Circular RNAs and Cardiovascular Regeneration

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circular RNAs (circRNAs) are a type of non-coding RNAs that are widely present in eukaryotic cells. They have the characteristics of stable structure, high abundance, and cell or tissue specific expression. circRNAs are single-stranded RNAs that are covalently back spliced to form closed circular loops. They may participate in gene expression and regulation through a variety of action modes. circRNAs can encode proteins or function by acting as miRNA sponges for protein translation. Since 2016, a growing number of research studies have shown that circRNAs play important role in the pathogenesis of cardiovascular disease. With the construction of circRNA database, the differential expression of circRNAs in the heart tissue samples from different species and the gradual elucidation of its mode of action in disease may become an ideal diagnosis biomarker and an effective therapeutic target. What can be expected surely has a broader application prospect. In this review, we summarize recent publications on circRNA biogenesis, expression profiles, functions, and the most recent studies of circRNAs in the field of cardiovascular diseases with special emphasis on cardiac regeneration.

Keywords: circular RNA, cardiovascular disease, pathogenesis, cardiomyocyte, regeneration

INTRODUCTION

Circular RNAs (circRNAs) are single-stranded RNAs that, unlike linear RNA, form a covalently closed continuous loop without 5' end caps or 3' Poly (A) tails. The concept of “circular RNA” was introduced by Sanger et al., when the team found that viroids are single-stranded covalently closed circRNA molecules (1). The cytoplasmic localization of circular RNA in eukaryotic cells was discovered by Hsu et al. through the electron microscope in 1979 (2). These pilot studies established the foundation of this research field.

Transcription of circRNAs had been a mystery for many years. The circular transcription of the Sry gene was discovered in mice in the early 1990s (3). In 2012, Salzman et al. (4) discovered that circRNA is a transformed transcript produced by reverse splicing of mRNA precursor and found that it is abundantly present in different types of human cells. As the field advances rapidly, a large number of circRNAs were discovered with the utilization of high-throughput sequencing technology, and their biological functions were intensively investigated. In 2016, Hansen et al. (5) found that circular RNA can act as a sponge of microRNA (miRNA) to regulate the growth and development of cells. This study shed new light on the circRNA field. Most recently, Li et al. developed a quickly screening and discovering tool for functional circular RNAs based on the CRISPR-Cas13d system, and discovered a set of functionalities that are important for cell growth and embryonic development (6). This technology provided a new research tool to the circRNA field.
Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide. Several lines of evidence showed that circRNAs play important roles in regulating cardiovascular function. Jakobi et al. were the first group to provide a comprehensive catalog of RNase R-resistant circRNA species for the adult murine heart and explored the circRNA landscape of heart tissue (7). Over the next years, studies had reported that circRNAs are involved in the regulation of the physiology and pathology of the cardiovascular system. In particular, it is noted that circRNAs are involved in the pathogenesis of CVDs, such as myocardial infarction (MI) (8–14), heart failure (15, 16) and coronary artery disease (CAD) (17–23). Some circRNAs served as potential biomarkers for the diagnosis of CVDs (24–26). These findings suggest that circRNAs may be the new target molecules for the diagnosis and treatment of CVDs. In this review, we summarize circRNA classification, biogenesis, properties, functions, and some new research progress in the field of CVDs.

CLASSIFICATION OF CIRC RNA

circRNAs can be divided into three types according to the different sources of the sequences: ecRNAs (exonic circRNAs) which are derived from single or multiple exons (4, 27), ciRNAs (circular intronic RNAs) which are derived from introns (28), EcliRNAs (exon-intron circRNAs) which are composed of exons and introns (29) and tricRNAs (tRNA intronic circRNAs) which are formed by splicing tRNA introns (30).

BIOGENESIS OF CIRC RNA

There are four primary models for the formation of circRNA loops from pre-mRNAs (Figure 1), namely lariat-driven circularization (exon skipping), intron-paired driving circularization (direct back-splicing), circular intronic RNAs, and RNA-binding protein (RBP)-driven circularization.

