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Circular RNAs in tumor immunity and immunotherapy

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Abstract

Circular RNAs (circRNAs) are unique noncoding RNAs that have a closed and stable loop structure generated through backsplicing. Due to their conservation, stability and tissue specificity, circRNAs can potentially be used as diagnostic indicators and therapeutic targets for certain tumors. Many studies have shown that circRNAs can act as microRNA (miRNA) sponges, and engage in interactions with proteins and translation templates to regulate gene expression and signal transduction, thereby participating in the occurrence and development of a variety of malignant tumors. Immunotherapy has revolutionized the treatment of cancer. Early researches have indicated that circRNAs are involved in regulating tumor immune microenvironment and antitumor immunity. CircRNAs may have the potential to be important targets for increasing sensitivity to immunotherapy and expanding the population of patients who benefit from cancer immunotherapy. However, few studies have investigated the correlation between circRNAs and tumor immunity. In this review, we summarize the current researches on circRNAs involved in antitumor immune regulation through different mechanisms and their potential value in increasing immunotherapy efficacy with the goal of providing new targets for cancer immunotherapy.

Keywords circRNAs, Tumor, Immunity, Cancer immunotherapy

Introduction

CircRNAs were originally thought to be splicing errors or byproducts of RNA transcription because they cannot encode proteins [1–3]. Developments in bioinformatics, RNA sequencing and genome sequencing, have led to preliminary discoveries about the production and function of circRNAs in recent years [4, 5]. In contrast to traditional linear RNAs, circRNAs lack a 3' poly(A) tail and a 5' cap structure, are closed into a ring in the form of covalent bonds and are not sensitive to exonucleases. This unusual structure provides circRNAs with an elevated level of stability. Most circRNAs are generated via back-splicing, which occurs mainly by base pairing between the intron reverse repeats of Alu transposition factors, which results in the formation of abnormal secondary structures in pre-mRNAs [6]. Dysregulation expression of circRNAs due to disorder of conventional splicing and back-splicing may cause tumors progression [7].

CircRNAs offer excellent preclinical diagnostic and therapeutic potential for a wide range of tumors owing to their universality, conservation, stability and differential expression in tumor patients and healthy people. The contribution of some circRNAs to tumor progression or immunotherapy have been preliminary demonstrated in preclinical models [8–10]. For example, circHIPK3,

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identified as an oncogene and autophagy regulator, significantly promotes malignant cell properties and inhibits autophagy in non-small cell lung cancer (NSCLC) and may be a prognostic marker and therapeutic target [11]. Circ-ZKSCAN1 is downregulated in bladder cancer (BCa) and hepatocellular carcinoma (HCC), and its absence results in malignant characteristics, such as cancer stemness, and is highly associated strongly correlated with low rates of survival overall and without recurrence, thus, circ-ZKSCAN1 may be a prognostic factor of BCa recurrence and a target for HCC treatment [12–14]. Circ-CPA4 is upregulated in NSCLC and can inhibit the functions of tumor immune cells (such as $CD8^+$ T cells) via the let-7/programmed death ligand 1 (PD-L1) axis, suggesting a new approach for exploring NSCLC therapeutic agents [15]. Exosomes contain some circRNAs, and exosomal circRNAs (exo-circRNAs) possess the capability of original biology roles of circRNAs and transferring to target sites [16, 17]. CircSKA3 in tumor-derived exosomes can mediate breast cancer metastasis, and targeting circSKA3 can suppress tumor formation capacity and tumor cells transfer [18]. CircRNAs can be found in liquid biopsy samples (such as plasma, urine and saliva) and have significant potential as diagnostic and prognostic biomarkers [19–22]. These results reveal that circRNAs constitute a novel and individual class of epigenetic regulators that are engaged in tumor immunity and immunotherapy.

Cancer immunotherapy is currently the fifth most popular type of tumor treatment, behind surgery, chemotherapy, radiation therapy and targeted therapy [23, 24]. However, only some patients can achieve effective treatment outcomes [25]. Thefunction of circRNAs in tumor microenvironment (TME) and immunotherapy has drawn a lot of attentions recently [26–29]. Our focus is on analyzing the tumor immune microenvironment (TIME) and enhancing the effectiveness of immunotherapy. For example, the cancer-derived exosome circUHRF1 induces malfunction in natural killer (NK) cells and drives anti-PD1 resistance in HCC, hence promoting tumor immunosuppression [30]. Increased circFAT1 expression can bind to STAT3 and promote its activation, then inhibit $CD8^+$ T cells infiltration into TME and reduce the sensitivity to PD1 blockade immunotherapy, which in turn stimulates cancer stemness and immune escape [31]. Focusing on circRNAs or managing alterations in immune cells and TME may can enhance the effectiveness of immunotherapy and survival of tumor patients.

In this review, understanding the roles of circRNAs in immunity and their application in immunotherapy may provide a new approach for guiding targeted clinical therapy.

The glance of circRNA

Researchers discovered circRNAs for the first time in a plant RNA virus in 1976 [32]. In 1979, the circRNA in eukaryotic cytoplasm was observed for the first time via electron microscopy, suggesting that RNA may occur in a circular form in the eukaryotic cells [33]. In 1991, endogenous spliced circRNAs derived from the human DCC gene were initially discovered by researchers. Numerous investigations conducted in the late 1990s and early 2000s revealed the ubiquitous presence of genes producing circRNAs in the eukaryotic cells, which include human cells and those of mammals and flies [34]. However, due to the limitations of scientific knowledge and technological development, circRNAs were commonly thought to be mis-spliced RNAs or byproducts of pre-mRNA processing for several decades. Until 2012, scientists found through high-throughput sequencing that hundreds of human genes can transcribe circRNAs and they are closely associated with a range of disease conditions, thus circRNA-related researches have gradually increased.

Many circRNAs are exhibited stable expression in saliva, blood, and exosomes, and they are more prevalent and more conserved than mRNAs in prokaryotes and eukaryotes [35]. CircRNA expression is 10% higher than mRNA expression in the majority of genes, and the expression abundance of some circRNAs is ten times greater than that of mRNAs [36]. According to other studies, the amount of circRNAs in human platelets is 17 ~ 188 times higher than that in nucleated tissues. CircRNAs are more stable in comparison to linear mRNAs and are not degraded easily. Additionally, circRNAs are easier to detect than miRNAs, which are present in smaller amounts [37]. These results demonstrated the enormous potential of circRNAs as diagnosis, prognostic, and predictive biomarkers. Unlike other noncoding RNAs, circRNAs are produced via the process of back-splicing, which creates a back-splice junction by looping the introns around the upstream and downstream splice acceptor and donor sites. Sequences with inverted intronic repeats (such as Alu elements) can help form loop structures [7]. Research has also revealed that RNA binding proteins (RBPs) may play important roles in the production of certain circRNAs. On the one hand, Muscleblind and Quaking can bind to the flanking introns of circularized exons in the mRNA precursors of their host genes to promote circRNAs production [38–40]. On the other hand, DHX9, nuclear RNA helicase, by explicitly binding to the inverted repeat Alu sequences, suppresses

the synthesis of circRNAs [6]. Adenosine deaminase 1 cleaves circRNAs by binding double-stranded RNA to the stem–loop structure [4].

There are three primary forms of circRNAs, exonic circRNAs (ecircRNAs), circular intronic RNAs (ciRNAs), and exonic-intronic circRNAs (EIciRNAs).EcircRNAs, which are generated from single or multiple exons, account for more than 80% of known circRNAs; the majority of ecircRNAs reside in the cytoplasm, they typically act as miRNA response elements and binding protein elements [41–43]. EcircRNAs can be formed in three ways: lasso-driven cyclization, intron pairing-driven cyclization and RNA-binding protein-promoted cyclization. Studies have shown that the ATP-dependent RNA helicase DDX39A and the spliceosome RNA helicase DDX39B can promote the release of ecircRNAs from the nucleus to the cytoplasm [44]. CiRNAs consist only of introns and thus are formed in a different manner than EcircRNAs. Under normal circumstances, the intron lassos released during the classical splicing process are degraded. However, ciRNAs are formed when the 5' end splicing site contains a 7-nt GU-rich base sequence and a nearby 3' end contains an 11-nt C-rich base sequence so that the intron lasso is protected, which promotes the formation of stable ciRNAs and functions as a transcription regulator in the nucleus [45, 46]. Exons and introns are present in EIciRNAs. In fact, lasso-driven and intron-driven cyclization can produce not only EcircRNAs but also EIciRNAs. EIciRNAs interact with U1 snRNPs, are mostly located in the nucleus, and stimulate the transcription of associated genes [16]. Some circRNAs that are enriched and highly expressed in exosomes can be packaged and released into the extracellular matrix to execute their functions. Exo-circRNAs not only can mediate tumor growth, invasion and metastasis and regulate antitumor immunity, but also have the potential to become efficient biomarkers for tumor diagnosis, monitoring and therapeutic targeting [47]. However, investigations into circRNAs in immunocytes are still in their early stages, and many new circRNAs have not yet been discovered to confirm their function.

Biogenesis and functions of circRNAs in tumors

CircRNAs can carry out their functions to affect the development and metastasis of malignancies tumors by various mechanisms [48–50]. Numerous studies have shown that abnormally expressed circRNAs may be crucial regulators of the development of tumors by sustaining proliferative signals, affecting invasion and metastasis abilities, regulating the formation of cancer stem cells, inducing angiogenesis and radioresistance and influencing the progression of antitumor immunity [51].

