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Circular RNA and its mechanisms in disease: From the bench to the clinic

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ABSTRACT

The emerging recognition of the functional roles of circular RNAs (circRNAs) has given rise to a new perspective regarding our understanding of cellular physiology and disease pathogenesis. Unlike linear RNAs, circRNAs are covalently closed continuous loops that act as gene regulators in mammals, and their sequence composition determines the mode of circRNA biogenesis. The availability and integrated use of advanced genome analysis platforms have allowed the identification of a large number of these molecules. Their high abundance, stability and evolutionary conservation among species endow circRNAs with numerous potential functions, such as acting as microRNA (miRNA) sponges or binding to RNA-associated proteins to form RNA-protein complexes that regulate gene transcription. Moreover, circRNAs have been shown to be expressed in a tissue-specific manner and in pathological conditions, which has stimulated significant interest in their role in human disease and cancer. In this concise review, we outline the characteristics, functions and mechanisms of action of circRNAs as well as their involvement in different diseases. Although their exact roles and mechanisms of gene regulation remain to be clarified, circRNAs have potential applications as disease biomarkers and novel therapeutic targets.

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Abbreviations: circRNAs, circular RNAs; miRNAs, microRNAs; RBPs, RNA-binding proteins; pre-mRNAs, precursor mRNAs; ecircRNAs, exonic circular RNAs; ciRNAs, circular intronic RNAs; EIciRNAs, exon-intron circular RNAs; tricRNA, tRNA intronic circular RNA; MBL, Muscleblind; QKI, quaking; ADAR1, adenosine deaminase acting on RNA 1; U1 snRNP, U1 small nuclear ribonucleoprotein; Pol II, RNA polymerase II; ceRNAs, endogenous RNAs; AD, Alzheimer's disease; FUS, fused in sarcoma; NP, neuropathic pain; MSA, multiple system atrophy; LPS, lipopolysaccharide; MI, myocardial infarction; GC, gastric cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; OSCC, oral squamous cell carcinoma; ESCC, esophageal squamous cell carcinoma; LAC, lung adenocarcinoma; ccRCC, clear cell renal cell carcinoma; PCa, prostate cancer; HDFs, human dermal fibroblasts; NASH, nonalcoholic steatohepatitis; HSCR, Hirschsprung's disease; PE, preeclampsia; OA, osteoarthritis; ECM, extracellular matrix; CTEPH, chronic thromboembolic pulmonary hypertension.

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1. Introduction

Protein-coding genes and their transcripts are the most studied sequences in eukaryotic cells (Esteller, 2011). However, protein-coding genes and RNAs comprise only a small fraction of genomes and transcriptomes (Alexander, Fang, Rozowsky, Snyder, & Gerstein, 2010). Indeed, the vast majority of sequences in the human genome do not encode proteins, and non-coding RNAs account for almost 95% of the total RNA transcribed from eukaryotic genomes (Warner, 1999). Non-cording RNAs, which are largely classified as transcribed ultraconserved regions, microRNAs (miRNAs), small nucleolar RNAs, PIWI-interacting RNAs, long non-cording RNAs, and circular RNAs (circRNAs), are being increasingly recognized as functioning in gene regulation and contributing to the development of many human disorders (Esteller, 2011; Mercer, Dinger, & Mattick, 2009). As a large proportion of the non-coding RNA family, circRNAs have drawn intense interest over the last few years. Unlike linear RNA molecules, circRNAs are closed circular molecules with a covalently closed loop structure that lack 5′-3′ polarity or a polyadenylated tail (Chen & Yang, 2015).

circRNAs were first identified in 1976 in an electron microscopy-based study of RNA viruses (Sanger, Klotz, Riesner, Gross, & Kleinschmidt, 1976) and have since been found in humans, mice, rats, fungi and other organisms (Capel et al., 1993; Cocquerelle, Daubersies, Majerus, Kerckaert, & Bailleul, 1992; Kolakofsky, 1976; Matsumoto, Fishel, & Wickner, 1990; Zaphiropoulos, 1996, 1997). Nonetheless, due to the lack of reliable high-throughput detection methods, only a handful of circRNAs have been identified in the past 30 years (Capel et al., 1993; Cocquerelle et al., 1992; Cocquerelle, Mascrez, Hetuin, & Bailleul, 1993; Kos, Dijkema, Arnberg, van der Meide, & Schellekens, 1986; Nigro et al., 1991; Zaphiropoulos, 1993, 1996). Based on their structural specificity, unknown functions and low abundance (Nigro et al., 1991), circRNAs were initially considered ancient and conserved molecules produced as errant byproducts of splicing and did not receive much attention.

However, advances in biotechnology, particularly bioinformatics and high-throughput sequencing technology have resulted in the discovery and identification of a large number of circRNAs. Indeed, circRNAs are abundant, diverse and conserved molecules that are often expressed in a tissue- and developmental stage-specific manner (Jeck et al., 2013; Rybak-Wolf et al., 2015; Salzman, Chen, Olsen, Wang, & Brown, 2013). Our knowledge regarding their functions has also expanded with their identification. Specifically, circRNAs might function as miRNA sponges to prevent mRNA translation (Hansen et al., 2013; Wang, Long, et al., 2016) and influence gene expression by regulating splicing (Ashwal-Fluss et al., 2014; Li, Huang, et al., 2015) or transcription and by interacting with RNA-binding proteins (RBPs) (Du et al., 2016). circRNAs play a critical role in biological processes and are reported to participate in multiple processes involved in disease progression, and thus, these molecules offer new potential opportunities for therapeutic intervention and might serve as diagnostic biomarkers. In this review, we briefly introduce the biogenesis, characteristics and function of circRNAs and highlight their roles in different diseases. Considering their ubiquitous presence and diversity, circRNAs might be major contributors to normal cellular physiological or pathological processes.

2. Characteristics of circRNAs

2.1. Broad presence and expression

Through analyses of transcriptome sequencing datasets with computational pipelines that specifically search for back-splicing junctions, the expression of circRNAs has been widely detected in a large number of metazoans and in diverse cell types and organisms, ranging from fruit flies to humans (Ivanov et al., 2015; Westholm et al., 2014; Zheng et al., 2016). circRNAs have also been found in plants (Lu et al., 2015; Sun et al., 2016; Ye, Chen, Liu, Zhu, & Fan, 2015) and other organisms, such as protists (Broadbent et al., 2015) and fungi (Wang et al., 2014). A total of 5.8% to 23% of actively transcribed human genes reportedly produce circRNAs (Conn et al., 2015; Kelly, Greenman, Cook, & Papantonis, 2015), and these circRNAs are dynamically regulated among tissues and cell types (Du et al., 2016; Li, Chen, et al., 2015). Although one study suggested that the levels of circRNAs and their linear counterparts are not highly correlated (Salzman et al., 2013), others have reported that expression changes between circRNAs and linear variants from the same gene are in fact largely correlated (Hansen et al., 2013; Jeck et al., 2013; Salzman, Gawad, Wang, Lacayo, & Brown, 2012). The potential functions of circRNAs are consistent with their widespread and regulated expression.

2.2. Stability

Due to their resistance to RNA exonucleases or RNase R, circRNAs are more stable than linear RNAs (Jeck et al., 2013; Suzuki et al., 2006), which might lead to the accumulation of circRNAs and thus a higher concentration of circRNAs than linear RNAs in quiescent and post-mitotic cells, such as neurons (Chen & Schuman, 2016). circRNAs also accumulate in some physiological processes, such as neuronal differentiation, fetal development and synaptic development (Rybak-Wolf et al., 2015; Szabo et al., 2015; Westholm et al., 2014; You et al., 2015), and this accumulation of circRNAs indicates that they might function in these processes. Furthermore, due to their high stability in blood and other body fluids, circRNAs are suitable biomarkers for disease diagnosis.

2.3. Conservation

circRNA expression appears to be conserved across mammals (Barrett & Salzman, 2016), and some are even conserved in evolutionarily distant *Drosophila* (Rybak-Wolf et al., 2015). In relatively closely related species, such as humans and mice, 4% of orthologous genes can generate circRNAs (Salzman et al., 2013), and approximately 5–30% of these circRNAs are completely conserved (Guo, Agarwal, Guo, & Bartel, 2014; Jeck et al., 2013; Memczak et al., 2013). In addition, approximately 5–10% of human brain circRNAs are expressed in the porcine brain (Barrett & Salzman, 2016; Veno et al., 2015), and 23% of circRNAs are conserved between mouse and rat (You et al., 2015). Taken together, the findings show that circRNAs are unlikely to be non-functional byproducts.

