability across generations, serving as custodians of germline integrity.[93] This exceptional stability could translate into prolonged effects within cells, potentially leading to an enduring and sustained immune response against cancerous cells, by choosing proper piRNAs.

e. Potential for Transgenerational Impact: In select organisms, piRNAs have shown the intriguing ability to traverse generational boundaries. While primarily studied in the realm of germline biology, this phenomenon hints at the prospect of harnessing long-lasting effects for therapeutic purposes.[66,93]
f. Taming Transposons: PiRNAs play a crucial role in silencing transposable elements (TEs) – sequences with the capability to relocate within a genome. This unique ability to govern TEs may indirectly influence the immune response by modulating the expression of immune-related genes.[94]

Thus, piRNAs seem to be able to provide more precise targeted therapeutic effects and fewer side effects. Recent evidence has further found that certain piRNAs can regulate the expression of immune checkpoint molecules and modulate the anti-cancer immunity,[63–65] indicating the strong potential of piRNAs for being a sensitizer for ICIs based therapy. Therefore, here we have summarized those candidates for a comprehensive discussion to reveal their value (Table 3).

Material and Methods

CIRCpedia V2 platform (http://yang-laboratory.com/circpedia/) was used to confirm the circID and species of selected genes. In addition, text mining diseases associations for selected genes were analyzed by using MalaCard database (www.malacards.org/); Moreover, the basic information of Genomic Length, Spliced Seq Length, and RNA-binding protein was gained from Circinteractome database (https://circinteractome.irp.nia.nih.gov). Furthermore, the predicted circle structures of exonic/intronic circRNAs were analyzed using the Cancer-specific circRNA Database (http://gb.whu.edu.cn/CSCD/). Finally, the theoretical three-dimensional structures of selected proteins corresponding to selected circRNAs were generated using the Protein Data

Table 3 Basic Information of Selected Piwi-RNAs

	Organism	Alias for Selected piRNAs	piRNA Code of NCBI	Sequence	Chromosomal Location		Length
					Start	End	
piRNA-932	Homo sapiens (human)	hsa-piRNA-31124; hsa-piR-000669; piR-30947; piR-hsa-1086	DQ570844.1	AGAGTGCATAAAAGAGT GTTGTCCAAGT	36601733	36,601,760	28
piR-Hep1	Homo sapiens (human)	NA	NA	GUUCCCUGGUGGUCUAG UGGUUAGAAAA	169096981	169,097,006	28
piR-23387	Homo sapiens (human)	hsa-piRNA-28660; hsa-piRNA-016913; piR-33411; piR-has-23,562	DQ593299.1	CGCCTACAAAGAGACTGT CTACCTTGCTAGA	28790886	28,790,916	31
piR-823	Homo sapiens (human)	hsa-piRNA-31224; hsa-piRNA-000592; piR-30847; piR-hsa-1000	DQ570735.1	AGAAGAAAGGTGGCGAG AAGATGGCTCT	141780444	14,780,471	28
piR-651	Homo sapiens (human)	hsa-piRNA-31396; hsa-piRNA-000458; piR-30675; piR-hsa-843	DQ570563.1	ACCTTCCCAGAGGATG GACCTCACGTGACT	5501155	5,501,184	30
piR-54265	Homo sapiens (human)	NA	DQ587153	TGGAGGTGATGAACTGT CTGAGCCTGACC	NA		29
piR-52207	Homo sapiens (human)	NA	DQ585095	TGCCTGTAATCCCAGCT ACTCAGGAGGCTG	NA		30

Abbreviations: NA, not available; NCBI, National center for biotechnology information.

Bank in Europe Knowledge Base (https://www.ebi.ac.uk/pdbe/pdbe-kb/). The information of selected piwi-RNAs was achieved from the piRNAdb database (https://www.pirnadb.org) and National center for biotechnology information (https://www.ncbi.nlm.nih.gov). The alias of selected piRNAs was also retrieved from ENA (European Nucleotide Archive), piRNABank, piRBase, piRNAQuest, and RNAdb (Table 1). PiRNA expression analysis was conducted using piRNAQuest V.2 platform. All databases and platforms applied in this manuscript are publicly free. Figures have been created using biorender (https://app.biorender.com).

Results

Twenty-two circRNAs and 7 piRNAs have been finally selected (Tables 1–3). Further basic information including location, genomic length, RNA-binding protein sites matching to circRNAs (binding sites), and RNA-binding protein sites matching flanking regions of selected circRNAs has also been gathered and displayed in those tables. The predicted circular structures of the selected circRNAs, and the predicted structures of proteins corresponding to these circRNAs, are shown in Figure 2, respectively. Moreover, the diseases associations for selected circRNAs have been displayed in Supplementary Table 1.

We further investigated the expression levels of two important piRNAs, namely piRNA-932 and piRNA-23387, across different cancer types. The results revealed intriguing insights into their expression patterns. For piRNA-932, we observed distinct expression levels in three different cancer types. In ovarian cancer, the expression level was measured at 0.1 piRNA counts per million (CPM). Meanwhile, bladder cancer exhibited a slightly higher expression level at 0.29 CPM, and cervical cancer demonstrated an even higher expression level at 0.32 CPM (Figure 3A). Additionally, we assessed the expression level of piRNA-23387 specifically in hepatocellular carcinoma. The results indicated a lower expression level of 0.05 CPM (Figure 3B).

Discussion

Selected circRNAs Regulating PD-L1 Expression

circIGF2BP3

N6-methyladenosine (m6A) modifications manipulate the stability of mRNA.[95] Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3) was reported to stabilize the PD-L1 mRNA by promoting the m6A modification of it.[67] Back-splicing between exons 4 and 13 of IGF2BP3 forms circIGF2BP3, the expression of which was increased in non-small-cell lung carcinoma (NSCLC) and negatively correlated with immune escape. Then, by sponging miR-328-3p and miR-3173-5p, circIGF2BP3 competitively upregulates the expression of plakophilin 3 (PKP3).[96] Furthermore, PKP3 forms protein-RNA complexes with Fragile X mental retardation syndrome-related protein 1(FXR1) to stabilize the mRNA of Otubain-1 (OTUB1). PKP3-mediated OTUB1 upregulation further elevates the expression of PD-L1 by facilitating its deubiquitination.[69] Therefore, the regulatory effect possessed by circIGF2BP3 on PKP3/OTUB1/PD-L1 axis may provide a novel mechanism for conquering immune escape in treating NSCLC.

circBART2.2

Epstein−Barr virus (EBV) expressed both exon-only and exon−intron circRNAs from the BamHI A rightward transcript (BART) locus (circBARTs).[97] As a member of circBARTs, circBART2.2 was found to be highly expressed in nasopharyngeal carcinoma (NPC) cells and promote the transcription of PD-L1 by binding the helicase domain of retinoic acid-inducible gene I (RIG-I), activating Interferon regulatory Factor 3(IRF3) and nuclear factor kappa-B (NF-κB), therefore inhibiting T-cell function in vitro and in vivo.[98] Thus, circBART2.2 can also act as a potential therapeutic target for cancer immunotherapy.

circCPA4 (Hsa_circ_0082374)

Hsa_circ_0082374 derives from carboxypeptidaseA4 (CPA4); thus, it is also named as "circCPA4".[99] Evidence has reported that circ-CPA4 is up-regulated in glioma tissues, and its expression is related to poor outcomes.[99] Moreover, in NSCLC cells, circ-CPA4 has been proven to be served as an RNA sponge for let-7 miRNA to upregulate PD-L1. In addition, PD-L1-containing exosomes derived from NSCLC cells have been found to inactivate CD8+ T cells. However, NSCLC cells with circ-CPA4 ablation were able to re-activate CD8+ T cells, indicating that circ-CPA4 is a robust immune regulator.[99] This mechanism may identify a new intervention target for NSCLC patients receiving immunotherapy.