CircRNAs act as miRNA sponges

Many circRNAs contain conserved miRNA target sites and can act as miRNA sponges that bind miRNAs [52]. By acting as competing endogenous RNAs (ceRNA), circRNAs competitively bind miRNAs and play essential parts in the progression and spread of malignancies. For example, the first circRNA proved to be a miRNA sponge was the antisense transcript of cerebellar degeneration-related protein-1 (CDR1as or ciRS-7), including 74 specifically conserved miRNA binding sites [41]. ciRS-7 can downregulate miR-7 expression by directly binding to miR-7 and inhibiting its activity, which is postulated to regulate tumor growth and metastasis. ciRS-7 is likely to become a clinical biomarker for tumor diagnosis and therapy [53]. Hsa_circ_0136666 droved PD-L1 phosphorylation via the miR-375/PRKDC axis, which promoted TME formation, suppressed immune function, and lead to tumorigenesis immune escape in gastric cancer (GC) [54]. Circ-CPA4 can decrease PD-L1 expression and $CD8^+$ T cells in TIME by sponging let-7, regulate stemness and drug resistance of NSCLC cells [15] (Fig. 1A). The expression of circ_0001005 can be upregulated by targeting androgen receptor and competitively sponged miR-200a-3p and PD-L1, which can enhance natural killer (NK) cell-mediated tumor-killing efficacy in breast cancer (BC) [55]. Has_circ_0007456 can significantly influence the NK cell-mediated cytotoxicity by miR-6852-3p/ICAM-1 axis, and regulate process of tumorigenesis and immune evasion in HCC [56] (Fig. 1B). Exo-circGSE1 can regulate the expansion of regulatory T cells by miR-324-5p/TGFBR1/Smad3 axis and promote immune escape of HCC [57]. Exo-circPOLQ can active interleukin-10 and STAT3 axis by targeting miR-379-3p to promote polarization of M2 macrophages in a colorectal cancer [58] (Fig. 1D).

CircRNAs interact with proteins as sponges, scaffolds or protein recruitment

Researches have demonstrated that circRNAs are capable of binding to RBPs and function as protein decoys or antagonists to regulate protein activity, which are crucial for the development of tumors [59]. RBPs are important regulators in tissue-specific circRNAs expression [60]. Many circRNAs have been reported to bind human antigen R (HuR) as sponges [61]. CircAGO2, which is derived from AGO2, interacts with the HuR protein to enable HuR-mediated inhibition of AGO2-miRNA complexes function and promote the GC progression [62]. CircDLC1 can bind to HuR, which decreases the interaction between HuR and MMP1 and consequently restricts MMP1 expression, thus leading to HCC [63]. Moreover, circRNAs are linked to proteins and act as scaffolds to form RNA–protein complexes, promoting

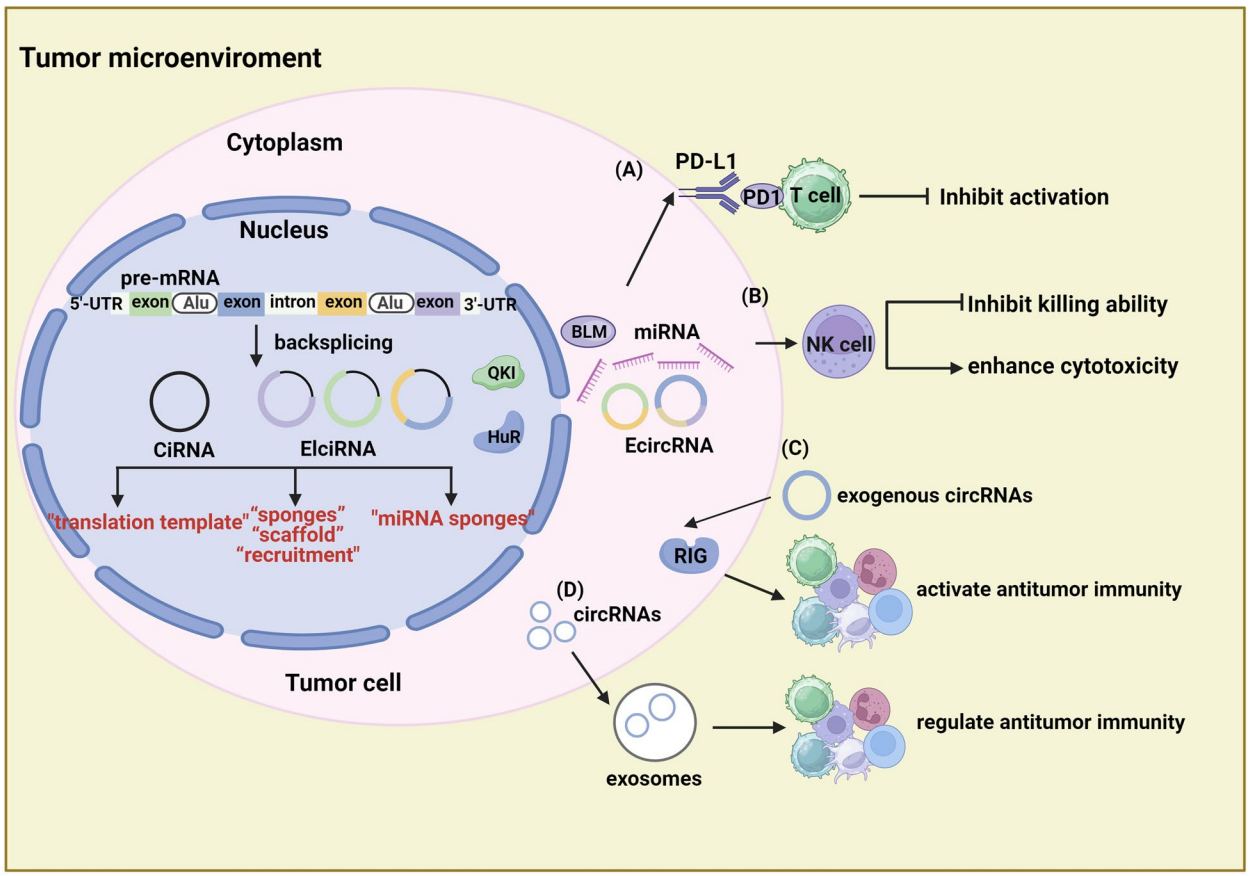

Fig. 1 Roles of circRNAs in the tumor microenvironment. **A** CircRNAs can as miRNAs sponge to regulate the expression of PD1/PD-L1 and inhabit activation of T cells. **B** CircRNAs can bind to miRNAs to influence the NK cell-mediated killing ability and cytotoxicity. **C** Exogenous circRNAs can regulate the function of antitumor immunity in RIG-dependent manner. **D** Exo-circRNAs can regulate antitumor immunity. ciRNAs: circular intronic RNAs; ecircRNAs: exonic circRNAs; ElciRNAs: exonic-intronic circRNAs; exo-circRNAs: exosomes circRNAs

enzyme–substrate colocalization to alter reaction kinetics [64]. For example, circ-Foxo3 facilitates p53 ubiquitination and degradation in an MDM2-dependent way, which can increase the amounts of the protein Foxo3 due to its low binding affinity and cause cancer cells to undergo apoptosis [65]. CircATG7 is located in pancreatic cancer cells of cytoplasm and nucleus. Cytoplasmic circATG7 plays a role by increase miR-766-5p/ATG7 axis. Nuclear circATG7 acts as a scaffold to enhance the interaction between HuR and ATG7, and regulate the growth and spreading of pancreatic cancer cells [66]. circREEP3 can accelerate tumorigenesis and metastasis of colorectal cancer by recruiting the CHD7 to FKBP10 promoter, and inhabit function of antitumor immunity in RIG-1-dependent manner [67]. CircKEAP1 upregulates of immune response genes and activates antitumor immunity of oral squamous cells by interacting with RIG-I [68] (Fig. 1C). circEIF3I, serving as a molecular scaffold, binds to the MH2 domain of SMAD3 and recruit SMAD3 to early endosomes, subsequently promotes pancreatic cancer progression via TGF-β signaling pathway [69]. Finally, circRNAs have ability to recruit particular proteins to certain cellular sites. For example, circular RNA FECR1,consisting of FLI1 exons 4-2-3 in FLI1 promoter chromatin complex, recruits TET1 demethylase to the FLI1 promoter region and downregulates DNMT1 in trans, which may coordinate the methylation and demethylation of DNA for regulating the metastasis of BC cells [70]. CircRHOT1 can bring TIP60 to the NR2F6 promoter and promote the transcription of the NR2F6 mRNA in HCC [71].

CircRNAs act as translation templates for peptides

Pamudurti first reported that circRNAs can be translated in 2017 [64]. This type of circRNA has an open reading frame, and its translation template mechanisms can be categorized as internal ribosome entry site (IRES)-dependent or IRES-independent ways [48, 65]. The translation of circ-SHPRH into the SHPRH-146aa protein is dependent on IRES [72]. Circ-ZNF609 has a

translational function, and can influence myoblast proliferation through its open reading frames and the IRESs [73]. The 5'-cap and poly(A) tails, which are necessary for cap-dependent translation, are absent from circRNAs. However, circRNAs can form through IRESs or following the incorporation of m6A RNA modifications in the 5'-untranslated region [74]. CircRNAs can translated into peptides by an m6A-driven progress, which may be involved in physiological reactions in tumors. Circ-FBXW7 is translated into circ-FBXW7-185AA by m6A modification and inhibits β-catenin ubiquitination, effectively inhibiting the proliferation of lung adenocarcinoma (LUAD) stem cells and reversing resistance to tyrosine kinase inhibitors by regulating Wnt pathway function [75]. To date, only a small number of endogenous circRNAs that function of protein templates have been discovered. The majority of peptides produced from circRNAs are still unknown to have any functional significance, despite the prediction that certain circRNAs will have a putative open reading frame with an upstream IRES.