Lariat-driven circularization (exon skipping) is formed by connecting the splice site of 30 nucleotides upstream of the exon to the site of 50 nucleotides downstream (Figure 1A). This connection leads to exon-skipping and the formation of an RNA lariat consisting of several exons and introns. The introns are then removed to generate circRNAs (27, 31).

Intron-paired driving circularization (direct back-splicing) is formed when pre-mRNA flanking introns contain inverted complementary sequences (Figure 1B). The complementary pairing on both sides of the intron can lead to alternative cyclization and then a generation of various circRNAs, including ecircRNAs and EcliRNAs (27, 32). Furthermore, longer introns can be found in the flanking sequences of circRNAs, and reverse complementary sequences in longer introns can aid the formation of circRNAs (29, 33).

Circular intronic RNAs are produced by eukaryotic spliceosome-mediated splicing (Figure 1C). The lariat intron generated from the splicing reaction evades normal debranching and degradation, and the 3' "tail" downstream from the branchpoint is trimmed leading to the formation of a stable circRNA. Conserved motifs at both ends, including the 7-nt GU-rich element near the 5' splice site and the 11-nt C-rich element near the branch point site, are combined to prevent introns form circular branches, which promote the formation of loop structures (28, 34).

Reverse complementary sequences, such as Alu repeats, are located in upstream and downstream introns. RBP-driven circularization is formed when certain transactivator RNA binding proteins that bind to each flanking intron trigger the splicing of the donor and acceptor sites close enough to form circRNA (Figure 1D) (32, 34–38).

FUNCTION OF CIRC RNA

Despite the rapid growth in the field, the biological functions of circRNAs in eukaryotic cells have not been fully understood. circRNAs share some common characters. First, circRNAs are widely distributed and abundantly expressed in a diverse of cells. circRNAs can be found in a large amount in the cytoplasm of eukaryotic cells derived from animals and plants (27, 39). In humans, more than 30,000 circRNAs have been discovered and are still increasing year by year (40, 41). Second, circRNAs are stable. Due to the covalently closed structure, circRNA is resistant to degradation by ribonuclease (RNase) or exonuclease and is more stable than linear RNA (42). The expression of circRNA differs according to time, tissues, or species (39, 43). circRNA profiles change at different stages of cardiac differentiation or during cardiogenic differentiation of induced pluripotent stem cells (44, 45). Moreover, circRNAs are evolutionarily conserved (43, 46). In 2016, Werfel et al. (47) reported high homology of 1288 circRNAs across humans, mice, and rats. However, many studies have also illustrated that circRNAs are species-specific (48, 49). circRNAs show different expression profiles between normal and diseased tissues (45, 47, 50). Increasing evidence suggests some circRNAs are derived from genomic loci associated with human diseases, and contribute to transcriptional, post-transcriptional, and translational regulations (51). To summarize, there are four main modes for circRNA function (Figure 2).

1) circRNAs can act as competitive endogenous RNAs (ceRNAs) to regulate gene expression by microRNAs (miRNAs) sponge effects (Figure 2A). miRNAs are important post-transcriptional regulators of gene expression that act by direct base pairing to target sites within untranslated regions of messenger RNAs (mRNAs) (52, 53). circRNAs contain miRNA response elements (MREs) that promote the binding between circRNAs and miRNAs. This binding can decrease the level of functional miRNAs and increase the expression of miRNA targets (53, 54). It has been reported that circRNAs regulate cell function by acting as miRNA sponges. For example, circFOXK2 promotes cell growth, migration, invasion, and apoptosis by binding to multiple sites and functioning as a sponge for miR-942 (55). Similarly, circRNA_100876 regulates the progression of triple-negative breast cancer by functioning as a sponge for miR-136 (56). Other circRNAs, such as circSLC26A4, circRNA_0000253, and circRNA_ANKIB1 can also function...
as the sponge of miR-1287-5p (57), miRNA-141-5p (58), and miR-195a-5p (59), respectively. circALMS1_6 may participate in the regulation of cardiac remodeling by functioning as a sponge for miR-133 (60).