3. circRNA biogenesis

Although it is known that circRNAs are derived from precursor mRNAs (pre-mRNAs), their biogenesis remains elusive. circRNAs differ from other RNAs in their remarkable continuous closed loop structure, which is covalently linked by free 3′ and 5′ ends (Granados-Riveron & Aquino-Jarquin, 2016). This closed loop structure, which is also called a "back-splicing" structure, is generated from the joining of an upstream 3′ splice site to a downstream 5′ splice site (Barrett & Salzman, 2016). Similar to canonical splicing, the formation of the 'back-splicing' structure requires not only a canonical splicing signal but also the canonical spliceosome machinery (Andres-Leon, Nunez-Torres, & Rojas, 2016). As shown in Fig. 1, several pathways participate in the circularization of circRNAs. circRNAs can mainly be classified in three categories: exonic circular RNAs (ecircRNAs) (Jeck et al., 2013; Memczak et al., 2013; Salzman et al., 2013; Zhang et al., 2013), circular intronic RNAs (ciRNAs) (Zhang et al., 2013), and exon-intron circular RNAs (EIciRNAs) (Li, Huang, et al., 2015). RBP pairing (Ashwal-Fluss et al., 2014; Conn et al., 2015) and intron pairing (Lee & Rio, 2015; Rybak-Wolf et al., 2015; Zhang et al., 2014) drive circularization in the direct back-splicing pathway of circRNA formation. Some RBPs, including Muscleblind (MBL) (Ashwal-Fluss et al., 2014), Quaking (QKI) (Conn et al., 2015) and adenosine deaminase acting on RNA 1 (ADAR1) (Ivanov et al., 2015), participate in the regulation of circRNA biogenesis. MBL and

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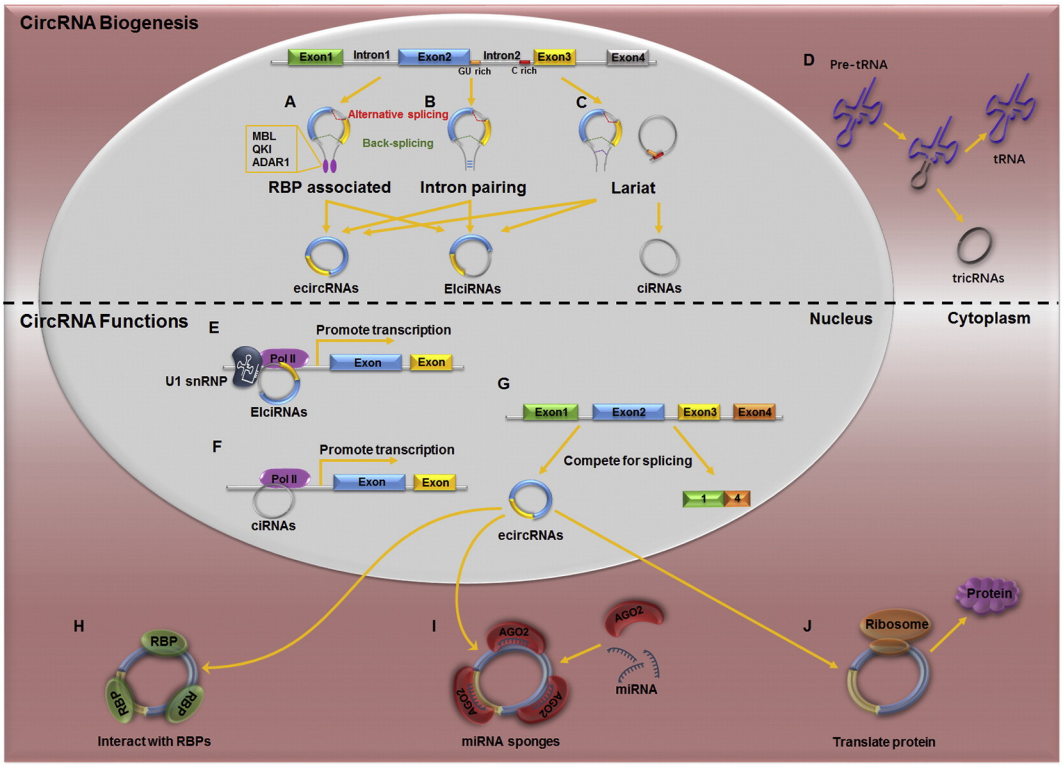

Fig. 1. circRNA biogenesis and function. (A) RBP-associated pathway of circRNA biogenesis. The flanking intronic reverse complementary sequences (e.g., Alu elements) can lead to the close location of introns and the subsequent connection of the exon ends. QKI and MBL can promote ecircRNA or EIciRNA generation. ADAR can antagonize ecircRNA or EIciRNA generation. (B) Intron pairing pathway of circRNAs biogenesis. The circular structure can be generated through the direct base-pairing of the introns flanking inverted repeats or complementary sequences. The introns are removed or retained to form ecircRNA or EIciRNA. (C) Lariat-driven pathway of circRNA biogenesis. In this circRNA formation pathway, a lariat structure containing skipped exons 2 and 3 is generated through the "head-to-tail" joining of the 3′ splice donor site of exon 1 to the 5′ splice acceptor of exon 4. This lariat is spliced internally, and the introns are removed or retained to form ecircRNA or EIciRNA. The lariat intron generated from the splicing reaction depends on the GU-rich sequences close to the 5′ splice site (orange box) and the C-rich sequences near the branch point (red box), avoiding debranching and degradation. A stable ciRNA is formed after the 3′ 'tail' downstream from the branch point is trimmed. (D) tricRNAs are synthesized from introns spliced from pre-tRNA. (E) EIciRNAs can interact with transcription complexes in the promoter region of their host gene to induce gene transcription by interacting with U1 snRNP. (F) ciRNAs can directly interact with transcription complexes on host genes to induce their transcription. (G) The back-splicing pattern of circRNA biogenesis can alter the expression of the linear gene product. The back-splicing and linear splicing can compete with each other during splicing. As a result, a linear RNA or an ecircRNA is generated. (H) circRNAs can interact with RBPs and affect their functions and translocations. (I) circRNAs can act as miRNA sponges to inhibit miRNA activity by interacting with miRNA-Ago2 complexes. (J) Some circRNAs have protein-coding capacity and can encode proteins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

QKI bind to the sequence motifs of flanking introns and link two flanking introns together, and thus, these RBPs regulate adjacent splice sites to promote circRNA formation. Conversely, ADAR1 inhibits circRNA formation by binding to double-stranded RNA and melting the stem structure, thus generating ecircRNAs and EIciRNAs (Fig. 1A). Intron pairing-driven and lariat-driven models of circRNA circularization have also been reported. With regard to intron pairing, alternative formation of inverted repeated Alu elements in flanking introns and competition between RNA pairings across flanking introns or within individual introns can lead to alternative circularization, resulting in the production of multiple circRNAs and linear transcripts from a single gene (Zhang et al., 2014). These circRNAs can be classified into two types of circRNAs: ecircRNAs and EIciRNAs (Fig. 1B). Moreover, circRNAs can be produced through an exon-containing lariat-driven pathway (Matera & Wang, 2014). As shown in Fig. 1C, a lariat containing an exon is created by an exon skipping event, and the flanking intronic sequence is removed though internal splicing of the lariat, ultimately producing ecircRNAs or EIciRNAs (Barrett, Wang, & Salzman, 2015). ciRNAs are also generated via a lariat-derived mechanism based on a consensus motif containing a 7-nt GU-rich element near the 5′ splice site and an 11-nt C-rich element near the branchpoint site (Zhang et al., 2013). In addition, John et al. found another conserved mode of circRNA biogenesis occurring in both archaea and eukaryotes. In this case, the tRNA splicing endonuclease complex cleaves an intron-containing pre-tRNA at the bulge-helix-bulge motif; the exon halves are then ligated, and the intron termini are also ligated to form a tRNA intronic circular RNA (tricRNA) (Noto, Schmidt, & Matera, 2017) (Fig. 1D).

4. circRNA function

Many of the functions of circRNAs have been elucidated over the last few years. For example, circRNAs can act as gene expression regulators via different regulatory modes (Fig. 1).

4.1. Regulation of transcription and alternative splicing

Almost all circRNAs distributed in the cytoplasm are generated from exons (Jeck et al., 2013; Salzman et al., 2012); in contrast, ciRNAs and EIciRNAs are primarily located in the nucleus (Hansen et al., 2013; Zhang et al., 2013) and most likely function at the transcriptional level. EIciRNAs have been demonstrated to interact with U1 small nuclear ribonucleoprotein (U1 snRNP) and RNA polymerase II (Pol II) via U1 snRNA-binding sites and perform similar *cis*-regulation function (Li, Huang, et al., 2015) (Fig. 1E). Moreover, circRNAs regulate the transcription of their parent genes in *cis* or *trans* and interact with the Pol II complex in the nucleus to activate transcription of their parent genes (Zhang et al., 2013) (Fig. 1F).

Certain circRNAs also act on gene expression *trans*-functionally by competing with linear splicing (Fig. 1G), and back-splicing might generate circRNAs and their corresponding linear RNAs (Ashwal-Fluss et al., 2014). For example, during circMbl formation, circMbl competes with

MBL pre-mRNA splicing and thus negatively affects canonical splicing (Ashwal-Fluss et al., 2014). Additionally, in the process of ecircRNAs formation, some ecircRNAs sequester the translation start site, leading to the production of non-coding linear transcripts and thereby reducing protein expression (Jeck & Sharpless, 2014). For example, some ecircRNAs are derived from exon 2, which contains the canonical translation start codon, of HIPK2/3. Circularization of the exon negatively affects the production of the canonical protein from the locus. Therefore, this form of "alternative splicing" might regulate the expression of the HIPK2/3 gene (Jeck et al., 2013; Jeck & Sharpless, 2014).

4.2. Interactions with RBPs

circRNAs also interact with RBPs, as evidenced by the co-localization of circRNAs and RBPs (Fig. 1H). Some circRNAs might bind, store or sequester RBPs to particular subcellular locations (Du, Zhang, et al., 2017). For example, circ-Amotl1 interacts with c-myc, STAT3, PDK1 and AKT1, facilitating their nuclear translocation and further affecting the expression of their targets (Yang, Awan, et al., 2017; Yang, Du, et al., 2017; Zeng et al., 2017). circRNAs also act as competing elements to regulate the function of RBPs. circPABPN1 and PABPN1 mRNA competitively bind to HuR, and the combination of circPABPN1 and HuR subsequently suppresses PABPN1 translation (Abdelmohsen et al., 2017). Moreover, it is possible that circRNAs not only bind a single RBP but also are more likely to act as scaffolds for the assembly of large protein complexes (Hansen, Veno, Damgaard, & Kjems, 2016; Jeck & Sharpless, 2014; Lasda & Parker, 2014). It has been reported that circ-Foxo3 combines with both CDK2 and p21 to form a ternary complex, and the resulting complex inhibits the function of CDK2 (Du et al., 2016). Furthermore, circ-Foxo3 interacts with ID-1, E2F1, FAK and HIFα and suppresses their anti-senescence and anti-stress roles (Du, Yang, et al., 2017).