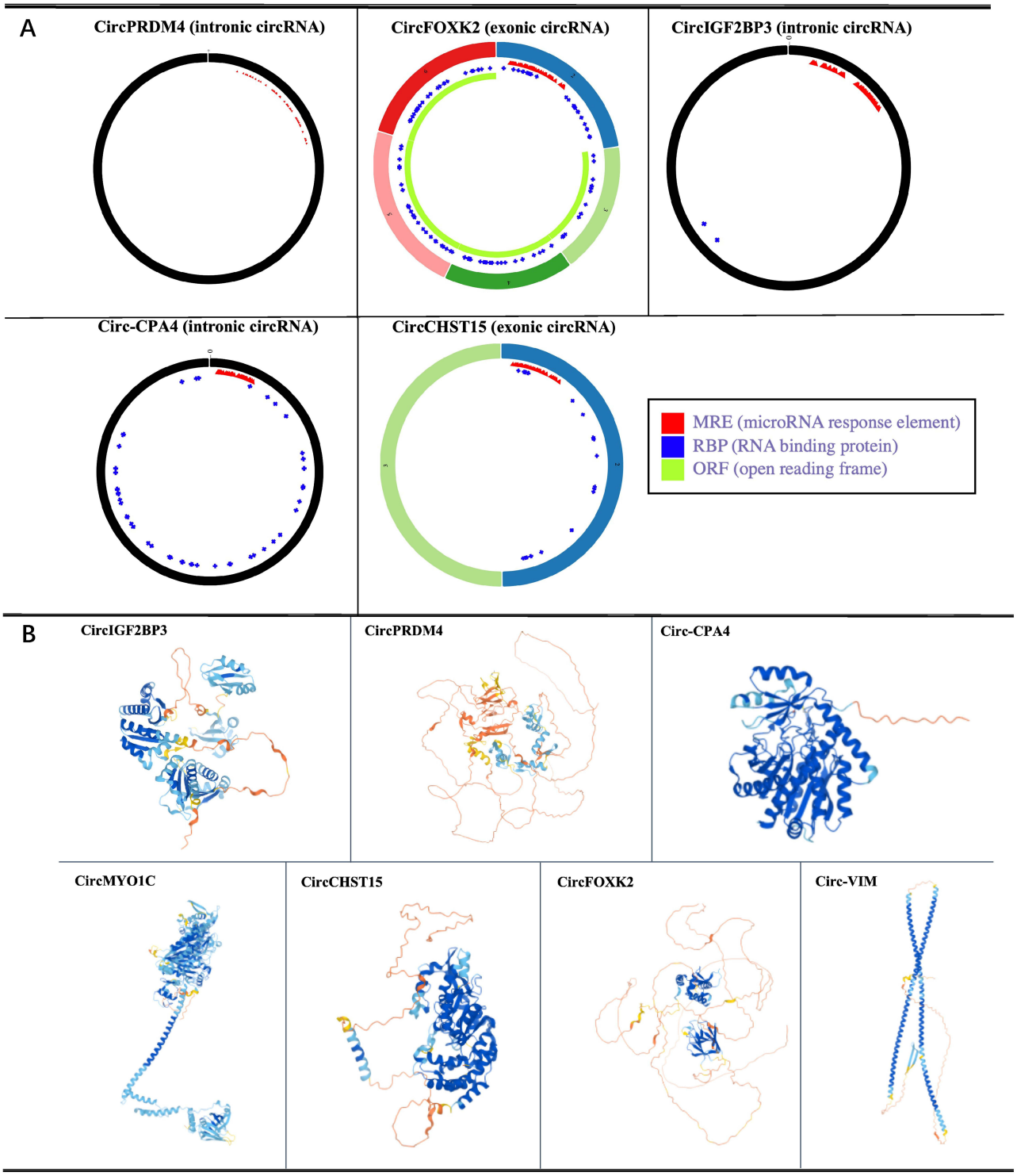

Figure 2 Predicted circle structure of selected circRNAs (**A**) and predicted structures of proteins corresponding to selected circRNAs (**B**).

circTMTC3 and circFAM117B

Recent evidence has revealed the clinical significance and regulatory networks of circRNAs in melanoma patients receiving immune checkpoint blockades (ICB).[100] For details, overall expression of circRNAs was detected by RNA-Seq (both in pre-treatment (PRE) collected biopsies, and during treatment (EDT) collected biopsies following ICB). An ICB-related circRNA signature score model based on progression-free survival-related circRNAs was applied to predict

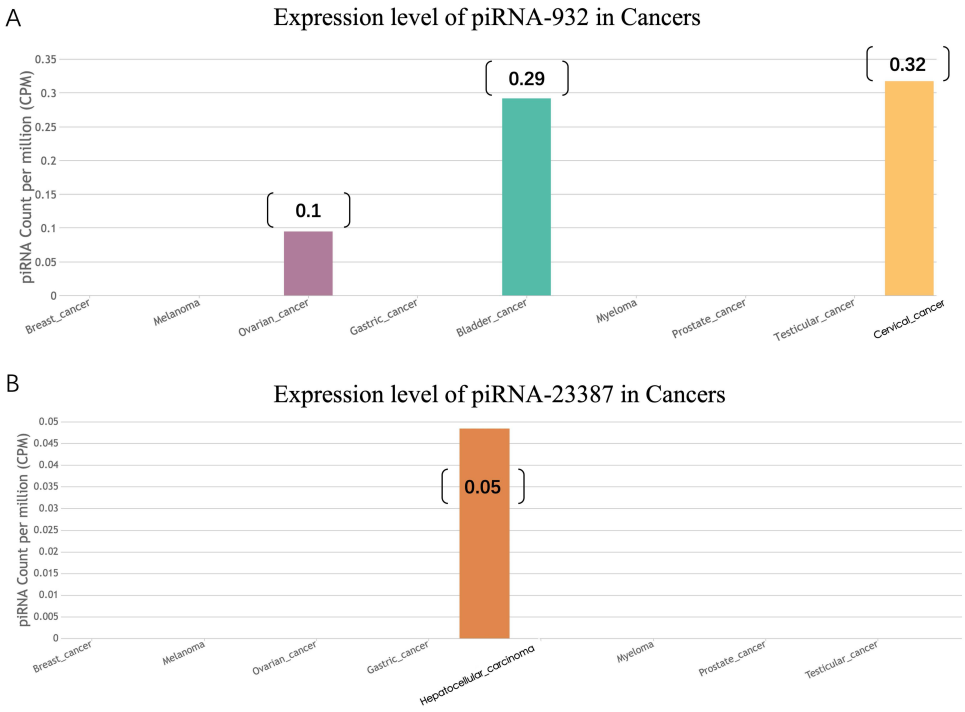

Figure 3 Expression levels of piRNA-932 and piRNA-23387 in Cancers. The expression levels of piRNA-932 in Ovarian cancer, bladder cancer and cervical cancer are 0.1, 0.29 and 0.32 piRNA counts per million (CPM)(**A**); Moreover, the expression levels of piRNA-23387 in hepatocellular carcinoma is 0.05 CPM (**B**).

immunotherapy efficacy. This research reported that upregulated circTMTC3 (transmembrane and tetratricopeptide repeat containing 3) and circFAM117B (family with sequence similarity 117, member B) are in the non-responder group at both the PRE and EDT time points, suggesting a potential association between circTMTC3 (or circFAM117B) and resistance to immunotherapy, respectively. Mechanistically, the overexpression of circTMTC3 and circFAM117B is reported to increase PD-L1 expression via the miR-142-5p/PD-L1 axis, thereby reducing T cell activity and inducing immune escape.[100] Collectively, the potential clinical utilities of circTMTC3 and circFAM117B should be highlighted.

circMYO1C

Myosin 1C (MYO1C) is an actin-binding motor protein that functions to maintain cell structure.[101] A m6A-modified circRNA from the MYO1C gene, circMYO1C, was found to be upregulated in the pancreatic ductal adenocarcinoma (PDAC) tissues.[56] circMYO1C targeted the m6A site of PD-L1 mRNA to enhance its stability, resulting in PDAC immune escape.[56] The oncogenic role of circMYO1C in PDAC via an m6A-dependent manner to elevate PD-L1 may provide a novel strategy for cancer immunotherapy.

circ_0010235

Circ_0010235 is transcribed from back-spliced aldehyde dehydrogenase 4 family member A1 (ALDH4A1).[71] It also has been reported to accelerate NSCLC progression by conferring cisplatin resistance, promoting proliferation and autophagy.[72,102,103] As shown by evidence,[104] circ_0010235 is overexpressed in NSCLC, and its downregulation has been found to enhance antitumor immune response via increasing IFN-γ and TNF-α, enhancing the cytotoxic activity of the cytokine-induced killer (CIK) cells against NSCLC. Additionally, circ_0010235 knockdown can further hamper the development of NSCLC.[104] More importantly, circ_0010235 has been found to increase the expression of PD-L1 via the miR-636/PD-L1 axis.[104] Thus, such candidate can potentially provide multiple anti-cancer effects in cancer treatment.

circPRDM4

PR domain zinc finger protein 4 (PRDM4) is a transcription factor that plays key roles in stem cell self-renewal and tumorigenesis.[105] Evidence has identified hypoxia-associated circRNAs in HCC (hepatocellular carcinoma) cells, and

found circPRDM4 was upregulated in responders to PD-1 blockade under hypoxia. For details, circPRDM4 recruited HIF-1α onto the promoter of CD274 and enhance the progression of transactivation, therefore elevating the PD-L1 expression, leading to the dysfunction of CD8+ T cells and tumor immune evasion.[106] Hence, targeting circPRDM4 is also a potential therapeutic strategy.

hsa_circ_0136666

Hsa_circ_0136666 was reported to promote the tumorigenesis in multiple solid tumors.[74–76] In colorectal cancer, hsa_circ_0136666 and PD-L1 were both remarkably upregulated in tumor tissues and cell lines. Briefly, Hsa_circ_0136666 has been found to directly target miR-497, and miR-497 decreased the expression of PD-L1 by binding to its mRNA 3'UTR.[76] In addition, hsa_circ_0136666 has also been reported to stimulate Treg cells mediated by miR-497/PD-L1 axis,[76] indicating its potential in cancer immunotherapy.

Circ-METTL15

CircRNA methyltransferase-like 15 (circ-METTL15), also known as hsa_circRNA_000791 and hsa_circ_0000282, has been verified to promote the development and malignancy of osteosarcoma.[107] In lung cancer samples, circ-METTL15 was reported as a screened upregulated circRNA derived from the GEO database.[108] In addition, circ-METTL15 directly modulated miR-1299, and PD-L1 was demonstrated to be a target of miR-1299. Therefore, circ-METTL15 could sponge to miR-1299 to increase PD-L1 expression, and decreased the cytotoxicity of CIK cells against lung cancer. Besides, circ-METTL15 downregulation blocked lung cancer tumour growth in vivo by regulating the miR-1299/PD-L1 axis.[108]

Circ-KRT6C

Circ-KRT6C, an oncogene, is derived from keratin 6C (KRT6C) gene, also named as hsa_circ_0026416.[77] Evidence has reported that the expression of circ-KRT6C is significantly elevated in colorectal cancer (CRC) cells and is also associated with the overall survival time of CRC patients.[77] For details, circ-KRT6C was found to increase PD-L1 expression by sponging to miR-485-3p and promoted tumor immune evasion. Therefore, circ-KRT6C may act as a novel therapeutic target for CRC immunotherapy.

circCHST15

CircCHST15 has been reported to act as an oncogene in lung cancer;[78] for instance, it can promote the expression of PD-L1 and regulate the distribution of T cell subtypes.[78] Previous evidence demonstrated that miR-155-5p inhibit the progression of lung cancer.[80] However, the expression of miR-155-5p and miR-194-5p was found to be decreased by circCHST15. In addition, PD-L1 was a functional target of miR-155-5p and miR-194-5p. Therefore, circCHST15 sponged miR-155-5p and miR-194-5p to increase the PD-L1 expression. Furthermore, circCHST15 was found to decrease the CD8+ T cells while increase the Tregs in mouse tumor. In clinical studies, patients receiving PD-L1 inhibitor with high level of circCHST15 showed more adverse events.[78] The above results indicate that inhibition of circCHST15 could be an alternative way to sensitize the efficacy of PD-L1 inhibitor.