2) circRNAs can function as RBP sponges and RBPs can also participate in back-splicing (Figure 2B) (61–66). RBPs are a group of proteins involved in gene transcription and translation. circRNAs can interact with RBP and inhibit their activities (66–68). circMbl absorbs MBL proteins and regulates the subsequent physiological processes (32). circPABPN1 can bind to HuR to suppress the translation of PABPN1 mRNA (69). circANRIL competitively recruits PES1 to inhibit ribosome biogenesis (70). circFoxo3 interacts with different RBPs to participate in the processes of cardiomyocyte senescence and cell cycle progression.
circuit RNAs can be translated into proteins via some modification (Figure 2D). As we know, the translation is performed by ribosomes and involves initiation, elongation, termination and ribosome recycling. Base-modification N6-methyladenosine (m6A) is a common form of base modification in RNAs. It can promote efficient initiation of protein translation from circRNAs in human cells. Legnini et al. revealed that m6A-driven translation of circRNAs is widespread with hundreds of endogenous circRNAs carrying the translation potential (76). circ-ZNF609 is an example of a protein-coding circRNA in eukaryotes. It is related to heavy polysomes and can be translated into a protein in a splicing-dependent and cap-independent manner (77). circRNAs play biological functions through the formation of complexes with proteins; otherwise, a novel protein circFAM188B-103aa encoded by circFAM188B that promotes the proliferation but inhibits the differentiation of chicken SMSCs was identified (78). Moreover, artificial (79) and endogenous circRNAs containing an internal ribosome entry site (IRES) that directly recruits ribosomes (80) can also be translated into protein. Additionally, circRNA with an infinite ORF has hundred-fold higher productivity than linear transcript by rolling circle amplification in an IRES-independent manner (81).

5) circRNAs may bind, store, sort, and sequester proteins to particular subcellular locations, can and act as dynamic scaffolding molecules that modulate protein-protein interactions (Figure 2E). circRNAs can bind to RNAs and can also bind, store, sort or sequester selected proteins such as RBPs to modulate their activity or localization (82). RNA-binding protein 3 (RBM3) dynamically adjusts the proliferation of hepatocellular carcinoma cells by regulating the production of SCD-circRNA2 encoded by the 3′-UTR of the stearoyl-CoA desaturase (SCD) gene (83). Recent studies have shown that RBP quaking could also modify the formation of circRNA through forming RNA-protein complexes (RPCs) (36). In addition to interacting with RBPs, circRNAs can function as protein sponges by adsorbing one or more proteins in binding sites, thereby acting as protein scaffolding by the mediating interaction between proteins. For example, CircFOXO3 could mediate the formation of circFOXO3-p21-CDK2 ternary complex and then serve as scaffolding, affecting the cell cycle progression of cancer (84).

SEQUENCING OF CIRCRNA

RNA-seq emerges as a powerful research tool to study the expression and function of non-coding RNAs including circRNAs (85). The technology of circRNA-seq generally includes library construction, computer sequencing, data analysis and processing, and function prediction (86). Either full transcriptome or circRNA profiling may be used to sequence circRNAs. The full transcriptome profiling is aimed to explore the expression patterns of both coding and non-coding RNA. This approach is suitable for the study of the biological function of circRNA. The circRNA profiling is focused on enriched circRNAs and this approach is appropriate to discover unknown circRNAs. Technically, the main difference between the two approaches is the construction of the sequencing library. The circRNA sequencing library not only requires the removal of most rRNA and poly (A), but also requires the use of ribonuclease RNase R to remove the interference of linear RNA. It has been reported that the abundance of circRNAs decreases after de-linear RNA because some circRNAs are sensitive to RNase R mediated digestion (87, 88). It is worthy to note that the alternative splicing of circRNA requires distinguishing the source of sense and antisense chains in the sequencing results. Therefore, constructing a chain-specific library is ideal as it may improve the accuracy of circRNA sequencing (87). Thus far, more than 100,000 unique human circRNAs have been discovered (89, 90).

