Circular RNAs: from biogenesis and function to diseases

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Abstract

Objective: Evidence suggests that various diseases may contribute to the circular RNAs (circRNAs) expression disorder. This review was aimed at looking for appropriate biomarkers for the treatment of diseases.

Data sources: The comprehensive search used online literature databases including PubMed of National Center for Biotechnology Information and Web of Science.

Study selection: The study selection was based on the following keywords: circRNAs, biogenesis, biologic function, and disease. The time limit for literature retrieval was from the year 1976 to 2019, with language restriction in English. Relevant articles were carefully reviewed, with no exclusions applied to study design and publication type.

Results: CircRNAs are one of the critical non-coding RNAs (ncRNAs), which are covalently closed continuous loops that do not possess 5′ and 3′ ends. This makes them resistant to exoribonuclease activity and potentially more stable than their cognate linear transcripts, thus making them ideal candidates for biomarker development. Due to the stable and extensive tissue-specific expression of circRNAs, they can function as microRNA sponges and bind to RNA-binding proteins, regulate transcription and splicing, and translate into proteins to participate in the regulation of physiologic and pathologic processes. Moreover, the expression disorders of circRNAs in diseases, such as neurodegenerative disease, cardiovascular disease, and cancer, make them have potential applications for the diagnosis and treatment of diseases.

Conclusions: Changes in circRNA expression profiles related to various diseases, and circRNAs often exhibit low expression in cancer tissues. In addition, circRNAs can be detected in patient's body fluids to indicate that circRNAs are effective biomarkers for disease diagnosis. These characteristics make circRNAs have potential applications as novel therapeutic targets for diseases.

Keywords: Circular RNAs; Biogenesis; Biological function; Neurodegenerative disease; Cancers

Introduction

In the human genome, the protein coding region accounts for 1.5% of the genome. However, in cells, about 80% of the DNA may be transcribed into RNA, which means that in an organism,[1] the amount of total RNAs is much more than mRNAs, suggesting that these RNAs that do not encode proteins may have many unknown functions. Circular RNAs (circRNAs) are a class of non-coding RNAs (ncRNAs) that are widely distributed in body fluids and tissues.[2,3] CircRNAs were initially identified in 1976 by Sanger *et al*[4] who found that some plant viroids are single-stranded covalently closed RNA molecules. The belief that circRNA is a product of genetic accident or mis-splicing, directly led to a few studies on circRNAs for the next 20 years. In 1990, Nigro *et al*[5] first found that 5′ exons were shuffled downstream of 3′ exons to form circRNA in a tumor suppressor gene (DCC), in human cells. However, with the development of high-throughput sequencing and the innovation of research methods, since 2012, research on circRNAs has increased immensely.[2,6] This has led to the recognition that circRNAs may play an important role in organisms.

CircRNAs mainly exist in the cytoplasm.[7] These RNAs mainly play a role as competitive endogenous RNA (ceRNA), while circRNA located in the nucleus primarily regulates transcription and splicing of parental genes.[8,9] Until now, circRNAs are known to have four main functions. Specifically, circRNAs might function as MicroRNA (miRNA) sponges to attenuate or prevent mRNA translation and regulate transcription and splicing of the parental gene.[10-12] In addition, circRNAs interact with

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000465

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Chinese Medical Journal 2019;132(20)

Received: 05-07-2019 **Edited by:** Yi Cui

RNA-binding proteins (RBPs) and translate into proteins to play important biologic functions.[13,14] Currently, there is increased recognition with respect to the widespread role of circRNAs that can influence the occurrence of many diseases. Therefore, circRNAs might have great prospects as biomarkers and targeted therapies.

In this review, we discuss the generation and function of circRNAs, and highlight their regulatory roles in different diseases, and their potential use as therapeutics for the treatment of diseases.