Roles and functions of circRNAs in tumor immunity and immunotherapy

The immune response is an important line of defense that monitor tumor occurrence and eliminate tumor cells, and tumor progression is accelerated after immune evasion of tumor cells [76, 77]. Immune evasion mechanisms continue to develop and become more diverse and complex in the process of tumor progression, leading to poor treatment efficacy in the clinic [78, 79]. CircRNAs mediate many signaling pathways, recruit and rewire essential TME components, thus influence carcinogenesis, the immune system, and the effectiveness of antitumor immunotherapy [76] (Table 1).

CircRNAs regulate cancer stemness and affect the antitumor immune response

Cancer stem cells (CSCs) are a special class of cells that can self-renew and maintain stemness; can induce tumorigenesis, tumor recurrence and progression in the TME; and are usually resistant to chemotherapy, radiotherapy, targeted therapy, and even immunotherapy [80]. Heterogeneous CSCs can interact with TME components and immune cells, thus playing an antitumor role and leading to treatment failure [81]. In the immunosuppressive TME, CSCs evade immune system surveillance by affecting immune cells and producing immunoregulatory factors, cytokines, and even exosomes [82]. CSCs can regulate immune checkpoints and immune evasion by producing extracellular vesicles (EVs) or exosomes. CSC-derived exosomes not only express PD-L1 but also increase the expression of PD1 on $CD8^+$ T cells, which promotes $CD8^+$ T cells exhaustion [83]. Additionally, CSCs can directly promote tumor resistance to immunotherapy via adoptive cytotoxic T cells transfer [84] (Fig. 2A). For example, circFAT1 plays an important role in sustaining CSC self-renewal and tumorigenicity in squamous cell carcinoma. Disruption of circFAT1 expression can impair the stemness of cancer cells and reverse tumor cell-intrinsic immunity in the tumor immunosuppressive microenvironment by binding STAT3 in the cytoplasm and promoting STAT3 activation, which significantly increases resistance to PD1 blockade immunotherapy by reducing $CD8^+$ T cells infiltration into the TME [31]. These findings suggest a significant relationship between circRNAs modulation of stemness in cancer and the establishment of an immunosuppressive microenvironment, suggesting that circFAT1 is a significant therapeutic target for overcoming immunotherapy resistance to PD1 blockade in head and neck squamous cell carcinoma. The TIP60 complex is recruited to the MAFF promoter by cia-MAF, which binds to the promoter, and induces liver tumor-initiating cell proliferation and self-renewal [85]. The miR-3908/TRIM59/p53 axis is the target of circLMP2A via an EBV-encoded circRNA, whose expression is increased and is essential for generating and sustaining stemness phenotypes in GC [86]. CircIPO11 binds TOP1 to the GLI1 promoter to stimulate its transcription, leading to the activation of Hedgehog signaling to maintain self-renewal of liver CSCs [87]. CircRGPD6 maintains the stem cell-like features of BC, resulting in the establishment of a TV-circRGPD6 nanoparticle that eliminates breast cancer metastases by specifically expressing circRGPD6 in metastatic BC [88]. In addition, these studies provide theoretical support for further investigations about the roles of circRNAs in CSC stem maintenance. However, the molecular mechanisms underlying the interactions among immune cells, CSCs, and immunotherapy response are poorly understood. Studying the interaction between tumor cells stemness and immune evasion according to circRNAs expression in CSCs will help improve a more knowledge of the underlying immunological mechanisms and design novel circRNAs CSC-targeted immunotherapy strategies to improve cancer prognosis.

CircRNAs regulate immune checkpoints

Immune checkpoints are a group of molecules located on immune cells that limit the level of immunological activation, act as gatekeepers during the immune response and prevent the body's defense system from becoming active. Increasing amounts of researches in molecular oncology have revealed the intricate regulatory mechanisms affecting immune checkpoint expression in recent years. A strong correlation exists between the expression of immunological checkpoints and circRNAs, which are

Table 1 The roles and functions of circRNAs in tumor immune

circRNA	Expression	Function	Mechanism	Tumor	PMID
circFAT1	up	Regulates cancer stemness and immune evasion	Binding to STAT3, preventing STAT3 dephosphorylation and reducing PD1 blockade immunotherapy and $CD8^+$ T cell infiltration	SCC	34258151
cia-MAF	up	Promotes liver TIC propagation, self-renewal, and metastasis	Binding to the MAFF promoter, recruiting the TIP60 complex to the MAFF promoter	TICs	34403373
circLMP2A	up	Generates and sustains stemness phenotypes in GC	Sponging to miR-3908, promoting theT-RIM59 expression and suppressing p53	GC	32790025
circIPO11	up	Maintains self-renewal of liver CSCs	Binding TOP1 to the GLI1 promoter to stimulate its transcription	HCC	34649567
circRGPD6	down	Regulates the CSCs maintenance and eradicates BC metastasis	Suppressing BCSC-mediated metastasis via the miR-26b/YAF2 axis	BCSCs	32950105
circFGFR1	up	Promotes NSCLC progression and resistance to anti-PD-1-based therapy	Interacting with miR-381-3p to increase CXCR4'expression	NSCLC	31815619
circRNA-002178	up	Induces T-cell exhaustion	As a ceRNA to promote PD-L1/PD1 expression	LUAD	31949130
circCHST15	up	Increases tumor development and immune evasion	Sponging miR-155-5p and miR-194-5p and suppressing the expression of PD-L1	LC	33777742
circTMTC3 circFAM117B	up	Promotes melanoma immune escape	Increasing PD-L1 expression by sponging miR-142–5, reducing the cytotoxicity of $CD8^+$ T cells	melanoma	37137884
circRNA_001678	up	Promotes NSCLC immune escape progression	Promoting the PD-1/PD-L1 pathway and triggering $CD8^+$T-cell apoptosis via the miR-326/ZEB1 axis	NSCLC	35848890
Hsa_circ_0067842	up	Promotes immune escape in BC	Interacting with HuR, improving the stability of CMTM6 and influencing the ubiquitination of PD-L1	BC	37592296
circPRDM4	up	Inhibits $CD8^+$T-cell cell infiltration and promotes PD-L1 expression in the TME	Regulating the interaction between HIF-1α and the promoter of CD274	HCC	36747292
circBART2.2	up	Inhibits T-cell function and promots tumor immune escape	Binding to RIG-I and activating the IRF3 and NF-κB, regulating of PD-L1 expression	NPC	34321242
circIGF2BP3	up	Reduces $CD8^+$ T-cell responses to promote NSCLC tumor immune evasion	Stabilizing OTUB1 mRNA in a PKP3-dependent way to reduce PD-L1 ubiquitination	NSCLC	34416901
circNFIX	up	Accelerates OC development and immune escape	Regulating miR-647/IL-6R/PD-L1 pathway	OC	37713785
circQSOX1	up	Facilitates Treg cells-based immune escape	Activating PGAM1/miR-326/miR-330-5p pathway to induce glycolysis	CRC	36370665
circUHRF1	up	Inhibits NK cell function and tumor infiltration, leads to NK cells exhaustion and drives resistance to anti-PD1 immunotherapy	Inhibiting the secretion of IFN-γ and TNF-α and upregulating the expression of TIM-3 by reducing of miR-449c-5p	HCC	32593303
circRERE	up	Promotes resistance to the chemotherapy drug bortezomib	Sponging miR-152-3p to upregulate the expression of CD47	MM	34307012
circHMGCS1-016	up	Induces ICC cell invasion and reshapes the TIME	Sponging the miR-1236-3p to regulate CD73 and GAL-8 expression	ICC	34526098
circSMARCC1	up	Leads to TAMs infiltration and M2 polarization in prostate cancer	Associating with colonization of $CD68^+$/$CD163^+$/$CD206^+$ TAMs and CD163 expression in macrophages via CCL20-CCR6 axis	PCa	36045408
circMAPK1	down	Promotes $CD8^+$ T-cell intratumoral infiltration in LUAD	Promoting the occupation of the 3'UTR of CCL5 by IGF2BP1	LUAD	38091827
circNDUFB2	down	Regulates immune response to NSCLC	Recruiting $CD8^+$ T cells and DCs to TME by the secretion and nuclear translocation of chemokines and IFNs	NSCLC	33436560
circ_0020710	up	Suppresses the immune system and reduces the invasion of CTLs	Sponging miR-370-3p to upregulate CXCL12 expression	MM	32381016

Table 1 (continued)

circRNA	Expression	Function	Mechanism	Tumor	PMID
circCsnk1g3 circAnkib1	up	Promotes tumor growth by shaping a pro-tumorigenic microenvironment	Repressing pro-inflammatory elements by buffering activation of the pathways mediated by RIG-I, the cytosolic viral RNA sensor	SCC	36433954
circMET	up	Induces TIME and anti-PD1 therapy resistance	Regulating levels of CXCL10 and less $CD8^+$ T cell infiltration	HCC	32430013
circCCAR1	up	Causes resistance to anti-PD1 immunotherapy	Promoting the exhaustion of $CD8^+$ T cells	HCC	36932387
circZNF451	up	Induces exhaustion of cytotoxic $CD8^+$T cells, and induces macrophages polarization	Reshaping the tumor immune microenvironment by TRIM56-mediated pathway of FXR1/ELF4/IRF4 axis,	LUAD	36209117
circTRPS1	up	Promotes the tumor proliferation, migration and invasion	Regulating glutamine metabolism through the circTRPS1/miR141-3p/glutaminase-1 axis, induce REDOX imbalance and $CD8^+$T cell exhaustion	BC	35038580

abundant noncoding RNAs in the human transcriptome that are involved in all cancer hallmarks. CircRNAs can be used not only as targets for regulating tumor progression but also as potential mediators of antitumor immunity and cancer immunotherapy targets. Recent studies have demonstrated that cancer-related circRNAs play crucial roles in the formation of the immunosuppressive tumor microenvironment and immunotherapy resistance and may regulate the expression of immune checkpoints, such as PD1/PD-L1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), T cells immunoglobulin and mucin domain-3 (TIM-3), and CD47, through the ceRNA mechanism [89–91] (Fig. 2B).

