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Review

Review on circular RNAs and new insights into their roles in cancer

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ABSTRACT

Circular RNAs (circRNAs) are a very interesting class of conserved single-stranded RNA molecules derived from exonic or intronic sequences by precursor mRNA back-splicing. Unlike canonical linear RNAs, circRNAs form covalently closed, continuous stable loops without a 5′end cap and 3′end poly(A) tail, and therefore are resistant to exonuclease digestion. The majority of circRNAs are highly abundant, and conserved across different species with a tissue or developmental-stage-specific expression. circRNAs have been shown to play important roles as microRNA sponges, regulators of gene splicing and transcription, RNA-binding protein sponges and protein/peptide translators. Emerging evidence reveals that circRNAs function in various human diseases, particularly cancers, and may function as better predictive biomarkers and therapeutic targets for cancer treatment. In consideration of their potential clinical relevance, circRNAs have become a new research hotspot in the field of tumor pathology. In the present study, the current understanding of the biogenesis, characteristics, databases, research methods, biological functions subcellular distribution, epigenetic regulation, extracellular transport and degradation of circRNAs was discussed. In particular, the multiple databases and methods involved in circRNA research were first summarized, and the recent advances in determining the potential roles of circRNAs in tumor growth, migration and invasion, which render circRNAs better predictive biomarkers, were described. Furthermore, future perspectives for the clinical application of circRNAs in the management of patients with cancer were proposed, which could provide new insights into circRNAs in the future.

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Abbreviations: RNA, ribonucleic acid; circRNAs, circular RNAs; RBP, RNA-binding protein; ncRNAs, noncoding RNAs; snRNA, small nuclear RNA; rRNA, ribosomal RNA; miRNAs, microRNAs; siRNAs, small interfering RNAs; lncRNAs, long ncRNA; RNase, ribonuclease; UTR, untranslated regions; ecircRNAs, exonic circular RNAs; ciRNAs, circular intronic RNAs; EIciRNAs, exon–intron RNAs; tricRNAs, tRNA intronic circRNAs; ceRNAs, endogenous RNAs; MER, miRNA response elements; ciRS-7, circular RNA sponge for miR-7; HCC, hepatocellular carcinoma; CRC, colorectal cancer; PCR, polymerase chain reaction; qPCR, quantitative PCR; RT-PCR, reverse transcription-PCR; GC, gastric cancer; TNM, tumor node metastases; AML, acute myloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; MM, multiple myeloma; LUAD, lung adenocarcinoma; EMT, epithelial-mesenchymal transition; NSCLC, non-small cell lung cancer; ccRCC, clear cell renal cell carcinoma; ISH, in situ hybridization; BSJ, back-splice junction; PDAC, pancreatic ductal adenocarcinoma.

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1. Introduction

Circular RNAs (circRNAs) were initially found in a plant-based virus in 1976 and described as "covalently closed circRNAs molecules"[1,2]. However, circRNAs were originally considered to be the byproducts of aberrant RNA splicing and did not attract much attention by researchers during the next decades[3,4]. Following developments in bioinformatics, a large number of circRNAs have been identified, and some of their features have become increasingly clear.

Due to the pressing need to understand the complex gene expression dynamics in various types of cancer, the cellular roles of RNA molecules with gene-regulatory potential have been widely revealed. The vast majority (>90%) of the mammalian genome could be transcribed into noncoding RNAs (ncRNAs), instead of coding RNAs[5-9]. ncRNAs are classified into five primary categories: Housekeeper ncRNAs (small nuclear RNA; snRNA), small nucleolar RNA, ribosomal RNA (rRNA), transfer RNA and regulatory ncRNAs. Regulatory ncRNAs could be categorized into: Small ncRNAs (<200 bp), including microRNA (miRNAs/miRs), small interfering (si)RNAs and PIWI-interacting RNAs, snRNAs and long ncRNAs (lncRNAs; >200 bp) [10,11]. circRNAs, a peculiar group of lncRNAs extensively existing in mammalian cells, have recently been regarded as an intriguing class of endogenous RNAs that form a closed continuous loop[12-14].

First, a number of studies have reported that circRNAs are strongly specific to tissues [15,16]. Secondly, due to their resistance to ribonuclease (RNase) activity, circRNAs are much more stable, as compared to their linear counterparts[15,17]. Thirdly, genome-wide analysis has discovered that circRNAs exhibit a higher sequence-conservation and more abundance than their linear counterparts [17,18]. In addition, circRNAs are regarded as competing endogenous RNAs that regulate alternative splicing or transcription, bind or sequester proteins, and are translated into functional peptides[19,20]. These features demonstrated that circRNAs may be capable of playing a role in pathological and biological cellular processes. Increasing evidence indicates that circRNAs are closely associated with the pathology of various diseases, including Alzheimer's disease[21] neurological dysfunction[22] osteoarthritis[23] diabetes[24] cardiac disease[25] and cancer[26].

In particular, circRNAs have been found to play crucial roles in cancer initiation, development and drug resistance[27,28]. Furthermore, circRNAs can have an impact on the tumor microenvironment through intercellular communication due to its abundance in exosomes and human fluids. Therefore, circRNAs can be visualized as promising biomarkers for cancer. In the present review, the current research on the clinical significance and functional mechanism of circRNAs in the biogenesis, biological functions, advances, challenges and clinical implications of various cancers was summarized.

2. Biogenesis of circRNAs

circRNAs are typically derived from the back-splicing of precursor mRNAs to form closed RNA transcripts. However, the mechanisms of circRNAs biogenesis and regulatory factors involved in circularization remains unclear [15,17]. circRNAs can also originate from exons, introns, 5′untranslated regions (UTR), 3′UTR or antisense sequences[15,17,29]. To date, circRNAs have been divided into four categories: Exonic circRNAs (ecircRNA), circular intronic RNAs (ciRNAs), exon–intron RNAs (EIciRNAs) and tRNA intronic circRNAs (tricRNAs)[30]. Among the various types of circRNAs, the most studied are ecircRNAs, which account for > 80% of all cir-

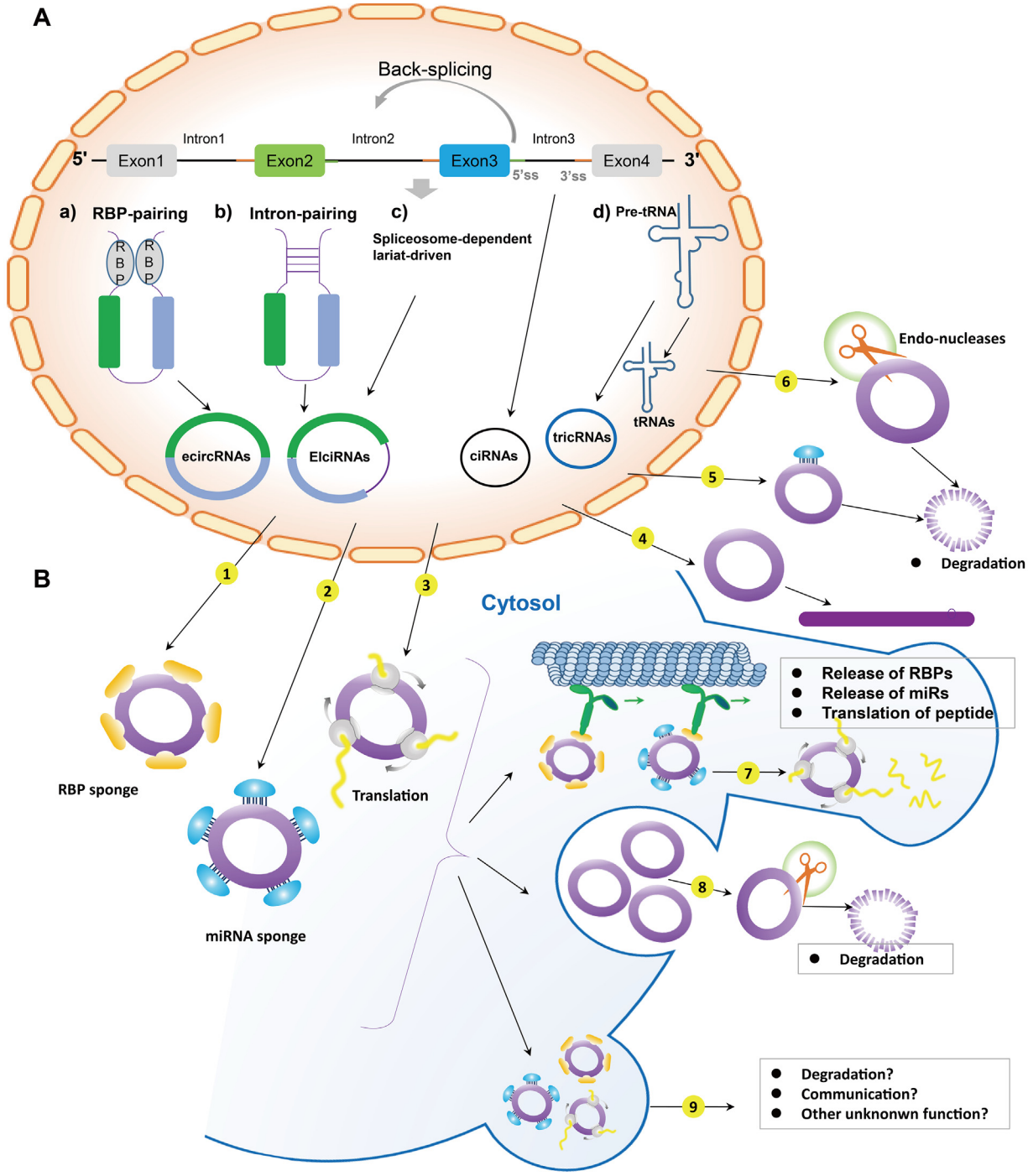

Fig. 1. Biogenesis, functions and degradation of circRNA. (A) Biogenesis of circRNAs. (a) circRNA formation through RBP-mediated pre-miRNA folding. (b) Pairing between the 2 introns flanking the circularized exons. (c) The back-splicing site promotes the joining of the downstream 5'donor sites with the upstream 3'acceptor sites. (d) tricRNA exon termini link to each other to form a mature tRNA, and intron termini are ligated together to form tricRNA. (B) Functions and degradation of circRNAs. circRNAs could (1) bind RBPs as transcription regulators, (2) function as miRNA sponges, (3) be translated into proteins/peptides, (4) generate pseudogenes, (5) sponge miRNA for direct degradation and (6) be degraded by endonucleases. (7) The circRNA-complex may diffuse in the cytoplasm or be actively transported into particular regions of the cell (e.g., the synapse) where it can release its bound cargo or start to be translated. (8) The enclosure of circRNAs or circRNA factor complexes in vesicles could be released into the extracellular space, which would remove circRNAs from the cytoplasm. (9) The circRNA or circRNA complexes could reach other cells or tissues and therefore act as messenger molecules or fulfill other unknown functions.

cRNAs. Four related biogenesis mechanisms are discussed below and the brief process of biogenesis is illustrated in Fig. 1.

