CircRNAs open a new era in the study of cardiovascular disease (Review)

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Abstract. Circular RNAs (circRNAs) are non-coding RNAs that are found in the cytoplasm or stored in the exosomes, where they are not affected by RNA exonucleases. CircRNAs are widely expressed in mammalian tissues and cells. A number of studies have suggested that circRNAs are associated with the physiology and pathology of cardiovascular diseases (CVDs). Therefore, circRNAs have been considered as effector molecules and biomarkers in the cardiovascular system. The present review article summarizes the biological origin and roles of circRNAs as well as the available databases and research methods for their identification. Furthermore, it describes their regulatory mechanisms in cardiovascular physiology and pathology, including the regulation of atherosclerosis, immunity, cell proliferation, apoptosis and autophagy. In addition, the current review discusses the unresolved problems in circRNA research and the application of circRNAs in the treatment of CVDs. Finally, the CVD-associated circRNAs are also reviewed.

Contents

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1. Introduction

It is estimated that only 20% of the nucleic acids in the human genome are encoded into proteins, while the rest, the so called non-coding nucleic acids, are considered as the 'dark matter' of the genome (1). However, several studies have shown that these non-coding RNAs are involved in important biological processes, such as cell proliferation, differentiation, apoptosis, metabolism, senescence, and especially in post-transcriptional regulation (1-3). Circular RNAs (circRNAs) were first discovered in the early 1970s (4,5), however, they have been poorly studied for nearly three decades due to the limited available technology. Recent advances in RNA sequencing technologies have promoted the identification of an increasing number of circRNAs (3,6-8). Several studies have shown that circRNAs can compete with mRNAs for the target binding sites on microRNAs (miRNAs) to affect gene expression. However, the function of the majority of circRNAs remains unclear. In 2013, Hansen *et al* (9) reported the overlapping co-expression of circRNA ciRS-7 and miR-7 in murine brain tissues. In addition, it was also demonstrated that the circRNA sex-determining region Y-9 (SRY-9) could act as a sponge for miR-138, thus providing the first solid evidence for the biological roles of circRNAs. To date, >183,000 circRNAs have been identified from human transcripts, to the best of our knowledge (10). The role of circRNAs in different tissues and different diseases have also been gradually revealed (11). The cardiovascular system is considered as one of the two major vascular systems in the human body. Cardiovascular diseases (CVDs) remain the leading cause of death worldwide. A study found that the circRNAs solute carrier family 8 member A1 (SLC8A1), ataxin 10 (ATXN10), SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5 (SMARCA5), chromodomain Y (CDY) and myoblast determination protein 1 (MyoD) are

associated with myocardial differentiation (12). In addition, several circRNAs are involved in the pathological process of atherosclerosis (AS) (13). Furthermore, stress stimuli such as hypoxia and high temperature have been associated with the expression of cardiovascular-related factors (14-16). Emerging evidence has shown that circRNAs are involved in the progression of CVD, and are therefore considered promising targets for diagnosis and treatment. The present review article aimed to summarize the current knowledge on the biological aspects of circRNAs and highlight the current research progress on the mechanisms underlying the function of circRNAs in the physiology and pathology of the cardiovascular system.

2. Formation and classification of circRNAs

It is considered that circRNAs are derived from the variable splicing of precursor mRNAs (pre-mRNA), mediated by RNA polymerase II (17-19). However, to the best of our knowledge, the mechanism underlying the formation of circRNAs remains to be elucidated.

CircRNAs can be cyclized by RNA binding protein (RBP) (20). For example, the RNA binding proteins, mannose-binding lectin (17), RBP quaking (21) and fused in sarcoma (22) are known to drive the folding and formation of precursor mRNAs of circRNAs. The aforementioned RBPs bind to introns near the splicing site to promote the formation of dimers upstream and downstream of the cyclic exons to aid their splicing, thus leading to the production of circRNAs.

CircRNAs may also be formed by complementary pairing of flanking introns located in dorsal exons, such as short interspersed nuclear elements or non-repetitive complementary sequences (23,24). Among them, Alu repetitive elements are widespread in the expressed RNA and in the non-coding parts (25,26). Alu repeats serve a key role in the formation of circRNAs, with >1 million copies in the genome (24). It is considered that the probability and intensity of the reverse intron folding, mediated by the repeats encompassed in the pre-mRNA, may also determine the frequency of reverse splicing (25,27).

Lariat-driven circularization is another important process of circRNA formation (28). Exon skipping occurs when the pre-mRNA is spliced, resulting in a lariat intermediate containing exons and introns. Subsequently, the internal splicing of the lariat leads to the removal or retention of introns, thus resulting in the formation of exon or exon-intron circRNAs (29-32).

Based on their biogenesis, circRNAs are divided into exon circRNAs, encompassing a single exon or multiple exons, exon-intron circRNAs, and intron circRNAs (Fig. 1). The majority of circRNAs belong to the first subtype and mainly exist in the cytoplasm (33). However, a few circRNAs are produced in the nucleus and can be secreted into the body fluids, such as the serum, saliva and cerebrospinal fluid (34-36). The exon-intron circRNAs are mainly located in the nucleus and interact with RNA polymerase II to promote the transcription of their parental genes (37). Finally, the intron circRNAs exist in the nucleus (38).

3. Biological functions of circRNAs

Gradually, circRNAs have been found to exert more specific biological functions, and their mechanism of action remains in the focus of attention (39,40). To date, several functions of circRNAs have been verified (Fig. 1).

MicroRNA sponges. Apart from a small number of circRNAs generated by intron circularization, which are located in the nucleus, the majority are mainly located in the cytoplasm (41). Several studies have shown that circRNAs can compete with mRNAs for binding to miRNAs located in the cytoplasm, thereby regulating the expression of mRNAs (3,9). ciRS-7/cerebellar degeneration-related protein 1 antisense RNA (CDR1as) sponging miR-7 and mouse SRY sponging miR-138 are considered as the most representative circRNAs supporting this biological function (3). Notably, only a few circRNAs have been identified to encompass multiple target binding sites for miRNAs (42). Furthermore, the binding of circRNAs by miRNAs may initiate circRNA decay (43). It has been also demonstrated that the CDR1as-miR-7 axis is modulated by Cyrano, a long non-coding RNA (lncRNA) (44). In the non-coding regulatory network, miR-671 shares a highly complementary sequence with CDR1as, which triggers the rare argonaute2-mediated RNA cleavage targeted by miRNAs in mammals and even vertebrates (43). The aforementioned findings indicate that circRNA-miRNA interactions not only mediate miRNA sequestration, but also exert other functions that are worth studying.