CircRNAs not only can regulate the expression of PD-L1 directly but can also affect the expression of downstream molecules to regulate the expression of PD-L1 via a sponge mechanism. CircFGFR1 can directly interact with miR-381-3p to increase the expression of target gene CXCR4, which promotes NSCLC progression and resistance to anti-PD1-based therapy [92]. Hsa-circRNA-002178 can cause T cells exhaustion in cancer cells by sponging miR-34, which increases PD-L1 expression [93]. circCHST15 can directly suppress the expression of PD-L1 as a sponge for miR-155-5p and miR-194-5p to increase tumor development and immune evasion in lung cancer [94]. Overexpression of circTMTC3 and circFAM117B can increase PD-L1 expression by sponging miR-142–5, thereby reducing the cytotoxicity of $CD8^+$ T cells and promoting melanoma immune escape [95]. circRNA_001678 actives the PD1/PD-L1 pathway and triggers $CD8^+$ T cells apoptosis via the miR-326/ZEB1 axis, thus promoting NSCLC immune escape progression [96].

CircRNAs also regulate PD1/PD-L1 through interacting with proteins or other unclarified mechanisms. Has_circ_0067842 interacts with HuR, altering its nuclear translocation and thus increasing the stability of CMTM6, influencing the ubiquitination of PD-L1, preventing its degradation and promoting immune escape in BC [97]. Hypoxia-related circPRDM4 is highly expressed in HCC, inhibits $CD8^+$ T cell infiltration and promotes PD-L1 expression in the TME by regulating the interaction between HIF-1α and the promoter of CD274 [98]. CircBART2.2, encoded by Epstein–Barr virus (EBV), can bind to RIG-I and activate the transcription factors IRF3 and NF-κB, which are essential for the regulation of PD-L1, thus inhibiting T cells function and promoting tumor immune escape in nasopharyngeal carcinoma [99]. N6-methyladenosine-modified circIGF2BP3 stabilizes the OTUB1 mRNA in a PKP3-dependent way to reduce PD-L1 ubiquitination, which in turn reduces $CD8^+$ T cells responses to promote NSCLC tumor immune evasion [100]. M6A-activated circNFIX increases ovarian cancer development and immune evasion by modulating the miR-647/IL-6R/PD-L1 pathway [101].

In addition to PD1/PD-L1, circRNAs regulate numerous other immune checkpoints. Other immunomodulatory receptors, such as CTLA-4 and TIM-3, bind to ligands on tumor cells and prevent antitumoral immunity in the surrounding tissue [102]. CircQSOX1 facilitates Treg cell-based immune escape in colorectal cancer by activating the PGAM1/miR-326/miR-330-5p pathway to induce glycolysis and inactivate the anti-CTLA-4 therapeutic response [103]. CircUHRF1 inhibits NK cell function and upregulates the expression of TIM-3 via the degradation of miR-449c-5p, and circUHRF1 may drive resistance to anti-PD1 immunotherapy in HCC patients [30]. circRERE (has_circ_0009581) promotes resistance to the chemotherapy drug bortezomib in multiple myeloma by sponging miR-152-3p to upregulate the

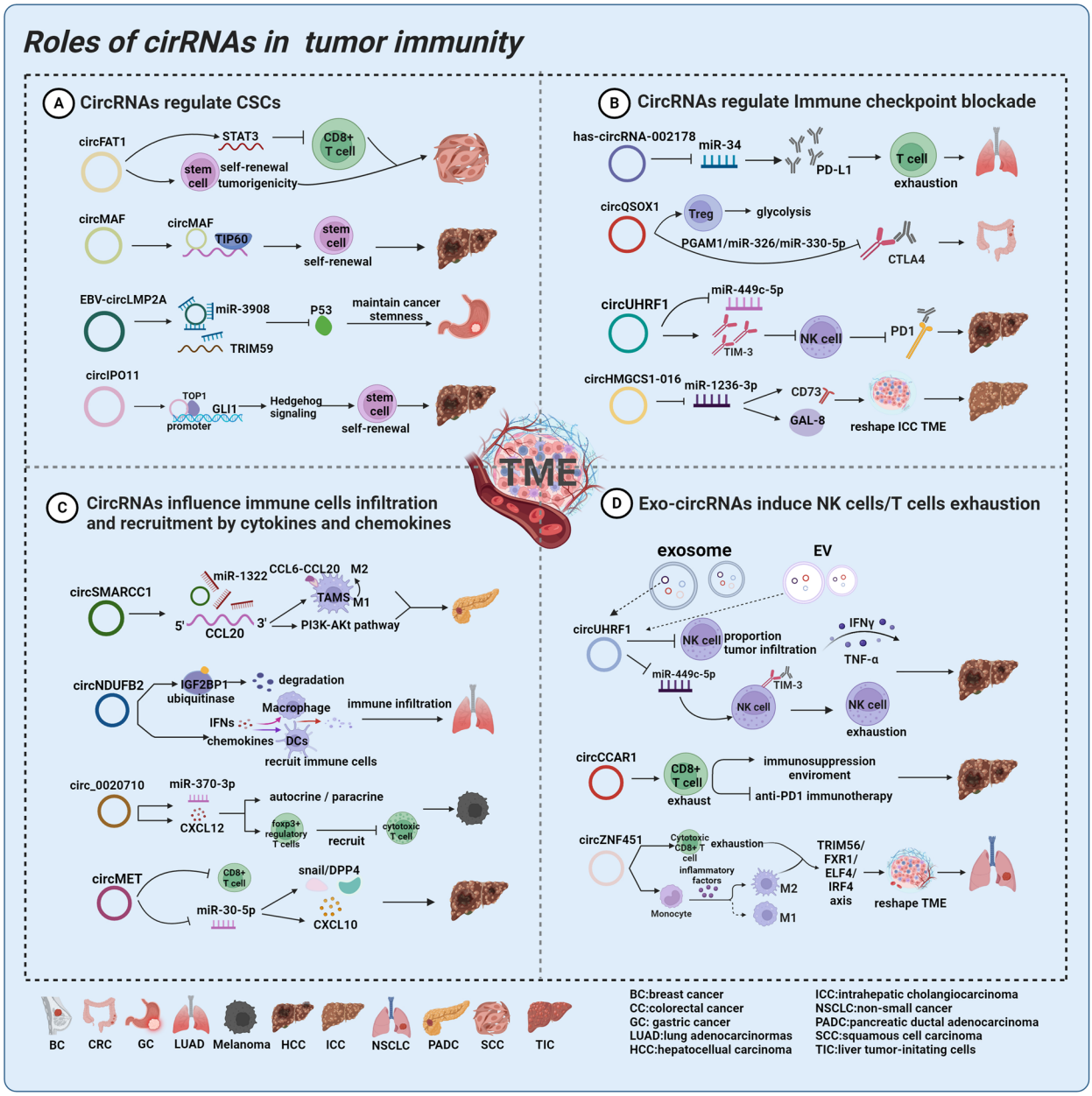

Fig. 2 Roles of circRNAs in tumor immunity. **A** CircRNAs regulate cancer stemness and affect the antitumor immune response. **B** CircRNAs regulate immune checkpoints. **C** CircRNAs influence immune cells infiltration and recruitment by cytokines and chemokines to shape the TIME. **D** Exo-circRNAs induce NK cells/T cells exhaustion. circRNAs: Circular RNAs; CSCs: Cancer stem cells; TME: tumor microenvironment

expression of CD47 [104]. CircHMGCS1-016 increases intrahepatic cholangiocarcinoma cells invasion and reduces $CD8^+$ T cells infiltration via miR-1236-3p to regulate CD73 and GAL-8 expression, thereby reshaping the TIME [105].

Immune checkpoint inhibitors (ICIs), such as PD1/PD-L1 inhibitors, are crucial strategies for cancer immunotherapy. By targeting immune checkpoints, ICIs can preferentially activate immune cells; however, their practical applicability in cancer immunotherapy is limited by primary and acquired resistance [106]. Combined utilization of piRNAs and circRNAs as innovative sensitizers to augment PD1/PD-L1 inhibitor therapy serves different purposes in regulating PD-L1 expression and modifying the immune response [107]. However, further studies are needed to verify whether circRNAs are involved in the regulation of immune checkpoints at the transcriptional, posttranscriptional, or protein levels or

at the protein modification and degradation levels. More researches are need to ascertain whether circRNAs can act as immune checkpoints directly to control immune responses against cancer cells or whether they facilitate ICI resistance by being engaged in cancer hallmarks such as cell death and proliferation; such studies could reveal whether these circRNAs can serve as new potential cancer immunotherapy targets.

