



# Circular RNA in cardiovascular disease

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**Abstract:** Although laudable approaches have been developed to improve the diagnosis and treatment of cardiovascular disease (CVD), they are still far from satisfactory. Thus, there is an increasing need for novel diagnostic biomarkers and new therapeutic interventions to efficiently decrease the morbidity and mortality of CVD. Circular RNAs (circRNAs) as a class of newly discovered endogenous noncoding RNAs, have been implicated in the development of CVD by multiple mechanisms including sponge-like regulation of microRNAs and proteins, indicating that circRNAs are promising therapy targets and potential biomarkers for CVD. In this review, we characterize the biogenesis and expression of circRNA, highlight the regulatory role of circRNA in different models of CVD. We also present perspectives of circRNAs as novel biomarkers and therapeutic targets for the treatment of CVD.

**Keywords:** Circular RNA (circRNA); cardiovascular disease (CVD); biomarker

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## Introduction

Over the past 20 years, different approaches have been developed to improve the diagnosis and treatment of cardiovascular disease (CVD) (1-3), among which translational medicine from basic research to clinical research plays an important role. However, they are still far from satisfactory. Thus, there is still a clinical need for investigating novel diagnostic biomarkers and new therapeutic targets to decrease the morbidity and mortality as well as improve the quality of life, considering the unsatisfactory outcome of the human disease.

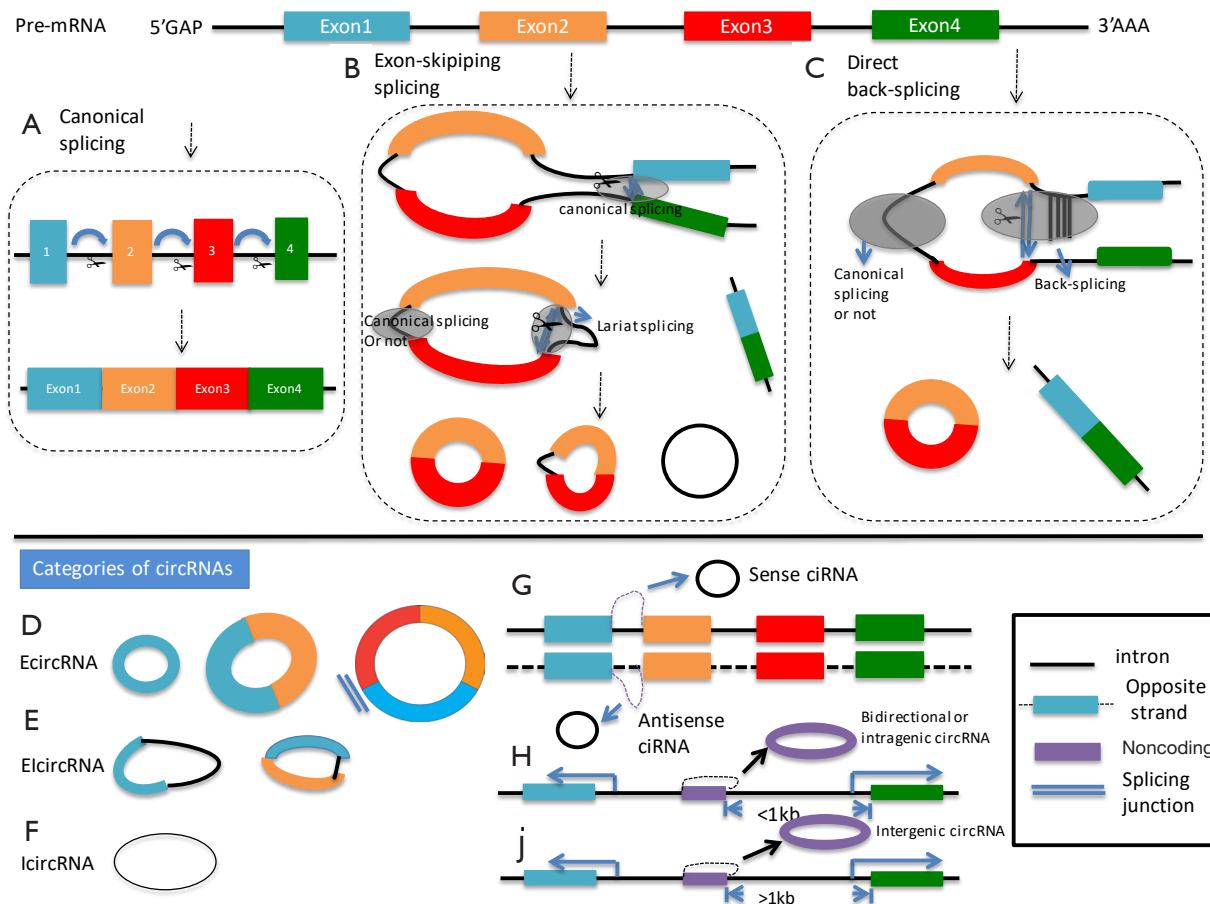
Circular RNAs (circRNAs) are a recently discovered non-coding RNAs, which have been shown to be involved in multiple physiological and pathological processes including early embryonic development (4), aging (5) and disease-related pathways. Moreover, circRNAs can not only be found in different tissues (6,7), organs (8,9) but also enriched in body fluids (10-12), such as blood, exosomes, saliva, seminal plasma, indicating that circRNAs are promising therapy targets and promising biomarkers for human disease.