2.1. RNA-binding protein (RBP)-induced circularization

circRNA biogenesis could be elicited by the mediation of RBPs of circularization. RBPs, such as Quaking, Muscleblind and Fused-in sarcoma, which are regarded as trans-acting factors, could enhance circularization by bridging related intronic sequences[31]. The dimerization of RBPs combined with the upstream and downstream of the circularized exons, can induce a closer link between 3′ and 5′ends of the circularized exons and facilitate splicing (Fig. 1Aa) [32].

2.2. Intron-pairing circularization

Pairing with a complementary inverted sequence could enhance back-splicing [33]. The unique intronic sequence allows the splice donor near the splice acceptor, finally promoting the nucleophilic attack and cleavage[34]. The competition of reverse complementary sequences at different locations results in one gene producing different circRNA isoforms (Fig. 1Ab)[35,36].

2.3. Spliceosome-dependent lariat-driven circularization

Exon circularization is spliceosome-dependent, as confirmed by the variation in 5′ splice sites[36]. At the back-splicing site, the spliceosomes are gathered to facilitate the connection between the 5′donor and 3′acceptor sites[37]. Internal splicing consequently occurs in the lariat, which leads to the release of ecircRNAs or EIciRNAs[38]. In addition, back-splicing covering single exons or several exons with intervening introns could occur post-transcriptionally and co-transcriptionally (Fig. 1Ac)[39].

2.4. tricRNA splicing pathway

The formation of tricRNA requires tRNA splicing enzymes to divide pre-tRNA into two parts: tricRNAs are derived from a 3′-5′ phosphodiester bond[40]. A structural motif resembling the archaeal bulge-helix-bulge is present in pre-tRNA. The leader and trailer are removed by RNase P and RNase Z, respectively. Cleavage of the pre-tRNA yields two exon halves and an intron, each bearing 5′OH and 2′,3′cyclic phosphate at the cut sites (Fig. 1Ad)[41].

3. Biological functions of circRNAs

circRNAs are known to have multiple functions, which include serving as miRNA sponges, interacting with RBPs, modulating alternative splicing and transcription, translation, generating pseudogenes, transportation and communication. In addition, circRNAs can regulate gene expression due to their role in aiding the process of translation.

3.1. circRNAs interacting with proteins

circRNAs function as protein antagonists or baits to inhibit the activity of proteins (Fig 1B.1). For instance, circ-Foxo3 could interact with cell cycle-related proteins, including p21 and p27, thereby blocking the roles of the proteins in cancer cell cycle progression [42]. Another circRNA, circPABPN1, has been shown to bind to HuR, a well-known RBP [43]. Similarly, the binding of circPABPN1 to the well-known RNA binding protein HuR reduced the translation of PABPN1 by preventing HuR from interacting with PABPN1 mRNA[44]. circANRIL, which was shown to bind to peccadillo homolo 1 (PES1), repressed PES1-mediated rRNA maturation[45].

3.2. circRNAs act as miRNA sponges or competing endogenous RNAs

To date, the majority of circRNAs have been reported to serve as miRNA sponges (Fig 1B.2) [29,46]. miRNAs play a pivotal role in tumor progression [47,48]. It has been reported that competitive endogenous RNAs (ceRNAs) can serve as sponges for miRNAs [49]. circRNAs are predominantly cytoplasmic and have multiple miRNA response elements (MER)[20] suggesting that circRNAs may competitively bind to miRNAs. Thus, circRNAs could regulate miRNA function through suppressing the effect of ceRNA.

The most well-known miRNA in the circRNA field is miR-7 [50,51]. miR-7 has been identified as either a tumor inducer or a tumor suppressor during tumorigenesis. circular RNA sponge for miR-7 (ciRS-7; also known as CDR1as), the most well-known circRNA, contains > 70 miR-7 binding sites, and is expressed in different tissues and organs[52]. By recruiting miR-7, ciRS-7 is capable of inhibiting the miR-7 function and upregulating the expression of related genes, such as IRS2 and EGFR[53]. In esophageal squamous cell carcinoma, ciRS-7 was shown to act as ceRNA to absorb miR-7 and regulate the NF-κB/p65 pathway[54]. In the mammalian brain, ciRS-7, a lncRNA cyrano, and miR-7 and miR-671, two miRNAs, can collaborate to form a sophisticated regulatory network [55]. In addition to ciRS-7, several circRNAs are considered to act as miRNA sponges. CircHIPK3 has been shown to act as ceRNA to absorb miRNAs, including the miR-379, miR-4288, miR-558 and miR-7[56-59]. circHIPK2 functions as an miR124-2HG sponge to modulate astrocyte activation through the interplay between autophagy and endoplasmic reticulum stress [60]. In addition, multiple lines of evidence have proven that circITCH is capable of sponging miR-214 in glioma and bladder cancer[61].

3.3. circRNA translation into proteins/peptides

A 5′cap and 3′poly(A)tail are required for linear mRNA translation[62]. Unlike mRNAs, circRNAs lack unique molecular structure [63]. However, circRNAs can be translated through N^6-methyladenosine modification or internal ribosome entry site (IRES) to promote direct binding of initial factors to the circRNAs, as demonstrated with engineered circRNAs [62,64-66]. Although the majority of circRNAs do not have the capacity to bind to ribosomes for translation, data have shown that a small proportion of endogenous circRNAs can be translated into proteins or peptides (Fig. 1B.3)[19,67-70]. It was reported by Pamudurti *et al*[19] that endogenous circMbl3 was translated into a small peptide in the fly head analyzed by mass spectrometry. In addition, it was indicated by Legnini *et al*[70] that circZNF609, an circRNA, regulated myogenesis and was translated into a protein.

The functional correlation between the majority of circRNA-originated proteins and the linear proteins remains unclear. Since circRNA-originated proteins are usually truncated versions of the linear proteins, the circRNA-originated proteins share the same start codons as their linear counterparts. However, they have a stop codon that is formed by the circular junction. This raises the question of whether circRNA-originated proteins share similar functions with, or act as competitors to, their linear counterpart-encoded proteins. Considering the rapid developments in the protein-coding circRNA field, the foremost aims of this research field are to expand our current understanding of the protein-coding ability of circRNAs and the function of the resulting proteins/peptides.

3.4. Pseudogenes derived from circRNAs

Pseudogenes are mainly derived from the reverse transcription of linear mRNAs, which are located in 10% of known gene loci inside the host genomes[71,72]. Myriad circRNA-originated pseudogenes have been characterized by checking the back-splicing junction sequences of the genomes (Fig. 1B.4)[73]. For example, by retrieving the corresponding circle locus in the mouse genome, 9 low-confidence circRFWD2-derived pseudogenes and 33 high-confidence circRFWD2-originated pseudogenes were identified. However, most circRFWD2-derived pseudogenes did not contain a poly(A)tail, indicating that the way in which circREWD2 is reverse-transcribed into cDNA remains unclear. Therefore, the molecular mechanism of generating pseudogenes is supposed to be explored in circRNA research.

3.5. circRNAs regulate alternative splicing or transcription

Most circRNAs in the cytoplasm are derived from exons. On the contrary, EIciRNAs are predominantly located in the nucleus and act as transcriptional regulators[17]. It was demonstrated by Li *et al*[74] that EIciRNAs interact with U1 snRNPs, and that the EIciRNA-U1 snRNPs complexes may regulate RNA polymerase II activity and promote the transcription of their parental genes [75]. In addition, circRNAs were shown to interact with the Pol II transcription compound to activate the transcription of their parent genes[75]. circSEP3, an ecircRNA, was confirmed to modulate the splicing of its parent gene. circSEP3 can bind intensively to the cognate DNA locus, while the linear counterpart interacts more weakly with DNA. The results identified the ability of circRNA to skew splicing preference and favor the cognate alternative splicing mRNA variant[76]. These studies together suggested that certain circRNAs could regulate gene expression at both splicing and transcription levels.

3.6. Other potential emerging functions of circRNAs

A compelling characteristic of circRNA is that it is extremely stable and accumulates over time. Threfore, circRNAs can act as "flight recorders" of cellular transcription history. In a physiological sense, long-lived circRNAs may act as a repository for translation. Considering that some of the circRNAs mentioned above encode proteins from IRES elements, this repository could be translated to respond to physiological changes or stress response. The local translation of circRNAs in synapses may be important, as other RNAs are also translated in synapses [77]. Since circRNAs can bind to RBPs[42] similar to miRNAs, circRNAs may work through binding, interacting, delivering and releasing their cargo to specific intracellular compartments. In addition, circRNAs may compete in specific subcellular locations for the limiting amounts of RBPs. However, further molecular biology experiments are urgently needed to validate these hypotheses.

Considering that some circRNAs are found in vesicles [78,79] and these vesicles could be transported to the target tissues, they may also act as a delivery capsule. Along with circRNAs, miRNAs and RBPs can be transferred to an organ or a tissue. At the targeted organ or tissue, the miRNAs and RBPs would be released from circRNA through circRNA degradation or other mechanisms. It was revealed by Liu *et al*[80] that *in vitro* synthesis of circRNAs can serve as a quick, convenient and effective strategy of inhibiting miRNA function.