After obtaining the circRNA sequencing data, the prediction and identification of circRNAs were carried out based on the identification software such as find circ, CIRCexplorer2, and CIRI (91–93). Real-time fluorescence quantitative PCR (quantitative real-time PCR, qRT-PCR), Northern blot hybridization (Northern blot), in situ hybridization (In situ hybridization, ISH), RPAD (RNase R treatment, polyadenylation, and poly (A) + RNA Depletion) and other techniques are used to validate the data of circRNA sequencing (94–97).

Microarray chip is another efficient tool for circRNA analysis, and it is commonly used in clinics for disease diagnosis. Compared to RNA-seq, microarray chip analysis is different in the following aspects: (2) microarray analysis of circRNA requires a known reference sequence, while RNA-seq can be utilized to analyze unknown circRNAs; (3) microarray chip analysis can be used to quantify circRNA expression when comparing with RNA-seq (98); and (4) microarray chip analysis can efficiently detect reverse splice site sequences and obtain a larger number of circRNAs than RNA-seq (99). However, some limitations of microarray chip analysis include: (2) high total RNA input is required during sample pretreatment; and
(3) unlike full transcriptome sequencing, microarray chip does not give the linear RNA data (100). If the reference sequence is unknown, many studies usually use RNA-seq to determine the full transcriptional sequence, then analyze the circRNAs by microarray.

**CIRCRNA AND CVDs**

With the development of deep sequencing technology, we can now understand the types and differential expression of circRNAs and their associated miRNAs in cardiovascular tissues (101–103). Many circRNAs have been reported to be associated with CVDs and their expression pattern are different between healthy and diseased human hearts (15, 19, 104, 105). Quantitative proteomics may be used to discover the regulatory networks of circRNAs in cardiovascular tissues (106). Here, we summarized recent publications on the roles of circRNAs in the development and treatment of CVDs (Table 1).

1) **Cardiac hypertrophy.** Cardiac hypertrophy is the heart's response to a variety of extrinsic and intrinsic stimuli that impose increased biomechanical stress and can be caused by various cardiovascular diseases. circRNA wwp1 exerts inhibitory roles of cardiac hypertrophy via down-regulation of ANF and miR-23a in isoproterenol hydrochloride-induced cardiac hypertrophy (107). A circRNA HRCR functions as an endogenous miR-223 sponge to sequester and inhibit miR-223 activity, resulting in an increase of ARC expression and protection of the heart from pathological hypertrophy and heart failure (108). Modulation of circRNAs levels may provide a promising therapeutic target for the treatment of cardiac hypertrophy.

2) **Cardiac fibrosis.** Activation and phenotypical transition of cardiac fibroblasts contribute to cardiac fibrosis. It was reported that circ_BMP2K enhances the regulatory effects of miR-455-3p on its target gene SUMO1 which leads to the inhibition of cardiac fibroblasts (109). MicroRNA-31-5p acts as a negative regulator of circPAN3 by directly suppressing QKI in doxorubicin-induced apoptosis of cardiomyocytes (117). circPAN3 also ameliorates myocardial ischemia and reperfusion injury by regulating miR-421/Pink1 axis-mediated suppression of autophagy (118). HECTD1 overexpression increases cell viability and decreases cell apoptosis and migration, and circDLPA/G4/HECTD1 mediates ischemia/reperfusion injury in endothelial cells via ER stress (119). Down-regulation of circFndc3b was observed in mice with myocardial infarction, and overexpression of circFndc3b increases angiogenic activity and reduces cell apoptosis in cardiac endothelial cells and cardiomyocytes which led to improved left ventricular functions (8). Other studies showed that miR-133 was regulated by circMAT2B. CircMAT2B knockdown attenuates oxygen-glucose deprivation-induced injury through up-regulating miR-133 in H9c2 cells (120). circNFI can serve as a pro-apoptosis factor in cardiomyocytes (121). The expression of circ ACAP2 is induced by myocardial infarction which leads to increased cardiomyocyte apoptosis by sponging miR-29 (109). Salidroside inhibits apoptosis and autophagy of cardiomyocytes by regulation of circular RNA hsa_circ_0000064 in cardiac ischemia-reperfusion injury (122). These data suggest that circRNAs may be the new targets for designing cardioprotective treatments against cardiomyocyte death.