Specifically, circRNAs are widely expressed in the heart of human (9,13), rats (13) and mice (13,14) and have been shown to be associated with CVDs. In this review, we summarize the biogenesis and expression of circRNA, their function mechanism, the common software or online tool for circRNA research. Specially, we discuss circRNAs and their potential roles in diagnosis, prognosis and therapeutic strategies for CVDs.

## Biogenesis and expression of circRNA (Figure 1)

In unicellular organisms, circRNAs mostly stem from self-splicing introns of pre-ribosomal RNA (15,16), but can also arise from protein-coding genes in archaea (15). In the validated circRNAs in animals, circRNAs are predominantly derived from exons, whose 5' and 3' ends are covalently linked to form a "head-to-tail" splice junction. Although spliceosomal machinery is implicated in the pre-mRNA splicing (17), the mechanisms involved in exon circularization remain elusive.

It is supposed that circRNA generated from the editing

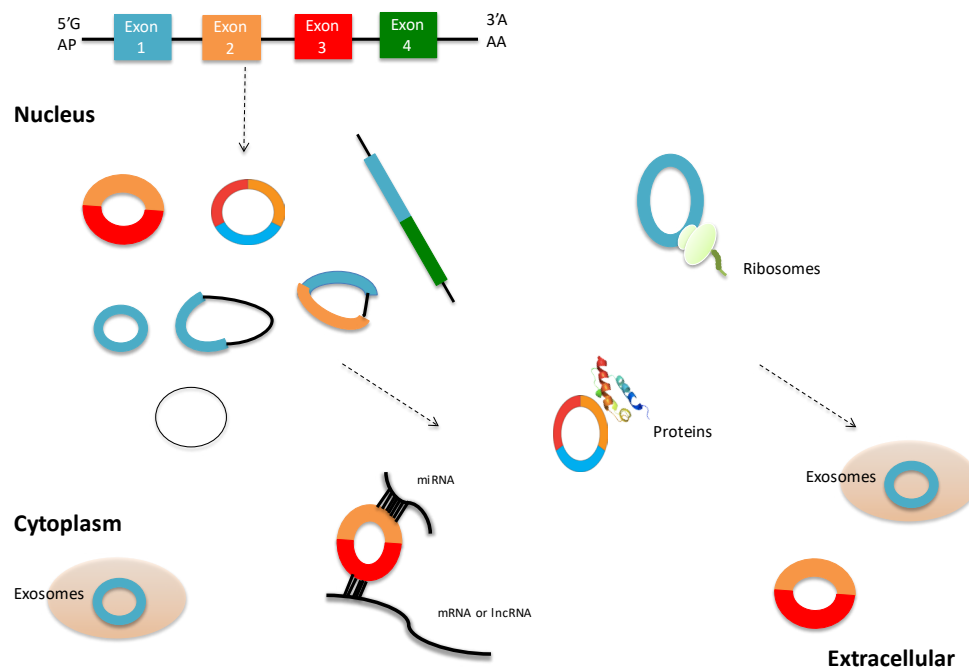


**Figure 1** Two mechanisms of circRNA formation and categories of circRNAs. (A) The process of canonical splicing; (B) the process of exon-skipping splicing; (C) the process of direct back-splicing; (D) ecircRNA: exon-circRNA; (E) EIcircRNA: exon-intron circRNA; (F) icircRNA: intronic circRNA; (G) sense circRNA and antisense circRNA; (H) bidirectional or intragenic circRNA; (J) intergenic circRNA.