CircRNAs shape the TIME

A growing body of research has demonstrated that circRNAs regulate numerous of biological processes within the TME. In addition to immune cells and immune checkpoints, the release of cytokines or chemokines also participates in the regulation of tumor immunity. Cancer-derived circRNAs are transferred into surrounding immune-related cells through transporters, thereby inhibiting the secretion of cytokines or chemokines and influencing immune cell infiltration and recruitment in tumors [108] (Fig. 2C). For example, circSMARCC1 was positively associated with the colonization of $CD68^+$/$CD163^+$/$CD206^+$ tumor-associated macrophages and CD163 expression in macrophages via the CCL20-CCR6 axis, which leads to tumor-associated macrophages infiltration and M2 polarization during prostate cancer progression [109]. CircMAPK1 can act as a scaffold that promotes the occupation of the 3'UTR of CCL5 by IGF2BP1, which significantly promotes $CD8^+$ T cells intratumoral infiltration in LUAD [110]. circNDUFB2 is involved not only in the IGF2BP3 ubiquitination and degradation, but also in the immune response to NSCLC by recruiting $CD8^+$ T cells and DCs to the TME through the secretion and nuclear translocation of chemokines and IFNs [111]. Circ_0020710 can serve as a miR-370-3p sponge to upregulate CXCL12 expression. It not only promotes the malignant activities of melanoma through autocrine and paracrine pathways, but also promotes the recruitment of immune-mediated suppressive cells ($foxp3^+$ regulatory T cells), which may suppress the immune system and reduce the invasion of cytotoxic T lymphocytes [112]. By regulating the expression of proinflammatory and interferon-related genes in sarcoma cells, circCsnk1g3 and circAnkib1 can drive the recruitment of immune cells into the tumor mass, hence promoting carcinogenesis by shaping the TME [113]. Reduced $CD8^+$ T cells infiltration and low CXCL10 levels are linked to circMET upregulation, which can induce a TIME and anti-PD1 therapy resistance in HCC [114, 115]. Chemokines bind to their receptors in the TME and can function as a link between host and tumor cells. These interactions aid in immune cell recruitment and accelerate angiogenesis and tumor progression. Blocking the binding of chemokines to receptors and downstream signaling pathways can improve immunotherapy sensitivity and efficacy, thus slowing tumor progression. Moreover, effector T cells need to respond quickly to produce sufficient cytokines, chemokines, and cytotoxic molecules to respond to inflammation and tumorigenesis [116]. Targeting circRNAs interferes with the TME by regulating cytokines, providing insights and ideas for clinical therapy to improve tumor inhibiting immune processes and drug resistance mechanisms.

Tumor cells secrete exo-circRNAs to regulate tumor immunity

Exosomes are extracellular vesicles with diameters ranging from 30 to 150 nm and have a lipid bilayer membrane structure. Both normal and pathological conditions cause nearly all cell types to produce exosomes, which are extensively distributed in the microenvironment [117–119]. The circRNAs expression levels in exosomes far exceed that of the corresponding linear RNAs. Exo-circRNA-mediated tumor cells can maintain antitumor immune processes by inducing immune cell exhaustion [120]. CircRNAs are associated with exosomes and EVs in the TIME. Certain exosomes and EVs can transfer circRNAs from donor cells to immunocytes, where circRNAs can function as tumor antigens to modulate immune responses by interfering with immune checkpoints expression and immune cells activation [121]. Furthermore, exo-circRNAs promote the entry of miRNAs into immunocytes to silence associated target genes when they are transferred from tumor cells to immunocytes (Fig. 2D). For example, cancer cell-derived exo-circUHRF1 inhibits NK cells function and tumor infiltration, inhibits the secretion of NK cell-derived IFN-γ and TNF-α and upregulates the expression of TIM-3 by reducing the expression of miR-449c-5p, which leads to NK cell exhaustion. CircUHRF1 may result in resistance to anti-PD1 immunotherapy, providing HCC patients with a potential therapeutic strategy [30]. Exo-circCCAR1 produced by HCC cells induces immunosuppression by promoting the exhaustion of $CD8^+$ T cells, which results in resistance to anti-PD1 immunotherapy. Targeting exo-circCCAR1 or CCAR1 may be a novel strategy to maximize immunotherapy efficacy in HCC patients [12]. NSCLC cells are the primary producers of exo-circUSP7, which promotes $CD8^+$ T cells exhaustion dysfunction and induces resistance to anti-PD1 immunotherapy, hence contributing to immunosuppression and providing a viable therapeutic approach for NSCLC patients [122]. Exo-circZNF451 can induce the anti-inflammatory function of macrophages and exhaustion of cytotoxic $CD8^+$ T cells and induce macrophage polarization by reshaping the TIME via the TRIM56-mediated pathway of the FXR1/ELF4/IRF4 axis, and one

potential novel biomarker for predicting LUAD sensitivity to PD1 blockade could be exo-circZNF451 [123]. Studies have shown that circTRPS1 derived from the exosomes of BC cells can regulate glutamine metabolism through the circTRPS1/miR-141-3p/glutaminase-1 axis, induce redox imbalance and $CD8^+$ T cells exhaustion in the TME, and promote the proliferation, migration and invasion of BC cells [124]. Inhibitory receptors such as PD1, T cell immunoglobulin and mucin domain 3, lymphocyte-activation gene 3, and T cells immunoreceptor with Ig and ITIM domains are expressed at high levels in both exhausted NK cells and exhausted $CD8^+$ T cells. Monotherapies that inhibit these receptors may be beneficial for cancer patients. Exo-circRNA-targeted monotherapies can improve the delivery of drugs and increase inhibitory receptor resistance to immunotherapy. One potentially effective tactic to reduce tumor spread and increase immunotherapy sensitivity is to target exo-circRNAs.

Applications and prospects of circRNA-based vaccines in cancer immunotherapy

Immunotherapy, including immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR) T cells therapy, tumor vaccines, and other innovative immunotherapy drugs, has been a prominent topic of tumor therapy research in the past few years both domestically and abroad [125, 126]. However, with the accumulation of more clinical data, these immunotherapies have been found to have many shortcomings, such as a low overall treatment response rate [127]. In clinical studies of cancer immunotherapies, tumor vaccines have demonstrated considerable promise. By customized design, these RNA vaccines can train the immune system to target specific neoantigens in individual cancer patients, thereby effectively attacking residual tumor cells [128]. Researchers are explored the use of mRNA vaccines and post-operative treatment, these vaccines are expected to train the immune system to identify and eliminated residual cancer cells (such as melanoma, colorectal cancer, and pancreatic cancer), thereby preventing disease from recurrence [129] The rapid development of mRNA technology and its applications in the fields of medicine, pharmacy, material science, may benefit from its advantages of not having to pass to the cell nucleus and not to interfere with the human genome [130]. However, mRNA vaccines still face some challenges. First, the stability of mRNA is an important issue, because mRNA is easily degraded by enzymes in the body, which affects its effectiveness. The large molecular mass and single chain structure of mRNA lead to its instability and therefore requires chemical modification to ensure its function. Secondly, mRNA vaccines may trigger an immune response, leading to side effects. In addition, mass production of mRNA vaccines is also a technical challenge, and production costs and supply chain issues need to be addressed.

The most promising cancer vaccine subtypes are circRNA-based vaccines. circRNA vaccines offer a novel solution to the limitations of mRNAs vaccines because that they have lower immunogenicity/cytotoxicity abilities and can boost the immune response to tumor antigens in terms of strength, breadth, quality, and duration [131, 132]. CircRNA vaccines have been developed to elicit effective both B cells and T cells immune responses against SARS-CoV-2 in mice and rhesus macaques [133]. Conventional CAR T cells therapy requires high-cost, long-term preparation, while having cleansing bone marrow, cell storm, targeted, non-tumor cell destruction, are not widely used. The pre-printed article published by Qian Yuan et. shown that circRNA-based in vivo CAR might enable higher and more durable CAR proteins on the cell membrane of functional immune cells than mRNAs. Combination of circRNA-based in vivo CAR and vaccines exhibited synergistically to enhance antitumor activity, holds the potential to become an upgraded off-the-shelf immunotherapy. According to a recent study, innate immunity and adjuvant action may be inhibited by m6A mutation of circFOREIGN, which is identified as "self" via YTHDF2 [134]. Clinically sophisticated carriers known as lipid nanoparticles (LNPs) can be utilized to package and transport RNA to specific organs [135]. An immune desert tumor model in B16 cells demonstrated that the circRNA-LNP vaccine can significantly suppress the immune-rejected tumor process, cause immune-desert tumors to regress, and prevent the spread of cancer cells. These findings demonstrate the superiority of cancer RNA vaccines over currently available cancer immunotherapies [136]. Chen et.al. developed a circRNA vaccine encoding HCC specific tumor neoantigen Ptpn2_I383T peptide (namely PTPN2), and proved the stability of the circRNA vaccine, and can continuously express the antigen protein, effectively stimulate a strong anti-tumor immune response, and significantly inhibit tumor growth [137]. The newly discovered circRNA vaccine represents a new safe, stable and simple immunotherapy option. CircRNA vaccines can increase the immunogenicity of cancer vaccines, which allows immunostimulatory proteins to more effectively activate the immune response during cancer immunotherapy. The potential of circRNAs as a source of new antigens in immunological agents was shown by an analysis of the candidate peptides from circRAPGEF and circMYH9, which are elevated in colorectal cancer and can trigger antigen-specific T cells reactions and proliferation [138]. CircRNAs also preserve antigen molecules (such

as cytokines and small antibody fragments) for extended periods, which may stimulate the infiltration of immune cells to the tumor location. Specific circRNA vaccines can be developed for individualized and precise cancer treatment. These findings provide crucial insights into the use of circRNAs as key therapeutic approaches and synergistic inhibitors of tumor development in conjunction with established tumor immunotherapies. Moreover, circRNAs have longer sequences than miRNAs and siRNAs, and off-target effects are greatly reduced when circRNAs are used for targeted tumor immunotherapy. The targeting of circRNAs could increase the antitumor response. Treatments that directly control the levels of certain circRNAs in tumors via gene-editing therapy may have fewer side effects and may be more effective than chemoradiotherapy or chemical drugs.