Competition with pre-mRNAs for cleavage and splicing during transcription. CircRNAs are formed as a result of the pre-mRNA atypical splicing, whereas mRNA is the final product of the pre-mRNA typical linear splicing (45). A study revealed that improving the efficiency of typical linear splicing could lead to a significant reduction in the number of generated circRNAs (17). When the length of the intron flanking the exon is longer, the efficiency of typical linear splicing is significantly decreased, and cyclization occurs (46). These findings indicate that circRNAs can compete with pre-mRNA during transcription.

CircRNAs regulate transcription and chromatin interactions, and act as protein sponges. CircRNAs located in the nucleus can bind RNA polymerase II or U1 small nuclear ribonucleoprotein to regulate the transcription of their parental genes (47,48). A study has shown that the expression of the parental genes is significantly attenuated when circRNAs are knocked out (47). Furthermore, back-splicing was observed in RNAs transcribed from centromeric retrotransposons in maize, while the resulting circular CRM1 RNAs could bind to maize centromeres through R-loops to promote the formation of chromatin loops. Previous studies have demonstrated that QKI attenuated doxorubicin (DOX)-induced cardiotoxicity via binding to titin (Ttn)-, formin homology 2 domain containing 3 (Fhod3)- and striatin 3 (Strn3)-derived circRNAs expressed in the heart (49). In addition, circRNAs contain binding sites for the RBPs of the host and can regulate their expression. For example, circRNA zinc finger protein 609 (ZNF609) can regulate the proportion of phosphorylated (p)-Rb/Rb and the levels of p-protein kinase B (Akt), thus affecting the G1/S phase progression in cells (50).

Translation of circRNAs. CircRNAs were originally defined as non-coding RNAs (51). However, it has been reported

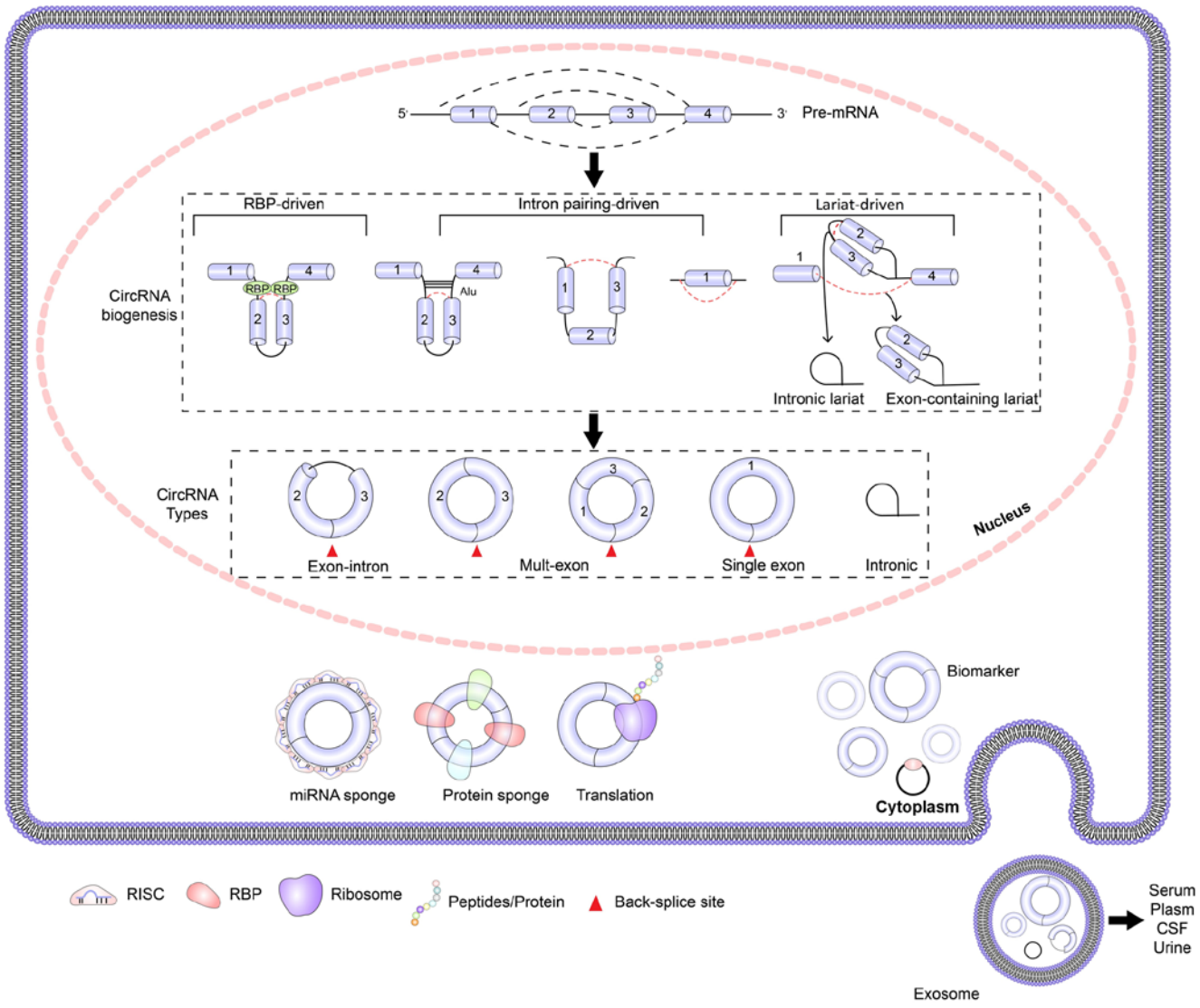

Figure 1. Schematic representation showing the biogenesis and function of circRNAs. CircRNA formation models: i) Intron pairing-driven circularization; ii) RBP-driven circularization; and iii) lariat-driven circularization. Types of circRNA: i) Single exon, intronic; ii) exon-intron; and iii) multi-exon. CircRNA functions: i) miRNA sponge; ii) scaffold for proteins, iii) translation; and iv) biomarker. CircRNA, circular RNA; RBP, RNA binding protein; miRNA, microRNA; RISC, RNA-induced splicing complex; CSF, cerebrospinal fluid.