process of pre-mRNA. There are two major mechanisms of circRNA formation: (I) direct back-splicing and (II) exon skipping (18,19). Direct back-splicing, where exons are spliced in non-canonical order, connects the downstream 5' splice site (splice donor) to an upstream 3' splice site (splice acceptor), thereby generating a circular transcript (20). The second mechanism is lariat splicing, which involves generation of a lariat intermediate containing exons, and then, the introns in the lariat are removed, generating exonic circRNA (ecircRNAs) (21). Both mechanisms function *in vivo* and correlate with the canonical spliceosome, but direct back-splicing may occur more frequently than exon skipping in ecircRNA biogenesis (22). Recent findings also indicate that RNA pairing, which can be formed by either repetitive elements (23) or non-repetitive but complementary sequences (15),

across flanking introns is positively associated with exon circularization. Additional observations have revealed that RNA binding proteins such as adenosine to inosine acting on RNA enzyme (ADAR), muscleblind (MBL) and Quaking (QKI) function in specific ecircRNA biogenesis (24-26). Of note, these three proteins are known to be implicated in alternative splicing. In addition, compared with all expressed exons, the exons generating single-exon circRNA are 3-fold longer in length (6,23). Therefore, the length of a given exon may also correlate with circularization.

Based on the above progress of circRNA formation, circRNAs have 5 categories: (I) ecircRNAs, spliced from the exonic segments; (II) intronic circRNA (icircRNAs), derived wholly from an intron of the linear transcript; (III) exon-intron circRNA (EIicRNA), consisted of both exons and introns; (IV) antisense, contained one or more



**Figure 2** Expression and biogenetic function of circRNA.

exons of the linear transcript on the opposite strand; (V) bidirectional or intragenic, transcribed from same gene locus of the linear transcript but in close genomic proximity and not classified as “sense” and “intronic”; and (VI) intergenic, when it is located in the genomic interval between two genes (*Figure 1*).

While most circRNAs are lowly abundant, some are ubiquitously expressed and are present at higher copy numbers (>10-fold) as compared to their linear transcripts (7,23). Besides, the levels of some circRNAs are significantly associated with the proliferative and differentiative stage (8,27,28), indicating that circRNAs may have important roles in organic development and functions. What's more, circRNAs are highly stable *in vivo* compared with their linear counterparts, and are predominantly in the cytoplasm and can be sorted into exosomes (11). Considering their widely distribution in body fluid (10-12), circRNAs can serve as a promising biomarker for human disease, including CVDs.

### Biogenetic function of circRNA: old tree with new flower

CircRNAs are attracting more attention owing to not only their abundance in the eukaryotic transcriptomes but also their functions. So far, it is regarded that circRNAs can

function in multiple biological processes, such as miRNA binding, protein binding and regulation of transcription and post-transcription (*Figure 2*).

#### *miRNA sponge*

The most exciting finding is that circRNAs could function as microRNA (miRNA) sponges. For instance, ciRS-7/CDR1as harbors >70 conserved binding sites for miR-7 (29,30). miR-7 activity could be dramatically reduced through tethering of this miRNA to ciRS-7/CDR1as, resulting in increased levels of miR-7 targets. Then, circRNA destruction could release a shower of miR-7. Sry, a testis-specific circRNA, is another validated miRNA sponge (30,31). This circRNA harbors 16 binding sites for miR-138. These findings changed the mechanistic understanding of miRNA regulatory networks and increased the complexity of competitive endogenous RNA (ceRNA) network (32). Although few circRNAs contain a majority of miRNA binding sites for a single miRNA, the depletion of polymorphisms at predicted miRNA binding sites implies that circRNAs could be efficiently targeted by miRNAs.