4. Degradation of circRNAs

It was reported by Enuka *et al*[81] that the majority of circRNAs have a longer half-life (18.8–23.7 h) than their full-length linear counterparts, according to an *in vitro* study of 60 circRNAs in cell culture following 4-thiouridine metabolic labeling (4.0–7.4 h). In addition, circRNAs may have an even longer half-life *in vivo* [82,83]. The accumulation of circRNAs in the brain is possibly due to the good stability of these circRNAs [82,83].

The mechanisms and rates of circRNA degradation *in vivo* remains unclear. In fact, the degradation of circRNA can be initiated by an endonuclease. The first study on circRNA degradation was performed using RNase H and Rrp44 to detect endonuclease activity *in vitro* [84,85].The authors demonstrated that the cleavage of artificial circRNA was very low. The best characterized circRNA degradation pattern is the small RNA-mediated degradation of circRNAs. For example, it was revealed by Hansen *et al*[52] that the degradation of *CDR1as* is mediatedby miR-671 through Argonaute 2 (Ago2)-mediated degradation. CDR1as, miR-671 and its binding site are highly conserved, and the deletion of one of these sites leads to a significant increase in *CDR1as* levels[55].

A recent study indicated that the RNA modification ofN6-adenosine methylation (m6A) promotes the recruitment of endonucleases to degrade circRNAs[86]. It was found by Liu *et al* [87] that the circRNAs are globally degraded by RNase L upon poly(I:C) stimulation or viral infection. The authors discovered that spontaneous RNase L activation, circRNA reduction and an increased phosphorylation of PKR in peripheral blood mononuclear cells (PBMCs) from patients with systemic lupus erythematosus [87].

In addition to degradation, circRNAs could be eliminated from cells by exocytosis. CircRNAs could be found in exosomes, but it remains unclear whether the secretion of circRNAs could lower their intracellular levels (Fig 1B.8)[88]. Moreover, circRNA secretion may become a communication mechanism (Fig 1B.9)[89,90]. Therefore, more attention should be pay on the degradation and extracellular transport of circRNAs. CircRNAs are abundant in the cytoplasm and are contained in exosomes during their formation. CircRNAs could be transferred from the cytoplasm into exosomes.

5. circRNA research database

With the rapid developments in bioinformatics, several useful databases have been developed to date to improve circRNA research. Online databases that are useful in circRNA prediction, identification, characterization, localization and investigation of the interaction of circRNAs with MER and RBP have been included in the present study. The online databases of circRNA research are shown in Table 1.

6. circRNAs in cancer

To date, various cancer-related circRNAs have been discovered and characterized (Fig. 2). Accumulating evidence indicates that these circRNAs function in a large number of cancers and play indispensable roles in their occurrence and progression [61,104,105].

6.1. Glioma

Emerging studies have confirmed that circRNAs play a pivotal role in glioma. circRNA is a (Table 2) double-edged sword in glioma. It was found by Yang *et al*[63] that circFBXW7, which contains a spanning junction open reading frame (ORF), could be translated into a 21-KDa protein, namely FBXW7-185aa. FBXW7-185aa could cooperate with the protein encoded by the linear FBXW7 to facilitate the degradation of c-Myc and suppress glioma cell growth. In addition, it was discovered by Zhang *et al*[69] that SHPRH-146aa, encoded by circSHPRH, protected its linear counterpart against degradation by the ubiquitin proteasome that functioned as a tumor inhibitor in human glioblastoma (GBM). In addition, the overexpression of circSMARCA5[106] increased the expression of serine and arginine rich splicing factor 3 to suppress tumorigenesis in GBM. As reported in a previous study, hsa_circ_0001649 and circITCH also acted as tumor suppressors in glioma[107]. Some circRNAs have also been found to play oncogenic roles in glioma. Another study showed that circNFIX functioned as ceRNA to absorb miR-34a-5p, and influenced the expression of targeted gene NOTCH1[108]. circNT5E directly sponged miR-422a, thus affecting the pathological development of GBM[109]. CircTTBK2 was found by Zheng *et al*[110] to be overexpressed in glioma tissues and cell lines, which facilitated glioma cell growth. In addition, hsa_circ_0000177[111] hsa_circ_0012129 [112] circCFH [113] and hsa_circ_0046701[114] could also acceler-

Table 1
Database for circRNA research.

Database	Website	Function	Ref
circRNADb	http://202.195.183.4:8000/circrnadb/circRNADb.php	Offering the detailed information of circRNAs, especially the exon splicing, IRES and ORF	[95]
CircPro	http://bis.zju.edu.cn/CircPro	Analysis of protein-coding potential of circRNAs	[96]
Circbase	http://www.circbase.org/	Providing circRNAs information from multiple species	[97]
Starbase v2.0	http://starbase.sysu.edu.cn/	Providing the RNA-RNA and protein-RNA interaction networks	[98]
CIRCpedia v2	http://www.picb.ac.cn/rnomics/circpedia/	Containing circRNA annotation across 6 different species	[99]
DeepBase v2.0	http://deepbase.sysu.edu.cn/	Containing 14,867 human circRNAs	[100]
Circnet	http://circnet.mbc.nctu.edu.tw/	Describing the regulation between circRNAs, miRNAs and genes	[101]
CircInteractome	http://circinteractome.nia.nih.gov/	Providing bioinformatic analysis of binding sites on circRNAs	[102]
CSCD	http://gb.whu.edu.cn/CSCD/	Predicting cellular distribution of circRNAs, MRE, RBP and variable splicing of related genes	[103]
Circ2Traits	http://gyanxet-beta.com/circdb/	Predicting the interaction among miRNAs, lncRNAs and circRNAs	[104]
CirclncRNAnet	http://app.cgu.edu.tw/circlnc/	Offering a “one-stop” resource for analysis of ncRNA biology	[105]
CircRNADisease	http://cgga.org.cn:9091/circRNADisease	Providing experimentally supported circRNA and disease associations	[106]
ExoRBase	http://www.exorbase.org/	Including annotation, expression level and possible original tissues about 58,330 circRNAs in human blood exosomes	[107]

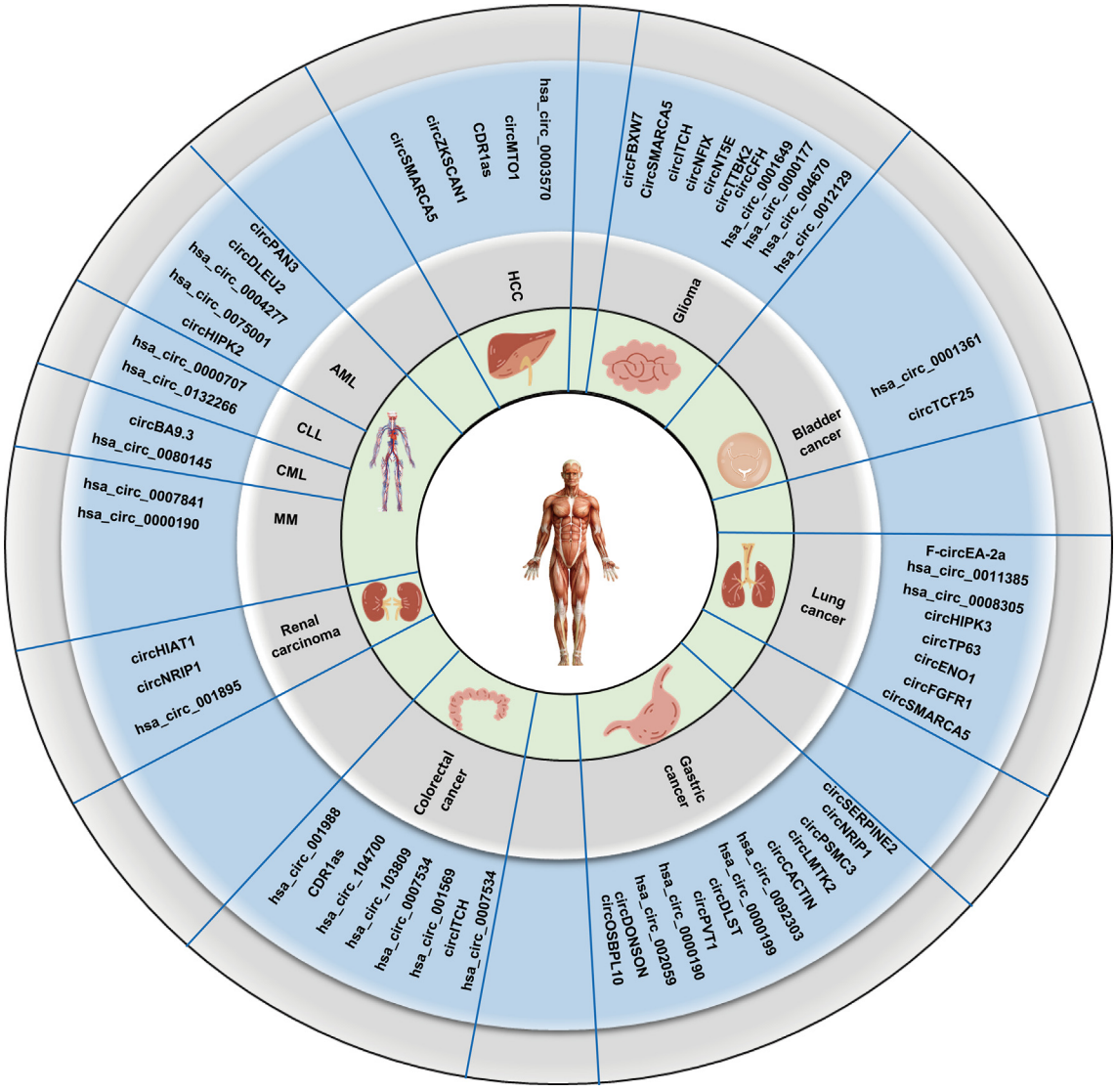

Fig. 2. Overview of functional circRNAs in various types of cancer. The map shows the circRNAs that have been confirmed to function in various types of cancer.