that translation may be carried out by the internal ribosome entry site (IRES) of circRNAs. For example, the untranslated region of endogenous circRNA ZNF609 can act as an IRES in a splicing-dependent manner, suggesting that circRNA ZNF609 can drive IRES-dependent translation (52). A study revealed that consensus N6-methyladenosine (m6A) motifs are enriched in circRNAs, while a single m6A site is sufficient to initiate translation. This m6A-dependent translation requires eukaryotic translation initiation factor 4F and the m6A reader, YTH N6-methyladenosine RNA binding protein 3 (53). Additionally, m6A-dependent translation can be enhanced by methyltransferase like 3/14, inhibited by demethylase obesity-associated protein and is upregulated in response to heat shock (53). m6A-driven circRNA translation commonly occurs, and hundreds of endogenous circRNAs exert translational capability (53). The translation capacity of circRNAs is not considered as their common function, however, further study on this field may provide revolutionary changes.

4. Databases and testing methods for circRNAs

Several laboratories and institutions have developed specialized databases and analytical tools to study circRNAs (10,54-65) (Table I). These databases provide the convenience to further investigate the already identified circRNAs. RNA sequencing analysis of rRNA-depleted total RNAs and microarrays are currently applied for the discovery of novel circRNAs (66). Nevertheless, the verification of the predicted circRNAs by *in vitro*/*in vivo* experiments is always necessary. Several widely used molecular biology techniques have been applied to detect and verify circRNAs, including northern blot analysis, which is considered as the gold standard for circRNAs verification (3,30), reverse transcription (RT)-PCR, which is the most commonly used and basic verification technique (17), quantitative PCR which is used to identify and quantitatively measure differentially expressed circRNAs, droplet digital PCR, which is performed to quantify the expression of circRNAs in challenging samples such as plasma (67,68), and fluorescence *in situ*

Table I. List of the main circRNA databases.

First author, year	Database	Function	URL	Last update	(Refs.)
Glažar *et al*, 2014	CircBase	Facilitates the identification of circRNA in sequencing data	http://www.circbase.org	2017	(54)
Panda *et al*, 2018	CircInteractome	Predicts the interaction between circRNA and RNA binding proteins and miRNA	http://circinteractome.nia.nih.gov/	2018	(55)
Liu *et al*, 2016	CircNet	Provides expression data of circRNA and regulatory network of circRNA-miRNA in different tissues	http://circnet.mbc.nctu.edu.tw	2016	(56)
Zheng *et al*, 2016	Deep Base	Provides small RNAs, long non-coding RNAs and circRNAs for deep sequencing of different species and tissues	http://rna.sysu.edu.cn/deepBase	2016	(57)
Liu *et al*, 2019	Circbank	Comprehensive database with circRNAs standard naming	http://http://www.circbank.cn	2019	(58)
Xia *et al*, 2018	CSCD	Database of specific circRNAs associated with cancer	http://gb.whu.edu.cn/CSCD	2016	(59)
Ghosal *et al*, 2013	Circ2Traits	CircRNAs database related to human diseases	http://gyanxet-beta.com/circdb	2013	(60)
Li *et al*, 2014	StarBase	Large-scale CLIP-Seq decoding the Interaction Networks	http://starbase.sysu.edu.cn/starbase2/index.php	2019	(61)
Dong *et al*, 2018	CIRCpedia	Allows comparison of circRNA expression in tissues and cell lines and classification of alternative reverse splicing and alternative splicing	http://www.picb.ac.cn/rnomics/circpedia/	2018	(10)
Wang, 2018	HppRNA	Meets the needs of large sample RNA-Seq system analysis for circRNAs research	https://sourceforge.net/projects/hpprna/	2018	(62)
Wu *et al*, 2020	CircAtlas2.0	Provides functional annotation and prioritization of circRNAs for further verification and functional research	http://circatlas.biols.ac.cn/	2020	(63)
Cai *et al*, 2020	VirusCircBase	The first comprehensive database of circular RNA molecules in viruses	http://www.computationalbiology.cn/ViruscircBase/home.html	2019	(64)
Chen *et al*, 2016	CircRNADb	Provides comments on chromosome location, source gene, genome length, protein coding	http://reprod.njmu.edu.cn/CircRNAdb	2016	(65)

circRNA, circular RNA.

hybridization, which is used to detect circRNA localization and interactions. Furthermore, overexpression and silencing techniques are performed to investigate the role of circRNAs *in vitro* or *in vivo*. For example, for the overexpression experiments, following amplification of a target DNA sequence encompassing the characteristic flanking Alu sequences, the sequence is digested with a restriction endonuclease and ligated to the pEGFP-C1 vector. Subsequently, the overexpression plasmid is transfected into the corresponding cell sample, thus resulting in the overexpression of the circRNAs (69,70). Additionally, for silencing experiments, small interfering RNA (siRNA) sequences complementary to the back-splice junction are synthesized and the RNA-induced silencing complex specifically targets circRNA, leading to its cleavage and degradation, thus silencing its expression (52,71). Notably, the effectiveness of siRNAs depends on transfection efficiency, and these RNAs only temporarily inhibit the expression of their corresponding targets (72). However, short hairpin RNAs (shRNAs) or Ago shRNAs exert fewer off-target effects, thus resulting in more stable target-circRNA knockdown (73). The progress on studies concerning the effects of circRNAs will promote the merging of specific versions of databases to expand research in the field of circRNAs.