The Generation of CircRNAs

CircRNAs are formed by the process of back-splicing, by the covalent joining of the 5′ and 3′ ends of the spliced RNAs,[15] including direct reverse splicing and lariat driven circularization [Figure 1]. CircRNAs are mainly divided into three categories, including exon-intron-circRNAs (EIciRNAs), circular intronic RNAs (ciRNAs), and exonic circular RNAs (ecircRNAs).[3,7]

Direct reverse splicing includes cis-regulatory elements, trans-acting factors, and spliceosome that control splicing.[15-17] Cis-regulatory elements, such as a reverse complement sequence flanking an intron through complementary pairing drives exon cyclization to form EIciRNAs and ecircRNAs [Figure 1A].[18] Trans-acting factors, such as RBPs, drive exon cyclization by binding to specific sites on the pre-mRNA flanking introns, ultimately forming EIciRNAs and ecircRNAs [Figure 1A].[16,18] Spliceosomes are mainly composed of small molecule nuclear RNA (snRNA) and protein, which can catalyze the reverse splicing of exons of pre-mRNAs to form EIciRNAs and ecircRNAs [Figure 1B].[17] Moreover, it can also catalyze the formation of 2′ to 5′ branched lariats of introns by collinear splicing.[17]

Lariat driven circularization includes exon skipping and intron cyclization.[12,19] During pre-mRNA maturation, exon skipping events result in the formation of a lariat containing an exon, followed by internal splicing of the lariat to produce EIciRNAs and ecircRNAs [Figure 1C].[19] Intron cyclization depends on a motif containing a 7-nucleotide (nt) GU-rich element near the 5′ splice site and an 11-nt C-rich element near the branch point site, and these sites protect the lariat from de-branching enzymes, thereby forming ciRNA [Figure 1D].[12]

In addition, pre-tRNAs can also be spliced to form tRNA intronic circular (tricRNA), which is dependent on a bulge-helix-bulge (BHB) motif by tRNA splicing endonuclease (TSEN) complex-specific cleavage [Figure 1E].[20] In summary, the formation of circRNA is very complicated, and there may be many unknown formation mechanisms.

The Functions of CircRNAs

The positional decision influences the different functions of circRNAs located in the nucleus and cytoplasm, and the functions of circRNAs are mainly divided into five categories [Figure 1].

Regulation of transcription and splicing

Located in the nucleus, circRNAs such as EIciRNAs and ciRNAs are involved in mRNA transcription and splicing [Figure 1F–H]. The ciRNA produced by *ANKRD52* gene can form a complex with RNA polymerase II (pol II), and the complex binds to the promoter region of *ANKRD52* gene to enhance its transcription [Figure 1G].[12] Similarly, EIciRNAs such as circEIF3J and circPAIP2 can also form complexes with U1 snRNP and pol II to promote transcription of the parent gene [Figure 1F].[21] In addition, the circRNA produced by *Arabidopsis SEP3* gene binds to its cognate DNA locus to affect SEP3 mRNA splicing efficiency [Figure 1H].[11]

MiRNA sponge

There is growing evidence that circRNAs act as miRNA sponges to regulate mRNA expression [Figure 1I]. The circRNAs that were first validated as miRNA sponges are CDR1as (ciRS-7) and circSry.[6] CDR1as is highly expressed in human and mouse brains, contains more than 70 conserved miR-7 binding sites, and binds to many miR-7 sites to inhibit its activity.[6] CircSry is a testis-specific circRNA containing 16 specific miR-138 binding sites that act as miR-138 sponge.[6] Taken together, these studies suggest the general ability of circRNAs to act as miRNA sponges.

RBPs adsorption

Similar to miRNA sponge, circRNAs can interact with RBPs [Figure 1J]. For example, circRNAs can form a 16- to 26-bp double-stranded RNA stem-loop structure to participate in the immune response in combination with double-stranded RNA-activated protein kinase (PKR).[22] Other examples, such as circ-Foxo3, binds to inhibitor of DNA binding 1 (ID-1), E2F transcription factor 1 (E2F1), focal adhesion kinase (FAK), and hypoxia inducible factor 1 subunit alpha (HIF1α) to promote cardiac senescence.[23]

Translation

Generally, linear mRNAs are translated into proteins and are dependent on the 5′-end cap structures.[24] However, circRNAs do not have a cap structure, suggesting that their translation into proteins may be more complicated [Figure 1K].[24] Early studies have reported the presence of internal ribosomal entry site (IRES) elements in circRNAs which promote translation of these RNAs into proteins. For example, there is a conservative IRES element in circ-FBXW7, which can be translated into FBXW7-185aa.[25] This protein can regulate the stability of c-Myc protein together with the protein translated by *FBXW7* gene, thereby inhibiting the progression of malignant glioma.[25] However, other studies have shown that even in the absence of IRES, circRNA can be translated into protein in a 5′ cap structure independent manner. For instance, m6A-modified circRNAs can be directly translated into proteins.[26]