Table 2
Summary of some tumor-related circRNAs.

Cancer	CircRNA ID	expression	Function	Mechanism	Refs.
Glioma	circFBXW7	Down-regulated	tumor suppressor	encoded peptides	[63]
	circSHPRH	Down-regulated	tumor suppressor	encoded peptides	[69]
	circSMARCA5	Down-regulated	tumor suppressor	circSMARCA5/SRSF1/SRSF3	[106]
	circITCH	Down-regulated	tumor suppressor	circITCH/miR-214	[107]
	circNFIX	Up-regulated	oncogene	circNFIX/miR-34a-5p	[108]
	circNT5E	Up-regulated	oncogene	circNT5E/miR-422a	[109]
	circTTBK2	Up-regulated	oncogene	miR-217/HNF1β/Derlin-1	[110]
	hsa_circ_0000177	Up-regulated	oncogene	hsa_circ_0000177/miR-638-FZD7/Wnt	[111]
	hsa_circ_0012129	Up-regulated	oncogene	hsa_circ_0012129/miR-661	[112]
	circCFH	Up-regulated	oncogene	circ-CFH/miR-149/AKT1	[113]
	hsa_circ_0046701	Up-regulated	oncogene	hsa_circ_0046701/miR-142-3p/ITGB8	[114]
HCC	circMTO1	Down-regulated	oncogene	circMTO1/miR-9	[117]
	circSMARCA5	Down-regulated	tumor suppressor	circSMARCA5/miR-17-3p	[118]
	circZKSCAN1	Down-regulated	tumor suppressor	PI3K pathway	[119]
	hsa_circ_0003570	Down-regulated	oncogene	Unknown	[120]
	Cdr1as	Up-regulated	oncogene	Cdr1as /miR-7	[121]
Colorectal	hsa_circ_0000069	Up-regulated	oncogene	Unknown	[124]
cancer	hsa_circ_0007534	Up-regulated	oncogene	Unknown	[125]
	hsa_circ_103809	Down-regulated	oncogene	Unknown	[126]
	hsa_circ_104700	Down-regulated	oncogene	Unknown	[126]
	CircCCDC66	Up-regulated	oncogene	CircCCDC66/miR-93	[127]
	Circular BANP	Up-regulated	oncogene	Circular BANP/p-Akt	[128]
	hsa_circ_001569	Up-regulated	oncogene	hsa_circ_001569/FMNL2	[129]
	Cdr1as	Up-regulated	oncogene	ciRS-7/miR-7 /EGFR	[130]
Gastric	hsa_circ_0000190	Down-regulated	oncogene	Unknown	[133]
	hsa_circ_002059	Down-regulated	oncogene	Unknown	[134]
cancer	hsa_circ_0000199	Up-regulated	oncogene	hsa_circ_0000199/miR-198	[135]
	circDLST	Up-regulated	oncogene	circDLST/miR-502-5p	[136]
	circPSMC3	Up-regulated	oncogene	circPSMC3/miR-296-5p	[137]
	hsa_circ_0092303	Up-regulated	oncogene	hsa_circ_0092303/miR-331-3p	[138]
	circNRIP1	Up-regulated	oncogene	circNRIP1/miR-149-5p	[139]
	circLMTK2	Up-regulated	oncogene	circLMTK2/miR-150-5p	[140]
	circSERPINE2	Up-regulated	oncogene	circSERPINE2/miR-375	[26]
	circDONSON	Up-regulated	oncogene	circDONSON/NURF complex	[141]
	hsa_circ_0008549	Up-regulated	oncogene	hsa_circ_0008549/miR-136-5p	[142]
Lung	hsa_circ_0008305	Down-regulated	oncogene	hsa_circ_0008305/miR-429	[144]
	F-circEA-2a	Up-regulated	oncogene	Unknown	[145]
cancer	hsa_circ_0011385	Up-regulated	oncogene	hsa_circ_0011385/miR-361-3p	[146]
	CircTP63	Up-regulated	oncogene	CircTP63/miR-873-3p	[147]
	circENO1	Up-regulated	oncogene	circENO1/miR-22-3p	[148]
	circFGFR1	Up-regulated	oncogene	circFGFR1/miR-381-3p	[149]
	circSMARCA5	Down-regulated	tumor suppressor	circSMARCA5/miR-19b-3p/HOXA9	[150]
AML	circDLEU2	Up-regulate	oncogene	circDLEU2/miR-496/PPKACB	[151]
	circHIPK2	Up-regulated	oncogene	circHIPK2/miR-124-3p	[152]
	circPAN3	Up-regulated	oncogene	circPAN3/miR-153-5p	[153]
CLL	circCBFB	Up-regulated	oncogene	circCBFB/miR-607/FZD3/Wnt	[154]
	hsa_circ_0132266	Down-regulated	tumor suppressor	hsa_circ_0132266/miR-337-3p/PML	[155]
	circ-RPL15	Up-regulated	oncogene	miR-146b-3p/RAF1 axis.	[156]
CML	circBA9.3	Up-regulated	oncogene	circBA9.3/c-ABL1	[157]
	hsa_circ_0080145	Up-regulated	oncogene	hsa_circ_0080145/miR-29b	[158]
	circHIPK3	Up-regulated	oncogene	circHIPK3/miR-124 axis	[159]
MM	hsa_circ_0007841	Up-regulated	oncogene	hsa_circ_0007841/miRNAs	[160]
	hsa_circ_0000190	Down-regulated	tumor suppressor	hsa_circ_0000190/miR-767-5p	[161]
	circITCH	Down-regulated	tumor suppressor	circITCH/miR-615-3p	[162]
BC	circTCF25	Up-regulated	oncogene	circTCF25/miR-103a-3p	[164]
	hsa_circ_0001361	Up-regulated	oncogene	hsa_circ_0001361/miR-491-5p	[165]
	circSLC8A1	Down-regulated	tumor suppressor	miR-130b/miR-494	[166]
kidney	circHIAT1	Down-regulated	tumor suppressor	circHIAT1/miR-195-5p/29a-3p	[167]
Cancer	hsa_circ_001895	Up-regulated	oncogene	hsa_circ_001895/miRNA-296-5p	[168]
	circNRIP1	Up-regulated	oncogene	circNRIP1/miR-505	[169]

(HCC: Hepatocellular carcinoma; AML:acute myloid leukemia; CLL: chronic lymphocytic leukemia; MM: multiple myeloma; BC: bladder cancer)

ate glioma tumorigenesis. These findings indicated that circRNA might have an marked effect on the progression of GBM, increasing its potential as a convenient biomarker for GBM screening.

6.2. Hepatocellular carcinoma (HCC)

HCC, which accounts for 90% of primary malignancies of the liver, is a major cause of cancer-related mortality worldwide [115,116]. circRNAs have been reported in several studies to be able to function as a tumor inhibitor in HCC. circMTO1 repressed HCC tumorigenesis by directly sponging miR-9 to elevate p21, suggesting that circMTO1 was associated with the prognosis of HCC [117]. In addition, circSMARCA5 increased TIMP3 expression by sponging miR-181b-5p to inhibit HCCprogression [118]. Furthermore, circZKSCAN1 could collaborate tightly with its linear mRNA to inhibit the growth, migration and invasion of HCC[119]. The downregulation of hsa_circ_0003570[120] has been shown to be closely linked to tumor size and neoplastic angiopoiesis in HCC. Of note, Cdr1as was found to be significantly overexpressed in HCC, as compared with the adjacent normal tissues, and Cdr1as

to be a sponge for miR-7, which was involved in the promotion of HCC cell growth and migration[121,122]. These findings indicated that circRNA was firmly interrelated with the progression and tumorigenesis of HCC.

6.3. Colorectal cancer (CRC)

CRC is the fourth leading cause of global mortality [123]. RNA sequencing was conducted toscrutinize the expression of circRNAs in tumor and normal tissues. In a study by Anna *et al*[82] 11 upregulated and 28 downregulated circRNAs were identified in CRC tissues. Furthermore, the ratio of selected circRNAs to their host gene in the CRC tissues (hsa_circ_0817/CUL5, hsa_circ_3204/USP3, hsa_circ_6229/METTL3 and hsa_circ_7374/TNS4) was smaller than that in the adjacent normal tissues. Similarly, microarray analysis showed that the expression of 412 circRNAs was upregulated, while 480 circRNAs were downregulated in CRC tissues, as compared with normal tissues[124]. In detail, quantitative polymerase chain reaction (qPCR) results of CRC patients indicated that the hsa_circ_0000069 expression was elevated and promoted cell proliferation, migration and invasion in CRC[124]. In addition, the expression of hsa_circ_0007534 was linked to tumor stage and lymphatic metastasis in CRC tissues [125]. In addition, it was found by Zhang *et al*[126] that the expression of hsa_circ_104700 and hsa_circ_103809 was clearly downregulated in CRC tissues and closely linked to cancer pathogenesis. It was shown by Hsiao *et al*[127] that the elevation of circCCDC66 in CRC was closely associated with tumor pathogenesis. CircCCDC66 can sponge miRNA-33b and miR-93 to protect MYC mRNA from degradation. On the other hand, circBANP was found to be elevated in CRC, the knockdown of which could significantly inhibit the growth of CRC cells [128]. In addition, hsa_circ_001569 could upregulate the expression of its functional targets FMNL2 and BAG4, subsequently exerting a strong effect on tumorigenesis[129]. Furthermore, CDR1as was aberrantly increased in CRC tissues. The expression of CDR1as was closely associated with tumor volume, tumor metastasis and survival rate [130]. The downregulation of CDR1as suppressed CRC cell growth and migration by blocking miR-7 targets [131]. Collectively, these findings revealed that circRNA is involved in the progression and pathogenesis of CRC.