5. Expression of circRNAs in the heart

Several studies have reported the comprehensive expression profiles of circRNAs in rat, murine and human hearts (74-76), including the first study on the development and expression of circRNAs in human induced pluripotent stem cell-derived cardiomyocytes (76). A study reported for the first time a catalog of 575 candidate circRNAs in murine hearts. Among them, several candidate genes were found to be associated with different diseases (74). Furthermore, another study extensively compared human, rat and murine circRNA conservation in a specific tissue (75). Only 10% of these circRNAs were conserved among the three species. In addition, this study demonstrated that the overall circRNA expression was significantly decreased in the heart of adult animals compared with neonatal rats. The expression of circRNAs in the human heart was approximately twice the baseline level of adult rats and mice. A study analyzed the characteristics of circRNA expression in 12 human hearts, 25 mouse hearts and across a 28-day differentiation time-course from human embryonic stem cell-derived cardiomyocytes (76). A total of 15,318 and 3,017 circRNAs were identified in human and murine myocardial tissue, respectively. The expression abundance of these circRNAs was basically associated with their cognate linear RNA. The parental genes of circRNAs with the highest content were also considered as key cardiac genes, such as ryanodine receptor 2 (Ryr2), Ttn and dystrophin. Among them, circRNA SLC8A1-1 was the most abundantly expressed circRNA in the myocardium, whereas 402 different circRNA isoforms were expressed from the Ttn gene locus. These circRNAs were dynamically and highly expressed during heart development. In addition, it has been reported that the circRNAs SLC8A1, circ-calcium voltage-gated channel subunit α1 D, SPHKAP and α kinase 2 are also specifically expressed in the heart (77). Another study showed that the circRNAs ATXN10, SMARCA5, CDY, MYOD, SLC8A1, ATXN7 and PHF2 were involved in cardiomyocyte differentiation (12). Furthermore, a notable study investigated the changes in the expression of lncRNAs, circRNAs and protein-coding genes via recording the RNA sequencing data at sequential stages of cardiomyocyte differentiation (78). The stages of differentiation studied were the following: Undifferentiated cardiomyocytes, mesoderm cardiomyocytes, cardiac progenitors and differentiated cardiomyocytes. This study described the dynamic expression pattern of non-coding RNAs during cardiogenesis, where the expression of circRNA SLC8A1 was gradually upregulated during cardiac differentiation. CircRNAs are derived from exons containing annotated start codons, which are also termed AUG circRNAs (79). Jakobi *et al* (80) described the AUG circRNAs in a heart model system for the first time, to the best of our knowledge. The authors demonstrated that m6A methylation was enriched in AUG circRNAs. They also reported the potential negative regulatory effect of AUG circRNAs on the translation of host genes. A recent study demonstrated that 40 ribosome-associated cardiac circRNAs could be translated into microproteins with different biological functions (81). Among them, the circRNAs CASP8 and FADD like apoptosis regulator, SLC8A1, myosin binding protein C3 and Ryr2 were reported for the first time. Therefore, the research on circRNAs will open a new chapter in heart biology.

6. Expression of circRNAs in cells of the cardiovascular system

It is understood that cardiomyocytes play an important role in the cardiovascular system (75,82). It has been reported that several circRNAs, such as hsa_circ_0001879, hsa_circ_0004104 and hsa_circTCF25, can be isolated from peripheral blood mononuclear cells, serving as biomarkers of coronary artery disease (CAD) (83,84). Another study revealed that the expression of circANRIL subtypes were inversely correlated with the risk and the severity of AS (85). Furthermore, a recent study documented for the first time the association between the expression of circRNAs and atrial fibrillation (86). Therefore, a total of five circRNAs were identified with notable biological significance, including chr9:15474007-15490122, chr16:75445723-75448593, hsa_circ_0007256, chr12:56563313-56563992 and hsa_circ_0003533. Regarding vascular endothelial cells, circRNAs may directly or indirectly affect the occurrence of CVDs through functional changes, such as endothelial cell apoptosis, endothelial-mesenchymal transition and angiogenesis (87-90). For example, *in vitro* results demonstrated that the expression of circRNA ZNF292 in human umbilical vein endothelial cells (HUVECs) was significantly upregulated, suggesting that and circRNA ZNF292 could exert pro-angiogenic effects (71). Li *et al* (91) reported that the expression of hsa_circ_0003575 and hsa_circ_0003204 were significantly increased in oxidized low-density lipoprotein (ox-LDL)-induced HUVECs. Furthermore, hsa_circ_0003575 silencing significantly inhibited apoptosis and promoted proliferation and angiogenesis of ox-LDL-induced HUVECs. Yang *et al* (92) found that the circRNA HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2 induced the expression of autophagy-related 5 (ATG5) in methamphetamine- or lipopolysaccharide (LPS)-induced human brain microvascular endothelial cells

via binding to miR-30d, and ATG5 was shown to affect the endothelial-mesenchymal transition in an autophagy-independent manner. Liu *et al* (93) revealed the mechanism underlying the microvascular dysfunction mediated by diabetes. This mechanism also mediates the biological changes in human retinal vascular endothelial cells following circRNA PWWP domain-containing protein 2A transfer from vascular pericytes to HUVECs through exosomes (93). Vascular smooth muscle cells (VSMCs) are another notable type of cardiovascular cells. A recent study reported that the circRNA low-density lipoprotein receptor-related protein 6 (Lrp6) can promote the phenotypic transformation of VSMCs via inhibiting miR-145, while silencing of circRNA Lrp6, using shRNA technology, attenuated intimal hyperplasia in a mouse carotid artery injury model (94). Additionally, Zheng *et al* (95) demonstrated that hsa_circ_000595 is upregulated in human aortic aneurysm patients and hypoxic VSMCs. In addition, hsa_circ_000595 can directly target cyclooxygenase 2, hypoxia-inducible factor 1α (HIF-1α) and nuclear factor-κB to promote its biological function in regulating cell apoptosis via targeting miR-19a. By contrast, hsa-circ-000595 knockdown significantly attenuated apoptosis (95). Sun *et al* (96) reported that angiotensin II inhibits apoptosis of mouse aortic smooth muscle cells through regulating the circNRG-1/miR-193b-5p/NRG-1 axis. Several circRNAs have been identified to regulate the phenotypic transformation of VSMCs, including WD repeat domain 77, diaphanous related formin 3 and actin α 2 (97,98). Regarding cardiomyocytes and cardiac fibroblasts, Gupta *et al* (49) demonstrated that QKI could inhibit DOX-induced cardiotoxicity via regulating the expression of the circRNAs Ttn, Fhod3 and Strn3. Furthermore, QKI could regulate circRNA expression to protect cardiomyocytes against adriamycin-induced injury in primary rat, H9C2 and HL-1 cardiomyocytes *in vitro*. CircRNAs are also considered as potential targets for treating cardiac fibrosis (99-101). Therefore, a study showed that the expression of circRNA_000203 of the myosin I9A transcripts was increased in the myocardium of diabetic mice and in angiotensin II-induced cardiac fibroblasts (102). It has been also revealed that the circRNA_000203/miR-26b-5p/collagen type I α 2 and connective tissue growth factor axes regulate the anti-fibrotic effects of cardiac fibroblasts (102). Wang *et al* (82) demonstrated that the mitochondrial fission and apoptosis-related circRNA (MFACR) could mediate cardiomyocyte mitochondrial fission and apoptosis via regulating the MFACR/miR-652-3p/mitochondrial trifunctional protein 18 axis. These findings provided novel knowledge for understanding the molecular events associated with mitochondrial fission. The circRNAs involved in the cardiovascular system are listed in Table II.