3) **Cardiomyocyte apoptosis.** It was reported that some circRNAs may be involved in injury-induced cardiomyocyte apoptosis. For example, circRNA ITCH mediates H2O2-induced myocardial cell apoptosis by upregulating miR-17-5p via wnt/β-catenin signaling pathway (115). circSAMD4A aggravates hypoxia/reoxygenation (H/R)-induced cardiomyocyte apoptosis and inflammatory response by sponging miR-138-5p (116).
| CVD type       | CircRNAs             | Source                  | Action mechanism | Regulation | References |
|---------------|----------------------|-------------------------|------------------|------------|------------|
| Cardiac Hypertrophy | circRNA wwp1         | Mouse myocardial tissue | Sponge miR-23a   | Down       | (107,)     |
|               | circRNA HnROR        | Mouse heart tissue      | Sponge miR-223   | Down       | (109,)     |
| Myocardial Fibrosis  | circ_LAS1L           | Human cardiac fibroblasts| Sponge miR-125b  | Down       | (12,)      |
|               | circ_BMP2K           | Cardiac fibroblast      | Sponge miR-455-3p| Down       | (109,)     |
|               | circ_PAN3            | Rat myocardial tissue   | Sponge miR-221   | Up         | (110,)     |
|               | circ_ACAP2           | Cardiac fibroblasts     | Sponge miR-29    | Up         | (109,)     |
|               | circRNA_010567       | Mouse cardiac fibroblasts| Sponge miR-141   | Up         | (111,)     |
|               | circRNA_000203       | Mouse cardiac fibroblasts| Sponge miR-26b-5p| Up         | (112,)     |
|               | circNFIB             | Mouse heart tissue      | Sponge miR-433   | Down       | (113,)     |
|               | circHIPK3            | Mouse cardiac fibroblasts| Sponge miR-29b-3p| Up         | (114,)     |
|               | circ-ITCH            | Rat cardiomyocytes cell lines| Sponge miR-17-5p| Down       | (115,)     |
| Cardiomyocyte   | circSAMD4A           | Rat cardiomyocytes cell lines| Sponge miR-138-5p| Up         | (116,)     |
|               | circ_PAN3            | Rat myocardial tissue / Rat cardiomyocytes cell lines| Sponge miR-31-5p| Down       | (117,)     |
|               | circDLGAP4           | Human endothelial cell lines| Sponge miR-143   | Down       | (119,)     |
|               | circFndc3b           | Mouse myocardial tissue | Sponge RBP FUS   | Up         | (8,)       |
|               | circMAT2B            | Rat cardiomyocytes cell lines| Sponge miR-134   | Up         | (120,)     |
|               | circNFIX             | Rat cardiomyocytes cell lines| Unknown     | Down       | (121,)     |
|               | circ_ACAP2           | Rat cardiomyocytes cell lines| Sponge miR-29   | Up         | (109,)     |
|               | hsa_circ_0000084     | Rat myocardial tissue   | Unknown     | Up         | (122,)     |
| Apoptosis      | circZNF609           | Human peripheral blood  | Unknown     | Down       | (19,)      |
|               | circMAP3K5           | Human coronary artery   | Sponge miR-22-3p| Down       | (23,)      |
|               | circRNA-100338       | Human endothelial cell lines| Sponge miR-200a-3p| Down      | (17,)      |
|               | hsa_circ_0089378     | Plasma                  | Sponge hsa-miR-130a-3p| Up | (21,)      |
| Coronary Heart Disease | circDHCR24         | Human aortic vascular smooth muscle cell | Sponge miR-149 | Up | (20,)      |
| Coronary Heart Disease | circZNF609         | Human peripheral blood  | Unknown     | Down       | (19,)      |
| Coronary Heart Disease | circMAP3K5         | Human coronary artery   | Sponge miR-22-3p| Down       | (23,)      |
| Coronary Heart Disease | circRNA-100338      | Human endothelial cell lines| Sponge miR-200a-3p| Down      | (17,)      |
| Coronary Heart Disease | hsa_circ_0089378    | Plasma                  | Sponge hsa-miR-130a-3p| Up | (21,)      |
| Coronary Heart Disease | hsa_circ_0083357    | Human peripheral blood  | Sponge Hsa_miR_609 | Up | (15,)      |
| Coronary Heart Disease | hsa_circ_0082824    | Human peripheral blood  | Sponge Hsa_miR_1294 | Up | (15,)      |
| Coronary Heart Disease | hsa_circ_0068942    | Human peripheral blood  | Sponge Hsa_miR_6799_5P | Down | (15,)      |
| Coronary Heart Disease | hsa_circ_0057576    | Human peripheral blood  | Sponge Hsa_miR_5000_5P | Up | (15,)      |
| Coronary Heart Disease | hsa_circ_0054537    | Human peripheral blood  | Sponge Hsa_miR_96_5P | Up | (15,)      |
| Coronary Heart Disease | hsa_circ_0051172    | Human peripheral blood  | Sponge Hsa_miR_5000_5P | Up | (15,)      |
| Coronary Heart Disease | hsa_circ_0032970    | Human peripheral blood  | Sponge Hsa_miR_5000_5P | Up | (15,)      |
| Coronary Heart Disease | hsa_circ_0063223    | Human peripheral blood  | Sponge Hsa_miR_5000_5P | Up | (15,)      |
| Heart Failure   | hsa_circ_0005565     | Human heart tissue      | Unknown     | Up         | (16,)      |
| Heart Failure   | hsa_circ_0097435     | Human peripheral blood  | Sponge Hsa_miR_609 | Up | (15,)      |
| Myocardial Regeneration | circTLK1           | Mouse myocardial tissue | Sponge miR-214   | Up         | (11,)      |
| Myocardial Regeneration | circRNA CDR1as      | Pig myocardial tissue   | Sponge miR-7     | Up         | (10,)      |
| Myocardial Regeneration | circ003593          | Cardiomyocytes cell lines| Unknown     | Up         | (13,)      |
| Myocardial Regeneration | circ-0001273        | Human umbilical cord mesenchymal stem cells (UMSCs) | Unknown     | Down       | (9,)       |
| Myocardial Regeneration | circCDYL            | Mouse myocardial tissue | Sponge miR-4793-5p| Down      | (14,)      |
| Myocardial Regeneration | circFASTKDI         | Human endothelial cell lines| Sponge miR-106a | Up         | (123,)     |
| Myocardial Regeneration | hsa_circ_0007623    | Human endothelial cell lines| Sponge miR-297 | Up         | (124,)     |
| Myocardial Regeneration | circHippk3          | Mouse heart tissue      | Sponge miR-133a | Up         | (104,)     |
| Myocardial Regeneration | circRNA_0001379     | Mouse myocardial tissue | Sponge miR-17-5p | Up         | (103,)     |
5) Heart failure. Despite the detailed roles of circRNAs in the progression of human heart failure remains elusive, recent high-throughput sequencing studies identified many circRNAs with changed expression in patients with heart failure. The top highly expressed EAT circRNAs corresponded to genes involved in cell proliferation and inflammatory responses. A recent study on circRNA expression profile in epicardial adipose tissue in patients with heart failure showed that EAT circRNAs may contribute to the pathogenesis of metabolic disorders (16). Another study showed that the upregulation of Hsa_circ_0097435 contributes to the pathogenesis of heart failure via sponging multiple microRNAs (15).