Packaging into exosomes

Exosomes are extracellular vesicles with a diameter of 30 to 100 nm, which can serve as carriers for transport of

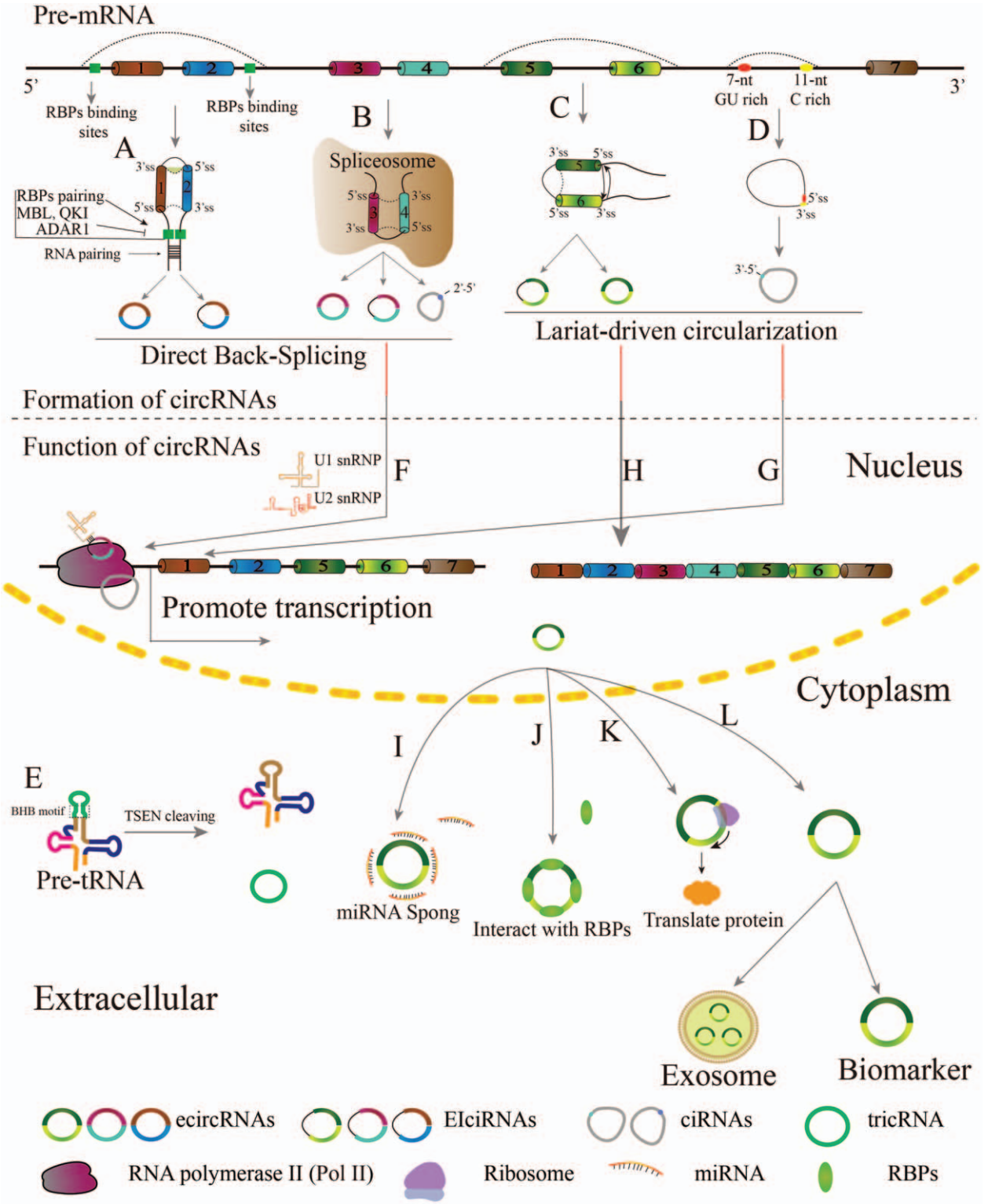

Figure 1: Generation and function of circular RNAs (circRNAs). (A) Intron pairing-driven circularization and RNA-binding proteins (RBPs), such as MBL, QIL, and ADAR1-driven circularization. (B) Spliceosome catalyzes pre-mRNA and forms ecircRNA, ElciRNA, and ciRNA by collinearly spliced or back-spliced mechanisms. (C, D) Lariat-driven circularization. (C) Exon skipping events result in the formation of exon-containing lariats, followed by the removal of the lariat to form ecircRNA and ElciRNA. (D) CiRNA generated depends on the GU-rich sequences close to the 5′ splice site (red oval) and the C-rich sequences near the branch point (yellow oval) to form a lariat to avoid degradation by the debranching enzyme. (E) The bulge-helix-bulge (BHB) motif of pre-tRNA can be spliced to form ciRNA by tRNA splicing endonuclease (TSEN) complex. (F, G) ElciRNA-Pol II-U1 snRNP complex and ciRNA-Pol II complex can regulate transcription of the parent gene. (H) circRNAs can regulate splicing of their linear cognates by exon skipping. (I) CircRNA can act as miRNA sponge to regulate mRNA expression by interacting with miRNA-Ago2 complex. (J) CircRNA can interact with RBPs to affect their function. (K) CircRNA plays a regulatory role by translating into protein. (L) CircRNA can be sorted into exosomes and transported to extracellular milieu or adjacent cells.