7. Regulatory mechanisms of circRNAs on CVD

It is understood that circRNAs are involved in numerous pathological mechanisms underlying CVDs, such as AS, immune response, apoptosis, cell proliferation, autophagy, cell senescence and myocardial repair (103-109). Emerging evidence has suggested that circRNAs play an important role in the occurrence and development of CVD (110). The documented regulatory mechanisms of circRNAs on CVDs are listed in Fig. 2.

Role of circRNAs in cell proliferation. The proliferation of cells involved in the cardiovascular system plays an important role in the development of CVDs, such as hypertension, AS and restenosis (110). CircRNAs serve an important role in the development of CVDs (103,111). Shen *et al* (103) demonstrated that circRNA-0044073 is highly expressed in patients with AS. In addition, it was shown that circRNA-0044073 promotes the proliferation and invasion of human umbilical vein smooth muscle cells and HUVECs, and accelerates the development of AS via targeting miR-107 and activating the Janus kinase/signal transducer and activator of transcription. Wang *et al* (112) found that the circRNA homeodomain interacting protein kinase 3 could act as a sponge to inhibit miR-29a activity, resulting in increased insulin-like growth factor 1 expression, which in turn could attenuate the dysfunction of oxidative stress-induced cerebral microvascular endothelial cells (CMVECs). Additionally, the circRNA HIPK3 promoted the proliferation and survival of CMVECs under hypoxic conditions. Another study demonstrated that HIPK3 could sponge miR-29b-3p to regulate the proliferation, migration and development of cardiac fibroblasts in angiotensin II-induced myocardial fibrosis (111).

Role of circRNAs in cardiomyocyte apoptosis. Cardiomyocyte apoptosis is an important factor affecting the occurrence and development of CVDs (104,113-115). Geng *et al* (104) investigated the effect of the co-expression of circ-Cdr1as with miR-7a in mouse myocardial infarction injury model. Cdr1as overexpression upregulated the expression of poly (ADP-ribose) polymerase and specific protein 1 to promote cardiomyocyte apoptosis, but was then reversed by miR-7a overexpression. Li *et al* (113) suggested that reactive oxygen species could upregulate circ-NCX1, which in turn could promote cardiomyocyte apoptosis. Mechanistically, circ-NCX1 could competitively bind to miR-133a-3p to increase the expression levels of the pro-apoptotic protein gene cell death-inducing protein 1 (CDIP1). Therefore, circNCX1 knockdown reduced the expression of CDIP1 in murine cardiomyocytes and heart tissues, thereby improving the condition of myocardial ischemia-reperfusion injury. Several other circRNAs have been identified to play a role in promoting cardiomyocyte apoptosis, including circ-MFACR and circRNA_0010729 (82,114).

Role of circRNAs in the regulation of AS. As early as 2010, a study revealed that the INK4/ADP ribosylation factor (ARF) gene locus is adjacent to the chromosome 9p21, which is associated with CAD (116). Circ-ANRIL, the antisense transcript of the INK4/ARF gene, was shown to be regulated by a single nucleotide polymorphism in the 9p21 chromosome and is associated with the risk of AS. Song *et al* (117) demonstrated that circ-ANRIL downregulation could prevent coronary AS via attenuating vascular endothelial cell apoptosis and the expression of inflammatory factors. However, another study suggested that circ-ANRIL exerts a protective effect against AS (85). The proportion of circ-ANRIL/lncRNA ANRIL in patients with coronary heart disease is higher, and patients with increased expression of circ-ANRIL rarely develop coronary heart disease. Furthermore, circ-ANRIL could induce nucleolar stress and p53 activation in VSMCs and macrophages, thus inducing and inhibiting apoptosis and proliferation,

Table II. List of circRNAs associated with cardiovascular diseases.

First author/s, year	Type of disease	CircRNA	Host gene	Cardiovascular cell types	Regulation (in disease)	Function	Regulatory mechanism	(Refs.)
Wang *et al*, 2016	Cardiac hypertrophy	CircHRCR	PWWP2A	Mouse cardiomyocytes	Downregulated	Overexpression attenuates cardiac hypertrophy	HRCR sponges miR-223 to regulate ARC expression to prevent cardiac hypertrophy	(69)
Lim *et al*, 2019		CircSLc8a1	SLc8a1	Mouse cardiomyocytes	Upregulated	Knockdown reduces myocardial hypertrophy caused by pressure overload	CircSLc8a1 acts as a sponge of miR-133a, which is related to cardiac hypertrophy	(108)
Tang *et al*, 2017		Circ_000203	Myo9a	Neonatal mouse ventricular cardiomyocytes	Upregulated	Overexpression aggravates cardiac hypertrophy	CircRNA_000203 can sponge miR-26b-5p and miR-140-3p, exacerbating cardiac hypertrophy	(102)
Huang *et al*, 2019	Myocardial infarction	CircNfix	NFIX	Cardiomyocytes	Downregulated	Downregulated expression promotes cardiomyocyte proliferation and angiogenesis	Dual function as a miR-214 sponge and interacts with YBX1 and NEDD4L to induce YBX1 degradation	(107)
Garikipati *et al*, 2019		CircFndc3b	Fndc3b	Mouse cardiac endothelial cells and human umbilical vein endothelial cells	Downregulated	Overexpression enhances endothelial cell function and reduces cardiomyocyte apoptosis	Overexpression of circFndc3b can significantly reduce FUS levels and upregulate VEGF-A levels to promote cardiac neovascularization and improve remodeling after myocardial infarction	(131)
Cai *et al*, 2019		CircTtc3	Ttc3	Rat cardiac fibroblasts and cardiomyocytes	Upregulated	Overexpression inhibits apoptosis of cardiomyocytes	Circ-Ttc3 as a miR-15b-5p sponge can increase Arl2 expression and inhibit myocardial cell apoptosis induced by myocardial infarction	(115)

Table II. Continued.