4.3. miRNA sponge

The majority of circRNAs are primarily localized in the cytoplasm (Jeck et al., 2013; Salzman et al., 2012), an observation that prompted researchers to study the function of circRNAs in post-transcriptional regulation. In 2013, two research groups provided the first demonstration that circRNAs might function as miRNA sponges or competitive endogenous RNAs (ceRNAs) to regulate the expression of miRNA targets. miRNAs can bind to the matched 3′-untranslated regions of mRNAs via the seed region, and circRNAs also contain miRNA target sites. By competing for miRNAs, circRNAs indirectly regulate the translation of mRNAs (Fig. 1I). These studies also found that the ciRS-7 circRNA contains more than 60 conserved miR-7 target sites and can serve as a sponge for miR-7, thereby regulating the expression of miR-7 target mRNAs. Shortly thereafter, two studies noted that most circRNAs do not function as miRNA sponges because the large majority of these molecules contain fewer miRNA binding sites than co-linear mRNAs (Guo et al., 2014; Jeck & Sharpless, 2014). However, an increasing number of studies have found that many circRNAs can perform this function despite lacking a large number of miRNA-binding sites. Because an increasing body of evidence indicates that this function is conserved in different species, the action of circRNAs as miRNA sponges is not an isolated phenomenon.

4.4. Translation

In addition to the non-coding functions of circRNAs, researchers have also studied the translation capacity of circRNAs (Fig. 1J). Initial findings revealed that synthetic exonic circRNAs that contain internal ribosome entry sites or prokaryotic binding sites have protein-coding capacity both in vivo and in vitro (Chen & Sarnow, 1995; Perriman & Ares, 1998; Wang & Wang, 2015), leading to the question of whether these translational products exist endogenously. Legnini et al. discovered that the circ-ZNF609, which functions in myogenesis, could be translated into a protein through a splicing-dependent, cap-independent mechanism (Legnini et al., 2017), providing the first indication that endogenous circRNAs can in fact encode proteins.

5. circRNAs in human diseases

Based on the functions of circRNAs, researchers have investigated the role of circRNAs in physiology and pathology. The available evidence shows that circRNAs are associated with autophagy (Huang et al., 2017; Zhang, Wang, Wan, Xu, & Pang, 2017), apoptosis (Du, Fang, et al., 2017), cell cycle (Du et al., 2016) and proliferation (Bachmayr-Heyda et al., 2015), indicating that circRNAs might function in diseases, and an increasing number of studies have revealed that circRNAs exert a regulatory function in different diseases (Fig. 2 and Table 1) via different mechanisms (Fig. 3). Moreover, circRNAs have the potential to serve as clinical diagnosis markers and therapeutic targets.

5.1. Neurological diseases

A previous study found that circRNAs are detected at a higher abundance in the mammalian brain than in other analyzed tissues (Rybak-Wolf et al., 2015; You et al., 2015), prompting many researchers to explore the role of circRNAs in diseases of the nervous system. Colocalization of the circRNAs ciRS-7 and miR-7 was observed, particularly in neocortical and hippocampal neurons (Hansen et al., 2013; Memczak et al., 2013). Thus, ciRS-7 can perform its "sponge" function to regulate miR-7, which has been implicated in Parkinson's disease and is involved in various cancer pathways. These researchers suggested that ciRS-7 serves as a crucial factor in neuron function and as a responsible candidate in neurological disorders and brain tumor development (Hansen et al., 2013). This previous study initiated research in the field of circRNAs and nervous system diseases.

5.1.1. circRNAs and Alzheimer's disease

Studies of Alzheimer's disease (AD) revealed that ciRS-7 levels were significantly reduced in AD hippocampal CA1 samples versus age-matched healthy controls. It was thus predicted that ciRS-7 deficiency might result in decreased expression of selective miR-7 targets, such as the AD-relevant target UBE2A, via the abovementioned "miRNA sponge" function (Lukiw, 2013). This conjecture was confirmed in the ensuing study (Zhao, Alexandrov, Jaber, & Lukiw, 2016), which revealed that ciRS-7 also inhibits NF-KB translation and induces its localization to the cytoplasm, and this effect de-represses the expression of UCHL1, which promotes APP and BACE1 degradation (Shi et al., 2017). Notably, APP and BACE1 function in the generation of amyloid β in AD (O'Brien & Wong, 2011; Zhang et al., 2015). Based on these results, we are confident that ciRS-7 serves as an effective target in the treatment of AD.

5.1.2. circRNAs and other neurological diseases

Fused in sarcoma (FUS) was recently reported to affect circRNA expression in motor neurons derived from murine embryonic stem cells (Errichelli et al., 2017). Considering the role of FUS in neurodegenerative disorders, including amyotrophic lateral sclerosis and frontotemporal dementia (Deng, Gao, & Jankovic, 2014), a previous study not only elucidated the mechanism of FUS-linked circRNA expression but also linked circRNA function to neurodegenerative processes. Zhou et al. performed second-generation sequencing to analyze differentially expressed ncRNAs in the spinal cord of rats with spared nerve injury-induced neuropathic pain (NP), and the results showed that 188 circRNAs were significantly dysregulated 14 days after spared nerve injury. The researchers then constructed a circRNA-miRNA-mRNA network based on the sequencing data and verified the relationship between rno_circ_0006928 and miR-184 through a luciferase assay, and the findings of the study suggested that circRNAs play a key role in the pathogenesis of NP (Zhou, Xiong, Chen, Yang, & Fan, 2017). Another group performed circRNA

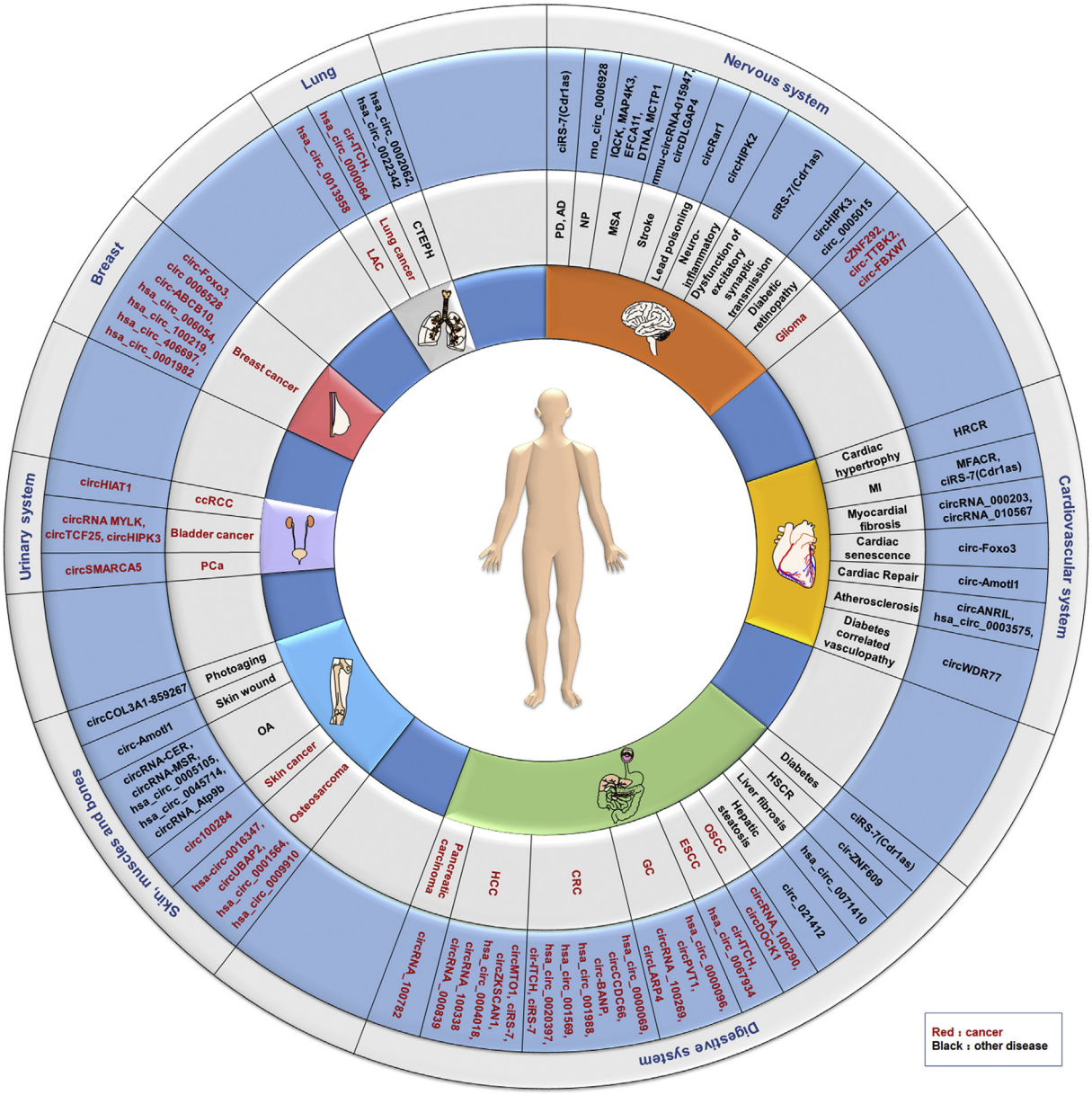

Fig. 2. Overview of functional circRNAs in different diseases. The map shows the circRNAs that have been confirmed to function in different diseases.