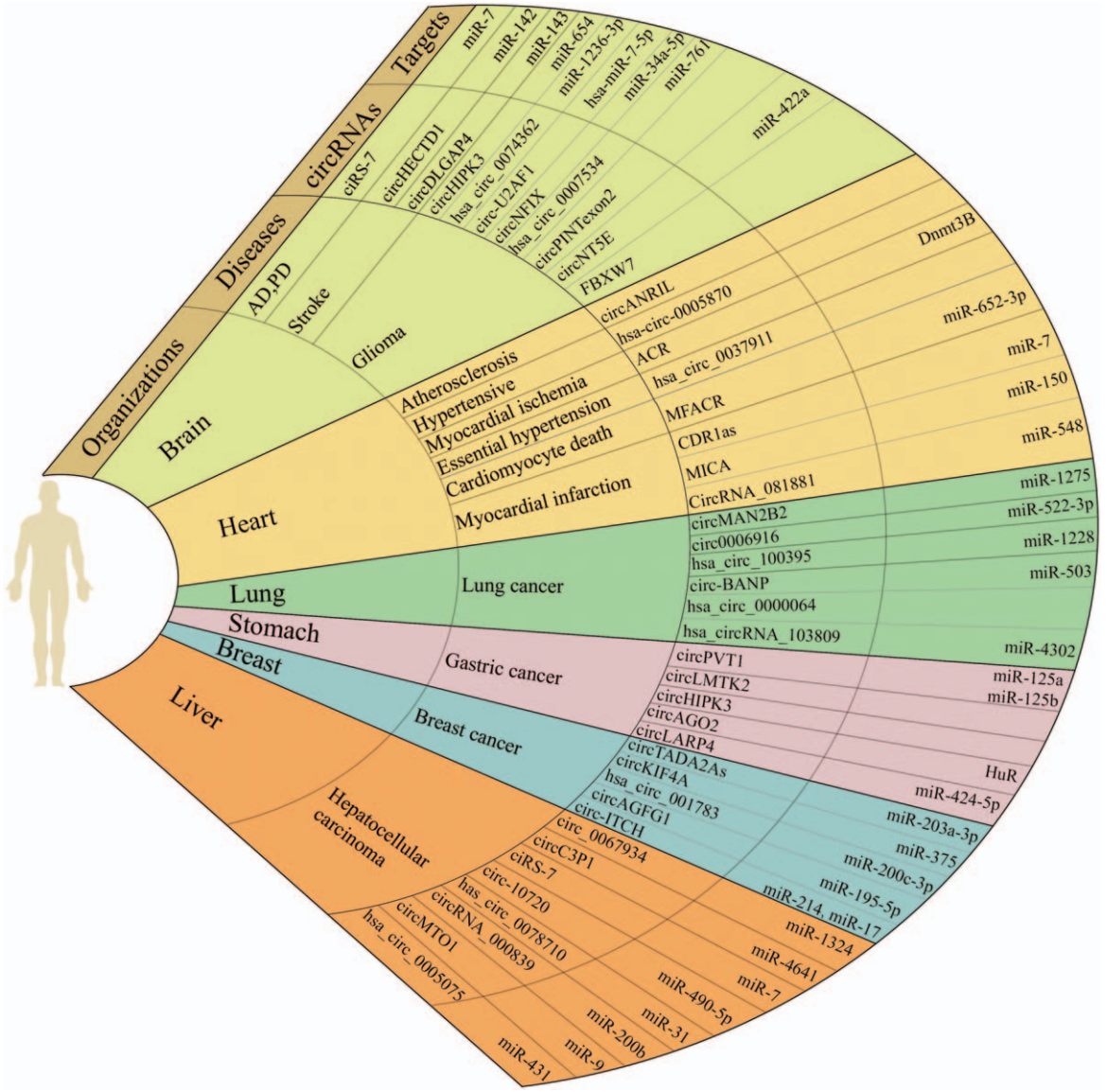

Figure 2: Overview of the circRNAs functions in various diseases. AD: Alzheimer disease; PD: Parkinson disease.

circRNAs to adjacent cells, distal cells, or body fluids [Figure 1L].[27,28] This allows circRNAs to be used as biomarkers to analyze the pathogenesis and development of disease.

CircRNAs and Diseases

Accumulating evidence has shown that circRNAs are widely involved in the initiation and progression of various human diseases [Figure 2], suggesting that circRNAs have broad prospects as biomarkers and targeted therapies.

Neurodegenerative diseases

CircRNAs are highly enriched in mammalian neuronal tissues, suggesting that they might play an important role in the regulation of the central nervous system. A study on Alzheimer disease (AD) found that ciRS-7 expression levels were significantly reduced in AD hippocampal CA1 samples, possibly due to decreased adsorption of miR-7 leading to an increased expression of AD-relevant target, ubiquitin conjugating enzyme E2A (UBE2A).[29] Another study showed that ciRS-7-miRNA-7-UBE2A circuit was significantly de-regulated in sporadic AD neocortex (Brodmann A22) and hippocampal CA1.[30] The expression of CiRS-7 decreases resulting in excess ambient miRNA-7 appear and miRNA-7-sensitive mRNA targets decrease, such as UBE2A.[30] UBE2A is a central effector of the ubiquitin-26S proteasome system and its decreased expression directly leads to amyloid accumulation and senile plaque deposits.