6.4. Gastric cancer (GC)

GC is the third most common cause of cancer-related mortality worldwide[132]. hsa_circ_0000190 Was found by Chen *et al*[133] to be downregulated in GC tissues. The low levels of hsa_circ_0000190 were correlated with tumor volume, metastasis and the tumor-node-metastasis stage. It was reported by Li *et al* [134] that the expression level of hsa_circ_002059 was closely associated with GC distant metastasis and tumorigenesis. Furthermore, Huang *et al*[135] reported that circAKT3 (hsa_circ_0000199) sponged miR-198 to promote PIK3R1 expression and DNA damage repair, consequently suppressing the apoptosis of GC cells. A recent study revealed that circDLST sponged miR-502-5p to facilitate cell proliferation, cell cycle and DNA synthesis both *in vitro* and *in vivo* [136]. Rong *et al*[137] identified circPSMC3 and reported that its expression was decreased in GC tissues, GC plasmas and GC cell lines. circPSMC3 sponged miRNA-296-5p with phosphatase and tensin homolog (PTEN) to promote GC proliferation.

Conversely, it was discovered by Zhang *et al*[138] that circCACTIN (hsa_circ_0092303) was a tumor inducer that promotes GC growth by modulating TGFBR1 mRNA expression and sponging miRNA-331-3p. It was found by Zhang *et al*[139] that circNRIP1 sponged miR-149-5p to enhance GC cell progression, migration and invasion. It was further proven that circNRIP1 could be transmitted between GC cells by exosomal communication. CircLMTK2 was found by Wang *et al*[140] to be elevated in GC tissues and correlated with poor prognosis, as well as poor tumor node metastasis (TNM) stage. circLMTK2 sponged miR-150-5p and eventually regulated the expression of c-Myc to promote GC tumorigenesis. YWHAZ and circSERPINE2 were found by Liu *et al*[26] to be upregulated, while miR-375 was clearly downregulated in GC tissues and cells. Mechanistically, circSERPINE2 sponged miR-375 and modulated YWHAZ expression to promote GC cell growth and cell cycle progression. It was discovered by Ding *et al*[141] that the expression of circDONSON was positively correlated with advanced TNM stage and poor prognosis. Mechanistically, circDONSON promoted GC proliferation by recruiting the NURF complex to initiate SOX4 expression. circOSBPL10 (hsa_circ_0008549) was found by Wang *et al*[142] to be markedly upregulated in GC tissues, and its decreased expression impaired GC tumorigenesis. Similarly, circOSBPL10 promoted GC cell growth through the circOSBPL10-miR-136-5p-WNT2 axis in GC cells. Therefore, circOSBPL10 may act as a novel predictor of prognosis and proliferation in GC. Collectively, these results revealed that circRNAs can serve as novel diagnostic and prognostic biomarkers of GC.

6.5. Lung cancer

Lung cancer has the highest cancer-related mortality rate worldwide[143]. Wang *et al*[144] confirmed that circPTK2 (hsa_circ_0008305) sponged miR-429, consequently favoring non-small cell lung cancer (NSCLC) cell invasion. F-circEA-2a was found by Tan *et al*[145] to be predominantly located in the cytoplasm and to facilitate cell migration and invasion. Chen *et al*[146] discovered that the oncogenic circRNA circHIPK3 (hsa_circ_0011385) could sequestrate miR-361-3p and interact with splicing factors. It was found by Cheng *et al*[147] that circTP63 sponged miR-873-3p and prevented the decrease of FOXM1, subsequently promoting cell cycle progression. Zhou *et al*[148] revealed that circENO1 and its linear counterpart were elevated in lung adenocarcinoma (LUAD) cells. The downregulation of circENO1 induced apoptosis, and suppressed cell growth, migration and EMT. CircENO1 sponged miR-22-3p and upregulated ENO1. Collectively, circENO1 may serve as a target of LUAD. circFGFR1 was found by Zhang *et al* [149] to be increased in NSCLC patients, an increase that was linked to poor prognosis. Mechanistically, circEGFR1 sponged miR-381-3p to elevate the expression of the downstream gene CXCR4, and finally accelerated NSCLC tumorigenesis. Nevertheless, Wang *et al*[150] showed that circSMARCA5 sponged miR-19b-3p, subsequently exerting its tumor inhibitory effects. Collectively, these results predicted that circRNAs can serve as a therapeutic target in lung cancer.

6.6. Hematological malignancies

A multitude of circRNAs have been used as diagnostic and prognostic biomarkers in hematological malignancies, such as acute myloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML) and multiple myeloma (MM).

AML. CircDLEU2 was reported by Wu *et al*[151] to act as a sponge to suppress the biological function of miR-496 and subsequently promoted AML tumorigenesis by targeting the miR-496/PPKACB channel. Li *et al*[152] discovered that circHIPK2 was elevated in other AML types, as compared to acute promyelocytic leukemia (APL). CircHIPK2 sponged miR-124-3p, which was closely associated with cell differentiation, thereby playing a crucial role in activating transcription. Therefore, circHIPK2 might act as an APL-associated biomarker. It was revealed by Shang *et al*[153] that oncogenic circRNA circPAN3 could be an important regulator of drug resistance, which is mainly due to the circPAN3-miR-153-5p axis in AML cells.

CLL. Xia *et al*[154] showed that circCBFB (hsa_circ_0000707) played a crucial role in CLL tumorigenesis. Mechanistically, circCBFB sponged miR-607 to elevate the expression level of FZD3 and subsequently activated the Wnt/β-catenin pathway. In a study by Wu *et al*[155] circMTO1 (hsa_circ_0132266) was shown to be markedly decreased in the peripheral PBMCs of CLL patients, as compared to the control group. Mechanistically, hsa_circ_0132266 facilitated CLL progression and pathogenesis mainly through the hsa_circ_0132266/miR-337-3p/PML signaling cascade. circ-RPL15 was identified as a novel oncogenic biomarker for CLL, with its mechanism working via the miR-146b-3p-mediated repression of the RAS/RAF1/MEK/ERK pathway[156].

CML. It was revealed by Pan *et al*[157] that circBA9.3 might efficiently facilitate the growth of cancer cells by suppressing apoptosis. circBA9.3 was mainly located in the cytoplasm and elevated the expression of c-ABL1 and BCR-ABL1. Therefore, circBA9.3 was linked to an increased tyrosine kinase activity, which promoted tyrosine kinase inhibitor treatment resistance. Liu *et al*[158] indicated that hsa_circ_0080145 functioned as a sponge to absorb miR-29b and subsequently favored the proliferation of CML. This study indicated that hsa_circ_0080145 could be a promising biomarker for CML treatment. As previously reported, the oncogenic role of circHIPK3 was confirmed in the development and treatment of CML. These oncogenic effects were achieved by sponging miR-124[159].

MM. It was confirmed by Gao *et al*[160] that the hsa_circ_0007841 expression was markedly elevated in MM cell lines, which was closely associated with disease prognosis. Bioinformatics analysis demonstrated that several miRNAs interacted with hsa_circ_0007841, suggesting that hsa_circ_0007841 may serve as a novel biomarker for MM. Feng *et al*[161] indicated that hsa_circ_0000190 suppressed cell growth and promoted apoptosis in MM by sponging miR-767-5P and regulating mitogen-activated protein kinase 4. It was discovered by Liu *et al*[162] that circITCH was downregulated in MM cells and circITCH acted as a sponge for miR-615-3p.

6.7. Other types of cancer

The ectopic expression of circRNAs has been validated in multiple types of cancer [163]. In bladder cancer, Zhong *et al*[164] speculated that the oncogenic circRNA circTCF25 could sponge miR-103a-3p/miR-107 based on the multiple bioinformatics approaches, which consequently increased 13 target genes associated with cell proliferation, migration and invasion. It was discovered by Liu *et al*[165] that an oncogenic circRNA, hsa_circ_0001361, was elevated in bladder cancer tissues. Hsa_circ_0001361 directly sponged miR-491-5p to elevate MMP9 and subsequently promoted the occurrence and progression of bladder cancer. In addition, circRNA circSLC8A1 has been reported to act as a sponge of miR-130b/miR-494 in preventing bladder cancer progression by regulating PTEN[166]. In kidney cancer, a decreased expression of circHIAT1 in clear cell renal cell carcinoma (ccRCC) tissues was identified by Wang *et al*[167]. CircHIAT1 can directly interact with miR-195-5p/29a-3p/29c-3p to elevate CDC42 expression. Androgen receptor (AR) inhibited circHIAT1 expression and subsequently led to the suppression of CDC42. The AR-circHIAT1-mediated miR-195-5p/29a-3p/29c-3p/CDC42 signaling pathway might provide an effective strategy for a more effective inhibition of ccRCC metastasis. It was found by Chen *et al*[168] found that hsa_circ_001895 sequestrated miR-296-5p, subsequently inducing ccRCC development. Dong *et al*[169] discovered that circNRIP1 was overexpressed in renal carcinoma tumors and circNRIP1 played oncogenic roles in the renal carcinoma cell lines by targeting miR-505 through the activation of the AMPK and PI3K/AKT/mTOR cascades. Collectively, the studies mentioned above demonstrated that circRNAs are potentially involved in tumorigenesis. However, the clinical implications of the use of circRNAs as novel therapeutic avenues require further research.

7. circRNAs are promising biomarkers in cancer

The clinical use of biomarkers is critical during all stages of cancer, and has become one of the major approaches for cancer diagnosis and prognosis. Different from linear mRNAs, the unique covalently closed-loop structures make circRNAs avoid RNaseR degradation and thus possess high stability[170]. In addition, circRNAs are widely distributed in the plasma, urine, tissue samples, cell-free saliva and other human components in a cell-specific manner[171,172]. The expression patterns and characteristics of circRNAs (high and selective abundance, high stability, high conservation and specific expression)could partly explain the ability of circRNAs as potential biomarkers or therapeutic targets. It was found by Memczak *et al*[173] that circRNAs were elevated, as compared with those of their linear counterparts in the blood. A diverse set of circRNAs exhibited a high level in the blood, which can be easily detected. However, the expression of their linear counterparts was very low. Therefore, blood circRNAs may offer disease-related knowledge that canonical RNA analysis cannot provide.