sequencing on brain samples from multiple system atrophy (MSA) patients and found five overexpressed circRNAs: IQCK, MAP4K3, EFCA11, DTNA and MCTP1. Further analysis revealed that these circRNAs are overexpressed in the white matter of MSA cortical tissue (Chen et al., 2016). To explore the relationship between circRNAs and cerebral ischemia-reperfusion injury, Lin et al. performed circRNA microarray analysis to investigate the expression of circRNAs in HT22 cells treated with oxygen-glucose deprivation/reoxygenation versus normal control cells, and these researchers found that 15 circRNAs were significantly altered and verified that mmu-circRNA-015947 was overexpressed. Combining their results with those from a bioinformatics analysis, these researchers concluded that mmu-circRNA-015947 might be involved in cerebral ischemia-reperfusion injury (Lin et al., 2016). Bai et al. found that circDLGAP4 was downregulated in the plasma of acute ischemic stroke patients and in a mouse stroke model. Overexpression of circDLGAP4 promoted HECTD1 expression by sponging miR-143, resulting in the amelioration of infarct areas and blood-brain barrier damage in the mouse stroke model. The investigation suggested that circDLGAP4 might serve as a novel therapeutic target in acute ischemic injury (Bai et al., 2018). Another study suggested that hsa_circRNA_103636 expression in peripheral blood mononuclear cells might serve as a novel diagnostic and therapeutic biomarker in major depressive disorder (Cui et al., 2016). In another study, Nan et al. investigated the toxic effects of lead on the developing nervous system and found that circRar1 mediates neuronal apoptosis in response to lead toxicity via miR-671 (Nan et al., 2017), which offers a new approach for the treatment of lead poisoning. circRNAs have also been associated with drug abuse and neuroinflammatory disorders. For example, circHIPK2 upregulation enhances astrocyte activation via autophagy and endoplasmic reticulum stress by targeting miR-124 and sigma-1 receptor in methamphetamine- or lipopolysaccharide (LPS)-injection mouse models (Huang et al., 2017). The regulatory role of circRNAs in diseases was first confirmed in circRNA-knockout mice, and the

Table 1
circRNAs associated with human diseases.

Diseases	Type of diseases	circRNAs	References (PMID)
Neurological diseases	Parkinson' disease	ciRS-7(Cdr1as)	23446346
	Alzheimer's disease	ciRS-7(Cdr1as)	24427167, 27929395
	Neuropathic pain	rno_circ_0006298	28420964
	Multiple system atrophy	IQCK, MAP4K3, EFCA1, DTNA, MCTP1	27470294
	Cerebral ischemia-reperfusion injury	mmu-circRNA-015947	26845359
	Lead poisoning	circRar1	27604105
	Neuroinflammatory	circHIPK2	28786753
	Dysfunction of excitatory synaptic transmission	ciRS-7(Cdr1as)	28798046
	Diabetic retinopathy	circHIPK3	28860123
	Ischemic stroke	circDLGAP4	29114076
Cardiovascular diseases	Cardiac hypertrophy	HRCR	26802132
	Myocardial infarction	MFACR	28498369
		ciRS-7(Cdr1as)	26998750
	Myocardial fibrosis	circRNA_000203	28079129
		circRNA_010567	28412345
	Cardiac senescence	circ-Foxo3	26873092
	Atherosclerosis	circANRIL	27539542
		hsa_circ_0003575	28946214
Cancers	Gastric cancer	hsa_circ_0000096	28081541
		circPVT1	27986464
		circ_100269	28657541
		circLARP4	28893265
	Colorectal cancer	hsa_circ_0000069	28003761
		circCCDC66	28249903
		circ-BANP	28103507
		hsa_circ_001988	26884878
		hsa_circ_001569	27058418
		hsa_circ_0020397	28707774
		cir-ITCH	26110611
		ciRS-7(Cdr1as)	28174233
	Hepatocellular carcinoma	circMTO1	28520103
		ciRS-7(Cdr1as)	27614453, 27391479
		circZKSCAN1	28211215
		circRNA_000839	28695771
		circRNA_100338	28710406
		hsa_circ_0004018	28938566
	Oral squamous cell carcinoma	circRNA_100290	28368401
		circDOCK1	29286141
	Esophageal squamous cell carcinoma	cir-ITCH	25749389
		hsa_circ_0067934	27752108
	Lung cancer	cir-ITCH	27642589
		hsa_circ_0000064	29223555
	Lung adenocarcinoma	hsa_circ_0013958	28685964
	Breast cancer	circ-Foxo3	26657152
		hsa_circ_0001982	28933584
		hsa_circ_0006528	28803498
		circ-ABCB10	28744405
		hsa_circ_006054, hsa_circ_100219, hsa_circ_406697	28484086
	Clear cell renal cell carcinoma	circHIAT1	28089832
	Bladder cancer	circTCF25	27484176
		circRNA MYLK	27363013
		circHIPK3	28794202
	Prostate cancer	circ-SMARCA5	28765045
	Papillary thyroid carcinoma	hsa_circRNA_100395	28288173
	Osteosarcoma	hsa-circ-0016347	28424426
		circUBAP2	28977896
		hsa_circ_0001564	29229385
		hsa_circ_0009910	29117539
	Glioma	cZNF292	27613831
		circ-TTBK2	28219405
		circ-FBXW7	28903484
	Pancreatic carcinoma	circRNA_100782	29255366
	Arsenite-induced skin oncogenesis	circ100284	28062277

Table 1 (*continued*)

Diseases	Type of diseases	circRNAs	References (PMID)
Immune diseases	Microbial infection	circRasGEF1B	27362560
Aging	Photoaging	circCOL3A1-859267	28286269
Diabetes	Diabetes	ciRS-7(Cdr1as)	26211738
	Diabetic retinopathy	circHIPK3	28860123
		circ_0005015	29288268
	Diabetes correlated vasculopathy	circWDR77	29042195
Digestive diseases	Hirschsprung's disease	cir-ZNF609	27903978
Skin, muscle and bone diseases	Osteoarthritis	circRNA-CER	26931159
		circRNA-MSR	28624198
		hsa_circ_0005105	28276108
		hsa_circ_0045714	28795385
		circRNA_Atp9b	29305974
	Skin wound	circ-Amotl1	28676341
Hepatopathy	Liver fibrosis	hsa_circ_0071410	28774651
	Hepatic steatosis	circRNA_021412	28717649
Hypertension	Chronic thromboembolic pulmonary Hypertension	hsa_circ_0002062, hsa_circ_0022342	28682884

miRNA sponge function of Cdr1as was verified using Cdr1as-knockout mice, as evidenced by that finding that Cdr1as participates in the regulation of synaptic transmission (Piwecka et al., 2017).

5.2. Cardiovascular diseases

Several studies have indicated that circRNAs play important roles in the initiation and development of cardiovascular diseases. For example, Wu et al. found that hsa-circ-0005870 was significant downregulated in hypertensive patients (Wu, Jin, & Cai, 2017). Moreover, hsa_circ_0002062 and hsa_circ_0022342 were downregulated in human blood samples from chronic thromboembolic pulmonary hypertension (CTEPH) patients. A further bioinformatics analysis indicated that these circRNAs might be crucial for CTEPH development (Miao et al., 2017). In addition to hypertension, increasing studies have focused on the role of circRNAs in heart and vascular function.

5.2.1. Function of circRNAs in the heart

The Syr circRNA, one of the first circRNAs that was found to act as an endogenous sponge, contains 16 binding sites for miR-138 (Hansen et al., 2013). Coincidentally, miR-138 protects cardiomyocytes from hypoxia-induced apoptosis (He et al., 2013); therefore, some researchers have predicted that the Syr circRNA might function in the regulation of hypoxia-induced cardiomyocyte apoptosis (Wang, Yang, Yang, Fan, & Yang, 2016). The first evidence confirming that circRNAs are involved in the regulation of heart physiology and pathology was reported in 2016 (Wang, Long, et al., 2016). In the study, the researchers discovered a circRNA, which was later named HRCR, that could bind to miR-223 and act as a sponge to sequester miR-223, removing the translation inhibition of apoptosis inhibitor with CARD domain (ARC). In agreement with the effect of reducing hypertrophic responses in ARC transgenic mice, the overexpression of HRCR attenuated the hypertrophic responses. The study suggested that circRNA HRCR might be an attractive target in cardiac hypertrophy and heart failure therapy. Subsequently, the researchers found the circRNA MFACR/miR-652-3p/MTP18 axis is involved in the regulation of mitochondrial dynamics, cardiomyocyte apoptosis, and myocardial infarction (MI), indicating that the circRNA MFACR might be a potential therapeutic target in cardiovascular disease (Wang, Gan, et al., 2017). Another study indicated that ciRS-7, which the researchers named Cdr1as, acts as a miR-7 sponge and increases the expression of SP1 and PARP, which indicates that Cdr1as overexpression aggravates the disease process of MI