[30] In addition to ciRS-7, circHDAC9 is also involved in the regulation of AD and decreased expression of circHDAC9 increases amyloid beta (Aβ) production by attenuating inhibition of miR-138, thereby regulating the development of AD.[31] A study on Parkinson disease (PD) found that miR-7 was mainly expressed in neurons and inhibited α-synuclein protein levels and that ciRS-7 regulation by miR-7 might be

associated with PD.[32] In addition to AD and PD, circRNAs are also closely associated with the development of amyotrophic lateral sclerosis (ALS). Armakola *et al*[33] found that deletion of Dbr1, which encodes an RNA lariat debranching enzyme, could strongly inhibit cytoplasmic TAR DNA-binding protein 43 (TDP-43) toxicity. The main reason is that the loss of debranching enzyme induces the formation of intronic lariats (ciRNA) and sequesters TDP-43, thereby inhibiting its toxicity. In addition to TDP-43, mutation in the RBP, fused in sarcoma (FUS), is also closely associated with ALS. FUS mutations cause translocation of proteins from the nucleus to the cytoplasm and form inclusion bodies in the cytoplasm and induce toxicity.[34,35] Not only that, FUS can regulate circRNA biogenesis by binding to the introns flanking the back-splicing junctions in N2a cells.[36] FUS depletion or its modification, so that it is localized to the nucleus will significantly reduce the formation of circRNAs, which may be associated with ALS development.[36] Taken together, circRNAs have been shown to regulate the occurrence and development of neurodegenerative diseases, suggesting that circRNAs might represent as useful targets for the development of therapeutic strategies for neurodegenerative diseases.