A large number of studies performed on HCC patients have shown that hsa_circ_0000798[174] hsa_circ_0027089[175] and hsa_circ_0058124[176] are upregulated in HCC tissues, whereas some circRNAs are downregulated, including hsa_circSMARCA5 [177] hsa_circ_0068669[178] hsa_circ_0028502[179] and hsa_circ_0076251[179]. These circRNAs are supposed to serve as potential biomarkers for HCC. Several circRNAs also serve as biomarkers in patients with CRC. Increased plasma levels of hsa_circ_0082182 and hsa_ circ_0000370[180] were significantly connected with lymph node metastasis, while the upregulation of hsa_circ_0004585[181] was correlated with patient tumor size. The downregulation of hsa_circ_0000567[182] was correlated with TNM stage. In GC, hsa_circ_0003159[183] hsa_circ_0000096[184] hsa_circ_002059[185] hsa_circ_0000190[133] and hsa_circ_0000181[186] were all downregulated and linked to distal metastasis or invasion, which may predict tumor metastasis. In addition, the expression level of hsa_circ_0000467 was higher in the GC tissues, plasma and GC cells, as compared with the healthy control, and was correlated with TNM stage in GC. The area under the curve (AUC) of hsa_circ_0000467 was 0.799, which was much higher than some already existing biomarkers. The sensitivity and specificity of hsa_circ_0000467 were 70.5 and 64.8%, respectively [187]. Furthermore, a circRNA downregulated in GC tissues, circPSMC3, was negatively correlated with TNM stage and lymphatic metastasis, and exhibited a high AUC (0.933). The sensitivity and specificity of circPSMC3 were 85.85 and 95.24%, respectively [137]. The expression level of hsa_circ_0001895 was correlated with GC cell differentiation, Borrmann type and carcinoembryonic antigen level. The AUC, sensitivity and specificity of hsa_circ_0001895 were 0.792, 67.8 and 85.7%, respectively[188]. The hsa_circ_0008673[189] increased in breast cancer patients, circASXL1[190] and hsa_circ_0137439[191] were increased in bladder cancer, and circBNC2 were decreased in ovarian cancer, which confirmed the diagnostic value of the circRNAs in neoplastic diseases. Other circRNAs serving as biomarkers in cancer are listed in Table 3. Besides, the AUC, sensitivity and specificity of plasma hsa_circ_0000520[192] (0.897, 82.4, 84.4%) were clearly higher than those in the tissue (0.613, 53.6 and 85.7%) respectively, indicating a relatively superior diagnostic value[192]. On the contrary, tissue hsa_circ_0000190[133] and hsa_circ_0000181[186] both exhibited a superior diagnostic value than their plasma counterparts in GC. In detail, tissue hsa_circ_0000190 was significantly

Table 3
The potential role of circRNAs as biomarkers in various cancers.

Cancer	Name of CircRNAs	Changes of expression	Diagnostic significance	ROC curve	Numbers of patients	Refs
HCC	CircSMARCA5	Down	Correlated with tumor differentiation, TNM stage, cancer invasion and cancer diameter	Plasma circRNA; AUC value: 0.938, 0.853, 0.711	133	[177]
	Hsa_circ_0000798	Up	Correlated with tumor size and cirrhosis	Plasma circRNA; AUC value: 0.703	102	[174]
	Hsa_circ_0068669	Down	Associated with microvascular invasion and TMN stages	Tissue circRNA; AUC value: 0.64; Sensitivity: 0.59; Specificity: 0.71	100	[178]
	Hsa_circ_0027089	Up	–	Plasma circRNA; AUC value: 0.794; Sensitivity: 0.578; Specificity: 0.848	239	[175]
	Hsa_circ_0058124	Up	Associated with tumor siz, tumor, node, TNM stage, and vascular invasion	Tissue circRNA; AUC value: 0.878	128	[176]
	Hsa_circ_0028502	Down	Related to TNM stage	Tissue circRNA; AUC value: 0.675; Sensitivity: 0.721; Specificity: 0.580	200	[179]
	Hsa_circ_0076251	Down	Related to Barcelona Clinic Liver Cancer (BCLC) stage and the presence of serum HbsAg	Tissue circRNA; AUC value: 0.738; Sensitivity: 0.713; Specificity: 0.640	200	
Colorectal cancer	Hsa_circ_0000567	Down	Correlated with tumor size, lymph metastasis, distal metastasis, and TNM stage	Tissue circRNA; AUC value: 0.8653; Sensitivity: 0.8333; Specificity: 0.7647	204	[182]
	Hsa_circ_0082182	Up	Connected with lymph node metastasis	Plasma circRNA; AUC value: 0.7371	156	[180]
	Hsa_circ_0000370	Up	Connected with lymph node metastasis	Plasma circRNA; AUC value: 0.8152	156	
	Hsa_circ_0035445	Up	Connected with the TNM stage	Plasma circRNA; AUC value: 0.7028	156	
	Hsa_circ_0004585	Up	Correlated with patient's tumor size	Plasma circRNA; AUC value: 0.731; Sensitivity: 0.851; Specificity: 0.511	284	[181]
	Hsa_circ-0004771	Up	Correlated with TNM stage and distant metastasis	Exosome circRNA; AUC value: 0.90	135	[195]
Gastric cancer	Hsa_circ_0003159	Down	Associated with gender, distal metastasis and TNM stage	Tissue circRNA; AUC value: 0.75; Sensitivity: 0.852; Specificity: 0.565	108	[183]
	Hsa_circ_0000096	Down	Associated with gender, invasion and TNM stage	Tissue circRNA; AUC value: 0.82	101	[184]
	Hsa_circ_002059	Down	Correlated with distal metastasis, TNM stage, gender and age	Plasma circRNA; AUC value: 0.73; Sensitivity: 0.81; Specificity: 0.62	101	[185]
	Hsa_circ_0000190	Down	Tissue circRNA: Related to tumor diameter, lymphatic metastasis, distal metastasis and TNM stage	Tissue circRNA; AUC value: 0.75; Sensitivity: 0.721; Specificity: 0.683; Plasma circRNA; AUC value: 0.6; Sensitivity: 0.414; Specificity: 0.875	208	[133]
	Hsa_circ_0000181	Down	Correlated with tumor diameter, lymphatic metastasis, distal metastasis	Tissue circRNA; AUC value: 0.756; Sensitivity: 0.852; Specificity:0.539 Plasma circRNA; AUC value: 0.582; Sensitivity: 0.206; Specificity: 0.99	115	[186]
	Hsa_circ_0001895	Down	Correlated with GC cell differentiation, Borrmann type, and CEA level	Tissue circRNA; AUC value: 0.792; Sensitivity: 0.678; Specificity:0.857	257	[188]
	Hsa_circ_0000467	Up	Correleated with TNM stage	Tissue circRNA; AUC value: 0.799; Sensitivity: 0.705; Specificity:0.648	102	[187]

(*continued on next page*)

Table 3 (*continued*)

Cancer	Name of CircRNAs	Changes of expression	Diagnostic significance	ROC curve	Numbers of patients	Refs
	CircPSMC3	Down	Associated with TNM stage and lymphatic metastasis	Tissue circRNA; AUC value: 0.933; Sensitivity: 0.536; Specificity:0.857	106	[137]
	Hsa_circ_0000520	Down	Tissue:associated with TNM stage Plasma: linked with CEA expression.	Tissue circRNA; AUC value: 0.613; Sensitivity: 0.852; Specificity:0.539 Plasma circRNA; AUC value: 0.897; Sensitivity: 0.824; Specificity: 0.844	112	[192]
Oral squamous cell carcinoma	Hsa_circ_0003829	Down	Correlated with lymph node metastasis status and TNM stage	Tissue circRNA; AUC value: 0.81; Sensitivity: 0.7; Specificity:0.8	120	[197]
	Hsa_circ_0001874	Up	Correlated with TNM stage and tumor grade	Salivary circRNA; AUC value: 0.863; Sensitivity: 0.744; Specificity:0.902	93	[196]
	Hsa_circ_0001971	Up	Correlated with TNM stage	Salivary circRNA; AUC value: 0.845; Sensitivity: 0.756; Specificity:0.878	93	[196]
Lung cancer	Hsa_circ_0001715	Up	Correlated with TNM stage and distant metastasis	Plasma circRNA; AUC value: 0.871; Sensitivity: 0.8772; Specificity: 0.7167	117	[198]
	Hsa_circ_0005962	Up	Related to EGFR mutations and gender	Tissue circRNA; AUC value: 0.73; Sensitivity: 0.719; Specificity:0.722	153	[199]
	Hsa_circ_0086414	Down	Related to gender	Tissue circRNA; AUC value: 0.81; Sensitivity: 0.778; Specificity:0.722	153	
	Hsa_circ_002178	Up	–	Exosome circRNA; AUC value: 0.9956	210	[194]
	Hsa_circ_0037515	Down	–	Tissue circRNA; AUC value: 0.81; Sensitivity: 0.57; Specificity:0.90	122	[200]
	Hsa_circ_0037516	Down	–	Tissue circRNA; AUC value: 0.82; Sensitivity: 0.65; Specificity:0.84	122	
Breast cancer	Hsa_circ_0008673	Up	Correlated with tumor size,distant metastasis, ER positive and PR positive	Plasma circRNA; AUC value: 0.833; Sensitivity: 0.550; Specificity: 0.971	378	[189]
Ovarian Cancer	CircBNC2	Down	Associated with histological grade , serous subtype, LNM, and distant metastasis	Tissue circRNA; AUC value: 0.879; Sensitivity: 0.964; Specificity:0.807	249	[201]
Bladder cancer	Hsa_circ_0001136	Up	Correlated with tumor grade, tumor stage, lymph node invasion and distant metastasis	Tissue circRNA; AUC value: 0.770; Sensitivity: 0.686; Specificity:0.769	122	[190]
	Hsa_circ_0137439	Up	Correlated with tumor stage, tumor grade, lymph node status	Tissue circRNA; AUC value: 0.890; Sensitivity: 0.886; Specificity:0.734	116	[191]
Papillary thyroid carcinoma	Hsa_circ_0137287	Down	Correlated with extrathyroidal extension, lymph node metastasis , advanced T stage and tumor size	Tissue circRNA;AUC value: 0.8973; Sensitivity: 0.792; Specificity:0.900	120	[202]
Pancreatic cancer	Circ-IARS	Up	Correlated with liver metastasis, vascular invasion and TNM stage	Exosome circRNA	92	[193]

linked to tumor diameter and TNM stage, whereas plasma hsa_circ_0000190 exhibited no linkage[133]. Hence, there is a need to compare circRNAs detected in different human components to identify a precise biomarker for distinguishing cancer patients from healthy controls.

