Circular RNAs as potential biomarkers and therapeutic for cardiovascular disease

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Circular RNAs (circRNAs) are a type of genetic regulators, which was earlier considered as “junk”. In contrast to linear RNAs, they have covalently linked ends with no poly-adenylated tails. CircRNAs can act as RNA-binding proteins, sequestering agents, transcriptional regulators, as well as microRNA sponges. In addition, some select circRNAs have been reported to translate into functional proteins. These RNA molecules always circularize through covalent bonds and their presence have been demonstrated across species. They are usually abundant and stable as well as evolutionarily conserved in tissue, saliva, exosomes, and blood. Therefore, it has been proposed as the “next big thing” in molecular biomarkers for several diseases, particularly in cancer. Recently, circRNAs have been investigated in cardiovascular diseases (CVD) and reported to play important roles in heart failure, coronary artery disease and myocardial infarction. Here, we review the recent literature and discuss the impact and the diagnostic and prognostic value of circRNAs in CVD.

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Abstract

Circular RNAs (circRNAs) are a type of genetic regulators, which was earlier considered as "junk". In contrast to linear RNAs, they have covalently linked ends with no poly-adenylated tails. CircRNAs can act as RNA-binding proteins, sequestering agents, transcriptional regulators, as well as microRNA sponges. In addition, some select circRNAs have been reported to translate into functional proteins. These RNA molecules always circularize through covalent bonds and their presence have been demonstrated across species. They are usually abundant and stable as well as evolutionarily conserved in tissue, saliva, exosomes, and blood. Therefore, it has been proposed as the "next big thing" in molecular biomarkers for several diseases, particularly in cancer. Recently, circRNAs have been investigated in cardiovascular diseases (CVD) and reported to play important roles in heart failure, coronary artery disease and myocardial infarction. Here, we review the recent literature and discuss the impact and the diagnostic and prognostic value of circRNAs in CVD.

Introduction

In human genome, noncoding RNAs (ncRNAs) account for the majority of transcripts and only 1~2% are protein-coding genes. ncRNA can be divided into two types: housekeeper ncRNAs and regulatory ncRNAs[1]. The latter consists of microRNAs (miRNAs), small nuclear RNAs (snRNAs), piwi-interacting RNA (piRNAs), small interfering RNA, (siRNAs), and long noncoding RNAs (lncRNAs). ncRNAs can also be categorized into two families based on size as large (> 200 nt) or small (< 200 nt)[2]. Between the two families, the large ncRNAs are produced by the same transcription and splicing machinery as mRNA, whereas small RNAs are always transcribed from genomic loci and processed by specific nucleases.

With the discovery that ncRNAs are necessary for heart physiology, the important roles of these molecules in cardiovascular disease (CVD) became evident[3,4]. The main groups of ncRNAs, including miRNA, lncRNA, and circular RNA (circRNA) have been reported to be

relevant for cardiovascular physiology and disease[5–7]. Particularly, in the past several years, miRNA and lncRNAs were shown to be critical contributors to cardiovascular pathophysiology. Moreover, they were reported to be potential biomarkers for several diseases, as they were abundant and stable as well as evolutionally conserved in tissue, saliva, exosomes, and blood[8–11].

CVDs are a major cause of death worldwide. Early diagnosis and treatment can reduce major adverse cardiovascular events (MACE)[12]. Thus, efficient biomarkers are important for diagnosis of CVD especially in cardiac injury. Cardiac troponins T/I (cTnT/I)[13] and creatine kinase myocardial band isoenzyme (CK-MB) have been widely used as biomarkers for myocardial infarction (MI), while brain natriuretic peptide (BNP) has been used for heart failure (HF) since long[14]. Ideal biomarkers must be sensitive and specific for specific diseases and should not be influenced by heterogeneity of the heart-associated disease, lifestyle, patient's age and genetic background[15,16]. Currently, highly sensitive biomarkers are urgently needed for earlier diagnosis of CVDs.

Cardiac physiology is a complex balance between electrical stimuli and chemicals related to the mechanism of contraction. Recently, ncRNAs were reported to be ideal regulators of cardiovascular system and circRNAs were linked with CVD[17–19]. CircRNAs are a class of ncRNAs that are more stable than linear RNAs[20], as they form a covalently[21] closed continuous loop, which is resistant to RNase R activity[22]. They were thought to be aberrant RNA splicing products with no functions[23] for nearly two decades until the development of bioinformatics method and RNA sequencing (RNA-Seq) technology indicated their association with specific biological processes[24]. These circRNAs are always stored in the cytoplasm[25] and only a small part exists in the nucleus[26]. Many studies have found that they participate in cell proliferation[27], migration and invasion[28–31], gene transcription[32], and function as expression regulators, miRNA sponges[32,33] and RNA-binding protein (RBP) sponges[34,35]. CircRNAs are highly stable and detectable in body fluids[36] as well as abundantly expressed[37,38] and evolutionarily conserved[39] in humans, making them better biomarkers than linear RNAs with potential value in clinical diagnosis, therapeutic, and prognostic applications[40–42].