circFOXK2

Fork head box K2 (FOXK2) plays an important role in the transcriptional regulation of cancer.[82] circFOXK2 derived from exon 2 to 8 of FOXK2 gene, and it was highly expressed in NSCLC.[83] Such RNA has been found to exert oncogenic effects on NSCLC by sponging miR-485-5p, and PD-L1 was a target of miR-485-5p.[83] In other words, circFOXK2 can sponge miR-485-5p to increase the PD-L1 expression.[83] Furthermore, circFOXK2 has also been reported to inhibit the cytotoxicity of CD8+T cells.[83] Oppositely, knockdown of circFOXK2 has been found to decrease the apoptosis of CD8+ T cells, which can be overturned by the upregulation of PD-L1.[83] Hence, the regulatory pathway modulated by circFOXK2 in NSCLC may help the development of clinical immunotherapy target for NSCLC treatment.

circ_0058058

Circ_0058058, also termed as Hsa_circRNA_102913, was reported to be involved in facilitating the development of pancreatic cancer (PC).[84] It was found to be increased in PC tissues and PC cell lines.[84] Evidence shows that knockdown

of circ_0058058 can increase the levels of TNF-α and IFN-γ in peripheral blood mononuclear cell (PBMC) and can also suppress the survival of PC cells when cocultured with CIK cells.[84] Moreover, circ_0058058 can act as a molecular sponge of miR-557, and PD-L1 was a target of miR-557, therefore circ_0058058 elevates PD-L1 expression by sponging to miR-557.[84] Thus, circ_0058058 itself may provide a novel target for cancer treatment.

hsa_circ_0068252

Compared to cisplatin-sensitive NSCLC patient samples, the expression level of hsa_circ_0068252 was found to be upregulated in cisplatin resistant NSCLC tissues and cell lines. Besides, high levels of circ_0068252 expression showed a shorter overall survival.[85] Moreover, a co-culture system of NSCLC cells with CD8+ T cells determined that circ_0068252 inhibited the cytotoxicity of T cells.[85] In addition, circ_0068252 has also been considered to up-regulate the expression of PD-L1 by modulating the expression of miR-1304-5p in NSCLC cells.[85] These results suggest that circ_0068252 is a potential diagnostic marker of immunotherapy for NSCLC.

hsa_circ_0001598

A study explored the oncogenic role of circRNAs in HER2-positive breast cancer (BC), and found that the expression level of circ_0001598 is elevated in BC samples, especially in trastuzumab-resistant BC tissues.[109] Circ_0001598 has been found to sponge to miR-1184 that can decrease the expression of PD-L1.[109] Besides, the oncogenic effects of circ_0001598 in promoting escaping of CD8+ T cell killing can be diminished after the restoration of miR-1184.[36] These results suggest that hsa_circ_0001598 may be a molecular target to treat HER2-positive BC patients by interfering miR-1184/PD-L1 axis.

CircKRT1

CircRNA keratin 1 (CircKRT1) has been found to be dysregulated in oral squamous cell carcinoma (OSCC).[86] In addition, the expression of CircKRT1 and PD-L1 was both elevated in OSCC tissues and cells.[86] CircKRT1 knockdown has been reported to inhibit OSCC cell growth, migration, invasion, and epithelial–mesenchymal transition (EMT). Besides, CD8+ T-cell apoptosis can also be inhibited by CircKRT1 knockdown, while the CD8+ T cell cytotoxicity was enhanced.[86] These effects of circKRT1 on OSCC immune evasion were related to the function of PD-L1. Mechanistically, CircKRT1 can sponge miR-495-3p that targets the 3'UTR of PD-L1 mRNA.[86] Therefore, CircKRT1 facilitated OSCC progression and immune evasion by affecting the miR-495-3p/PDL1 axis.

Hsa_circ_0003528

Abnormal expression of Hsa_circ_0003528 in NSCLC samples was observed.[110] Briefly, as compared to matching normal lung tissues, the expression of circ_0003528 was significantly upregulated in NSCLC tissues.[110] Besides, NSCLC cell lines had a higher expression of circ_0003528 than the normal lung epithelial cell lines.[110] Patients with high expression level of circ_0003528 showed a worse 5-year overall survival.[110] Moreover, silence of circ_0003528 has been found to inhibit NSCLC in vivo and repress immune escape, cell viability, EMT, angiogenesis in vitro.[38] Evidence has displayed that circ_0003528 can regulate PD-L1 expression by sponging to miR-511-3p. Thus, the effects of circ_0003528 knockdown on NSCLC cell immune escape can be recovered after miR-511-3p inhibition.[110] Furthermore, miR-511-3p upregulation-mediated suppression on NSCLC cell immune escape can be partially counteracted by PD-L1 overexpression.[110] Hence, the proper way of using circ_0003528 can potentially help to augment the anti-cancer immunity.

Hsa_circ_0046523

Hsa_circ_0046523 is an oncogenic factor in PC.[79] The expression of Hsa_circ_0046523 was found to be upregulated in PC tissues and PC cell lines.[79] High expression of hsa_circ_0046523 has also been found to be correlated with advanced pathological stage and worse prognosis.[79] Overexpression of Hsa_circ_0046523 can promote the proliferation of PC cells while decrease the proportion of CD4+ and CD8+ T cells, alongside increasing the proportion of Tregs among PBMCs, and it promoted the exhaustion of CD8+ T cells.[79] Additionally, overexpression of Hsa_circ_0046523 has been reported to decrease the secretion of immune effector cytokines, including IFN-γ and IL-2, and increased the secretion of some

immunosuppressive cytokines, such as IL-10.[79] Moreover, hsa_circ_0046523 was found to bind to miR-148a-3p that can be sponged to the 3'UTR of PD-L1 mRNA, thus leading to the upregulation of PD-L1 expression in PC.[79]

circ_0001005

The initiation and progression of bladder cancer (BCa) may be tightly related to the androgen receptor (AR).[111] After analyzing BCa survival based on TCGA database, it was found that PD-L1 expression negatively correlated to survival in male BCa patients but not in female patients. Thus, AR has been speculated to regulate PD-L1 expression.[81] Research also found that the RNA-editing gene ADAR2 could be inhibited by AR, and ADAR2 decreased the expression of circ_0001005.[81] Moreover, knockdown of AR has been reported to significantly inhibit the expression of circ_0001005, while knockdown of ADAR2 could increase the PD-L1 expression level.[81] Mechanistically, AR has been proven to inhibit the expression of ADAR2 to further upregulate circ_0001005 which can competitively sponge the miR-200a-3p to promote PD-L1 expression.[81] Thus, the cascade can finally lead to a weaker killing ability of NK cells for conquering BCa cells.[81] Collectively, targeting the ADAR2/circ_0001005/miR-200a-3p/PD-L1 pathway may boost immunotherapeutic efficacy in BCa male patients.

Circ-VIM

Circ-VIM is a predictor for poor prognosis of acute myeloid leukemia.[112] Besides, it acts as a multiple oncogenic factor for esophageal cancer (EC).[112] The expression of circVIM has also been found to be significantly higher in EC tissues and cell lines than in normal esophageal epithelial tissues and cell lines, respectively.[112] Moreover, higher circ-VIM expression is a significant indicator for advanced TNM stage and lymph node metastasis.[112] In contrast, miR-124 expression level has been reported to be significantly decreased in EC tissues or cell lines, and lower miR-124 expression has been proven to be correlated with worse survival.[112] However, Pearson analysis showed that the expression of circ-VIM was negatively correlated with miR-124.[112] Furthermore, phenotype analyses showed that circ-VIM promoted the cell viability, clone formation, migratory and invasive capacities of EC cells.[112] Besides that, circ-VIM has been displayed to significantly inhibit the killing capacity of CD8+ T cells in the EC cell and CD8+ T cell co-culturing system.[112] In addition, circ-VIM can sponge miR-124 that targets the downstream PD-L1 mRNA.[112] Inhibition of circ-VIM has also been found to induce the inactivation of Ras/ERK signaling pathway.[112] Thus, silencing circ-VIM presented strategy to reverse immune escape and multiple malignant phenotypes of EC progression.

Circ-HSP90A

The current study selected 16 circRNAs that derived from host gene heat shock protein 90 alpha (HSP90A) and measured their expression levels in NSCLC cells and normal human bronchial epithelial cells,[113] and found that only hsa_circ_0033393 (circ-HSP90A) was upregulated in all selected NSCLC cells. In addition, silencing of circ-HSP90A has been found to inhibit cell viability, migration, invasion, and stemness in NSCLC cells.[113] Circ-HSP90A has also been found to recruit the USP30 (an enzyme that de-ubiquitinates proteins) to stabilize HSP90A protein.[113] Overexpression of HSP90A can recover the migration, invasion, and stemness in NSCLC cells that are repressed by circ-HSP90A inhibition.

Moreover, HSP90A overexpression can also promote the phosphorylation level of STAT3 which is an important regulator of immune escape.[114] It further confirmed that the cytotoxicity function of CD8+ T cells can be enhanced by circ-HSP90A inhibition.[113] Besides, circ-HSP90A can also affect the alteration of CD8+ T cells via sponging miR-424-5p to up-regulate PD-L1.[113]

circ_001678

CircRNA high-throughput sequencing identified circ_001678 is up-regulated in NSCLC tissues.[115] Besides, immunohistochemical staining highlighted an inverse relationship between high circ_001678 expression and the reduction of CD8+ T cells in NSCLC tissues.[115] It was further confirmed that overexpression of circ_001678 promoted the viability, migration, and invasiveness in NSCLC cells, decreased the percentage of CD8+ T cells, while inhibition of circ_001678 led to opposing effects.[115] Mechanistically, circ_001678 served as a sponge of miR-326, and miR-326 inhibited the E-box binding homeobox 1 (ZEB1), a regulator that can augment the expression of PD-L1.[116] Besides, miR-326 mimics

diminished the increase of PD-L1 that induced by overexpression of circ_001678.[115] Collectively, these findings indicated that circ_001678 regulates PD-1/PD-L1 by miR-326/ZEB1.