Conclusions and outlook

Increasing researches have demonstrated that circRNAs are essential for several in processes in tumors. CircRNAs have been confirmed to be critical for tumor-related processes, including growth, angioplasty and metastasis, and can further regulate the TME and promote antitumor immunity. Recently, circRNAs were discovered to be involved in antitumor immune responses in several ways. For example, circRNAs can activate immune cells, especially CSCs, or shape the TIME by regulating the expression of immune checkpoints and secretion of cytokines and chemokines to regulate antitumor immunity. Tumor cells that secrete exo-circRNAs can also induce NK cells/T cells exhaustion in the TME to cause an immune evasion. The broad importance of circRNAs in cancer immunotherapy is gradually becoming known. The combination of circRNAs and ICIs for sensitizing tumors to immunotherapy or the use of circRNA vaccines alone represent key steps forward in our understanding of tumor immunity and immunotherapy resistance. CircRNA vaccines form bench to bedside will bring benefit to more tumor patients.

From a clinical perspective, circRNAs have shown promise as valid biomarkers for diagnosis and treatment in various tumors, including early detection, diagnosis, prognosis, and therapeutic response. Due to their stability in circulation, circRNAs may serve as early indicators of tumor, allowing for timely diagnosis before the onset of symptoms. Certain circRNAs may be specific to particular tumor, helping to confirm diagnoses when traditional methods yield ambiguous results. Expression levels of specific circRNAs may correlate with tumor aggressiveness, offering prognostic insights and helping to stratify patients according to risk. Some circRNAs may predict how tumors respond to specific therapies, allowing for more personalized treatment approaches. Engineered circRNAs could be designed to restore or enhance tumor-suppressive pathways, potentially counteracting oncogenic signals. However, there are some questions and challenges related to circRNAs as therapeutic target.

First, tumor immune escape is a difficult problem in tumor therapy. Several treatments, including ICIs, have failed to address this problem. By making full use of the functions of circRNAs in tumor immunity, the combination of circRNAs with existing clinical treatment methods (such as PD1/PD-L1 ICIs to enhance cancer immunotherapy sensitivity), more effectively activates the host immune system and prevents the immune escape of tumors, highlighting a direction worthy of further research. Second, the stability, conservation and tissue specificity of circRNAs can be fully utilized to produce targeted drugs against certain components of tumor cells or the TME to greatly limit adverse effects on normal cells in the body, improve the recovery rate of patients, and reduce adverse effects for treatment while improving patients' quality of life. For example, LNP-coated circRNA-targeting vectors regulate circRNA expression in tumors. CircRNAs are persisitent markers producedin response to immunotherapy and can also be used as tumor vaccine targets to improve the antitumor immune response.

Although the circRNA sector shows significant prospects for clinical transformation, several scientific problems and challenges in immune processes must be solved before this translational promise can be fulfilled. 1) Only 10%-20% of circRNAs are conserved between humans and mice. This property limits the translation of findings on poorly conserved circRNAs in animal models to patients. 2) Current technical restrictions on circRNA synthesis include the low cyclization efficiency and costly enzymes and reagents. The special technical problems related to the cyclic structure include the low reproducibility of biomarkers and the increased complexity of cancer immunotherapy development. 3) Many circRNA biomarkers have been poorly developed. At present, the determination of tumor markers mostly depends on comparisons of tumor-adjacent tissues or normal tissues, while changes in the TME, such as the secretion of immune cells, inflammatory factors and cytokines, are rarely comprehensively considered. Whether the presence of ferroptosis-related circRNAs or immune-related circRNAs, similar to long non-coding RNAs, can predict tumor prognosis, immune cell infiltration, and sensitivity to immunotherapy remains unclear.

Whether circRNAs are engaged in the regulation of tumor immune checkpoints and the TIME at multiple levels (transcription, posttranscription, translation, and protein modification), as well as their effects on the

evolution of immune cells themselves, remains to be further explored. Strategies for utilizing these characteristics and roles of circRNAs to boost the immune response, prevent the immune escape of tumors, improve the efficacy of immunotherapy, and ultimately realize the translation of clinical still need to be further explored.

Abbreviations

BC	Breast cancer
BCa	Bladder cancer
CDR1as/ciRS-7	Cerebellar degeneration-related protein-1
CeRNA	Competing endogenous RNA
CiRNAs	Circular intronic RNAs
CircRNA	Circular RNA
CSCs	Cancer stem cells
EcircRNAs	Exonic circRNAs
ElciRNAs	Exonic-intronic circRNAs
EVs	Extracellular vesicle
GC	Gastric cancer
HCC	Hepatocellular carcinoma
HuR	Human antigen R
IRES	Internal ribosome entry site
LNP	Lipid nanoparticles
LUAD	Lung adenocarcinomas
miRNA	MicroRNA
NK cell	Natural killer cell
NSCLC	Non-small cell lung cancer
PD1	Programmed death
PD-L1	Programmed death ligand 1
RBPs	RNA binding proteins
TIME	Tumor immunosuppressive microenvironment
TME	Tumor microenvironment

Authors' contributions

WJ Z, C X, wrote the paper; ZP Y, JS Z, W P, HM L, SB Q edited the paper; WJ Z, X Z, SB Q made the figures; SB Q and KS T final edited the paper.

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Data availability

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Declarations

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References

1. Li X, Yang L, Chen LL. The biogenesis, functions, and challenges of circular RNAs. Mol Cell. 2018;71(3):428–42.
2. Santer L, Bär C, Thum T. Circular RNAs: a novel class of functional RNA molecules with a therapeutic perspective. Mol Ther. 2019;27(8):1350–63.
3. Qu S, et al. Circular RNA: a new star of noncoding RNAs. Cancer Lett. 2015;365(2):141–8.
4. Kristensen LS, et al. The emerging roles of circRNAs in cancer and oncology. Nat Rev Clin Oncol. 2022;19(3):188–206.
5. Dong Y, et al. Circular RNAs in cancer: an emerging key player. J Hematol Oncol. 2017;10(1):2.
6. Aktaş T, et al. DHX9 suppresses RNA processing defects originating from the Alu invasion of the human genome. Nature. 2017;544(7648):115–9.
7. Vo JN, et al. The landscape of circular RNA in cancer. Cell. 2019;176(4):869-881.e13.
8. Wang J, et al. ncRNA-encoded peptides or proteins and cancer. Mol Ther. 2019;27(10):1718–25.
9. Li J, et al. Circular RNAs in cancer: biogenesis, function, and clinical significance. Trends Cancer. 2020;6(4):319–36.
10. Qadir J, et al. CircRNAs regulate the crosstalk between inflammation and tumorigenesis: the bilateral association and molecular mechanisms. Mol Ther. 2023;31(6):1514–32.
11. Chen X, et al. Circular RNA circHIPK3 modulates autophagy via MIR124-3p-STAT3-PRKAA/AMPKα signaling in STK11 mutant lung cancer. Autophagy. 2020;16(4):659–71.
12. Zhu YJ, et al. Circular RNAs negatively regulate cancer stem cells by physically binding FMRP against CCAR1 complex in hepatocellular carcinoma. Theranostics. 2019;9(12):3526–40.
13. Bi J, et al. Circular RNA circ-ZKSCAN1 inhibits bladder cancer progression through miR-1178-3p/p21 axis and acts as a prognostic factor of recurrence. Mol Cancer. 2019;18(1):133.
14. Bi J, et al. Correction to: circular RNA circ-ZKSCAN1 inhibits bladder cancer progression through miR-1178-3p/p21 axis and acts as a prognostic factor of recurrence. Mol Cancer. 2020;19(1):148.
15. Hong W, et al. Circular RNA circ-CPA4/ let-7 miRNA/PD-L1 axis regulates cell growth, stemness, drug resistance and immune evasion in non-small cell lung cancer (NSCLC). J Exp Clin Cancer Res. 2020;39(1):149.
16. Li Z, et al. Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol. 2015;22(3):256–64.
17. Shi X, et al. circRNAs and exosomes: a mysterious frontier for human cancer. Mol Ther Nucleic Acids. 2020;19:384–92.
18. Du WW, et al. Promotion of tumor progression by exosome transmission of circular RNA circSKA3. Mol Ther Nucleic Acids. 2022;27:276–92.
19. Lin Z, et al. Exosomal circRNAs in cancer: implications for therapy resistance and biomarkers. Cancer Lett. 2023;566:216245.
20. Tuo B, et al. Roles of exosomal circRNAs in tumour immunity and cancer progression. Cell Death Dis. 2022;13(6):539.
21. Beilerli A, et al. Circular RNAs as biomarkers and therapeutic targets in cancer. Semin Cancer Biol. 2022;83:242–52.
22. Wen G, Zhou T, Gu W. The potential of using blood circular RNA as liquid biopsy biomarker for human diseases. Protein Cell. 2021;12(12):911–46.
23. Singh AK, McGuirk JP. CAR T cells: continuation in a revolution of immunotherapy. Lancet Oncol. 2020;21(3):e168–78.
24. Xu S, et al. Immunotherapy for glioma: current management and future application. Cancer Lett. 2020;476:1–12.
25. Zhang M, et al. Monitoring checkpoint inhibitors: predictive biomarkers in immunotherapy. Front Med. 2019;13(1):32–44.
26. He Y, et al. The role of circular RNA in tumor microenvironment and immunotherapy. Int J Biol Macromol. 2023;242(Pt 4):124929.
27. Guan L, et al. Regulation of the tumor immune microenvironment by cancer-derived circular RNAs. Cell Death Dis. 2023;14(2):132.
28. Xu Z, et al. Role of exosomal non-coding RNAs from tumor cells and tumor-associated macrophages in the tumor microenvironment. Mol Ther. 2022;30(10):3133–54.
29. Ma Y, et al. The role of circular RNAs in regulating resistance to cancer immunotherapy: mechanisms and implications. Cell Death Dis. 2024;15(5):312.
30. Zhang PF, et al. Cancer cell-derived exosomal circUHRF1 induces natural killer cell exhaustion and may cause resistance to anti-PD1 therapy in hepatocellular carcinoma. Mol Cancer. 2020;19(1):110.