Li *et al*[193] was the first to identify that exosomes contained large amounts of circRNAs, due to the fact that > 1,000 circRNAs were found in human serum exosomes. Of note, circRNAs have been found to be stably overexpressed in exosomes, by at least 2-fold, as compared to producing cells such as circIARS, circRASSF2

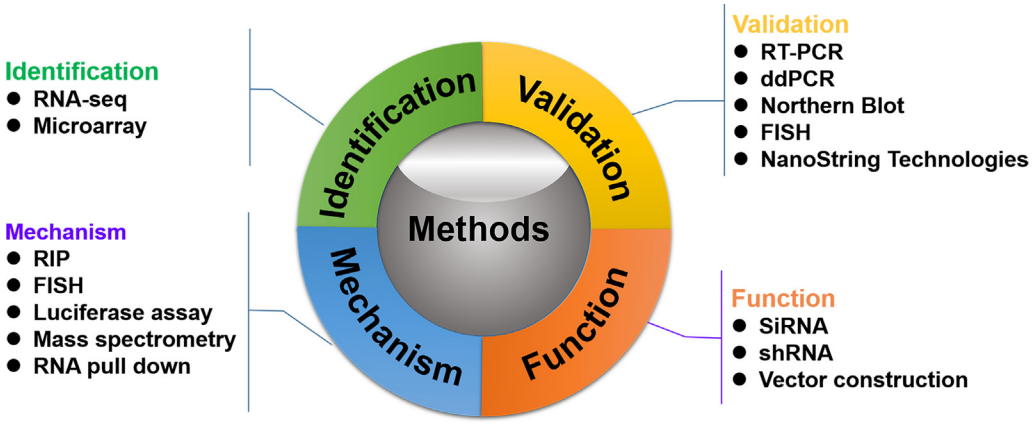

Fig. 3. Strategies of circRNA research (identification, validation, function and mechanism).

and circPTGR1[172]. Therefore, circRNAs could be placed in the novel category of exosomal cancer biomarkers [172]. In pancreatic cancer tissues and plasma exosomes, the expression of exosomal circRNA IARS was higher than that of the control group. The results of the study indicated that the presence of exosomal circRNA may be a useful diagnostic marker for pancreatic ductal adenocarcinoma (PDAC)[193]. The amplification of hsa_circ_002178[194] in the exosomes was found to function as a novel diagnostic biomarker for lung cancer, with a reported AUC value of 0.9956. In addition, the AUC value of exosomal hsa_circ_0004771[195] is 0.9 in CRC, serving as an invasive diagnostic biomarker for CRC treatment.

A total of 422 salivary circRNAs were discovered by Bahn *et al* [172] which were confirmed to play a key role in signal transduction and inflammatory response in human cell-free saliva. As the occurrence and development of tumors are largely influenced by inflammation, it is believed that circRNAs originating from saliva could play an essential role in tumorigenesis; 2 such circRNAs are hsa_circ_0001874 and hsa_circ_0001971[196] in oral squamous cell carcinoma. In addition, circRNAs could also be found in gastric juice. Shao *et al*[172] discovered that hsa_circ_0014717 have favorable stability in gastric juice. This team proved that the expression levels of hsa_circ_0014717 in gastric juice did not change under freeze–thaw for 8 h. Collectively, circRNAs may serve as effective biomarkers for cancer diagnosis.

8. Available strategies in circRNA research

There are several challenges in cancer-related circRNAs research that are often neglected. Most have to do with the fact that most circRNA sequences are the same as the host gene sequence. Therefore, circRNA identification, characterization, quantification, overexpression and knockdown methods all depend on the specific junction site (Fig. 3)[197] .

8.1. circRNA identification

8.1.1. RNA-seq

By using algorithms designed to examine "out-of-order" splicing, a variety of circRNAs, such as exonic, intronic and intergenic circRNAs, have been broadly discovered according to the total RNA-seq data[198]. The methodologies used included find_circ, circRNA_finder, CIRCexplorer and CIRI[198]. However, the accurate quantification of circRNAs from the total RNA-seq datasets frequently requires a high sequencing depth, and at least 100-bp sequencing was recommended to ensure the accurate prediction of circRNAs[199]. Currently, there are regular advancements in novel bioinformatics algorithms, as they are attractive tools for identifying circRNAs. For instance, the analysis of circRNAs can be performed by 6 algorithms: ACSF, CIRCexplorer2, CIRI2, DCC, KNIFE and Uroborus[199]. In addition, the mapper like STAR is capable of annotating more complex RNA sequence arrangements with the features of a high accuracy and speed, such as chimeric and circRNA[200]. Alternatively, the BWA-MEM algorithm has been found to detect circRNAs with fast and low RAM requirements[201]. Segemehl, which exploited an improved suffix array for seeding, was found to outperform its competitors on splice site validation[202].

8.1.2. Microarrays

The application of microarrays is an attainable supplement to RNA-seq for validating circRNAs, since they require less bioinformatics expertise[203]. The first commercial microarrays were manufactured from Arraystar Inc. (https://www.arraystar.com/arraystar-human-circular-rna-microarray), which contains > 10,000 circRNAs that have been selected from publications. Microarray analysis can eliminate the uncertainty of RNA-Seq analysis, due to lack of generalization. As previously reported, when manufacturers ensure reproducibility and efficiency, the process is highly targeted, and relevant standard analysis methods can be used regardless of the type of hybridized[202]. In a recent study, 87,935 circRNA sequences covering most circRNAs characterized to date in circBase have been integrated to design microarray probes, which is clearly more accurate than RNA-seq. Furthermore, the majority of circRNAs measured by this microarray could be further confirmed through reverse transcription (RT)-qPCR or RNA-seq[204].

8.2. circRNA validation and characterization

8.2.1. RT-qPCR

RT-qPCR of circRNAs has been broadly employed for the detection, validation and sometimes even quantification of circRNAs [205]. However, detecting the putative circRNA junction by harnessing qPCR does not guarantee the existence of a circRNA, as certain linear RNAs share the same sequences through junction sites. Currently, divergent primers are particularly designed to extend the circRNA back-splice junction (BSJ) sequence, which was found to exhibit high specificity on the amplification of the circRNAs and not target the linear RNA, allowing direct and precise detection and quantification of circRNAs[205]. In detail, total RNA was digested by RNaseR, reverse-transcribed into cDNA, and subsequently amplified by divergent and convergent primers. In a previous study, RNase R could degrade most linear RNAs, but had no effect

on circRNAs[206]. Both divergent and convergent primers could produce a band in the RNaseR(-) group. In the RNaseR(+) group, divergent primers produce a band, while the convergent primers did not. Furthermore, the amplification product should be detected by sanger sequencing to ensure its true presence.

8.2.2. Droplet digital PCR (ddPCR)

ddPCR is a novel technology for the accurate quantification of RNA, which exhibits a higher sensitivity. A previous study tested the application of ddPCR in circRNA quantification and determined the stability of circRNA, as well as compared RT-qPCR with ddPCR [207]. It was observed that a prolonged RT incubation time would result in the circRNA accumulating a variety of PCR products, which would lead to a relatively low accuracy of RT-qPCR in the quantification of circRNA. DdPCR can overcome this shortcoming and be used instead of qPCR for the quantification of circRNA [207]. In addition, it has been shown that plasma levels of secretory circRNAs are detectable by RT-ddPCR in advanced lung cancers[208].

8.2.3. Northern blot

Evidence has demonstrated that northern blot hybridization is the gold standard for circRNA analysis, convincingly indicating the circular configuration of putative circRNAs[209]. Strictly, circRNA validation generally requires the northern blots to be combined with other tools, such as RNase R and RNase H treatments [210]. In the RNaseR(-) group, both circRNA and linear mRNA could be detected, while in the RNaseR(+) group only the band of circRNA could be found, due to linear mRNA digestion[189]. However, northern blotting is not without drawbacks, including the requirement of a very large number of RNA, and the amount required and frequency of radioactively-labelled probes[199]. Nowadays, various northern gels and detection systems are under investigation, so they can be improved.

8.2.4. Fluorescence in situ hybridization (FISH)

Visualizing circRNAs in cells is extremely critical for studying their biology. An oligonucleotide probe coupled with alkaline phosphatase, fluorescent dyes or an antigen can be used to visualize an circRNA in fixed and permeabilized cells, as shown by the use of digoxigenin in ISH[199]. In a previous study a simple smRNA FISH protocol was used to measure the circRNAs produced from identical genetic loci and coexisted with overlapping, non-circular mRNA isoforms[211]. Most importantly, the BSJ site needs to be extended by the designed hybridization probe.

8.2.5. NanoString technology

NanoString, a relatively new digital counting technology, is precise in quantifying linear mRNAs without any enzymatic reactions. Recent studies have found that after designing color-coded probes spanning the specific back-spliced junctions of circRNAs, NanoString technology was used for the detection of circRNAs in both high- and low-quality RNA samples from cell lines and samples from patients with B-cell malignancies, a method that is sensitive, specific and quantitatively accurate[212].

8.3. Overexpression of circRNAs

The biological functions of circRNAs can be investigated by overexpressing the selected circRNAs. circRNAs could be produced *in vitro* using self-splicing introns or splint ligation methods [31,213]. To construct stable cell lines overexpressing the selected circRNA, cells could be transfected using a linearized circRNA-producing plasmid[35]. However, this approach often leads to the random insertion of a circRNA expression locus. The change of intronic sequences could make circRNA circulation more accurate under certain circumstances[214]. Therefore, the amount of circRNA generation is supposed to be detected and confirmed by Northern blotting.