To sum up, the above evidence demonstrated that certain circRNAs increase the expression of PD-L1 with multiple mechanisms in various cancers. The main mechanism of these circRNAs is to increase the expression of PD-L1 mRNA by interacting with miRNAs, such as circ_001678, which interacted with miR-326 to up-regulate ZEB1 and subsequently led to the activation of the PD-1/PD-L1 pathway and inhibited CD8+ T cells, and these effects could be diminished by miR-326 mimics (Figure 4).[115] Besides, circ-CPA4 sponges let-7 miRNA to upregulate PD-L1, and circ-CPA4 ablation could re-activate CD8+ T cells that are inhibited by PD-L1-containing exosomes in NSCLC.[99] Moreover, circ_0001005 is elevated in BCa male patients, the androgen receptor inhibited the RNA-editing gene ADAR2, and ADAR2 decreased the expression of circ_0001005, circ_0001005 further sponged the miR-200a-3p to promote PD-L1 expression, leading to a weaker killing ability of NK cells, so circ_0001005 is a promising candidate for gender-dependent biomarker of BCA immunotherapy[81] (Figures 4 and 5).

CircRNAs also increase the expression of PD-L1 mRNA by forming protein complex stabilization, such as circBART2.2 could bind to RIG-I, activated IRF3 and NF-κB, thus promoting the transcription of PD-L1 in NPC (Figure 4).[98] Besides, circPRDM4 recruited HIF-1α onto the promoter of CD274 and enhance the transactivation, subsequently elevated the PD-L1 expression in HCC.[106] In addition, circIGF2BP3 can competitively upregulate the expression of PKP3, which can form protein-RNA complexes with FXR1 to stabilize the OTUB1 mRNA, eventually inhibiting the PD-L1 ubiquitination.[96] Moreover, circMYO1C targeted the m6A site of PD-L1 mRNA to enhance its stability.[56] These studies indicated that circRNAs play multiple roles in up-regulating PD-L1 expression.

Additionally, it is worth noting that certain circRNAs may exert multiple functions in cancer development and treatment beyond simply regulating the PD-L1 checkpoint. For instance, circ-HSP90A not only compromised the

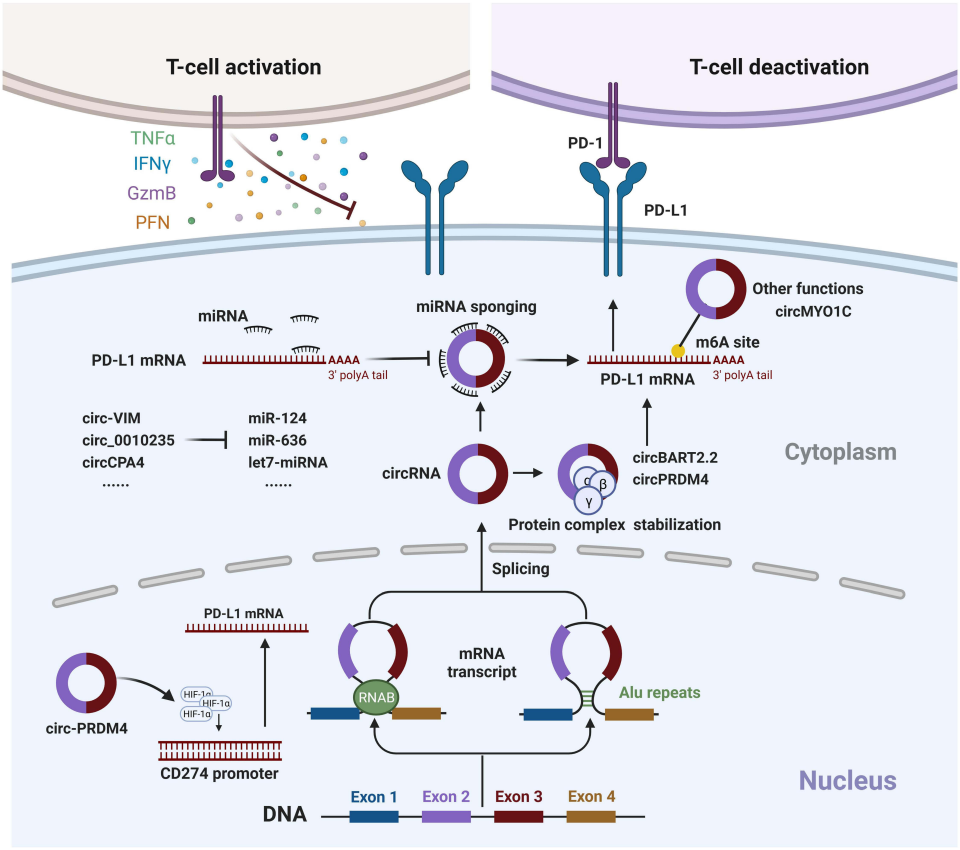

Figure 4 Selected circRNAs which can regulate immune checkpoints PD-L1 by (1) miRNA sponging, such as circ-VIM/miR-124, circ_0010235/miR-636, circ-CPA4/let7-miRNA, etc. (2) Protein complex stabilization, such as circBART2.2, circPRDM4, etc. (3) Other functions such as circMYO1C, etc.

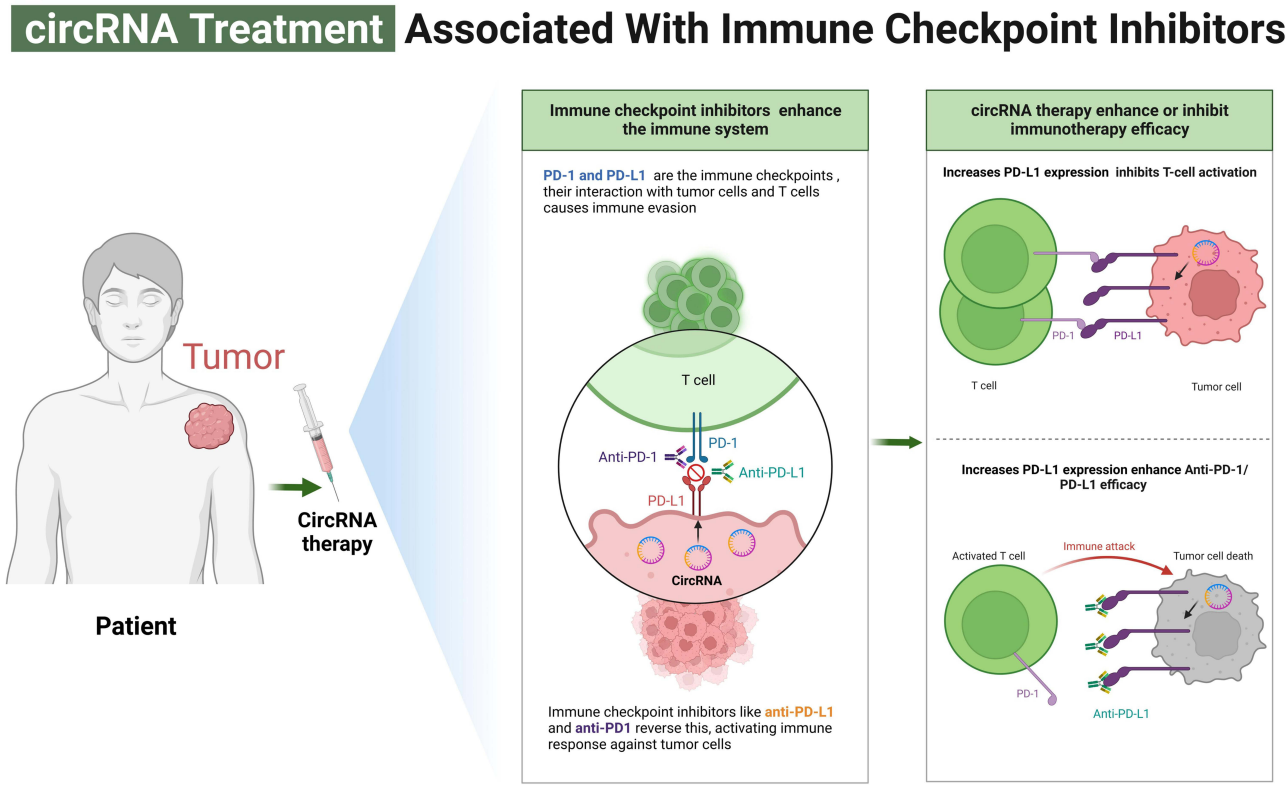

Figure 5 The potential value of circRNAs treatment in regulating the efficacy of PD1/PD-L1 inhibitor is a double sword: increase PD-L1 expression inhibits T cell activation or increase PD-L1 expression enhance anti PD-1/PD-L1 efficacy.

cytotoxicity of CD8+ T cells but also promoted cell viability, migration, invasion, and stemness in NSCLC cells.[113] Similarly, circ-VIM not only inhibited the killing capacity of CD8+ T cells but also facilitated cell viability, clone formation, and migratory and invasive abilities of endometrial cancer (EC) cells through the activation of the Ras/ERK signaling pathway.[112] Besides, some other immune checkpoints,[117] such as PD-L2, CTLA4, B7H6, TIGIT may also be regulated by circRNA, which needs to be explored by further research.[118–120]

Selected piRNAs Candidates

PD-L1 Regulation

piR-823

PiR-823 has demonstrated potential in the realm of regulating anti-cancer immunology. For instance, such RNA has been found to regulate the development of colorectal cancer cells by affecting the progress of both cell apoptosis and mitophagy.[121] Moreover, abnormally low expression of piR-823 have been found in different types of cancers, including gastric cancer[122] and renal cell carcinoma.[121] More importantly, in gastric cancer, restoration of piR-823 has been found to suppress the activity of cancer cells.[122] Furthermore, research has illustrated that piR-823 can facilitate the expedited degradation of Phosphatase and Tensin Homolog Deleted on Chromosome Ten (PTEN)-induced kinase 1 (PINK1).[123] This is particularly significant since PINK1-mediated mitochondrial quality control subsequently triggers the increased expression of Programmed Death-Ligand 1 (PD-L1).[124] In addition, piR-823 has been identified as a promoter of the phosphorylation and transcriptional activity of Heat Shock Factor 1 (HSF1).[125] The expression of HSF1, in turn, has also been found to be negatively associated with the expression of PD-L1[126] (Table 3). Hence, piR-823 not only showcases promise as a potential tumor suppressor gene but also operates as a sensitizer for immune checkpoint inhibitors (ICIs). However, how to effectively and properly apply such genes in cancer treatment, requires a lot of exploration (Figure 6).