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(Geng et al., 2016). Additionally, a research team from Luxembourg reported lower expression of the circRNA MICRA in peripheral blood samples from MI patients compared with those from healthy controls, and patients with low levels of MICRA were at high risk of left ventricular dysfunction (Vausort et al., 2016). Another study performed a microarray analysis to obtain the circRNA expression profiles in the heart tissue of mice with MI and discovered a number of circRNAs that were deregulated during heart failure (Wu, Zhang, Zhang, Chang, & Wang, 2016). Some researchers also found that circRNA_000203 and circRNA_010567 are upregulated and function through the ceRNA mechanism in myocardial fibrosis (Tang, Zhang, et al., 2017; Zhou & Yu, 2017). Another research group investigated the relationship between circRNAs and aging and found that the expression of circ-Foxo3 is increased in aged hearts of both humans and mice. In addition, circ-Foxo3 functions as a positive regulator of cellular senescence, and circ-Foxo3 has been found to interact with ID-1, E2F1, FAK and H1F2α, retaining these proteins in the cytoplasm and limiting their anti-stress and anti-senescence roles to promote cardiac senescence (Du, Yang, et al., 2017). Similarly, circ-Amotl1 enhances cardiac repair by binding to PDK1 and AKT1, which leads to AKT1 phosphorylation and nuclear translocation (Zeng et al., 2017). Stanislas et al. performed RNA-Seq analysis using samples from rats (neonatal and adult), mice (sham or after transverse aortic constriction) and humans (failing, non-failing) and detected circRNAs derived from the titin gene, which suggested that circRNA formation might be involved in the regulation of titin splicing (Werfel et al., 2016). In support of this hypothesis, an increasing body of evidence from China and Canada indicates that *Ganoderma lucidum* protects the cardiovascular system by regulating circ-Foxo3 expression (Xie, Yang, et al., 2016), indicating a novel potential therapeutic strategy for cardiac disease.

Jakobi et al. (Jakobi, Czaja-Hasse, Reinhardt, & Dieterich, 2016) provided a comprehensive catalog of circRNAs that are resistant to RNase R in the adult murine heart, and many of the circRNAs coincided with cardiovascular disease-associated genetic loci, such as Hectd1, Ppp2r3a and Pry2. Moreover, a detailed analysis of the circRNA expression landscape in heart tissue revealed a high abundance of cardiac-specific circRNAs (Tan et al., 2017). All these studies indicate that circRNAs might serve as biomarkers of heart disease.

5.2.2. Function of circRNAs in blood vessels

circRNAs also play an important role in vascular disease. To explore the effects of circRNAs in endothelial cells under normoxic or hypoxic conditions, Boeckel et al. performed computational analyses, validated the hypoxia-induced circRNAs cAFF1, cZNF292 and cDENND4C and the hypoxia-downregulated circRNA cTHSD1 and found that cZNF292 exhibits proangiogenic activities (Boeckel et al., 2015). Another study observed aberrant high expression of hsa_circ_000595 in hypoxic aortic smooth muscle cells. Furthermore, the knockdown of hsa-circ-000595 in human aortic smooth muscle cells decreased the apoptotic rate via miR-19a (Zheng et al., 2015). A group from Germany and the USA studied blood vessels under hypoxic conditions, focusing on the role of circRNAs in atherosclerosis, and showed that circANRIL binds to PES1 to control ribosomal RNA maturation and induce nucleolar stress and p53 activation. As a result, circANRIL confers atheroprotection via the induction of apoptosis and inhibition of proliferation (Holdt et al., 2016), demonstrating that circRNAs can alter RNA function and ultimately affect human disease. Moreover, it has been reported that hsa_circ_0003575 suppresses vascular endothelial cell proliferation and angiogenesis induced by oxLDL, providing a new potential therapeutic target in atherosclerosis-induced vascular endothelial cell injury (Li, Ma, & Yu, 2017). Lending credence to this study, Chen et al. found that circWDR77 promotes vascular smooth muscle cell proliferation and migration through the miR-124-FGF2 axis (Chen, Cui, Yuan, Zhang, & Sang, 2017), indicating that circRNAs might be a novel therapeutic target in diabetes mellitus-correlated vasculopathy. Because the peripheral blood level of hsa_circ_0124644 can function as a diagnostic biomarker of coronary artery disease, circRNAs can also be used as biomarkers of blood vessel diseases (Zhao, Li, Gao, et al., 2017).

5.3. Cancers

Many studies have shown that circRNAs are closely associated with a variety of tumors. These studies can be divided into two main categories: those that detect the different expression patterns of circRNAs to identify potential biomarkers for cancer diagnosis and those that examine the regulatory role of circRNAs in cancer development.

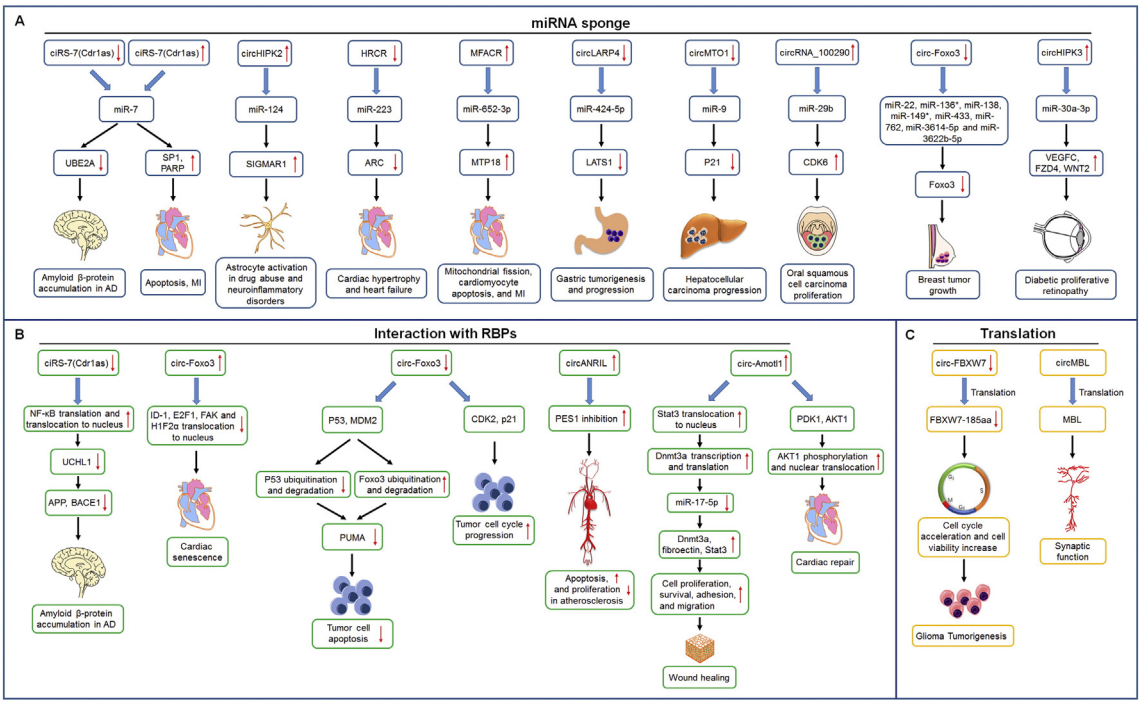

Fig. 3. circRNA-mediated mechanisms in different diseases. There are three main types of mechanisms: miRNA sponge (A), interaction with RBPs (B) and translation (C).

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5.3.1. Cancer diagnosis

Early diagnosis is very important in cancer therapy, and the field of cancer diagnosis has long been a research focus of global scientists. New cancer diagnostic methods are constantly emerging, and over the past several years, a growing awareness of circRNAs has led researchers to recognize the potential role of circRNAs in this crucial process. As biomarkers, circRNAs have several remarkable characteristics. 1) High and selective abundance: circular RNA isoforms of many human transcripts are present at comparable levels relative to their canonical linear counterparts (Salzman et al., 2012). In addition, the abundance of circRNAs is higher in low-proliferating cells or organs, such as the brain and blood (Bachmayr-Heyda et al., 2015; Memczak, Papavasileiou, Peters, & Rajewsky, 2015; Rybak-Wolf et al., 2015). 2) High stability: due to their covalently closed loop structures, which lack 5′-3′ polarity and polyadenylated tails, circRNAs likely resist RNase R or RNA exonuclease activation, which results in higher stability than that exhibited by linear RNAs (Suzuki & Tsukahara, 2014). 3) Conservation: most circRNAs are conserved in different species (Jeck et al., 2013; Wang et al., 2014; Zhang et al., 2014). 4) Specific expression: circRNA expression is tissue specific and/or developmental stage specific (Guo et al., 2014). 5) circRNAs can be detected not only in tumor tissues but also in blood and saliva (Bahn et al., 2015; Bonizzato, Gaffo, Te Kronnie, & Bortoluzzi, 2016). All these features make circRNAs suitable for use as potential biomarkers in cancer diagnosis, and there is an abundance of literature on the role of circRNAs in the clinical diagnosis of various cancers (Table 2).

Table 2
circRNAs reported to be potential biomarkers for detecting cancer.