First author/s, year	Type of disease	CircRNA	Host gene	Cardiovascular cell types	Regulation (in disease)	Function	Regulatory mechanism	(Refs.)
Geng *et al*, 2016		Circ-CDR1as	Cdr1	Rat cardiomyocytes	Upregulated	Overexpression in MCM cells promotes cell apoptosis	Circ-CDR1as can bind to miR-7a, resulting in increased expression of PARP and SP1, which increases myocardial infarct size	(104)
Zhou *et al*, 2019		CircMFACR	Smyd4	Mouse cardiomyocytes	Upregulated	Overexpression attenuates autophagy and cell death in cardiomyocytes	circMFACR represses autophagy and myocardial infarction by targeting Pink1-mediated phosphorylation of FAM65B	(128)
Zhu *et al*, 2019	Myocardial fibrosis	CircNFIB	Nfib	Mouse cardiomyocytes	Downregulated	Overexpression inhibits the proliferation and differentiation of mouse myocardial fibroblasts	CircNFIB as a sponge for miR-433 inhibits myocardial fibroblast proliferation induced by TGF-β	(99)
Ni *et al*, 2019		CircHIPK3	Hipk3	Cardiac fibroblasts	Upregulated	Overexpression attenuates the proliferation and migration of cardiac fibrosis	CircHIPK-3 as a sponge for miR-29b-3p changes the expression of α-SMA, COL1A1 and COL3A1 to regulate cardiac fibrosis	(111)
Sun *et al*, 2020		Circ_Las1l	Las1l	Human cardiac fibroblasts	Downregulated	Overexpression inhibits cardiac fibroblast proliferation and migration, and promotes apoptosis	Circ_Las1l as a sponge of miR-125b promotes the expression of SFRP5, and inhibits the activation, proliferation and migration of cardiac fibroblasts	(100)
Zhou and Yu, 2017		CircRNA_ 010567	Zswim	Diabetic mouse cardiomyocytes	Upregulated	Silencing inhibits the translation of fibrosis	circRNA_010567 silencing can upregulate miR-141 and downregulate TGF-β1 expression	(101)
Holdt *et al*, 2016	Atherosclerosis	CircANRIL	INK4	Human monocytic	Downregulated	Overexpression increases apoptosis and decreases proliferation	CircANRIL governs pre-rRNA maturation and nucleolar stress through interaction with multiple RBPs	(85)

Table II. Continued.

First author/s, year	Type of disease	CircRNA	Host gene	Cardiovascular cell types	Regulation (in disease)	Function	Regulatory mechanism	(Refs.)
Zhang *et al*, 2020		Circ_0003204	USP36	Human aorta endothelial cells	Upregulated	Ectopic expression inhibits proliferation, migration and tube formation of ox-LDL-induced human aorta endothelial cells	Circ_0003204 as a sponge of miR-370 promotes protein expression of TGFβR2 and its downstream phosphorylated-SMAD3 and exacerbate deactivation of HAECs in response to ox-LDL	(109)
Chen *et al*, 2017		CircWDR77	WDR77	Human vascular smooth muscle cells	Upregulated	Silencing inhibits vascular smooth muscle cells proliferation and migration	CircWDR77 regulates vascular smooth muscle cells proliferation and migration by targeting miR-124/FGF2	(97)
Li *et al*, 2017		Circ_0003575	CHMP5	Human umbilical vein endothelial cells	Unchanged	Silencing enhances human umbilical vein endothelial cells proliferation and angiogenesis	Unknown	(91)
Zeng *et al*, 2017	Cardiac senescence	CircAmotl1	Amotl1	Mouse cardiomyocytes	Downregulated during aging	Ectopic expression induces primary cardiomyocyte proliferation	CircAmotl1 induces Akt phosphorylation and phosphorylated-Akt nuclear transport by binding Akt and PDK1, and activates the Akt signaling pathway to promote cell survival and proliferation	(130)
Du *et al*, 2017		CircFOXO3	FOXO3	Mouse cardiomyocytes	Upregulated	Ectopic expression induces senescence	CircFOXO3 interacts with id-1, E2F1 and FAK to inhibit their effects and accelerate cell senescence	(15)
Liu *et al*, 2017	Coronary heart disease	CircZNF609	ZNF609	Human umbilical vein endothelial cells	Upregulated	Silencing increases endothelial cell viability, proliferation, migration, and tube formation	CircZNF609 sponges miR-615-5p leading to increased MEF2A expression to reduce migration, proliferation, and apoptosis of endothelial cells	(90)

Table II. Continued.

First author/s, year	Type of disease	CircRNA	Host gene	Cardiovascular cell types	Regulation (in disease)	Function	Regulatory mechanism	(Refs.)
Hall *et al*, 2019		CircLrp6	Lrp6	Mouse vascular smooth muscle cells	Unchanged	Silencing prevents intimal hyperplasia in mouse carotids.	Circ_LRP6 sponges miR-145 to inhibit its mediated regulation of vascular smooth muscle cell migration, proliferation, and differentiation	(94)
Sun *et al*, 2019		CircNrg1	Nrg1	MASMCs	Downregulated	Knockdown inhibits the apoptosis of MASMCs	Angiotensin II downregulates the expression of circNrg-1 and sponges miR-193b-5p to regulate the expression of Nrg-1 to inhibit the apoptosis of MASMCs	(96)
Zou *et al*, 2018		CircTCF25	TCF25	Human umbilical vein endothelial cells	Downregulated	Downregulated expression in coronary heart disease	CircTCF25 is a miR-103 sponge. Coronary artery disease risk factors inhibit the expression of circTCF25 in human umbilical vein endothelial cells	(84)

circRNA, circular RNA; MASMCs, mouse aortic smooth muscle cells; miR, microRNA; ox-LDL, oxidized low-density lipoprotein; FOXO3, forkhead box O3.