6) Cardiac regeneration and repair. Recent studies indicated that modulating circRNA activity may have great therapeutic potential for myocardial regeneration and repair. Up- or down-regulation of circRNAs and miRNAs and circRNA-miRNA coexpression had been shown to change the expression of the genes associated with myocardial ischemia and reperfusion injuries (101, 103). It has been reported that circRNAs can regulate inflammatory factors to improve myocardial ischemia and reperfusion injury. The circTLK1 exacerbates myocardial ischemia and reperfusion injury via targeting miR-214/RIPK1 through TNF signaling pathway (11). circ003593 has also been shown to confer cardioprotection through NLRP3 inflammasome myocardial infarct rats (13). circRNA CDR1as was identified in pig hearts. Elevated circRNA CDR1as in the infarction region of the pig heart is negatively associated with infarct size and positively associated with improved heart function (10). circ-0001273 can remarkably inhibit myocardial cell apoptosis and promote repair in myocardial infarction hearts (9). circCDYL was downregulated in myocardial tissues and hypoxia myocardial cells after acute myocardial infarction. circCDYL overexpression and downregulation can promote and inhibit the proliferation of cardiomyocytes in vitro, respectively. Additionally, circCDYL can promote the proliferation of cardiomyocytes through the miR-4793-5p/APP pathway (14). The downregulation of circFASTKD1 induces angiogenesis and improves cardiac function and repair after myocardial infarction (123). Hsa_circ_0007623 can bind to miR-297 and acts as a sponge of microRNA-297 which promotes cardiac repair after acute myocardial ischemia and protects cardiac function (124). circHipk3 was overexpressed in the fetal or neonatal heart of mice and functioned to promote the proliferation of cardiomyocyte and endothelial cells which leads to angiogenesis. Further study showed that circHipk3 regulates cardiac regeneration in mice post myocardial infarction by interacting with Notch1 and miR-133a (104). These findings highlight the physiological role of circRNAs in cardiac repair and indicate that modulation of circRNA may represent a potential strategy to promote cardiac function and remodeling after myocardial injuries.