8.4. circRNA-knockdown

The key means of investigating the biological function of circRNAs is a loss-of-function study by RNA interference. Gene-knockdown through the use of siRNAs specifically targeting the BSJ is widely used as a method of reducing the expression of circRNAs. Of note, the design space is extremely restricted when targeting the BSJ, and the passenger disabled siRNA could be beneficial [215]. The construction of siRNAs relies on a high transfection efficiency, since these RNAs merely knock down the targets transiently. The more stable knockdown method is the use of AGO shRNAs or vectors expressing shRNAs [216,217]. The RNA-targeting Cas13 system is a useful tool for degrading circRNAs [218,219]. The Cas13 enzymes belong to the class 2 type VI CRISPR/Cas effectors. Efficient Cas13 knockdown requires 28–30-nt long spacers and is intolerant to mismatches in spacers. Therefore, CRISPR RNAs carrying spacers that specifically target and span the BSJ site, in principle, should be able to discriminate circular and linear RNAs.

8.5. Mechanistic study

For mechanistic studies, bioinformatics prediction, luciferase reporter assay, RNA immunoprecipitation and RNA pull-down followed by mass spectrometry are conducted to explore circRNA-miRNA and circRNA-protein interactions. For instance, circRNA-miRNA interactions can be predicted by employing Arraystar's homemade miRNA target prediction software based on TargetScan and miRanda to establish a circRNA-miRNA-mRNA coexpression network of hsa_circ_0044556[220]. In the study performed on the role of circSLC8A1 in bladder cancer, RNA pull-down and luciferase reporter assays were performed to explore the interactions between the specific circRNA, miRNA and mRNA[166]. In addition, it has been shown that researchers from the South China University of Technology determined circRNA-miRNA interactions via AGO cross-linking and immunoprecipitation, along with CLIP-Seq and RNA-Seq data. However, these techniques do not distinguish between circRNA and linear RNA. Therefore, the circRNAs should be further quantified using a circRNA-specific method, such as RT-qPCR, with a divergent primer[199]. It was reported in recent studies that the RNase protection assay can be employed to map protein-RNA interactions, which will block the cleavage of circRNAs via RNase H [209]. A site of interaction between the protein and RNA is then illustrated under the condition of a protein binding to the RNA at the target sequence[221]. An RNA pull-down assay is another attractive method of investigating putative protein-binding partners by employing probes for known circRNAs, followed by confirmation by western blotting and mass spectrometry. As compared with the RNA pull-down assay, the RBP immunoprecipitation assay discovers the RNAs by targeting the protein[222]. In order to study the circRNA protein-coding ability, circRNA N6-methyladenosin, IRES and ORF should be predicted by bioinformatic analysis[70,104,171].

9. Challenges and future perspectives

The functions and properties of circRNAs have been elucidated little by little through the advances in high-throughput screening [20]. A plethora of circRNAs have been found to participate in tumorigenesis through multiple molecular mechanisms, such as their interaction with RBPs, which serve as miRNA sponges that

translate into small peptides and regulate the expression of parental genes[69,104]. Despite the fact that great progress has been made in the study of circRNAs, there are several aspects of circRNAs need to be investigated before it can be incorporated into clinical practice.

A noteworthy characteristic of circRNAs is subcellular distribution. circRNAs are mainly distributed in the cytoplasm [15,49]. Of note, certain circRNAs exhibit a modulated switch in their nucleocytoplasmic localization during their development [223]. The distribution of circRNAs at synaptosomes, dendrites and axons is appealing [224,225]. It remains unclear whether the accumulation of circRNA is due to a directed transport or diffusion of the molecules in the spots (e.g. by binding to membrane proteins). Further studies are required to investigate what drives the subcellular distribution of circRNAs. Since circRNAs and mRNAs share 5′UTR regions in most cases[19] it is conceivable that these sequences show that the distribution of both the circular and linear forms originate from a given locus. In addition, mRNA and circRNA may compete for the interaction with effector or transport proteins, which is a way for circRNAs to modulate gene expression. Further studies are required to verify these assumptions and screen circRNA biogenesis and transportation using live-cell imaging. In addition, this area is still lacking an accurate description of the amount and classification of circRNAs.

Emerging evidence confirms that epigenetics is associated with tumorigenesis. To date, several circRNAs have been proven to regulate epigenetic changes, such as histone modifications and DNA methylation[226]. However, little is known about how circRNAs are transported inside the cells and their degradation mechanisms of circRNAs. First, although circRNA may serve as a miRNA sponge, miRNA-mediated circRNA degradation has rarely been explored. The expression of CDR1as is regulated by miR-671 via AGO2-mediated degradation[29]. Meanwhile, CDR1as levels are modulated by miR-7 possibly through slicing [55]. Secondly, variations in the m^6A modifications of circRNAs may have an effect on RNA stability, cell-specific expression and the length of single exons [227]. Furthermore, m^6A modification identified the binding of YTHDF2 to the molecular target and interacted with HRSP12 to regulate the cleavage of circRNA, indicating its positive effect on the degradation of circRNAs[228]. In addition, in a study by Chen *et al*[229] the m^6A modification of circNSUN2 was found to facilitate its export from the nucleus to the cytoplasm.

It's worth noting that circRNAs are enriched in extracellular vesicles, so that cells can eliminate circRNAs through extracellular vesicles[230]-[231]. In addition, extracellular vesicles or microvesicles may have an impact on the tumor microenvironment through intercellular communication [232,233]. For example, in a study by Li *et al*[234] it was found that tumor-excreted circPDE8A diffused into blood circulation by exosome transportation, and plasma exosomal circPDE8A was strongly associated with tumor invasion in pancreatic ductal adenocarcinoma (PDAC). This suggested that exosome communication indeed occurs in PDAC cells. In addition, UAP56 or URH49 were confirmed to play a key role in the nuclear exportat of circRNAs in HeLa cells [235,236]. In addition, exosomal circRNAs also existed in platelet-derived extracellular vesicles[237] pancreatic cancer cells[193,238] and hepatic cells[238]. Exosomes can be received by many types of cells, including macrophages, and they could function as messengers for cell-to-cell communication. Collectively, the extracellular transport and degradation of circRNAs should be studied in detail in future studies, which will contribute to a novel insight of circRNAs biology.

Despite the aforementioned exciting progress in circRNA research, there are still challenges in the clinical application of exosomal circRNAs. Firstly, it is difficult to detect circRNA exosomes due to its low abundance. Secondly, the conformation and sequence of circRNA overlap with linear mRNA counterparts, making the accurate assessment of circRNA expression challenging. In addition, the mechanism through which circRNAs are enriched during exosomal formation remains unclear.

Most reports of circRNAs as essential regulators of cancer have provided limited information about their function. CeRNA could not represent the primary function of circRNAs, since most circRNAs are short in length and low in abundance[239]. The function of circRNAs in cancer pathogenesis is still not fully understood, particularly with regards to drug resistance. The functional study of these newly selected circRNAs may not only broaden our knowledge of the non-conding RNA and eukaryotic transcriptome, but also offer new insights into the diagnosis and treatment of cancer. circRNAs could be appropriately modified to change the key binding sites associated with cancer, and specific targeting molecular drugs can be developed to alter downstream gene expression for the purpose of treating cancer.

Meanwhile, improved methods of artificially overexpressing or silencing circRNAs make it possible to regulate the expression of circRNAs, which is crucial to further investigating the functions of circRNAs. In addition, nanoparticles are closed spherical lipid vesicles and have been widely used in the clinic as drug carriers. For instance, due to passive targeting of drug carriers, stable nucleic acid lipid particles accumulate in the tumor tissue, so nanocarriers of a suitable size can easily pass through the tumor [240]. Of note, it was found by Du *et al*[18] that circFoxo3 can be delivered through plasmid conjugated with gold nanoparticles to induce tumor apoptosis. Future research, in combination with materials science, should focus on delivering circRNA to target cells in an efficient manner. Furthermore, a way in which to control therapeutic circRNAs once they have been delivered, as well as a mechanism of blocking the functions of an circRNA once it has completed exerting its therapeutic effects need to be identified. Stimulus-responsive nanoparticles may be a potential approach for delivering circRNAs. Presumably, circRNAs could be delivered as a promising drug for the clinical treatment of cancer in the next few years.

Despite the natural sponges acting as efficient miR sponges in tumor cells, synthetic circRNA sponges are also worth investigating. The synthetic circRNA sponges can obtain therapeutic loss-of-function targeted against miRNAs more conveniently and steadily, thereby controlling tumor progression. It has been shown that synthetic circRNA can function as an miR-21 sponge to hamper gastric carcinoma cell proliferation, which indicates the potential broad application of synthetic circRNA in the treatment of human cancer [80]. In addition, a new type of artificial circular multi-miR sponge exhibiting miR-21 and miR-93 loss-of-function was synthesized to inhibit cellular proliferation and migration in esophageal carcinoma cells[241]. However, the synthetic technology is not complete and still has drawbacks, such as a limited number of miR binding sites, an altered yield of ligation for RNA circularization and an appearance of potential toxicity, which require further investigations.

circRNA is a new important player in the ncRNA network, which has been identified as a key regulator of various types of cancer. Furthermore, circRNAs have been confirmed to participate in anticancer drug resistance. The latest studies on circRNAs in anticancer drug resistance were summarized by Xu *et al*[242] ranging from traditional chemotherapeutic drugs to targeted and immunotherapeutic drugs, which will expand their clinical potential and serve as a research hotspot. Hence, the appropriate and precise use of circRNAs is essential in the field of cancer studies, as well as the new foothold for precision medicine in the near future.

10. Conclusions

The study of circRNAs is a novel research field that has emerged with the rapid development of technology. Research on circRNAs has led to several surprising findings, indicating that circRNAs govern a wide spectrum of physiological and pathological processes, particularly in tumorigenesis. What we know so far suggests that circRNA-based diagnostic and therapeutic strategies may play promising role in cancer management.

Declaration of Competing Interest

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

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