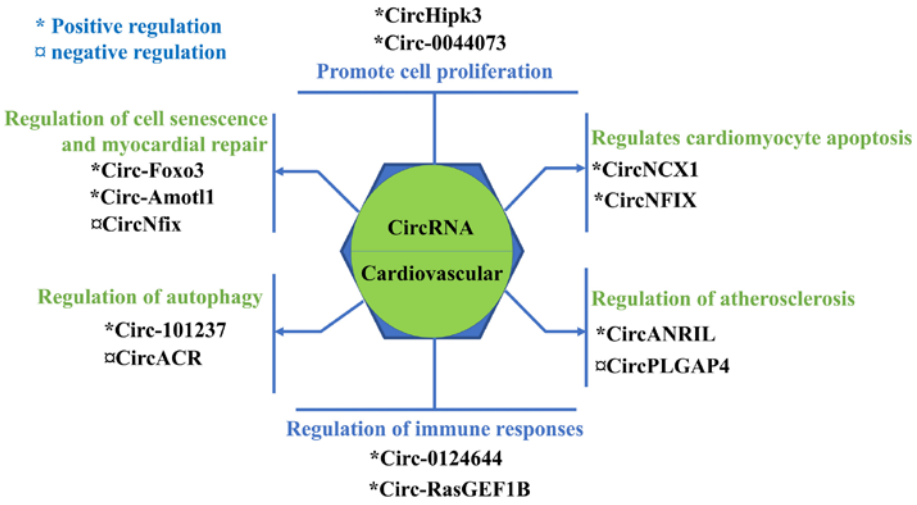

Figure 2. CircRNAs as therapeutic agents, targets and/or biomarkers in cardiovascular. The possible regulatory mechanisms linking circRNA to cardiovascular diseases. Circ/CircRNA, circular RNA; FOXO3, forkhead box O3; Nfix, nuclear factor 1 X-type; RasGEF1B, Ras-GEF domain-containing family member 1B.

respectively. Therefore, the role of circ-ANRIL in regulating AS should be further investigated. In addition, a study revealed that circ-DLG associated protein 4 (DLGAP4) is negatively associated with the risk of coronary heart disease (118). Therefore, it was demonstrated that circ-DLGAP4 was down-regulated in ox-LDL-induced foam cells in a time-dependent manner. In addition, circ-DLGAP4 could sponge endogenous miR-143 to reduce the size of the atherosclerotic plaque and macrophage infiltration in mice (119,120). This finding indicated that circ-DLGAP4 could be considered as a protective factor against monocyte-macrophage foaming, and as a potential inhibitory factor of AS plaque forming.

Role of circRNAs in the regulation of immune responses. CircRNAs are involved in innate immunity (121). A study showed that circ_0124644 could affect the progression of CAD through regulating the immune responses such as cell adhesion, therefore, it is considered as a biomarker for CAD (105). Another study investigated the expression of circRNAs in bone marrow-derived macrophages under two distinct polarization conditions, namely M1 macrophages stimulated with interferon-γ and LPS, and M2 macrophages induced by interleukin-4 (122). The results revealed an interaction between circRNA-010231 and miR-141-5p, miR-145a-5p, miR-1964-5p, miR-19b-2-5p and miR-6950-5p. circRNA-010231 regulated the expression of the aforementioned miRNAs via participating in the differentiation and polarization of macrophages, thereby regulating the immune responses. The circRNA Ras-GEF domain-containing family member 1B (RasGEF1B) predominantly exists in the cytoplasm of murine macrophages (123). Ng *et al* (123) demonstrated that the expression of intercellular adhesion molecule-1 (ICAM-1) is downregulated following circ-RasGEF1B knockout in LPS-induced macrophages, indicating that ICAM-1 is a downstream regulator of circ-RasGEF1B-associated immune responses. In addition, circ-RasGEF1B mediates the immune responses via regulating the stability of mature ICAM-1 mRNAs. Additional studies have also reported the role of different circRNAs on the regulation of the immune responses (121,124,125). Therefore, the effects of circRNAs on the immune function in CVDs are worth further investigation.

Role of circRNAs in the regulation of autophagy. CircRNAs are involved in autophagy by regulating the transcription and post-transcriptional modifications of autophagy-related genes (126). Cell autophagy is considered as a double-edged sword in health and disease, while it exerts protective effects on restoring homeostasis, it has also been reported that excessive autophagy mediates cardiomyocyte death (127). Zhou *et al* (128) demonstrated that circ-acrosin (ACR) attenuates autophagy and cell death in cardiomyocytes. In addition, ACR protects against ischemia-reperfusion injury and reduces myocardial infarct sizes in a myocardial ischemia/reperfusion injury model. The same study revealed that circ-ACR could induce the expression of putative kinase 1 (PINK-1) via directly binding to DNA methyltransferase 3B (Dnmt3b), thus blocking the Dnmt3b-mediated DNA methylation of the PINK-1 promoter. Furthermore, PINK-1 could phosphorylate its downstream target, Rho family-interacting cell polarization regulator 2 on serine 46 and inhibit cardiac autophagy and cell death. Another study on autophagy demonstrated that circRNA_101237 acts as a sponge of let-7a-5p to regulate insulin like growth factor 2 mRNA binding protein 3 (IGF2BP3)-dependent autophagy (106). Therefore, circRNA_101237 downregulation decreases the expression of IGF2BP3, which in turn attenuates the apoptosis of primary cardiomyocytes and inhibits hypoxia/reoxygenation-induced autophagy. The aforementioned studies indicated that autophagy could serve an important role in the regulation of CVDs.