Cardiovascular diseases

Cardiovascular disease is one of the leading causes of human death worldwide.[37] Studies on human heart circRNA expression profiles revealed that there were 7000 to 16,000 different circRNAs,[38,39] suggesting that circRNAs may be involved in the development and progression of cardiovascular disease. Cardiovascular disease, such as atherosclerosis is reported to be regulated by circular ANRIL (circANRIL). Burd *et al*[40] found that the expression of *ANRIL* isoforms containing exons proximal to the *INK4/ARF* locus is closely associated with atherosclerosis. The mechanism might be that the PcG complex targets the coding locus of *ANRIL* to form circANRIL by exon skipping, thereby regulating *INK4/ARF* gene expression. Another study found that circANRIL also binds to pescadillo homologue 1 (PES1) leading to impaired ribosome formation and pre-rRNA production, which finally results in nucleolar stress and p53 activation leading to induction of apoptosis and inhibition of proliferation.[41] This increases the risk of atherosclerosis in humans. In another study, the expression level of circANRIL in the serum of patients with Kawasaki disease was significantly different from those in healthy individuals,[42] suggesting a correlation between circANRIL and Kawasaki disease. Another research group found that circRNA MFACR directly sequesters miR-652-3p in the cytoplasm and up-regulates mitochondrial protein 18 kDa (MTP18) expression, thereby regulating mitochondrial dynamics, cardiomyocyte apoptosis, and myocardial infarction.[43] In summary, all these studies indicate that circRNAs might serve as biomarkers for cardiovascular diseases.

Cancers

Growing evidence has shown that circRNAs contribute to various aspects of cancer progression,[44-46] suggesting their role in cancer. The current research on circRNAs in cancer can be divided into two categories. Changes in circRNA expression profiles help in finding suitable biomarkers or to explore the regulatory role in cancer development.

CircRNA expression profile in cancers

The occurrence of cancer is often accompanied by changes in the expression profile of circRNAs. Chen *et al*[47] identified a total of 76,311 distinct circRNAs by sequencing and annotating 144 localized prostate tumors, and these circRNAs are associated with the aggressiveness of cancer cells. In addition, among the 76,311 circRNAs, there are 171 circRNAs essential for prostate cancer cell proliferation, indicating that localized prostate cancer is widely regulated by circRNAs. Earlier, Bachmayr-Heyda *et al*[48] found that the ratio of circular and linear RNA isoforms was always lower in colorectal cancer tissue compared to normal tissue. They believe that the abundance of circRNAs is negatively correlated with cancer cell proliferation. More recently, another research group found similar results. They compared normal tissue and non-matched tumor, including osteosarcoma, colorectal adenocarcinoma, renal cell carcinoma, hepatocellular carcinoma, lung adenocarcinoma, and gastric adenocarcinoma, and found that circRNA abundance was lower in these cancers,[49] suggesting that down-regulation of circRNA might be a common phenomenon in cancer. However, despite a general decrease in the abundance of total circRNAs in cancers, a small number of circRNAs are still highly expressed in cancers. This could be due to the high expression of the parent genes resulting in high expression of circRNAs (as mentioned earlier, circRNAs are derived from pre-mRNA).[49] In other cancers, such as gastric cancer, tongue cancer, and breast cancer,[50-52] researchers have also detected changes in the expression profile of circRNAs, including high-abundant circRNAs and low-abundant circRNAs.

In summary, cancer can cause changes in the expression profile of circRNAs. These RNAs play a negative regulatory role in the process of cancer cell proliferation. Cancer-induced high-abundance of circRNAs could be due to the increased expression of their parent genes. Detection of circRNA expression profiles in cancers is important for the identification of effective biomarkers.

Regulation of circRNAs in cancers

Although analysis of circRNA expression profiles might be useful in identifying suitable biomarkers, only an in-depth study of the selected circRNAs can reveal the mechanism of cancer development, thus provide a theoretical basis for the clinical application of circRNAs. Cancer cells exhibit three characteristics, namely proliferation, metastasis, and invasion.[53-56] The current research on the molecular mechanism of circRNAs mainly focuses on these three aspects. Recent lines of evidence support the involvement of circRNAs in the regulation of cancer cell proliferation. Zhang *et al*[57] found that circ-MTO1 was significantly down-regulated in lung adenocarcinoma (LUAD), which served as miR-17 sponge to increase the expression of KH