#### *Protein sponge*

CircRNAs can also act as protein sponges. For example,

circMbl, derived from the muscleblind (MBL/MBNL1) in flies and humans, harbors multiple muscleblind binding sites (25). When MBL proteins are in excess, circMbl could sponge out the redundant ones. By this way, MBL levels could be well regulated. And circ-Foxo3, which is downregulated in cancer cells and is associated with cell cycle progression, also interacts with some proteins. For examples, circ-Foxo3 binds cyclin-dependent kinase 2 (CDK2) and p21 (CDKN 1a), forming an RNA-protein complex that disrupts the interactions of CDK2 with cyclins A and E, required for cell cycle progression (33). Circ-Foxo3 also interacts with proteins inhibitors of differentiation (ID1), E2F transcription factor 1 (E2F1), focal adhesion kinase (FAK) and hypoxia inducible factor-1 (HIF-1), retaining them in the cytoplasm and thus promoting cardiac senescence (34).

#### ***Alternative splicing, mRNA trap and transcriptional regulation***

CircRNAs can affect their parental genes in cis- or trans- actions. Circularization and splicing compete against each other, enabling ecircRNAs to function in alternative splicing (25). Once a back-splice occurs, it removes the internal exons, causing an alternative splicing. EcircRNAs may serve as an “mRNA trap” by sequestering the translation start site, making the truncated linear mRNA fail to translate. For instance, the mice formin (Fmn) gene could generate ecircRNAs acting as mRNA trap. Deletion of specific exons caused a failure to produce these ecircRNAs, but appeared to produce normal amounts of the linear mRNAs. The inability to generate ecircRNAs from the Fmn locus caused aberrant expression of the formin protein and changed the phenotype as a result (25).

Moreover, ciRNAs can function as a positive regulator of Pol II transcription by interacting with Pol II machinery (35). More recently, a study has revealed that EIciRNAs use a specific interaction with U1 snRNA to fine-tune the expression of their parental genes in the nucleus (36). This discovery not only reveals a role for circRNAs in transcriptional control but also highlights a regulatory strategy through specific RNA–RNA interaction between EIciRNA and U1 snRNA.

Collectively, the interplay between circRNAs and the transcriptional machinery provides new insights into the

strategies to regulate gene expression in cells.

#### **circRNAs and cardiovascular disease: common molecules with huge potentials (Table 1)**

Since *Nature* firstly ascribed an actual function to one of these circRNA molecules (29), circRNAs become a research hotspot in the field of RNA. Though circRNAs are old molecules, the huge potential role of circRNAs are recently being discovered from the laboratory to the clinic. CircRNAs have been shown to be associated with various diseases in both patients’ cohorts and animal models including CVDs. *Table 1* summarizes circRNAs that have been reported in CVDs.

#### ***CircRNAs-miRNAs axes in CVDs***

As the miRNA sponge, several studies demonstrate the key role of circRNAs in regulating miRNA in CVD.

#### **Heart failure**

A heart-related circRNA (HRCR) was reported to be able to attenuate the development of cardiac hypertrophy and heart failure in mice by targeting miR-223 and ARC (apoptosis repressor with caspase recruitment domain) (41). This study revealed a novel cardiac hypertrophic regulating model that was composed of HRCR, miR-223 and ARC. miR-223 promote the process of cardiac hypertrophy and heart failure, while ARC is the downstream of miR-223. However, the pathological effects of miR-223 can be inhibited by HRCR, which is identified as an upstream regulator of miR-223.

#### **Myocardial infarction (MI)**

In MI mice, Cdr1as (or CiRS-7) (37) was reported to be upregulated with increased cardiac infarct size. In Geng’s study, Cdr1as and miR-7 were both overexpressed in MI mice, but the function of Cdr1as can be downregulated by miR-7. SP1 and poly (ADP-ribose) polymerase (PARP) were the downstream targets of miR-7.

#### **Aortic aneurysm**

Aortic aneurysm is a leading cause of cardiovascular mortality. CircRNA has-circ-000595 was highly expressed in tissues from aortic aneurysm patients and hypoxic aortic smooth muscle cells (42). Knockdown of hsa-circ-000595 resulted in decreased apoptotic rate of human aortic smooth