31. Jia L, Wang Y, Wang CY. circFAT1 promotes cancer stemness and immune evasion by promoting STAT3 activation. Adv Sci (Weinh). 2021;8(13):2003376.
32. Sanger HL, et al. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proc Natl Acad Sci U S A. 1976;73(11):3852–6.
33. Hsu MT, Coca-Prados M. Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells. Nature. 1979;280(5720):339–40.
34. Pisignano G, et al. Going circular: history, present, and future of circRNAs in cancer. Oncogene. 2023;42(38):2783–800.
35. Li R, et al. CircRNA: a rising star in gastric cancer. Cell Mol Life Sci. 2020;77(9):1661–80.
36. Jeck WR, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA. 2013;19(2):141–57.
37. Alhasan AA, et al. Circular RNA enrichment in platelets is a signature of transcriptome degradation. Blood. 2016;127(9):e1–11.
38. Kramer MC, et al. Combinatorial control of Drosophila circular RNA expression by intronic repeats, hnRNPs, and SR proteins. Genes Dev. 2015;29(20):2168–82.
39. Xi Y, et al. CircBCAR3 accelerates esophageal cancer tumorigenesis and metastasis via sponging miR-27a-3p. Mol Cancer. 2022;21(1):145.
40. Conn SJ, et al. The RNA binding protein quaking regulates formation of circRNAs. Cell. 2015;160(6):1125–34.
41. Memczak S, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013;495(7441):333–8.
42. Huang A, et al. Circular RNA-protein interactions: functions, mechanisms, and identification. Theranostics. 2020;10(8):3503–17.
43. Lyu L, et al. Regulatory mechanisms, functions, and clinical significance of CircRNAs in triple-negative breast cancer. J Hematol Oncol. 2021;14(1):41.
44. Huang C, et al. A length-dependent evolutionarily conserved pathway controls nuclear export of circular RNAs. Genes Dev. 2018;32(9–10):639–44.
45. Zhang Y, et al. Circular intronic long noncoding RNAs. Mol Cell. 2013;51(6):792–806.
46. Chen L, Shan G. Circular RNAs remain peculiarly unclear in biogenesis and function. Sci China Life Sci. 2015;58(6):616–8.
47. Preußer C, et al. Selective release of circRNAs in platelet-derived extracellular vesicles. J Extracell Vesicles. 2018;7(1):1424473.
48. Kristensen LS, et al. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet. 2019;20(11):675–91.
49. Feng XY, et al. New insight into circRNAs: characterization, strategies, and biomedical applications. Exp Hematol Oncol. 2023;12(1):91.
50. Della BE, Koch J, Baerenfaller K. Translation and emerging functions of non-coding RNAs in inflammation and immunity. Allergy. 2022;77(7):2025–37.
51. Rong Z, et al. Circular RNA in pancreatic cancer: a novel avenue for the roles of diagnosis and treatment. Theranostics. 2021;11(6):2755–69.
52. Yang L, Wilusz JE, Chen LL. Biogenesis and regulatory roles of circular RNAs. Annu Rev Cell Dev Biol. 2022;38:263–89.
53. Hansen TB, Kjems J, Damgaard CK. Circular RNA and miR-7 in cancer. Cancer Res. 2013;73(18):5609–12.
54. Miao Z, et al. Hsa_circ_0136666 stimulates gastric cancer progression and tumor immune escape by regulating the miR-375/PRKDC axis and PD-L1 phosphorylation. Mol Cancer. 2023;22(1):205.
55. Liu Q, et al. Targeting the androgen receptor to enhance NK cell killing efficacy in bladder cancer by modulating ADAR2/circ_0001005/PD-L1 signaling. Cancer Gene Ther. 2022;29(12):1988–2000.
56. Shi M, et al. Hsa_circ_0007456 regulates the natural killer cell-mediated cytotoxicity toward hepatocellular carcinoma via the miR-6852-3p/ICAM-1 axis. Cell Death Dis. 2021;12(1):94.
57. Huang M, Huang X, Huang N. Exosomal circGSE1 promotes immune escape of hepatocellular carcinoma by inducing the expansion of regulatory T cells. Cancer Sci. 2022;113(6):1968–83.
58. Sun Z, et al. Exosomal circPOLQ promotes macrophage M2 polarization via activating IL-10/STAT3 axis in a colorectal cancer model. J Immunother Cancer. 2024;12(5):e008491.
59. Wei J, et al. Understanding the roles and regulation patterns of circRNA on its host gene in tumorigenesis and tumor progression. J Exp Clin Cancer Res. 2023;42(1):86.
60. Ji P, et al. Expanded expression landscape and prioritization of circular RNAs in mammals. Cell Rep. 2019;26(12):3444-3460.e5.
61. Chen G, et al. Circular RNA circStag1 promotes bone regeneration by interacting with HuR. Bone Res. 2022;10(1):32.
62. Chen Y, et al. Circular RNA circAGO2 drives cancer progression through facilitating HuR-repressed functions of AGO2-miRNA complexes. Cell Death Differ. 2019;26(7):1346–64.
63. Liu H, et al. Circular RNA circDLC1 inhibits MMP1-mediated liver cancer progression via interaction with HuR. Theranostics. 2021;11(3):1396–411.
64. Li F, et al. Circular RNAs in cancer: limitations in functional studies and diagnostic potential. Semin Cancer Biol. 2021;75:49–61.
65. Du WW, et al. Induction of tumor apoptosis through a circular RNA enhancing Foxo3 activity. Cell Death Differ. 2017;24(2):357–70.
66. He Z, et al. Autophagy-associated circRNA circATG7 facilitates autophagy and promotes pancreatic cancer progression. Cell Death Dis. 2022;13(3):233.
67. Chen Z, et al. circREEP3 drives colorectal cancer progression via activation of FKBP10 transcription and restriction of antitumor immunity. Adv Sci (Weinh). 2022;9(13):e2105160.
68. Zhang Y, et al. A tumor suppressor protein encoded by circKEAP1 inhibits osteosarcoma cell stemness and metastasis by promoting vimentin proteasome degradation and activating anti-tumor immunity. J Exp Clin Cancer Res. 2024;43(1):52.
69. Zhao Z, et al. circEIF3I facilitates the recruitment of SMAD3 to early endosomes to promote TGF-β signalling pathway-mediated activation of MMPs in pancreatic cancer. Mol Cancer. 2023;22(1):152.
70. Chen N, et al. A novel FLI1 exonic circular RNA promotes metastasis in breast cancer by coordinately regulating TET1 and DNMT1. Genome Biol. 2018;19(1):218.
71. Wang L, et al. Circular RNA circRHOT1 promotes hepatocellular carcinoma progression by initiation of NR2F6 expression. Mol Cancer. 2019;18(1):119.
72. Zhang M, et al. A novel protein encoded by the circular form of the SHPRH gene suppresses glioma tumorigenesis. Oncogene. 2018;37(13):1805–14.
73. Legnini I, et al. Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. Mol Cell. 2017;66(1):22-37.e9.
74. Yang Y, et al. Extensive translation of circular RNAs driven by N(6)-methyladenosine. Cell Res. 2017;27(5):626–41.
75. Li K, et al. Enhancement of TKI sensitivity in lung adenocarcinoma through m6A-dependent translational repression of Wnt signaling by circ-FBXW7. Mol Cancer. 2023;22(1):103.
76. Ma Z, et al. Circular RNAs in the tumour microenvironment. Mol Cancer. 2020;19(1):8.
77. Xu Y, et al. A circular RNA, cholangiocarcinoma-associated circular RNA 1, contributes to cholangiocarcinoma progression, induces angiogenesis, and disrupts vascular endothelial barriers. Hepatology. 2021;73(4):1419–35.
78. Kristensen LS, et al. Circular RNAs in cancer: opportunities and challenges in the field. Oncogene. 2018;37(5):555–65.
79. Abdelmoez A, et al. Screening and identification of molecular targets for cancer therapy. Cancer Lett. 2017;387:3–9.
80. Loda A, et al. Cancer stem-like cells in uveal melanoma: novel insights and therapeutic implications. Biochim Biophys Acta Rev Cancer. 2024;1879(3):189104.
81. Clara JA, et al. Targeting signalling pathways and the immune microenvironment of cancer stem cells - a clinical update. Nat Rev Clin Oncol. 2020;17(4):204–32.
82. Alqarni A, et al. Challenges and opportunities for cancer stem cell-targeted immunotherapies include immune checkpoint inhibitor, cancer stem cell-dendritic cell vaccine, chimeric antigen receptor immune cells, and modified exosomes. J Biochem Mol Toxicol. 2024;38(6):e23719.
83. Gholami A. Cancer stem cell-derived exosomes in CD8(+) T cell exhaustion. Int Immunopharmacol. 2024;137:112509.
84. Miao Y, et al. Adaptive immune resistance emerges from tumor-initiating stem cells. Cell. 2019;177(5):1172-1186.e14.