Role of circRNAs in the regulation of cell senescence and myocardial repair. The senescence and repair of tissues or cells is closely associated with their function (107,129-131). A study showed that the circRNA forkhead box O3 (FOXO3) is significantly upregulated in heart samples of elderly patients and mice and is associated with cell senescence (15). Therefore, circ-FOXO3 is mainly distributed in the cytoplasm, where it can interact with the anti-senescence protein inhibitor of DNA binding-1, the transcription factor E2F1 and the anti-stress proteins focal adhesion kinase and HIF-1α, thereby promoting cardiomyocyte senescence. Additional mechanistic studies suggested that the pro-senescent effect of circ-FOXO3

could be associated with the regulation of the cell cycle. Therefore, in this study, the ectopically expressed circ-FOXO3 could form a ternary complex by binding with cyclin kinase 2 (CDK2) and cycle dependent kinase inhibitor 1 (p21). The formed circ-FOXO3-p21-CDK2 ternary complex blocks the function of CDK2, thereby inhibiting the progression of the cell cycle (129). Zeng *et al* (130) investigated the role of circRNA in myocardial repair by screening the myocardial tissues of patients of different ages. The results revealed that circRNA angiomotin Like 1 (Amotl1) is significantly upregulated in myocardial tissues, thus enhancing the myocardial function in the neonatal heart. Furthermore, circ-Amotl1 induces the phosphorylation of Akt and its nuclear transportation via binding Akt and phosphoinositide dependent kinase 1. This process promotes the activation of the Akt signaling pathway, resulting in enhanced cell survival and proliferation. In addition, circ-Amotl1 could play a protective role in the DOX-induced cardiomyopathy (130). In another study, the authors investigated the positive and negative regulatory effects of the signaling pathways Meis1/circ-nuclear factor 1 X-type (Nfix)/miR-214/Gsk3β/β-catenin and circ-Nfix/Ybx1/cyclin A2 and cyclin B1 by functional verification experiments (107). The results revealed a close association between the expression of circ-Nfix and heart regeneration and repair. The aforementioned studies indicated that circRNAs could serve an important role in regulating cell senescence and cell regeneration, and could be used as targets for treating heart failure and myocardial infarction in the future.

8. Perspectives and conclusions

Nowadays, the interest of the scientific community regarding circRNAs is increasing with their comprehensive exploration, and the mystery of circRNAs has been slowly revealed. Although several functions of circRNAs have been identified, numerous remain to be elucidated. Due to their high stability and resistance to RNases, circRNAs may accumulate in quiescent or post-mitotic cells, such as neurons (132). Several studies revealed that the expression of circRNAs in proliferative cells and tissues, including cancer cells, is lower compared with terminally differentiated cells, indicating a negative association between circRNA abundance and cell proliferation (133). Previous studies have shown that circRNAs are enriched in extracellular vesicles and are secreted by cells in the form of vesicles (133-137). Notably, smaller circRNAs are more prone to vesicle-mediated transport (35). In addition, circRNAs maintain their circular structure in vesicles and perform their activities in recipient cells, suggesting that circRNA transport may be important for cell-to-cell communication (136). In cardiac metabolic diseases, serum markers in peripheral blood, such as cardiac troponin and creatine kinase, serve a significant role in the diagnosis of several diseases. It has been reported that certain linear non-coding RNAs can be used as biomarkers in the diagnosis of CAD (137,138). Therefore, circRNAs could be considered as potential biomarkers for the diagnosis and treatment of CVDs. However, whether circRNAs selectively enter into exosomes, and the mechanism and significance of exosomal circRNA formation should be verified first. Certain circRNAs accumulate in the nucleus, while the majority are effectively transported to the cytoplasm (41). The accumulation of circRNAs in the nucleus is mediated by the ATP-dependent RNA helicase DexH/D-box, and their nuclear export depends on the length of the mature circRNA (139). m6A modification widely occurs on circRNAs (140). In view of the influence of m6A-binding protein YTH domain-containing 1 (YTHDC1) on the nuclear export of methylated mRNA (141), the effect of m6A on the export of circRNAs remains to be elucidated, to the best of our knowledge. Several circRNA-mediated degradation mechanisms have been reported (121,142,143). Therefore, the binding of circRNAs by miRNAs can initiate circRNA decay (121). Furthermore, circRNA can also be degraded by the cytoplasmic endonuclease RNase L, which is activated in the presence of pathogenic double-strand RNA during viral infection (142). In addition, it has been reported that the YTHDF2-HRSP12-RNase P/MRP and UPF1-G3BP1 complexes mediate m6A-modified and high-structured circRNAs, respectively (143). However, to the best of our knowledge, how the degradation mechanisms of circRNAs affect the disease course remains to be investigated. It is understood that circRNAs are conserved among various species (75), and are specifically expressed in different tissues and different developmental stages (12,76). Nevertheless, the reasons for the conservatism and specificity of circRNAs and how they affect the functional development of tissues and organs have not been reported to date. Although it has been documented that circRNAs exert a folded secondary structure (144), to the best of our knowledge, the association between their secondary structure and their function remains unclear, and it is worthy of further investigation. There are a number of limited reports regarding the translational function of circRNAs, including the IRES and m6A modification pathways (52,53,81,145). Recently, researchers have combined the stability of circRNAs and their potential for continuous translation to develop a new expression system that could produce a protein in a sustainable and stable manner (146). This finding indicated that the translational function of circRNAs could be applied in the future for biological therapy.

A number of circRNAs have been identified, discovered and applied in the CAD (84,105,118). It has been suggested that active pharmaceutical ingredients can interfere with the differential expression of circRNAs in CAD (147). Several networks of circRNA-miRNA signaling pathways in the CAD have been also reported (103,148). These networks are systematic and three-dimensional and can truly and dynamically reflect the occurrence and development of CAD. A study revealed that circRNAs could be involved in chemoresistance, thus providing a novel approach for circRNAs in drug intervention and targeted therapy for CVDs (149). In addition, a research team used RNA polymers and poly (lactic-co-glycolic acid) (PLGA) coated stents containing and releasing therapeutic factors to treat local lesions in AS (149). The results demonstrated that these stents could promote endothelial cell regeneration and reduce the risk of coronary artery restenosis. Another study suggested that modern transfection methods could be used to transfer circRNAs during cardiovascular operations (150). Recently, a study introduced the circmiRs technology, opening a new chapter in the *in vivo* treatment of CAD using circRNAs (151). In this study, a circmiR sponge was constructed targeting the known cardiac pro-hypertrophic miRs-132 and miRs-212. Therefore, the hypertrophic

characteristics of the disease were attenuated, and the cardiac function was preserved in transverse aortic constriction-treated mice, thus supporting the potential of circmiRs as novel therapeutic tools. By exploiting the characteristics of circRNAs in CVDs through drug intervention, artificial overexpression or knockout, and circRNA sequencing, it is possible to achieve a more accurate treatment strategy for CVDs.

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Availability of data and materials

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Authors' contributions

ZY, HW and ZZ contributed to the conception of the study, performed the literature search and wrote the manuscript. QH, QZ and HW edited the manuscript, assisted in the literature search and critically revised the article for important intellectual content. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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