piR-54265

PiR-54265 has been identified as an oncogenic piRNA with substantial influence on the onset and progression of colorectal cancer (CRC).[127] To elaborate, piR-54265 is found to be overexpressed in CRC and is implicated in promoting both the proliferation and invasiveness of CRC cells.[127] Furthermore, piR-54265 binds to P-element induced wimpy testis like 2 (PIWIL2), thus triggering the activation of the signal transducer and activator of transcription 3 (STAT3) signaling pathway. This, in turn, fosters the metastasis and chemoresistance of CRC cells.[127] Moreover, STAT3 activation leads to an increase in PD-L1 expression in non-small cell lung cancer (NSCLC) cells,[128] thereby facilitating immune evasion and resistance to chemotherapy.[129] Importantly, research has demonstrated that inhibiting STAT3 can effectively lower PD-L1 expression and induce apoptosis in HCT-15 cells (a CRC cell line)[130] (Table 3). Consequently, piR-54265 is acknowledged as an oncogene, and the suppression of such genes can potentially regulate the expression of PD-L1 by affecting STAT3 activity. Further research is essential in this field (Figure 6).

piR-Hep1

PiR-Hep1 has been considered to be an oncogene and has been observed to be abnormally up-regulated in cancers such as hepatocellular carcinoma (HCC).[131] Such gene has also been found to provoke the phosphatidylinositol 3-kinase (PI3K)/v-Akt Murine Thymoma Viral Oncogene (AKT) signaling pathway, thereby causing tumorigenesis, accelerating the growth, expansion, and proliferation of cancer cells.[131–137] In addition, PIWIL2 can increase the expression of piR-Hep1 therefore elevating the activity of the phosphatidylinositol 3-kinase (PI3K)/v-Akt Murine Thymoma Viral Oncogene (AKT) signaling pathway.[131–133] Activation of PI3K/AKT signaling pathway can further contribute to tumorigenesis.[134,135] Conversely, inhibiting the PI3K/AKT pathway may suppress the growth, expansion, and proliferation of cancer cells.[136,137] Moreover,

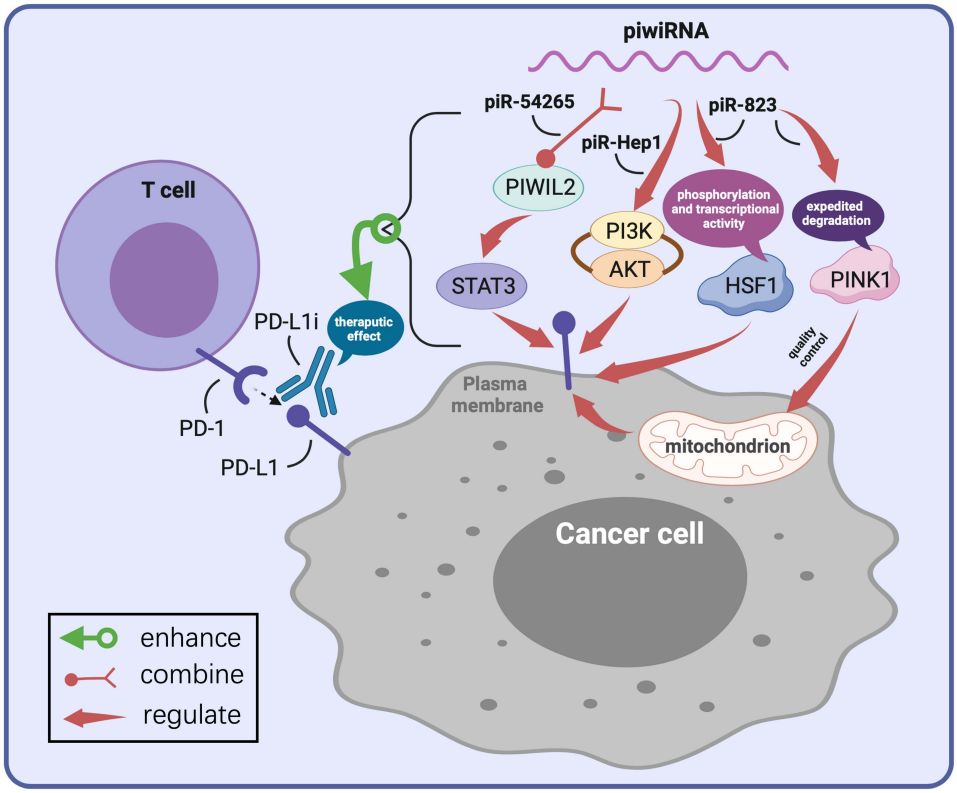

Figure 6 Selected piRNAs and their roles in PD-L1 regulation. piR-823 is involvement in cancer cell development through apoptosis and mitophagy regulation. The figure also highlights the role of piR-823 in degrading PINK1, impacting mitochondrial quality control and PD-L1 expression, and promoting HSF1 phosphorylation and transcriptional activity, inversely related to PD-L1 expression. PiR-54265 can regulate the cancer cell proliferation and invasiveness, and its interaction with PIWIL2 leading to STAT3 activation. This activation contributes to metastasis, chemoresistance, and increased PD-L1 expression, facilitating immune evasion. It can also inhibit STAT3 to reduce PD-L1 expression and induce apoptosis in cancer cells. In addition, piR-Hep1 can regulate the PI3K/AKT signaling pathway, therefore affecting the tumorigenesis, and cancer cell proliferation.

inhibiting PI3K/AKT has been found to increase the levels of interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α), while reducing the levels of IL-10, all of which collectively inhibit tumor growth[64] (Table 2). Thus, piR-Hep1 can act as an oncogene. Moreover, piR-Hep1 has the capability to enhance the activity of the PI3K/AKT signaling pathway, consequently decreasing the levels of IL-2 and TNF-α, this underscores the potential of piR-Hep1 in regulating tumor immunotherapy.[64] Additionally, it has been demonstrated that the elevation of PD-L1 expression can be influenced by the PI3K/AKT pathway.[63] The activation of the PI3K/AKT signal pathway can result in an upregulation of PD-L1 expression[63] (Table 2). Therefore, piR-Hep1 holds the potential to regulate the expression of PD-L1 through the activation of the PI3K/AKT pathway, making it a crucial therapeutic target in cancer therapy (Figure 6).

Immunoregulation

piR-932

Evidence has shown that the expression of piR-932 is significantly higher in breast cancer cells that undergo the process of epithelial–mesenchymal transition (EMT), compared to those that have not undergone such a process.[88] The interaction between piR-932 and PIWIL2 leads to latexin methylation, thereby promoting EMT.[88] EMT is the process by which epithelial cells lose their cell polarity and cell–cell adhesion and gain migratory and invasive properties to become mesenchymal stem cells.[138] This transition promotes the activation of cancer stem cells,[139] stimulates angiogenesis,[140] and facilitates distant metastases.[141] Furthermore, the EMT process can enhance the infiltration of various immune cell subsets, leading to immune evasion.[142] Therefore, piR-932 can be regarded as a potential oncogene, and the down-regulation of piR-932 can suppress EMT and the infiltration of immune cells, ultimately inhibiting the generation, growth, and distant metastasis of tumors. Moreover, aberrantly high expression of piR-932 has been found in ovarian, bladder, and cervical cancer by our expression analysis (Figure 7), making the unrevealed role of such piRNA in these cancers more intriguing.

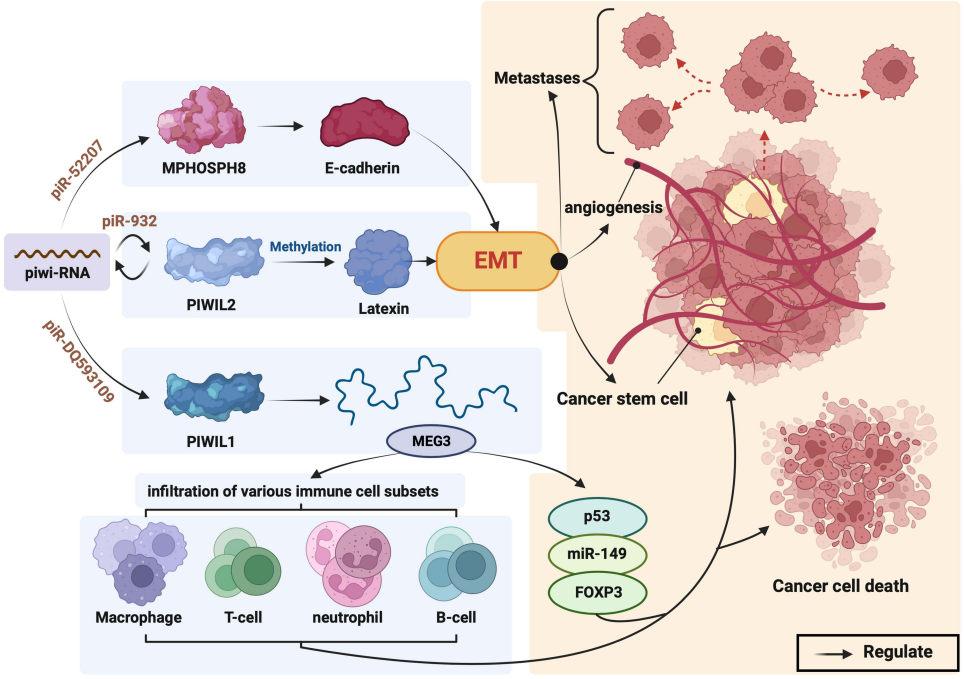

Figure 7 Roles of selected piRNAs in antitumor-immunoregulation. PiR-932 is involved in epithelial–mesenchymal transition (EMT) therefore regulating cancer stem cell activation, angiogenesis, and distant metastases. PiR-932's interaction with PIWIL2 promotes EMT, leading to immune evasion. PiR-Hep increases the activity of the PI3K/AKT signaling pathway, contributing to tumorigenesis. Inhibiting this pathway can suppress cancer cell growth and promote anti-cancer immunity by altering cytokine levels. **PiR-DQ593109 (piR-23387)** forms a complex with PIWIL1, leading to the degradation of MEG3, a long non-coding RNA important in immune dynamics. MEG3 hinders cancer cell proliferation and promotes immune cell infiltration, making piR-DQ593109 a potential immunomodulator. PiR-52207 targets MPHOSPH8, initiating EMT and contributing to immune evasion. As an immunomodulator, its suppression could prevent EMT and restore anti-cancer immunity.