Type of cancer	circRNAs	Expression	References (PMID)
Gastric cancer	hsa_circ_0000096	Downregulated	28081541
	circPVT1	Upregulated	27986464
	hsa_circ_002059	Downregulated	25689795
	hsa_circ_0000190	Downregulated	28130019
	hsa_circ_0001649	Downregulated	28167847
	hsa_circ_0001895	Downregulated	28443463
	circRNA_100269	Downregulated	28657541
	hsa_circ_0000026	Downregulated	28737829
	circLARP4	Downregulated	28893265
	hsa_circ_0074362	Downregulated	29240459
	hsa_circ_0000520	Downregulated	29103021
Colorectal cancer	circCCDC66	Upregulated	28249903
	hsa_circRNA_103809	Downregulated	28349836
	hsa_circRNA_104700	Downregulated	28349836
	circ-BANP	Upregulated	28103507
	ciRS-7(Cdr1as)	Upregulated	28174233
	hsa_circ_001988	Downregulated	26884878
	hsa_circ_0000069	Upregulated	28003761
	hsa_circ_001569	Upregulated	27058418
	hsa_circ_0020397	Upregulated	28707774
	circRNA0003906	Downregulated	29123417
Hepatocellular carcinoma	hsa_circ_0001649	Downregulated	26600397
	hsa_circ_0005075	Upregulated	27258521
	circZKSCAN1	Downregulated	28211215
	hsa_circ_0004018	Downregulated	28938566
	ciRS-7(Cdr1as)	Upregulated	27391479
	circRNA_100338	Upregulated	28710406
Laryngeal squamous cell cancer	hsa_circRNA_100855	Upregulated	27158380
	hsa_circRNA_104912	Downregulated	27158380
Esophageal squamous cell carcinoma	cir-ITCH	Downregulated	25749389
	hsa_circ_0067934	Upregulated	27752108
Oral squamous cell carcinomas	circRNA_100290	Upregulated	28368401
	circDOCK1	Upregulated	29286141
Osteosarcoma	hsa_circ_0016347	Upregulated	28424426
	circUBAP2	Upregulated	28977896
	hsa_circ_0001564	Upregulated	29229385
	hsa_circ_0009910	Upregulated	29117539
Breast cancer	hsa_circ_0001982	Upregulated	28933584
	hsa_circ_103110	Upregulated	28484086
	hsa_circ_104689	Upregulated	28484086
	hsa_circ_104821	Upregulated	28484086
	hsa_circ_006054	Downregulated	28484086
	hsa_circ_100219	Downregulated	28484086
	hsa_circ_406697	Downregulated	28484086
	circ-ABCB10	Upregulated	28744405
Non-small cell lung cancer	circRNA_100876	Upregulated	28343871
Lung cancer	cir-ITCH	Downregulated	27642589
	hsa_circ_0000064	Upregulated	29223555
Lung adenocarcinoma	hsa_circ_0013958	Upregulated	28685964
Glioma	circ-TTBK2	Upregulated	28219405
	circ-FBXW7	Downregulated	28903484
Clear cell renal cell carcinoma	circHIAT1	Upregulated	28089832
Bladder cancer	circTCF25	Upregulated	27484176
	circRNA-MYLK	Upregulated	28687357
Prostate cancer	circSMARCA5	Upregulated	28765045
Papillary thyroid carcinoma	hsa_circRNA_100395	Downregulated	28288173
Pancreatic carcinoma	circRNA_100782	Upregulated	29255366
Acute myeloid leukemia	hsa_circ_0004277	Downregulated	28282919

5.3.2. Cancer therapy

Considering the large number of circRNAs identified to date, circRNAs might regulate cancer proliferation, metastasis and invasion. It has been demonstrated that circRNAs can regulate the growth, apoptosis, and cell cycle progression of tumor cells(Du et al., 2016; Du, Fang, et al., 2017; Yang, Du, Li, Yee, & Yang, 2016), indicating that circRNAs might be a novel therapeutic target in cancer.

5.3.2.1. Gastric cancer. As one of the most common human cancers, gastric cancer (GC) is a steady contributor to cancer deaths worldwide (Li, Mo, Fu, Xiao, & Guo, 2016). Recent lines of evidence support the involvement of circRNAs in the regulation of GC occurrence and development (Chen, Li, et al., 2017; Gao et al., 2017; Li, Chen, et al., 2017; Liang, Zhang, Liu, Jia, & Li, 2017; Sui et al., 2017). Li et al. found that hsa_circ_0000096 was significantly downregulated in GC tissues compared with adjacent non-tumorous tissues. A further analysis showed that hsa_circ_0000096 can inhibit GC cell proliferation and migration by suppressing the expression of cyclin D1, CDK6, MM9-2 and MMP-9 (Li, Chen, et al., 2017). However, this study did not clarify the molecular regulatory mechanism. More recently, another research group found a series of circRNAs that were differentially expressed in GC tissues compared with matched normal tissues, as revealed by circRNA sequencing data. Among these circRNAs, circPVT1 was found to be upregulated in GC patients and to promote cell proliferation by sponging members of the miR-125 family (Chen, Li, et al., 2017). In addition, another study showed that circ_100269 was downregulated in GC tissues and inhibits cell proliferation by targeting miR-630 (Zhang, Liu, et al., 2017). Experimental evidence also demonstrated that circLARP4 is downregulated in GC tissues and suppresses cell growth and tumor invasion by upregulating the miR-424 target gene LATS1 (Zeng et al., 2017).

5.3.2.2. Colorectal cancer. Colorectal cancer (CRC) is the third most common cancer and leads to high mortality (Siegel, Miller, & Jemal, 2016). Guo et al. found that hsa_circ_0000069 was highly expressed in 30 CRC tissues compared with paired adjacent non-cancerous tissues, and a subsequent functional analysis demonstrated that hsa_circ_0000069 knockdown inhibited cell proliferation, migration, and invasion and affected the cell cycle by inducing the G0/G1 phase, which suggests that hsa_circ_0000069 might be a promising target in CRC therapy (Guo et al., 2016). circCCDC66 and circBANP are also overexpressed in CRC cancerous tissues and play similar roles in CRC (Hsiao et al., 2017; Zhu, Xu, Chen, & Yan, 2017). Conversely, hsa_circ_001988 is downregulated in CRC tumor tissues and plays a negative role in differentiation and perineural invasion (Wang, Zhang, et al., 2015). Another study reported that hsa_circ_001569 acts as a positive regulator of cell proliferation and invasion in CRC by targeting E2F5, BAG4 and FMNL2 via miR-145 (Xie et al., 2016). Similarly, hsa_circ_0020397 regulates CRC cell viability, apoptosis and invasion by sponging miR-138 and promoting TERT and PD-L1 expression (Zhang, Xu, & Wang, 2017). The downregulation of cir-ITCH in CRC tissues inhibits the Wnt/β-catenin

pathway and promotes ITCH expression, whereas upregulation of the expression of cir-ITCH reduces cell proliferation (Huang et al., 2015). ciRS-7 is an important molecule that functions in many biological and disease processes: upregulation of ciRS-7 activates the EGFR and RAF1 oncogenes by inhibiting miR-7, which indicates that ciRS-7 might function in CRC (Weng et al., 2017). These results suggest that circRNAs could be envisioned as new targets in the treatment of CRC.

5.3.2.3. Hepatocellular carcinoma. Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer (Yu et al., 2016). Approximately 740,000 people are newly diagnosed with HCC each year, and the disease accounts for approximately 700,000 annual deaths worldwide (Marquardt, Andersen, & Thorgeirsson, 2015). Multiple lines of evidence indicate that circRNAs are correlated with HCC. circMTO1 (hsa_circRNA_0007874/hsa_circRNA_104135) is significantly downregulated in HCC tissues and suppresses HCC progression by sponging miR-9 to promote p21 expression. In addition, HCC patients with reduced circMTO1 expression showed decreased survival, suggesting that circMTO1 might be a potential target in HCC therapy (Han et al., 2017). Yu et al. investigated the relationship between ciRS-7 and HCC and found that ciRS-7 is significantly overexpressed in HCC tissues compared with adjacent non-tumor tissues ($n = 35$). ciRS-7 also promotes CCNE1 and PIK3CD expression by targeting miR-7, with consequent enhancement of HCC cell proliferation and invasion (Yu et al., 2016). These studies suggest that ciRS-7 might serve as a novel therapy target in HCC. However, another study found no significant difference in ciRS-7 expression between HCC tissues and matched non-tumor tissues ($n = 108$); among the samples, ciRS-7 was upregulated in 39.8% (43/108) and downregulated in 60.2% (65/108). This research also showed that ciRS-7 was correlated with hepatic microvascular invasion levels and serves as an independent factor of hepatic microvascular invasion (Xu et al., 2017), which is closely related to some clinicopathological features of HCC (Lei et al., 2016). Additionally, the expression of circZKSCAN1 was significantly lower in HCC tissues than in matched adjacent non-tumorous tissues ($n = 102$). circZKSCAN1 inhibits HCC cell growth, migration and invasion by mediating several cancer-related signaling pathways (Yao et al., 2017), and hsa_circ_0004018, circRNA_100338, and circRNA_000839 might also play roles in HCC development (Fu et al., 2017; Kong et al., 2017; Peng, Shi, et al., 2017).

5.3.2.4. Digestive tract cancer. Mounting evidence indicates that circRNAs are involved in digestive tract cancer. For example, Chen et al. found that circRNA_100290, which increases CDK6 expression by sponging members of the miR-29b family, is upregulated in oral squamous cell carcinoma (OSCC) tissues compared with match non-cancerous tissues, indicating that circRNA_100290 might be a potential target in OSCC therapy (Chen, Zhang, et al., 2017). In addition to circRNA_100290, circDOCK1 could be another novel potential therapeutic target in OSCC because this circRNA significantly suppresses OSCC cell apoptosis by regulating the miR-196a-5p target BIRC3 (Wang, Wei, et al., 2017). In addition, Li et al. investigated cir-ITCH in 684 esophageal squamous cell carcinoma (ESCC) and paired adjacent non-tumor tissue samples and found that its expression was low in ESCC. cir-ITCH might increase the level of ITCH by sponging miR-7, miR-17 and miR-214 and thus plays a tumor-suppressive role in ESCC (Li, Zhang, et al., 2015). Another circRNA, hsa_circ_0067934, is also overexpressed in ESCC tissues compared with paired adjacent normal tissues ($n = 51$); it inhibits ESCC cell proliferation and migration and blocks cell cycle progression, indicating that hsa_circ_0067934 might be a therapeutic target in ESCC (Xia et al., 2016).