85. Chen Z, et al. Circular RNA cia-MAF drives self-renewal and metastasis of liver tumor-initiating cells via transcription factor MAFF. J Clin Invest. 2021;131(19):e148020.
86. Gong LP, et al. Epstein-Barr virus-derived circular RNA LMP2A induces stemness in EBV-associated gastric cancer. EMBO Rep. 2020;21(10):e49689.
87. Gu Y, et al. Circular RNA circIPO11 drives self-renewal of liver cancer initiating cells via Hedgehog signaling. Mol Cancer. 2021;20(1):132.
88. Lin X, et al. TV-circRGPD6 nanoparticle suppresses breast cancer stem cell-mediated metastasis via the miR-26b/YAF2 axis. Mol Ther. 2021;29(1):244–62.
89. Hu T, et al. Cancer-derived non-coding RNAs endow tumor microenvironment with immunosuppressive properties. Wiley Interdiscip Rev RNA. 2023:e1822. https://doi.org/10.1002/wrna.1822.
90. Jiang W, et al. The role of lncRNAs and circRNAs in the PD-1/PD-L1 pathway in cancer immunotherapy. Mol Cancer. 2021;20(1):116.
91. Shibru B, et al. Detection of immune checkpoint receptors - a current challenge in clinical flow cytometry. Front Immunol. 2021;12:694055.
92. Zhang PF, et al. Circular RNA circFGFR1 promotes progression and anti-PD-1 resistance by sponging miR-381-3p in non-small cell lung cancer cells. Mol Cancer. 2019;18(1):179.
93. Wang J, et al. circRNA-002178 act as a ceRNA to promote PDL1/PD1 expression in lung adenocarcinoma. Cell Death Dis. 2020;11(1):32.
94. Yang J, et al. Circular RNA CHST15 sponges miR-155-5p and miR-194-5p to promote the immune escape of lung cancer cells mediated by PD-L1. Front Oncol. 2021;11:595609.
95. Dong Y, et al. Identification of CircRNA signature associated with tumor immune infiltration to predict therapeutic efficacy of immunotherapy. Nat Commun. 2023;14(1):2540.
96. Tian Q, et al. Immunomodulatory functions of the circ_001678/miRNA-326/ZEB1 axis in non-small cell lung cancer via the regulation of PD-1/PD-L1 pathway. Hum Mol Genet. 2022;31(23):4094–106.
97. Li J, et al. Circular RNA hsa_circ_0067842 facilitates tumor metastasis and immune escape in breast cancer through HuR/CMTM6/PD-L1 axis. Biol Direct. 2023;18(1):48.
98. Chen ZQ, et al. Hypoxia-associated circPRDM4 promotes immune escape via HIF-1α regulation of PD-L1 in hepatocellular carcinoma. Exp Hematol Oncol. 2023;12(1):17.
99. Ge J, et al. Epstein-Barr virus-encoded circular RNA CircBART2.2 promotes immune escape of nasopharyngeal carcinoma by regulating PD-L1. Cancer Res. 2021;81(19):5074–88.
100. Liu Z, et al. N(6)-methyladenosine-modified circIGF2BP3 inhibits CD8(+) T-cell responses to facilitate tumor immune evasion by promoting the deubiquitination of PD-L1 in non-small cell lung cancer. Mol Cancer. 2021;20(1):105.
101. Wang R, et al. m6A-modified circNFIX promotes ovarian cancer progression and immune escape via activating IL-6R/JAK1/STAT3 signaling by sponging miR-647. Int Immunopharmacol. 2023;124(Pt A):110879.
102. Olaoba OT, et al. Of immune checkpoint maladies and remedies: the throwing of jabs in the oncogenic ring of PDAC. Biochim Biophys Acta Rev Cancer. 2021;1875(1):188483.
103. Liu Z, et al. N6-methyladenosine-modified circular RNA QSOX1 promotes colorectal cancer resistance to anti-CTLA-4 therapy through induction of intratumoral regulatory T cells. Drug Resist Updat. 2022;65:100886.
104. Fang W, et al. CircRERE confers the resistance of multiple myeloma to bortezomib depending on the regulation of CD47 by exerting the sponge effect on miR-152-3p. J Bone Oncol. 2021;30:100381.
105. Xu YP, et al. circHMGCS1-016 reshapes immune environment by sponging miR-1236-3p to regulate CD73 and GAL-8 expression in intrahepatic cholangiocarcinoma. J Exp Clin Cancer Res. 2021;40(1):290.
106. Meng L, et al. Mechanisms of immune checkpoint inhibitors: insights into the regulation of circular RNAS involved in cancer hallmarks. Cell Death Dis. 2024;15(1):3.
107. Han R, et al. Synergistic immunoregulation: harnessing CircRNAs and PiRNAs to amplify PD-1/PD-L1 inhibition therapy. Int J Nanomedicine. 2024;19:4803–34.
108. Zhang Q, et al. Roles of circRNAs in the tumour microenvironment. Mol Cancer. 2020;19(1):14.
109. Xie T, et al. CircSMARCC1 facilitates tumor progression by disrupting the crosstalk between prostate cancer cells and tumor-associated macrophages via miR-1322/CCL20/CCR6 signaling. Mol Cancer. 2022;21(1):173.
110. Zhao F, et al. CircMAPK1 promoted CD8 + T cell infiltration in LUAD by improving the IGF2BP1 dependent CCL5 upregulation. Int Immunopharmacol. 2024;127:111267.
111. Li B, et al. circNDUFB2 inhibits non-small cell lung cancer progression via destabilizing IGF2BPs and activating anti-tumor immunity. Nat Commun. 2021;12(1):295.
112. Wei CY, et al. Circular RNA circ_0020710 drives tumor progression and immune evasion by regulating the miR-370-3p/CXCL12 axis in melanoma. Mol Cancer. 2020;19(1):84.
113. Piras R, et al. circCsnk1g3- and circAnkib1-regulated interferon responses in sarcoma promote tumorigenesis by shaping the immune microenvironment. Nat Commun. 2022;13(1):7243.
114. Huang XY, et al. Circular RNA circMET drives immunosuppression and anti-PD1 therapy resistance in hepatocellular carcinoma via the miR-30-5p/snail/DPP4 axis. Mol Cancer. 2020;19(1):92.
115. Huang XY, et al. Correction: circular RNA circMET drives immunosuppression and anti-PD1 therapy resistance in hepatocellular carcinoma via the miR-30-5p/snail/DPP4 axis. Mol Cancer. 2024;23(1):44.
116. Salerno F, Turner M, Wolkers MC. Dynamic post-transcriptional events governing CD8(+) T cell homeostasis and effector function. Trends Immunol. 2020;41(3):240–54.
117. Ruan S, et al. Extracellular vesicles as an advanced delivery biomaterial for precision cancer immunotherapy. Adv Healthc Mater. 2022;11(5):e2100650.
118. Zhang M, et al. Engineered exosomes from different sources for cancer-targeted therapy. Signal Transduct Target Ther. 2023;8(1):124.
119. Pan Y, et al. Extracellular vesicles as delivery shippers for noncoding RNA-based modulation of angiogenesis: insights from ischemic stroke and cancer. Small. 2023;19(17):e2205739.
120. Liu QW, He Y, Xu WW. Molecular functions and therapeutic applications of exosomal noncoding RNAs in cancer. Exp Mol Med. 2022;54(3):216–25.
121. Muskan M, et al. Therapeutic potential of RNA-enriched extracellular vesicles: the next generation in RNA delivery via biogenic nanoparticles. Mol Ther. 2024:S1525-0016(24)00094-7. https://doi.org/10.1016/j.ymthe.2024.02.025.
122. Chen SW, et al. Cancer cell-derived exosomal circUSP7 induces CD8(+) T cell dysfunction and anti-PD1 resistance by regulating the miR-934/SHP2 axis in NSCLC. Mol Cancer. 2021;20(1):144.
123. Gao J, et al. Exosomal circZNF451 restrains anti-PD1 treatment in lung adenocarcinoma via polarizing macrophages by complexing with TRIM56 and FXR1. J Exp Clin Cancer Res. 2022;41(1):295.
124. Yang C, et al. Exosome-derived circTRPS1 promotes malignant phenotype and CD8+ T cell exhaustion in bladder cancer microenvironments. Mol Ther. 2022;30(3):1054–70.
125. Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. Annu Rev Pathol. 2021;16:223–49.
126. Hong E, Dobrovolskaia MA. Addressing barriers to effective cancer immunotherapy with nanotechnology: achievements, challenges, and roadmap to the next generation of nanoimmunotherapeutics. Adv Drug Deliv Rev. 2019;141:3–22.
127. Schoenfeld AJ, Hellmann MD. Acquired resistance to immune checkpoint inhibitors. Cancer Cell. 2020;37(4):443–55.
128. Chehelgerdi M, Chehelgerdi M. The use of RNA-based treatments in the field of cancer immunotherapy. Mol Cancer. 2023;22(1):106.
129. Dolgin E. How personalized cancer vaccines could keep tumours from coming back. Nature. 2024;630(8016):290–2.
130. Lorentzen CL, et al. Clinical advances and ongoing trials on mRNA vaccines for cancer treatment. Lancet Oncol. 2022;23(10):e450–8.
131. Bowen WS, et al. Current challenges for cancer vaccine adjuvant development. Expert Rev Vaccines. 2018;17(3):207–15.
132. Niu D, Wu Y, Lian J. Circular RNA vaccine in disease prevention and treatment. Signal Transduct Target Ther. 2023;8(1):341.
133. Qu L, et al. Circular RNA vaccines against SARS-CoV-2 and emerging variants. Cell. 2022;185(10):1728-1744.e16.

134. Chen YG, et al. N6-methyladenosine modification controls circular RNA immunity. Mol Cell. 2019;76(1):96-109.e9.
135. Loan YT, et al. Clinical delivery of circular RNA: lessons learned from RNA drug development. Adv Drug Deliv Rev. 2023;197:114826.
136. Li H, et al. Circular RNA cancer vaccines drive immunity in hard-to-treat malignancies. Theranostics. 2022;12(14):6422–36.
137. Wang F, et al. Circular RNA-based neoantigen vaccine for hepatocellular carcinoma immunotherapy. MedComm (2020). 2024;5(8):e667.
138. Ren Y, et al. Circular RNA as a source of neoantigens for cancer vaccines. J Immunother Cancer. 2024;12(3):e008402.

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