piR-Hep1

PiR-Hep1 has been considered as an oncogene and has been observed to be up-regulated in hepatocellular carcinoma (HCC) tissues when compared to adjacent non-tumor liver cells.[131] PIWIL2 can increase the expression of piR-Hep1, thereby elevating the activity of the phosphatidylinositol 3-kinase (PI3K)/v-Akt Murine Thymoma Viral Oncogene (AKT) signaling pathway.[131–133] Activation of PI3K/AKT signaling pathway can further contribute to tumorigenesis.[134,135] Conversely, inhibiting the PI3K/AKT pathway may suppress the growth, expansion, and proliferation of cancer cells.[136,137] Moreover, inhibiting PI3K/AKT has been found to increase the levels of interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α), while reducing the levels of IL-10, all of which collectively inhibit tumor growth[64] (Table 3). Thus, piR-Hep1 can act as an oncogene. Inhibition on such piRNA might positively improve the anti-cancer immunity (Figure 7).

piR-DQ593109 (piR-23387)

PiR-DQ593109 has the capacity to form a complex with PIWIL1, resulting in the degradation of the long non-coding RNA, maternally expressed 3 (MEG3), which holds significance in both immune infiltration and immune evasion dynamics.[89] The heightened expression of MEG3 can hinder the proliferation of bladder cancer cells, trigger cellular apoptosis, and notably decelerate the cell cycle progression.[143] Research has documented that in glioblastoma multiforme (GBM) cells increased MEG3 expression fosters CD8+ T cell infiltration and showcase characteristics of tumor suppression.[144] Additionally, evidence also demonstrated that MEG3 has the capability to suppress the development of regulatory T cells in esophageal cancer mice, operating through the p53/miR-149-3p/forkhead box protein P3 (FOXP3) axis, thereby curbing immunological evasion[145] (Table 3). Thus, piR-DQ593109 holds the potential to function as an immunomodulator. Suppressing its expression might exert multiple anti-cancer effects by modulating the augmentation of MEG3. Furthermore, an aberrantly high expression of piR-23387 has been found in HCC cells by our expression analysis (Figure 3).

piR-52207

piR-52207 has been found to promote cell proliferation, migration, and tumorigenesis.[146] Research has revealed that, in endometrioid ovarian cancer (ENOCa) cells, M-phase phosphoprotein 8 (MPHOSPH8) stands as a potential target for piR-52207 and can be downregulated by piR-52207.[146] Downregulation of MPHOSPH8 can further foster the repression of E-cadherin, leading to the initiation of EMT.[146–148] As previously mentioned, EMT possesses the capability to bolster the infiltration of various immune cell subsets and contribute to immune evasion (Table 3).[142] Hence, piR-52207 has been considered as a potential immunomodulator. By suppressing the expression of such genes, the onset of EMT could be curbed, leading to the restoration of anti-cancer immunity.

In light of the findings discussed above, the identified piRNAs demonstrate promising potential for various applications in the realm of cancer immunology and therapy. These piRNAs exhibit distinct roles in immune regulation, immune checkpoint modulation, and as potential immune biomarkers.[149,150] Applications of these piRNAs present intriguing opportunities for further exploration and utilization in precision medicine approaches targeting cancer. For instance, given that piR-823 is commonly down-regulated in various tumors and has the capability to down-regulate the expression of PD-L1, it can be regarded as both a tumor suppressor gene and a sensitizer for ICIs. On the one hand, supplementing piR-823 can reduce the activity of tumor cells; on the other hand, it can decrease PD-L1 expression, potentially enhancing the effect of ICIs treatment. Consequently, its application holds substantial promise. Moreover, the silencing of piR-Hep1 and piR-54265 can also contribute to the reduction of PD-L1 expression, potentially enhancing the efficacy of ICIs. Notably, piR-651 can serve as an indicator not only for poor prognosis in cases of NSCLC but also for the potential responsiveness to ICIs treatment.

Future Research Direction and Challenges

Combination Therapies

Adding circRNAs and/or piRNAs to ICIs-Based Therapy

CircRNAs and piRNAs are both types of non-coding RNAs, meaning they are involved in gene silencing mechanisms, although through different pathways. piRNAs are primarily known for silencing transposable elements in the germ line, while circRNAs can act as microRNA sponges, thereby regulating gene expression post-transcriptionally. Both have been

implicated in the development of cancers, and are being researched for their potential roles in diagnosis, prognosis, and therapy, also including PD-L1 expression regulation.[151,152] In addition, circRNAs are notably stable due to their covalently closed-loop structures, and piRNAs are also relatively stable compared to other RNA molecules. Thus, theoretically, circRNAs and piRNAs could be applied simultaneously in ICIs-based therapies.

Each ncRNA has a unique role in gene regulation, and combining them could potentially target multiple pathways associated with cancer progression. This approach could enhance the therapeutic effects by simultaneously regulating different aspects of cancer cell biology and the immune response. For instance, piR-823 and piR-Hep1 emerge as piRNAs that can be potentially used in conjunction with circRNAs to synergistically regulate PD-L1 expression and enhance the efficacy of PD-1/PD-L1 inhibitors. piR-823 has been shown to facilitate the degradation of PINK1, which is linked to PD-L1 expression. Meanwhile, piR-Hep1 elevates PD-L1 expression through the PI3K/AKT signaling pathway. These piRNAs could potentially be paired with circRNAs like circIGF2BP3 or circBART2.2, which also regulate PD-L1 expression through various mechanisms, to create a combinatorial approach targeting multiple pathways for an enhanced anti-tumor immune response. However, the feasibility, safety, and efficacy of such combination therapies would need to be thoroughly evaluated through further research to determine the optimal types of RNAs (circRNAs or/and piRNAs), and dosing strategies that work synergistically without adverse effects.

Safety of circRNAs and/or piRNAs Therapy

The phenomenon of "too many targets for miRNA effect" (TMTME) refers to the issue wherein microRNAs can target multiple genes, leading to widespread effects that may be difficult to predict and control. This can be problematic in therapeutic contexts where precise targeting is crucial. TMTME is also considered a key factor contributing to the adverse effects of miRNA therapeutics.[117,153] However, using circRNAs and piwi-RNAs (piRNAs) in cancer treatment offers a promising alternative to miRNAs with potentially greater specificity.

As mentioned before, circRNAs have a stable structure due to their closed loop, which may provide a longer-lasting effect compared to the more transient nature of miRNAs.[154] They can also act as 'sponges' for miRNAs, selectively inhibiting their activity and thereby modulating gene expression in a more targeted fashion. This means they could offer a way to fine-tune the miRNA activity without the TMTME issue.[155] Besides that, piRNAs are typically involved in the regulation of gene expression at the epigenetic level and are primarily known for their role in silencing transposable elements in germ cells.[156] They could potentially offer a more targeted approach to gene regulation in cancer cells, affecting specific aspects of the cancer epigenome or transcriptome, leading to fewer off-target effects against TMTME (Figure 8).

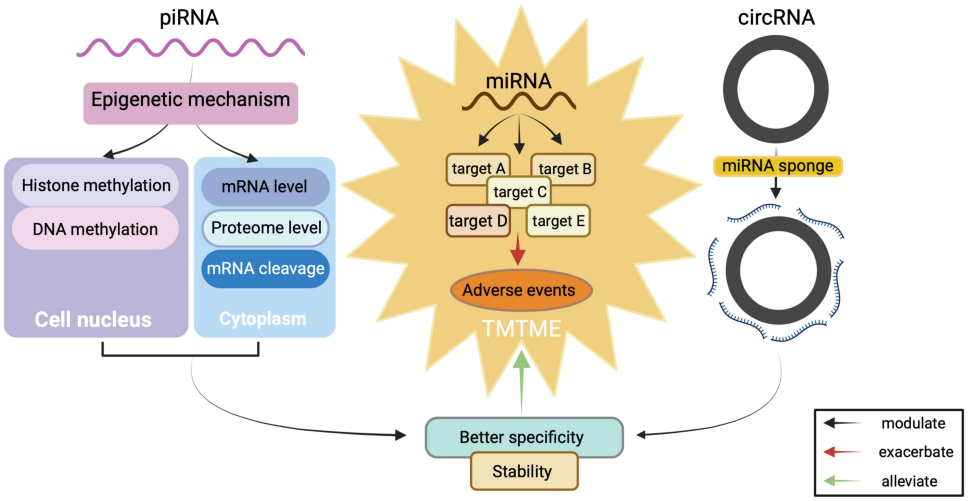

Figure 8 Application of circRNA and/or piRNA therapy can alleviate the effects of TMTME. A single miRNA can bind to multiple targets, therefore leading strong adverse event. However, piRNAs, which are involved in the regulation of gene expression at the epigenetic level, can offer a more targeted approach to gene regulation in cancer cells, affecting specific aspects of the cancer epigenome or transcriptome. In addition, circRNAs possess stable circle structure, and can selectively regulate gene expression. Application of piRNA and/or circRNA can lead to fewer off-target effects against TMTME.
Abbreviation: TMTME, too many targets for miRNA effect.

However, the therapeutic use of circRNAs and piRNAs is still in the early stages, and more research is needed to understand their mechanisms of action and to develop safe and effective delivery methods for clinical applications.