5.3.2.5. Osteosarcoma. Accumulating lines of evidence indicate that circRNAs play a critical role in the pathogenesis of osteosarcoma. For example, Jin et al. reported that hsa-circ-0016347 upregulates the expression of caspase-1 by targeting miR-214 and promotes the proliferation, invasion and metastasis of osteosarcoma cells (Jin, Jin, Zhang, & Wang, 2017). Additionally, circUBAP2 promotes osteosarcoma development by enhancing the expression and function of the miR-143 target Bcl-2 (Zhang, Wang, Ding, et al., 2017). Moreover, the level of hsa_circ_0001564 is increased in osteosarcoma tissues and contributes to tumorigenicity by sponging miR-29c-3p (Song & Li, 2018). A similar study was conducted by Deng et al., who revealed that hsa_circ_0009910 promotes carcinogenesis in osteosarcoma through regulation of the expression of miR-449a with its downstream target IL6R (Deng et al., 2018).

5.3.2.6. Other cancers. circRNAs have also received attention in studies of other cancers. For instance, Wan et al. investigated the role of cir-ITCH in lung cancer and, similar to the results of a previous study (Li, Zhang, et al., 2015), found that cir-ITCH acts as a sponge of oncogenic miR-7 and miR-214, resulting in ITCH overexpression and suppression of Wnt/β-catenin signaling activation in lung tumor tissues (Wan et al., 2016). Furthermore, Luo et al. indicated that hsa_circ_0000064 is upregulated in lung cancer tissues and promotes cell proliferation and metastasis (Luo et al., 2017). Moreover, the circ_0013958/miR-134/cyclin D1 axis is involved in lung adenocarcinoma (LAC) (Zhu, Wang, et al., 2017). circ-Foxo3 expression is downregulated in mouse breast tumor tissues compared with normal breast, skin and lipid tissues and acts as a suppressor of tumor growth and cancer cell survival and proliferation (Yang, Du, et al., 2016). Additionally, Tang et al. found that hsa_circ_0001982 promotes breast cancer cell carcinogenesis by targeting miR-143 (Tang, Zhou, et al., 2017). Accumulating evidence suggests that hsa_circ_0006528, circ-ABCD10, hsa_circ_006054, hsa_circ_100219 and hsa_circ_406697 might also play roles in breast cancer (Gao et al., 2017; Liang et al., 2017; Lu et al., 2017). Wang et al. examined the relationship between circRNAs and clear cell renal cell carcinoma (ccRCC) and found that circHIAT1 exerts a function as a metastatic inhibitor in suppressing androgen receptor-enhanced ccRCC cell migration and invasion. This regulation was found to be based on androgen receptor-circHIAT1-mediated miR-195-5p/29a-3p/29c-3p/CDC42 signals (Wang, Sun, Tao, Fei, & Chang, 2017). Through a circRNA microarray study, quantitative reverse transcription polymerase chain reaction identification and multiple bioinformatics approaches, Zhong et al. found that circTCF25 overexpression can increase CDK6 expression by downregulating miR-103a-3p and miR-107 in bladder cancer tissues, resulting in increased tumor cell proliferation and migration (Zhong, Lv, & Chen, 2016), and Huang et al. found that the circRNA MYLK/miRNA-29a-3p/DNMT3B/VEGFA/ITGB1 network might be relevant in bladder carcinoma (Huang et al., 2016). Similarly, circHIPK3 can suppress bladder cancer growth and metastasis by targeting miR-558 and heparanase (Li, Zheng, et al., 2017). It has been demonstrated that circ-SMARCA5 acts as an oncogene in prostate cancer (PCa) by inhibiting apoptosis and promoting the cell cycle (Kong et al., 2017). Microarray and bioinformatics results show that the hsa_circRNA_100395/miR-141-3p/miR-200a-3p axis might be involved in the regulation of papillary thyroid carcinoma tumors (Peng, Shi, et al., 2017). Moreover, cZNF292 and circ-TTBK2 reportedly regulate glioma though the Wnt/β-catenin and miR-217/HNF1β/Derlin-1 pathways, respectively (Yang, Qiu, et al., 2016; Zheng et al., 2017), and circ-FBXW7I might have potential prognostic implications in glioma by encoding the functional protein FBXW7-185aa (Yang et al., 2018). Moreover, a recent study indicated that circ_100782 promotes pancreatic carcinoma proliferation by sponging miR-124 through the IL6-STAT3 pathway (Chen, Shi, Zhang, & Sun, 2017). Through a study of the mechanism of arsenite-induced cell cycle progression and carcinogenesis acceleration, Xue et al. found that circ100284 expression is increased by arsenite and that this circRNA acts as an upregulator of EZH2 by sponging miR-217 in human keratinocyte cells. circ100284 might use this pathway to upregulate cyclin D1 and CDK4 and accelerate the cell cycle, ultimately leading to malignant transformation (Xue et al., 2017).

5.4. Other diseases

5.4.1. Immune diseases

The involvement of circRNAs in other diseases has also been reported. For example, Cardamone et al. found that an ecircRNA derived from exons 4 and 5 of the GSDMB gene is upregulated in the peripheral blood mononuclear cells of relapsing-remitting multiple sclerosis patients compared with those from healthy controls ($n = 30$) (Cardamone et al., 2017). The study indicated that this ecircRNA might serve as a diagnostic biomarker of multiple sclerosis. circRNAs also play critical roles in finetuning immune responses and protecting cells against microbial infection. A study by Ng et al. indicated that mcircRasGEF1B, the human homolog of which is named hcircRasGEF1B, regulates the stability of ICAM-1 mRNA and induces LPS-induced ICAM-1 expression in macrophage cells, which suggests that circRasGEF1B is a positive regulator of ICAM-1 in the TLR4/LPS pathway (Liu et al., 2016). circRNAs also regulate macrophage differentiation and polarization. Macrophages are activated through two different routes: classical activation (M1) and alternative activation (M2) (Mosser, 2003). Zhang et al. confirmed that the expression of circRNAs differs between these two activation states, and among the studied circRNAs, circRNA-003424, circRNA-013630, circRNA-001489 and circRNA-018127 are downregulated and circRNA-003780, circRNA-010056, and circRNA-010231 are upregulated in M1 compared with M2 (Wu, Zhang, Zhang, & Wang, 2017). Based on their analysis and inferences from the literature, Li et al. proposed that circRNAs are associated with systemic lupus erythematosus (Lan et al., 2016; Li, Zhang, et al., 2017), even though there is a lack of direct evidence. However, the results of microarray and bioinformatics analyses performed in another study suggested that the 100,783 circRNA might be a biomarker for the longitudinal tracking of CD28-related CD8(+) T cell aging and global immunosenescence (Wang, Yu, Luo, & Han, 2015).

5.4.2. Aging

The role of circRNAs during photoaging has also been evaluated. Twenty-nine circRNAs that showed significant differential expression between UVA-irradiated and non-irradiated human dermal fibroblasts (HDFs) were identified by deep sequencing of RNA, and among these circRNAs, circCOL3A1-859267 exhibited the most significant downregulation in photoaged HDFs. A follow-up study showed that type I collagen is regulated by circCOL3A1-859267 in photoaged HDFs, suggesting that this circRNA might be a new therapeutic target in photoaging (Peng, Song, Zheng, Wang, & Lai, 2017). In another aging study, Gruner et al. discovered that circRNAs accumulate in the aging mouse brain, but this age-related accumulation did not show a global trend, such as in the aged heart. These findings suggest that circRNAs might play biological roles in the age-related decline in neural function (Gruner, Cortes-Lopez, Cooper, Bauer, & Miura, 2016). Another aging study demonstrated that circPVT1 is downregulated in senescent fibroblasts and functions as a let-7 sponge, resulting in reduced expression of several proliferative proteins, such as IGF2BP1, KRAS and HMGA2 (Panda et al., 2017).

5.4.3. Diabetes

Diabetes is one of the most important health issues worldwide (Yoo, 2017). Approximately 46.5% of the nearly 410 million diabetic patients worldwide have not been diagnosed (Rahelic, 2016). The level of hsa_circ_0054633 in peripheral blood was recently found to be associated with diabetes and might be a diagnostic biomarker of pre-diabetes and type 2 diabetes mellitus (Zhao, Li, Jian, et al., 2017). Another circRNA, Cdr1as, can improve insulin production and secretion by targeting Pax6 and Myrip in mouse β cells, respectively, via miR-7 as a mediator (Xu, Guo, Li, & Yu, 2015), which indicates that this circRNA might be a novel therapeutic target in diabetes. Diabetic vascular complications are the major causes of disability and high mortality among patients with diabetes (Sena, Pereira, & Seica, 2013), and circHIPK3 can increase VEGFC, FZD4 and WNT2 expression by sponging miR-30a-3p. In contrast, the silencing of circHIPK3 alleviates retinal vascular disorders, suggesting that circHIPK3 silencing is a potential therapy target for controlling diabetic proliferative retinopathy (Shan et al., 2017). In addition, circ_0005015 is upregulated in the plasma, vitreous samples, and fibrovascular membranes of diabetic retinopathy patients. circ_0005015 also facilitates retinal endothelial angiogenic function because it increases the expression of MMP-2, XIAP, and STAT3 by acting as a miR-519d-3p sponge (Zhang, Chen, et al., 2017).