FUTURE PERSPECTIVES

The studies we discussed in the paper highlight the significance of circRNAs in the pathogenesis of CVDs. circRNAs are stable and abundantly present in the circulatory system which enables them to serve as biomarkers for the diagnosis and treatment of CVDs; however, there are some critical issues to be addressed. Firstly, there is no reliable methodology for the detection of circRNAs. In terms of circRNA detection, newer, simpler, and more reliable methods will be expected to appear. This will provide convenience for us to study circRNAs, facilitate the faster output of research results, and obtain more target circRNAs with diagnostic and therapeutic significance. Secondly, some circRNA biomarkers come from small samples and populations. This makes us question the reliability and representativeness of the research results. Thirdly, the mechanism underlying circRNA functions in the cardiovascular system remain largely elusive. For the function and mechanism studies, the current research methods are limited and difficult to operate. New research protocols need to be further explored. Rapid development can be achieved only by breaking through the technological bottleneck in the field of circRNA research. Lastly, there is a lack of efficient approaches for modulating circRNA expression in the cardiovascular system. It is supposed to be that in the future there will be new and more diverse methods in modulating the overexpression and inhibition of circRNAs. This will promote the development of the mechanism underlying circRNA functions. Then the research results can be quickly used for clinical diagnosis and treatment in the field of vascular diseases.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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