Other Combinations

Recent findings suggest that combining three medications, including a PD-1/PD-L1 inhibitor, may enhance the anti-cancer efficacy beyond that of traditional two-drug regimens. This emerging area could be pivotal in refining PD-1/PD-L1 inhibitor therapy when used alongside piRNA or circRNA strategies.[157] For instance, the TRICOTEL study highlighted that adding atezolizumab to vemurafenib (a BRAF inhibitor) and cobimetinib (a MEK inhibitor) showed promising results for patients with BRAFV600-mutated melanoma with CNS metastases.[158] Additionally, a systematic review and meta-analysis on Stage III–IV melanoma revealed that a triple combination therapy, including anti-PD-1/PD-L1 immunotherapy and BRAF/MEK inhibitors, notably improved progression-free survival (PFS) and 2-year overall survival (OS) in 1266 patients across five randomized controlled trials.[159] Furthermore, a Phase I clinical trial is exploring the effectiveness of combining atezolizumab (a PD-1/PD-L1 inhibitor), tiragolumab (a TIGIT monoclonal antibody), and stereotactic body radiation therapy for metastatic multi-organ cancer.[160] Furthermore, recent evidence has pointed out that carbonic anhydrase XII (CAXII) inhibitor and histone deacetylases (HDAC) inhibitor can also act as promising sensitizers for PD-1/PD-L1 inhibitors-based combination therapy.[6,9,161]

Therapeutic approaches like chemotherapy, radiotherapy, angiogenesis inhibitors, other types of ICIs, co-stimulatory molecule agonists, and targeted therapies could all potentially complement PD-1/PD-L1 inhibitor therapy in conjunction with piRNA or circRNA.[19] However, the optimal combination remains undetermined. The feasibility, safety, and efficacy of such combination therapies need comprehensive evaluation through ongoing research (Figure 9).

Approaches of circRNAs/piRNAs Regulation

Delivery Systems for circRNAs/piRNAs Regulation

Undoubtedly, an efficient delivery system that protects, guides, and delivers these RNAs to target cancer cells is indispensable for an effective gene therapy.[162] Nanoparticles, however, are integral to the efficient delivery of RNA

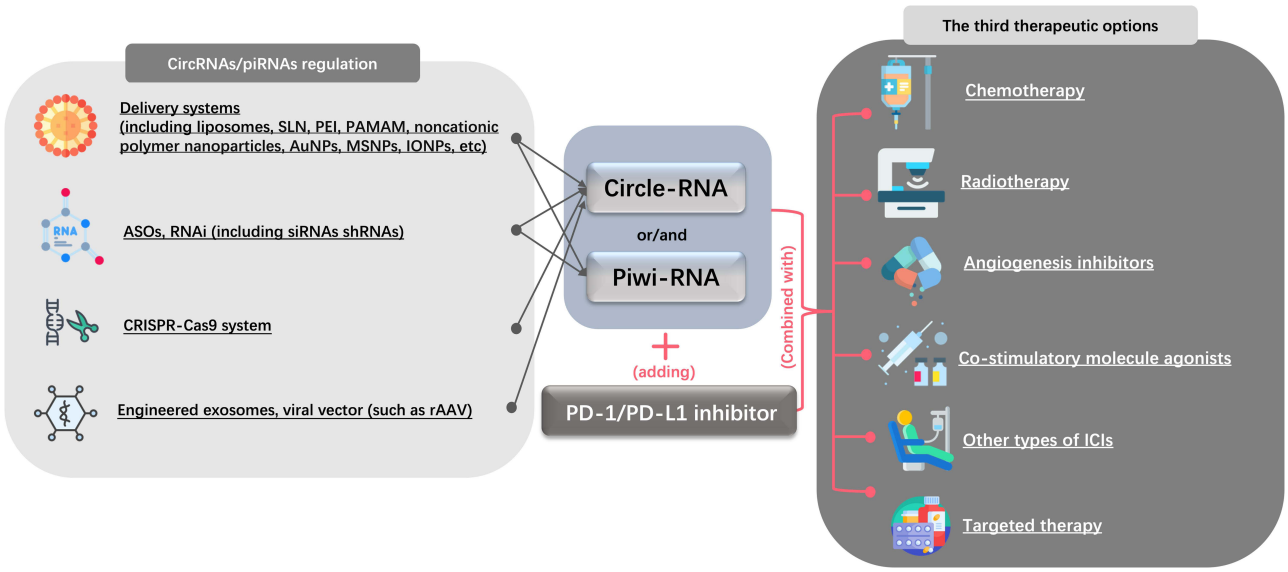

Figure 9 Various therapeutic strategies and delivery systems utilized PD-1/PD-L1 inhibitors plus circRNAs (or piRNA) based-combination therapy. The potential methods of regulation for circRNAs and piRNAs have been displayed including delivery systems like liposomes, solid lipid nanoparticles (SLN), polymeric nanoparticles, and inorganic nanoparticles (AuNPs, MSNPs, IONPs, etc.). Antisense oligonucleotides (ASOs), RNA interference techniques (including siRNAs and shRNAs), the CRISPR-Cas9 gene-editing system, and engineered exosomes and viral vectors (such as recombinant adeno-associated virus, rAAV) are also listed as tools for gene regulation. Moreover, a combination therapy approach is also illustrated, showing the addition of Circle-RNA or/and Piwi-RNA with PD-1/PD-L1 inhibitors to potentially enhance therapeutic efficacy. The extra therapeutic options also include mainstream cancer treatments such as chemotherapy and radiotherapy, along with more targeted approaches like angiogenesis inhibitors, co-stimulatory molecule agonists, other types of immune checkpoint inhibitors (ICIs), and targeted therapy.

Abbreviations: SLN, liposomes; solid lipid nanoparticles; micelles; PEI, polyethyleneimine; PAMAM, poly amidoamine; AuNPs, gold nanoparticles; MSNPs, mesoporous silica nanoparticles; IONPs, iron oxide nanoparticles; ASOs, antisense oligonucleotides; RNAi, RNA Interference; RNAi, RNA Interference; siRNAs, small interfering RNAs; shRNAs, short hairpin RNAs; rAAV, adeno-associated viral vector (rAAV); CRISPR-Cas9 system, CRISPR associated protein 9 system.

interference therapeutics due to their distinctive size, allowing for higher targeting and safety. Various types of nanoparticles are used for this purpose, including liposomes, solid lipid nanoparticles (SLN), micelles, polyethyleneimine (PEI), poly(amidoamine) (PAMAM), noncationic polymer nanoparticles, gold nanoparticles (AuNPs), mesoporous silica nanoparticles (MSNPs), iron oxide nanoparticles (IONPs), and up-conversion nanoparticles.[163–168] Some types of nanoparticles have been proven to be proper carriers for circRNAs or piRNAs (Table 4). For instance, lipid-based nanoparticles (LNPs) are adept at encapsulating circRNAs for precise cellular targeting.[57,169,170] LNPs facilitate the internalization of circRNAs through endocytosis, subsequently releasing them into the cellular cytoplasm.[171] This protective encapsulation within nanoparticle carriers not only shields circRNAs from potential degradation but also enhances their uptake by target cells.[172] Such characteristics contribute to elevating the efficiency of circRNA therapy, allowing for high levels of absorption and utilization. Thus, developing a proper delivery system for circRNA therapy is indispensable. circRNAs can be engineered or selected for their ability to target specific cellular pathways or genes relevant to cancer immunotherapy. This specificity allows for precision in therapeutic interventions (Figure 9).

Moreover, various types of lipid nanoparticles, including multiarmed ionizable lipids (AX4-LNPs) and FDA-approved commercial versions, are effectively used to package and deliver circular RNA (circRNA) vaccines. These vaccines, when administered intramuscularly, induce antigenic coding and immune responses. Notably, AX4-LNPs are quickly broken down in spleen cells, which helps in the faster release of circRNA vaccines.[57,183–186] Other types of delivery systems such as polymeric nanoparticles, inorganic nanoparticles, and exosome-based delivery have all been pointed out to be the proper vehicle for circRNA delivery (Table 4).

Similarly, there are certain types of nanoparticles that have been developed for piRNA delivery (Table 4). In one example, nano-sized amphiphilic poly-(N-vinylpyrrolidone) (PVP) complexes, loaded with miRNA-152 and piRNA-30074, have shown the ability to elevate apoptotic markers such as caspase 9 and mTOR, while simultaneously reducing the proliferation marker ki-67 (MKI-67) in CaCo2 colorectal adenocarcinoma cells.[187] Additionally, a separate study with animals revealed that magnetic nanoparticles, when used to carry anti-piR-823-mimic, can hinder the development of luminal breast cancer in mice. This effect is achieved through the regulation of DNA methyltransferases (DNMTs), the methylation of the adenomatous polyposis coli (APC) gene, and the modulation of Wnt signaling pathways.[188] Furthermore, liposomal and polydopamine nanoparticles (PDA-NPs) designed to target the MUC12 transmembrane protein and carry a piR-1742 inhibitor have demonstrated effectiveness in curbing the progression of renal cell carcinoma cells. This is accomplished by manipulating the piRNA-1742/USP8/MUC12 axis.[189]

However, the use of nanoparticles for delivering circRNA and piRNA in cancer treatment is still a novel and evolving field within nanomedicine and cancer therapy. For developing more ideal nanoparticles, the key challenges include ensuring the stability and bioavailability of the RNA molecules, achieving targeted delivery to cancer cells while minimizing off-target effects, and assessing the long-term safety and efficacy of these treatments. Moreover, clinical trials and further research are necessary to fully understand the potential and limitations of existing nanoparticle-mediated circRNA and piRNA delivery in cancer therapy (Figure 9).