domain RBP (QKI-5) and inhibit LUAD proliferation. Another study found that circ_0026134 was significantly up-regulated in non-small-cell lung cancer (NSCLC) tissues and cell lines.[58] Decreased expression of circ_0026134 could attenuate NSCLC cell proliferation and metastasis by acting as miR-1256 and miR-1287 sponge.[58] Moreover, circ-DONSON could recruit the NURF complex to the SOX4 promoter and initiate its transcription and silencing of circ-DONSON expression could significantly inhibit the proliferation, migration, and invasion of gastric carcinoma.[59] Additionally, circTADA2A is involved in the regulation of multiple types of cancer.[60,61] The circTADA2A/miR-203a-3p/CREB3 axis is involved in osteosarcoma progression and metastasis.[60] On the contrary, circTADA2A-E6/miR-203a-3p/SOCS3 axis is involved in the inhibition of breast cancer progression and metastasis.[60]

Thus far, studies regarding the regulatory mechanisms of circRNAs in cancer have increased tremendously. One of the main functions of circRNAs is to act as miRNA sponges in the development and progression of cancer. Overall, circRNAs might be important targets for cancer treatment.

Other diseases

CircRNAs have also been reported to participate in other diseases. For example, Cheng *et al*[62] found that circVMA21 was significantly reduced in the nucleus pulposus (NP) tissue of patients and can attenuate inflammatory cytokines-induced NP cell apoptosis by acting as miR-200c sponge. The study indicated that this circVMA21 might provide a potentially effective therapeutic strategy for intervertebral disc degeneration (IVDD). CircRNAs also play critical roles in acute myeloid leukemia (AML). A study by Wu *et al*[63] indicated that circRNA-DLEU2, which was up-regulated in AML tissues and cells, promoted AML cell proliferation and inhibited cell apoptosis by suppressing miR-496 and promoting PRKACB expression; this ultimately accelerated the ALM process. Other circRNA, such as circ_0009910, which was significantly up-regulated, had a poor risk and outcome with patients with AML.[64] Silencing of circ_0009910 expression could inhibit AML cell growth through interacting with miR-20a-5p.[64] In addition, circ-ANAPC7 has also been reported to be up-regulated in patients with AML, which may act as a sponge for the miR-181 family by bioinformatics analysis.[63] The exact function of circ-ANAPC7 needs further research. CircRNAs also regulate hepatic steatosis process. Overall, circRNAs might be important therapy targets in disease.

Future Perspectives

Although circRNAs were thought to be products of incorrect splicing, a large number of these RNAs have been identified and discovered, and it has been well established that circRNAs are involved in important regulatory processes in various diseases. Several studies have found that circRNAs are closely related to tumor TNM stage.[66,67] In addition, circRNAs can be detected in exosomes and body fluids, that is, circRNAs can be detected in peripheral blood and urine. All these observations indicate that circRNAs have potential applications as disease biomarkers and therapeutic targets. A latest study showed that low starting amounts of RNA (50 ng) could detect 1092 circRNAs in urine samples of prostate cancer patients by exome capture RNA-seq method.[49] This method not only facilitates the diagnosis and treatment of prostate cancer but also provides new insights into the diagnosis of other diseases. In addition, in cancer, low-abundant circRNAs are much more than high-abundant circRNAs. The low-abundance of circRNAs might be due to the down-regulated expression of the parent genes, but there are still many other low-abundant circRNAs and the reason for their low-abundance cannot be explained. This suggests that the occurrence of cancer might be more complicated than we think. It is difficult to determine the occurrence and development of cancer by studying the function of a single circRNA. The development of a single disease is often associated with many circRNAs, or a single circRNA can regulate the progression of many diseases. In the future, it might be beneficial to use network analysis to study the relationship between circRNAs and diseases.

Funding

This study was supported by grants from the Fundamental Research Funds of China West Normal University (Nos. 416535 and 403627), Institute of Environmental Science of China West Normal University, and Environmental Education Center of China West Normal University.

Conflicts of interest

None.

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How to cite this article: Li D, Yang Y, Li ZQ, Li LC, Zhu XH. Circular RNAs: from biogenesis and function to diseases. Chin Med J 2019;132:2457–2464. doi: 10.1097/CM9.0000000000000465