5.4.4. Digestive diseases

To investigate the pathogenesis of nonalcoholic steatohepatitis (NASH), Jin et al. investigated the circRNA and mRNA profiles for biomarkers that could be used for the diagnosis of NASH and predicted the potential circRNA-miRNA-mRNA network to analyze the pathogenesis of NASH (Jin, Feng, Xiang, Chen, & Li, 2016). Peng et al. studied the relationship between circRNAs and Hirschsprung's disease (HSCR) and found that cir-ZNF609 participates in the pathogenesis of HSCR through the miR-150-5p/AKT3 pathway (Peng, Chen, et al., 2017). Moreover, Chen et al. found that hsa_circ_0071410 promotes hepatic stellate cell activation in radiation-induced liver fibrosis by targeting miR-9-5p (Chen, Yuan, et al., 2017). A similar study was undertaken by Guo et al., who found that circRNA_021412 induces hepatic steatosis through miR-1972/LPIN1 signaling (Guo et al., 2017).

5.4.5. Reproductive system diseases

By profiling circRNA expression in human testis tissue via high-throughput sequencing, Dong et al. predicted more than 15,000 circRNAs, of which 67% were novel circRNAs. The genes encoding these circRNAs are mostly related to reproduction, and these researchers also found that circRNAs exist stably and can be detected in seminal plasma. According to this study, circRNAs might participate in the regulation of spermatogenesis and could serve as biomarkers of male infertility diseases. Preeclampsia (PE) is a common pregnancy disorder with high maternal and fetal mortality and morbidity (Lopez-Alarcon et al., 2015). Two different groups analyzed the results from circRNA microarrays of blood corpuscles (Zhang, Yang, Long, & Li, 2016) and placental tissues (Qian et al., 2016) from PE patients and age-matched pregnant women, and the findings indicated that circRNAs might serve as biomarkers in PE diagnosis and potential targets in PE treatment. Regardless, Prefumo commented that there is still a long way to go from the laboratory to the clinical use due to the restrictions of detection techniques and the classification of clinical samples (Prefumo, 2016).

5.4.6. Skin, muscles and bones

The relationship between circRNAs and skin wound healing has also been explored. For example, ectopic circ-Amotl1 can increase the fibronectin, Dnmt3a and Stat3 levels by decreasing miR-17-5p, but the mechanism through which circ-Amot1 regulates miR-17-5p does not ceRNA (Yang, Awan, et al., 2017). circ-Amotl1 facilitates Stat3 nuclear translocation and enhances the transcription and translation of Dnmt3a, which in turn decreases miR-17-5p expression by inducing miR-17 promoter methylation. As the targets of miR-17-5p, fibronectin, Dnmt3a and Stat3 are upregulated, contributing to skin wound repair (Shan et al., 2009; Yang, Awan, et al., 2017). As a prevalent degenerative joint disease (Vinatier, Merceron, & Guicheux, 2016), osteoarthritis (OA) causes great pain in patients (Mesci, Mesci, & Kulcu, 2016), but the pathogenesis of OA has not been clarified to date. Focusing on circRNA function in OA, Liu et al. found that 71 circRNAs showed differential expression between OA and normal articular cartilage. Among these circRNAs, the knockdown of the upregulated circRNA CER can suppress MMP13 expression by sponging miR-136, which increases chondrocyte extracellular matrix (ECM) formation (Liu et al., 2016). Similarly, the knockdown of overexpressed circRNAs-MSR in chondrocytes under mechanical stress suppresses TNF-α expression and ECM formation (Liu

et al., 2017). Another research group found that hsa_circ_0005105 expression was increased during this process, resulting in the upregulation of nicotinamide phosphoribosyltransferase by targeting miR-26a to promote ECM formation (Wu, Zhang, et al., 2017). hsa_circ_0045714 also regulates ECM synthesis as well as chondrocyte proliferation and apoptosis by promoting the miR-193b target gene IGF1R (Nan et al., 2017). Moreover, the knockdown of circRNA_Atp9b inhibits ECM catabolism and inflammation in OA by targeting miR-138-5p (Zhou, Du, Huang, Chen, & Zhu, 2018). The results of these studies provide new targets for OA therapy. Lan et al. performed ncRNA and mRNA microarray analyses of lumbar disc samples from 10 adults and revealed the RNA interaction machinery through a bioinformatics analysis. Their study suggested that circRNAs might be involved in the regulation of human intervertebral disc degeneration (Lan et al., 2016). Dou et al. found that that expression of 24 circRNAs was changed at different stages during osteoclast differentiation, which suggests the participation of circRNAs during osteoclastogenesis (Dou et al., 2016). Overall, circRNAs might be important therapy targets in diseases.

6. Therapeutic advantage of circRNAs

Considering the abovementioned results, circRNAs might be useful therapeutic agents. Controlling the expression of natural circRNAs in specific tissues and cells of the human body might yield greatly reduced side effects compared with those obtained with synthetic molecules, such as modified chemical drugs and RNA interference constructs, which would increase the value of circRNAs. In addition, this control might be a meaningful starting point for future gene therapy. To the best of our knowledge, a general phenomenon of circRNAs and one of their main functions are acting as a miRNA sponge. Thus, potent artificial sponges can be designed and developed by studying endogenic circRNA sponge structures to ultimately regulate miRNA function in disease. Artificial sponges constitute a new prospect in the development of drugs targeting miRNA. Another advantage of circRNA therapy is their potentially low off-target effect, whereas the off-target effects of miRNAs and siRNAs are very high due to their short lengths. Indeed, off-target effects are an important problem that restricts the clinical application of small molecule RNAs. In contrast, this problem will not hinder the progress of circRNA therapy due to the specific and stable structure of circRNAs.

7. Future perspectives

Over the past several years, a large number of circRNAs have been identified in biological systems. However, as new circRNAs continue to be discovered, there is no unified nomenclature for standardizing the names of circRNAs. Currently, most circRNAs are named according to their function, such as ciRS-7 (circRNA sponge for miR-7) (Hansen et al., 2013) and MFACR (mitochondrial fission and apoptosis-related circRNA) (Wang, Gan, et al., 2017), or their host genes, such as circMTO1 (Han et al., 2017) and circ PAIP2 (Li, Huang, et al., 2015). However, both approaches are flawed. Because more than one circRNA with related functions or transcripts are produced from the same host gene, these methods cannot be used to name each circRNA based on respective characteristics. circBASE, a database that merges and unifies datasets of circRNAs (Glazar, Papavasileiou, & Rajewsky, 2014), provides formal names for circRNAs in the form of “species_circ_7-digit number”, such as hsa_circ_0101582/mmu_circ_0010155. However, this complex form is not conducive to information transfer. Therefore, the question of how to design a simple form for the differential naming of all circRNAs is very important and needs to be addressed.

Thus far, researchers have closely investigated the biogenesis and expression of circRNAs, but little is known about circRNA clearance, and circRNAs might accumulate in quiescent and post-mitotic cells, such as neurons, due to their high stability and RNase resistance (Westholm et al., 2014). Regarding the mechanisms for circRNA clearance, Lasda et al. showed that cells can eliminate circRNAs via extracellular vesicles (Lasda & Parker, 2016), which might be one route for alleviating circRNA accumulation. We believe that additional mechanisms of circRNA degradation or expulsion exist, and these should be continually investigated.

The observed differential expression of circRNAs in human tissues and diseases suggests that circRNAs might play important physiological and pathological roles. Considering the characteristics of circRNAs that are absent in other forms of RNA, it is thought that with the development of technology and continuous research, the earliest use of circRNAs in clinical practice will be as diagnostic biomarkers of various diseases. However, whether circRNAs can serve as targets in disease treatment remains to be determined. Although many studies have demonstrated the potential role of circRNAs in disease therapy, their application appears to be a future endeavor.

The most reported mechanism of circRNA function in disease occurrence or development is the role of circRNAs as miRNA sponges in regulating targeted mRNA translation. However, there are at least three types of molecules in the circRNA axis, and the question of whether circRNAs, as opposed to other molecules, should be targeted in disease treatment needs to be addressed. No clinical data are available for reference. In addition to this mechanism, the involvement of other mechanisms in the same functional control should be considered. Whether circRNAs can simultaneously exert the same functional control through different mechanisms should be investigated, and if so, therapy targeting circRNAs might be better than alternative options.

Some studies have had surprising findings, for example, the possibility that circRNAs are translated in vivo and in vitro (Abe et al., 2015; Pamudurti et al., 2017; Yang, Fan, et al., 2017). However, there is no evidence that every endogenous circRNA can produce a protein, and whether only some circRNAs have this ability needs to be further researched. It is also necessary to determine whether the proteins originating from circRNA translation are functional. The results reported by Cortés-López and Miura suggest that proteins generated from circRNAs might have important implications in disease by undergoing multiple rounds of translation (Cortes-Lopez & Miura, 2016).

A genetic disease is caused by one or more abnormalities in an individual's genome. For example, the expression of circRNAs tends to be associated with certain diseases with a genetic predisposition, such as diabetes and breast carcinoma. Regardless, the correlation between circRNAs and genetic diseases has not been studied. This fact inspired us to assess whether abnormalities in circRNA-producing genes are linked to genetic diseases with the aim of providing new insights into the treatment of genetic diseases.

The ultimate goal of medical research is to contribute to clinical diagnosis or treatment, and the interest in translational medicine has experienced an upsurge worldwide (Zhang, 2012). In particular, how the results of circRNA research can be applied in the clinic to regulate the expression of circRNAs in specific human tissues or cells without side effects should be investigated. Researchers need to study the design of molecularly targeted therapeutic drugs and the delivery of drugs to specific areas. We expect that the development of biotechnology and basic research will lead to the discovery of more physiological and pathological circRNA functions and that therapeutic strategies based on circRNAs will be formulated, ultimately leading to the development of safe and effective strategies for use in clinical practice.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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