Other Strategies for circRNAs/piRNAs Regulation

These are other approaches that can be applied for circRNAs/piRNAs regulation. For inhibiting circRNA expression, antisense oligonucleotides (ASOs), as a synthetic RNA molecule designed to target and bind to specific circRNAs, can be applied. Such binding can lead to degradation of the circRNA or inhibit its splicing.[190] RNA Interference (RNAi), such as small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs), can also be used to target and degrade specific circRNAs by inducing cleavage of the RNA molecule.[191] Moreover, the CRISPR-Cas9 system can potentially be engineered to target and edit the genomic regions responsible for circRNA production, effectively reducing their expression.[192] In addition, small molecules that interfere with the biogenesis of certain circRNA can be developed to reduce their production.[193] Engineered exosomes that can deliver inhibitory molecules to specific cells or tissues can also act as a promising approach to regulate circRNA expression.[194]

For promoting the expression of circRNA, some potential approaches are recommended. For instance, adeno-associated viral vector (rAAV) could be used to achieve overexpression of circRNAs.[195] Besides, vectors for over-expressing circRNAs, such as pLCDH-ciR or pCD-ciR, carry optimized flanking loop-forming frameworks containing

Table 4 Different Types of Nanoparticles That Can Be Applied for circRNAs/piRNAs Delivery in Cancer Treatment

Types of Nanoparticles	The Transportee	Advantages	Disadvantages	Common Application	References
Lipsomes	piRNAs	Good biocompatibility, reduced toxicity, stability, enhanced permeability and retention effect, precise targeting	Potential for leakage or fusion with other vesicles, variable shelf life.	Widely used for drug delivery	[173]
Solid Lipid Nanoparticles (SLN)	piRNAs	Improved stability, controlled drug release	Risk of drug expulsion during storage, potential toxicity due to lipid degradation products	Drug delivery, especially for poorly soluble drugs.	[158]
Polymeric Nanoparticles (eg, PLGA, Dendrimers, Polymeric Micelles):	piRNAs/ circRNAs	Biodegradability, controlled release of circRNA or piRNA, and the ability to modify the surface for targeted delivery	Potential toxicity, immune responses, complexity in manufacturing and variability in degradation rates affecting circRNA release	Delivering therapeutic agents, including anticancer drugs	[174,175]
Inorganic Nanoparticles (eg, Gold Nanoparticles, Carbon Nanotubes, Silica Nanoparticles, Magnetic Nanoparticles):	piRNAs/ circRNAs	High surface area to volume ratio, easily modified surface for targeted delivery, and good stability. Gold nanoparticles: They can be used for targeted delivery of circRNAs to cancer cells, often guided by specific ligands or antibodies; Magnetic nanoparticles: Targeted delivery through magnetic guidance, potential for use in hyperthermia therapy; Carbon nanotubes: High surface area for circRNA loading, potential for penetration through cellular barriers.	Poorer biocompatibility and biodegradability, potential toxicity. Gold nanoparticles: High cost of production, potential toxicity at higher concentrations; Magnetic nanoparticles: Possible issues with aggregation, and concerns about long-term biocompatibility; Carbon nanotubes: Concerns about toxicity and biocompatibility, potential for causing inflammation.	Drug delivery, thermal ablation, gene therapy	[176–179]
Hybrid Nanoparticles (eg, Lipid-Polymer Hybrid Nanoparticles, Liposome-Silica Hybrid Nanoparticles)	piRNAs	Combines advantages of organic and inorganic nanoparticles, high biocompatibility, structural integrity, effective internalization by cancer cells	Complexity in design and synthesis, potential for unexpected biological interactions	Delivering a range of therapeutic agents, enhancing treatment efficacy	[180]
Lipid-Based Nanoparticles (LNPs)	circRNAs	High encapsulation efficiency, protection of circRNA from nuclease degradation, and relatively safe with low immunogenicity	Potential for toxicity and accumulation in non-target tissues, challenges in targeting specific cell types	Gene delivery. A well-known example is the lipid nanoparticle used in mRNA vaccines.	[53]
Exosome-Based Delivery	circRNAs	High biocompatibility, reduced immune response, natural targeting abilities	Complex isolation and purification processes, potential for carrying unwanted cellular components	Encapsulating certain RNAs and deliver them to specific cells, exploiting their natural cell-targeting properties	[181,182]

https://doi.org/10.2147/IJN.S461289

modified binding sites for RBPs, and use newly designed circularization-mediating sequences can ensure efficient circularization of inserted circRNA.[170] Moreover, plasmids or viral vectors have also been reported to be applied to overexpress specific circRNAs in cells or tissues, thereby increasing their levels.[59,196] The method of modifying the epigenetic marks on the genomic regions (Epigenetic Modification) associated with certain circRNA production to promote circRNA expression, is also a potential solution.[197] Additionally, manipulating the activity of transcription factors that regulate circRNA biogenesis can be a way to enhance circRNA expression.[198] Another potential strategy is to develop compounds or chemicals that enhance the biogenesis or stability of circRNAs.[199] Moreover, RNA editing technologies such as CRISPR-based approaches can also be applied to modify and increase circRNA expression.[192,200,201]

Regulating the expression of piRNAs is also a complex area of study. However, some methods have been proven to successfully regulate piRNA expression.[68] For instance, in a study involving neurodegeneration, knocking down piRNA biogenesis genes using RNA interference (RNAi) has shown improvements in movement and behavior in model organisms. Briefly, by using a Caenorhabditis elegans model of Lewy body disease, RNAi was used to knock down genes involved in piRNA biogenesis, such as tofu-1, tofu-3, tofu-4, and tofu-6. This approach successfully led to improved movement and amelioration of neurodegenerative phenotypes.[70,73]

Moreover, the deletion of genes involved in piRNA processing has been found to downregulate piRNA expression and improve phenotypes related to neurodegenerative diseases. In the same study on C. elegans, the deletion of the tofu-1 gene, which is involved in piRNA processing, resulted in downregulation of piRNA expression and improved behavioral phenotypes.[70] Furthermore, analysis of the piRNA transcriptome in the human placenta showed that a significant number of piRNAs were expressed from the DLK1-DIO3 imprinted region, suggesting their potential roles in placental biology and gene regulation. This may also provide more ideas for the development of methods for regulating piRNAs.[173]

However, regulation of circRNA/piRNA expression is a complex and emerging field. The choice of method depends on the specific RNA of interest and the desired regulatory outcome, and these approaches still require a lot of investigation.

Limitation

The data we have gathered from publicly available databases for the analysis of selected circRNAs and piRNAs is constrained. This limitation affects both the foundational information and the predictive outcomes related to circRNA and piRNA candidates. Such constraints were inevitable given the nascent nature of circRNAs and piRNAs therapy as a field. Nevertheless, as this field continues to evolve, subsequent research endeavors will progressively enhance the availability of relevant data. This article's primary objective is to furnish readers with as comprehensive and detailed information as possible about the chosen RNA candidates. It also seeks to broaden the research perspective to the fullest extent possible.

Conclusion

The investigation into the role of circRNAs and piRNAs has pioneered a transformative approach to PD-1/PD-L1 inhibition therapy, as detailed in our manuscript. These non-coding RNAs have emerged as powerful regulators of the immune response in oncology, a domain once underestimated but now at the forefront of personalized cancer treatment. Our in-depth review has revealed their profound impact on PD-L1 expression and their capacity to modulate anti-cancer immunity, thereby potentiating the efficacy of immune checkpoint inhibitors (ICIs).

CircRNAs are derived from diverse sources, exhibit stability, possess evolutionary conservation, display tissue-specificity, and are implicated in numerous potential biological functions. These functions include sequestering miRNAs, regulating transcription, facilitating protein transport, and mediating protein–protein interactions. Fortunately, accumulating evidence has pointed out that certain circRNAs with immune modulating function can regulate the expression of PD-L1. Moreover, circRNAs are more resilient to degradation compared to linear non-coding RNAs, enabling them to persist in the cellular environment, potentially providing longer-lasting therapeutic effects. CircRNAs can also contain multiple binding sites for miRNAs, allowing them to indirectly regulate the expression of genes that are involved in cancer progression and immune evasion. Moreover, some circRNAs can even affect various cellular processes, such as cell viability, migration, invasion, and stemness, making them versatile candidates for targeted cancer therapies. In

addition, due to their stability and potential to affect cellular processes, circRNAs may offer a longer duration of therapeutic impact compared to some other non-coding RNAs.

PiRNAs, such as piR-823, piR-54265, and piR-Hep1, have also shown immense promise as both tumor suppressors and enhancers of the immune response. These findings are now echoed by the recent outcomes of the KEYNOTE-942 trial, where the addition of an mRNA-based cancer vaccine to pembrolizumab significantly improved recurrence-free survival in patients with high-risk melanoma, indicating the potential of RNA-based treatments to improve immunotherapy results regardless of tumor mutational burden. Furthermore, the 2023 Nobel Prize in Medicine, awarded to Katalin Karikó and Drew Weissman for their groundbreaking work on mRNA technology, underlines the significance of RNA in medical innovations, validating the concepts explored in our manuscript. Their discoveries concerning nucleoside base modifications have led to effective mRNA vaccines, showcasing the therapeutic promise of RNA modalities in enhancing patient-specific treatment strategies.

CircRNAs and piRNAs are emerging as pivotal modulators of immune checkpoint blockade therapy. Their ability to regulate PD-L1 expression through intricate pathways, such as the CXCL12/CXCR4/CXCR7/miR-370-3p axis, the RP11-424C20.2/UHRF1 interaction, and the miR-382-3p/STAT1/PD-L1 pathway, significantly enhances the efficacy of PD-1/PD-L1 inhibitors.[174–176] These findings also underscore the potential of leveraging circRNAs and piRNAs to overcome resistance and optimize immunotherapeutic strategies against cancer.[177–179] The synergy between these ncRNAs and ICIs suggests a promising strategy for overcoming resistance mechanisms that often limit the success of current cancer treatments. By integrating circRNAs and piRNAs into combination therapies, we can potentially harness their ability to fine-tune immune responses and target multiple pathways involved in tumor evasion.[178,180,181] This approach not only exemplifies the complexity of tumor-immune interactions but also emphasizes the need for personalized therapeutic strategies that consider the individual tumor microenvironment and the specific ncRNA profiles of patients.

Apparently, the synergy of circRNAs and piRNAs with ICIs opens new avenues for tailored therapies, offering hope for more effective and individualized treatments. Yet, there are challenges in their application. To conquer other issues, key areas of focus should include the development of efficient and targeted delivery systems for these ncRNAs, the identification of specific circRNAs and piRNAs that can serve as biomarkers for predicting therapy response, and the exploration of their roles in regulating other immune checkpoints beyond PD-1/PD-L1.[182,202] Additionally, the integration of circRNA and piRNA profiling into precision medicine frameworks could offer novel insights into patient stratification and the customization of immunotherapy regimens, ultimately enhancing treatment outcomes and reducing adverse effects.

In conclusion, the evidence summarized and displayed in this article underscores the imperative for continued exploration in this domain. The potential of circRNAs and piRNAs to enhance cancer immunotherapy is substantial, with these non-coding RNAs poised to offer innovative therapeutic targets and deepen our understanding of tumor immunology, shaping the future of cancer therapy.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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