**Table 1** CircRNAs in CVD

Disease	circRNAs	Model	Expression	Possible targets	Potential action	Potential value
MI (37)	Cdr1as (CiRS-7)	Mice	Increased	SP1, miR-7a; PARP	Overexpression of Cdr1as aggravated MI injuries with upregulated PARP and SP1, which could be rescued by the co-overexpression of miR-7a	Therapeutic, target
AMI (38)	Circ-081881	Patients	Decreased	miR-548	CircRNA_081881 could compete for miR-548 with PPAR $\gamma$	Biomarker and Therapeutic
MI with or without left; ventricular dysfunction (39)	MIRC	Patients	Decreased	–	MICRA, can predict LV dysfunction after 3 to 4 months	Biomarker
CAD (40)	ANRIL	Patients	Increased	PES1	circANRIL confers atheroprotection by controlling ribosomal RNA (rRNA) maturation and modulating pathways of atheroprotection	Therapeutic, target
Heart failure (41)	HRCR	Mice	Increased	miR-223, ARC	HRCR inhibits cardiac hypertrophy through miR-223	Therapeutic, target
Cardiomyopathy (34)	circFOX-3	Patients	Increased	ID-1, E2F1, FAK, HIF-1 $\alpha$	circ-Foxo3 induce senescence by interacting with the anti-senescent protein ID-1 and the transcription factor E2F1, as well as the anti-stress proteins FAK and HIF-1 $\alpha$	Therapeutic, target
Aortic aneurysm (42)	Circ-000595	Patients	Increased	miR-19a	hsa-circ-000595 was highly expressed aortic tissues of patients with aortic aneurysm	Therapeutic, target
Hypoxic conditions (43)	cZNF-292	Endothelial cell		–	Exhibits proangiogenic activities	Therapeutic, target

MI, myocardial infarction; PARP, poly (ADP-ribose) polymerase; AMI, acute myocardial infarction; CAD, coronary artery disease; PES1, Pescadillo homologue 1; HRCR, heart-related circRNA; ARC, apoptosis repressor with caspase recruitment domain; ID1, inhibitors of differentiation; E2F1, E2F transcription factor 1; FAK, focal adhesion kinase; HIF-1, hypoxia inducible factor-1.

muscle cells. It was determined that miR-19a was a target of hsa-circ-000595.

### Cardiac fibrosis

Individuals with diabetes are at significantly risk of developing diabetic myocardial fibrosis. CircRNA\_000203 was up-regulated in the diabetic mouse myocardium and in Ang-II-induced mouse cardiac fibroblasts (44).

CircRNA\_000203 containing two potential binding sites for microRNA-26b-5p(miR-26b-5p), which exerted the anti-fibrotic effect by targeting Colla2 and CTGF. Overexpression of circRNA\_000203 could eliminate the anti-fibrotic effect of miR-26b-5p in cardiac fibroblasts. Furthermore, blocking the function of miR-26b-5p contributed to the pro-fibrosis effect of circRNA\_000203 in cardiac fibroblasts.



### ***CircRNA-protein axes associated with CVDs***

#### **Coronary atherosclerosis**

ANRIL (antisense non-coding RNA in the INK4 locus) was differentially expressed by the genotype at 9p21 (45) and increased linear ANRIL (linANRIL) was associated with increased atherosclerosis (46). ANRIL can also be expressed as circular transcripts, such as circANRIL. CircANRIL can protect the tissue from atherosclerosis by controlling ribosomal RNA (rRNA) maturation and modulation pathways of atherogenesis (40). Pescadillo homologue 1 (PES1), which was an essential 60S-preribosomal assembly factor, was the downstream target of circANRIL. CircANRIL can impair exonuclease-mediated pre-rRNA processing and ribosome biogenesis in vascular smooth muscle cells and macrophages through binding to PES1. Overall, these findings demonstrated that circANRIL plays an important role in regulating ribosome biogenesis and conferring atheroprotection.

#### **Cardiomyopathy**

circ-Foxo3 was highly expressed in heart samples of aged patients and mice, and was shown to be correlated with cellular senescence (34). Doxorubicin-induced cardiomyopathy was aggravated by ectopic expression of circ-Foxo3 but was relieved by silencing endogenous circ-Foxo3. circ-Foxo3 induce senescence by interacting with the anti-senescent protein ID-1 and the transcription factor E2F1, as well as the anti-stress proteins FAK and HIF-1 $\alpha$ . Strikingly, in their study, *in vivo* delivery of circ-Foxo3 targeted siRNA into mouse embryonic fibroblasts and primary cardiac myocytes resulted in lower levels of circ-Foxo3 expression and beta-gal staining, which is a standard marker for senescence, suggesting a potential therapeutic approach for myocardial protection.

Except for those circRNAs that have been shown the interaction with miRNAs or proteins, there're also some circRNAs correlated with CVDs, which have not been found the functional mechanisms (14,43). cZNF292 is a hypoxic-induced circRNA, which has been shown to be regulated hypoxia and have biological functions (43), however, no validated microRNA-binding sites for cZNF292 were detected, suggesting that cZNF292 does not act as a microRNA sponge. The circRNA landscape of heart tissue can be explored using a novel DCC software (14). Among those circRNAs, several circRNAs can be directly attributed to host genes that have been previously described as associated with CVD. Further studies of these candidate

circRNAs are needed to reveal disease-relevant properties or functions of specific circRNAs.

### **CircRNA as biomarker for CVD**

In addition to participate to the pathophysiological processes of CVD, circRNA has also been shown to be biomarkers for human disease, including circRNAs from pathological section (47,48), blood plasma (49,50), saliva (51) or seminal fluid (12). Several studies have been taken out to find out whether circulating circRNAs can be biomarkers for CVD (38,39).

For example, circRNA have the potential to predict outcome after reperfusion (39). In this study, they enrolled patients diagnosed as MI with or without left ventricular dysfunction, and finally identified a circRNA, MICRA, can predict LV dysfunction after 3 to 4 months. CircRNA can be also used as a potential target for the precision diagnosis and therapy of AMI (38). This study demonstrated that circRNA\_081881 regulated PPAR $\gamma$  expression by functioning as a competing endogenous RNA (ceRNA) of miR-548. Considering that circRNA\_081881 was detected in the plasma samples, circRNA\_081881 may be used as a potential target for AMI.

In addition, some other circRNAs have been shown to be associated with CAD. For instance, that the expression of hsa\_circ\_0124644 was moderately correlated with the CAD (52) implying that hsa\_circ\_0124644 might be deeply involved in the pathologies of CAD. As hsa\_circ\_0124644 can be easily determined using peripheral blood at a relatively low cost, yet the specificity and sensitivity are relatively high, and the diagnostic value would slightly increase after introducing hsa\_circ\_0098964, making hsa\_circ\_0124644 a powerful tool in the diagnosis of CAD.

### **Characters of circRNAs as biomarker for CVD**

CircRNAs are widely found in blood, saliva, and seminal fluid, and they are stable due to their circular structure, which are pre-conditions for their usage as biomarkers for various diseases. Furthermore, the expression levels of circRNAs vary under different conditions, making them possible to be biomarkers for diseases.

At present, molecular biomarkers used in the clinic are always proteins that have low organ specificity. For example, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are common in heart failure, and cardiac troponin T (cTnT) is common in MI. If circRNAs can be used in the clinic as biomarkers of CVD, their specific

expression may assist in solving the problem of existing markers' low organ specificity.

However, there're still some questions remains to be solved. First, the expression of some circRNAs only differs in patients' tissue, but not in patients' blood, which may cause some trauma for patients. Second, the relationship between the expression levels of circRNAs and the severity of CVD remains unknown, and thus we cannot estimate the severity of disease according to the expression levels of circRNAs. Third, the reliability of using circRNAs for diagnosis still needs to be proven. Furthermore, due to the enormous number of circRNAs, their functions may be complex and different from each other. Current studies mainly focus on the interactions between RNAs and proteins, but the function of their secondary structures and their roles in DNA genome are still unknown.

## Conclusions

Considering the facts that circRNA can act as miRNA or protein sponges and are widely expressed in the heart of human, mice and rats, it is plausible that some circRNAs can act as local regulators of gene expression. Although some circRNAs have been proved to be the regulators of pathophysiological processes in CVD, much more functional circRNA studies are needed to dissect their roles and mechanism-of-action in the CVD and development of cardiovascular biology.

In addition to a void of data on the functional roles of circRNAs, circRNAs also have the potential to be a biomarker for CVD. The specific expression of circRNAs may assist in solving the problem of existing markers' low organ specificity. Although not discussed in detail in the current review, circRNAs are also viewed as targets for designing therapeutic approaches in CVD. For example, circANRIL induced apoptosis and inhibited proliferation by binding to PES1 $\Delta$ 446-588 (40), therefore, it is plausible that a drug can be produced to competitive bind PES1 $\Delta$ 446-588 and thus escape such a negative effect.

In conclusion, circRNAs may play an important role in CVD, and are potential biomarkers or therapy targets for CVDs.

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## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/ncri.2017.08.04>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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