Multiple recent studies have reported the potential of circRNAs as biomarkers[41–44] in *Drosophila* and humans to detect diseases especially in cancer. Here, in this review, we discuss the characteristics and function of circRNA. Moreover, we review the current research on circRNAs in CVDs, providing evidence for the significance of circRNAs in CVD diagnosis and clinical treatment.

Survey Methodology

Scholarly articles that were reviewed in this paper were searched in journal databases and subject-specific professional websites. The search terms that were used when searching for articles included circular RNAs ,potential biomarkers ,therapeutic and cardiovascular disease. Inclusion criteria for selected articles required that articles be directly related to the topic on circular RNAs and be peer reviewed. Both qualitative and quantitative articles were reviewed. Qualitative articles provide insights into the problem by helping understand reasons, opinions, and motivations. Quantitative articles on the other hand use measurable data to formulate facts and discover patterns in research.

Biogenesis of circRNAs

In contrast to liner RNA, circRNAs lack a 5' cap and 3' polyadenylated tail. They are spliced from pre-mRNA and specially formed with closed covalent bonds[21]. There are three different types of circRNAs: exonic, intronic, and exon-intron circRNAs[45]. Recent evidence indicated that exonic circRNA was formed when a 3' splice donor attached to the 5' splice acceptor of a single exon. This type of circRNA accounted for more than 80% of all types of circRNAs. Occasionally, the start of an upstream exon splices and attaches to the other end of a downstream exon, which called back-splicing process. Thus, a spliced donor joins an acceptor site to form a circular transcript, after which introns are spliced out[46]. However, if the intron is retained during this process, an exon–intron circRNA is formed. The intronic circRNAs always form from intron lariats, which contain a single unique 2' –5' linkage. GU-rich sequences near the 5' splice site and

C-rich sequences near the branch point bind into a circle, then exonic and intronic sequences are cut out with the remaining introns brought together to form intronic circRNA[47] (Fig. 1).

Emerging studies report another mode of circRNA biogenesis that depends on RBPs such as Quaking (QKI)[48] and Muscleblind (MBL)[49], which bridge two flanking introns close together. Another RBP, adenosine deaminases acting on RNA-1 (ADAR1)[50], can stop circRNA formation by melting the stem structure. Meanwhile, some studies have revealed that heterogeneous nuclear ribonucleoprotein (hnRNP) and serine-arginine (SR) proteins regulate the expression of circRNAs in *Drosophila*[51], suggesting that RBPs also play an important role in regulating levels of circRNAs.

CircRNAs as miRNA sponges

CircRNAs have been reported to function as miRNA sponges by mediating the downregulation or upregulation of miRNA target gene expression[52]. They are found to negatively regulate the expression by absorption and sequestration of miRNA molecules. miRNAs are a class of small ncRNAs that play an important role in regulating gene expression through the repression of mRNAs. ciRS-7/CDR1as and Sry are representative circRNAs that function as miRNA sponges[53,54]. They have 16 miR-138 and 74 miR-7 binding sites, respectively. Overexpression or knockdown of ciRS-7/CDR1as or Sry results in synchronous increased or decreased expression of the relevant miRNAs, respectively. It is interesting that some circRNAs contain multiple binding sites for a single miRNA. For example, circHIPK3 contains 9 binding sites for growth-suppressive miRNAs[55]. On the other hand, some circRNAs, unlike circHIPK3, contain only two binding sites for miR-124, and yet are able to regulate the function/expression of the miRNA. Thus, although multiple binding sites may not be a prerequisite for its regulatory function, circRNAs with multiple binding sites may affect the expression of more miRNA targets. It is as yet unclear whether a single miRNA binding site is sufficient for miRNA sponging function of circRNAs.

CircRNAs as RBP sponges

RBPs play an important role in post-transcriptional regulatory processes associated with cell differentiation, proliferation, apoptosis, and oxidative stress[56]. Study shows that specific RBPs can influence the lifecycle of an mRNA. Recent research showed that circRNAs could function as 'super-sponges' with a given RBP and thereby regulate the target gene[57]. The hsa_circ_0000020 contains 6 binding sites for human antigen R (HuR) and 10 sites for fragile mental retardation protein (FMRP), while the hsa_circ_0024707 contains 85 binding sites for argonaute 2 (AGO2). These circRNAs can store, sort, deliver, and regulate RBPs owing to the high density of binding sites[58]. Another example shows that circPABPN1 can competitively bind to HuR to prevent polyadenylate binding protein 1 (PABPN1) mRNA from binding to HuR in order to influence the translation process[59]. Another recent study suggests that circular antisense non-coding RNA in the INK4 locus (ANRIL) transcripts may regulate the INK4/ARF coding transcripts by competitive splicing[60].

CircRNAs as transcriptional regulators

Misregulation of alternative splicing is associated with the aberrant expression of splicing factors. Research shows that circRNAs can act as splicing isoforms and function in regulating alternative splicing. Nuclear exon-intron circRNAs (EIciRNAs) can regulate transcription. The intronic sequence of these specific circRNAs can interact with the U1 component of the spliceosomal machinery and promote expression of the target genes through recruiting RNA polymerase (Pol) II to the promoter region of genes[65]. For example, circRNA ciankrd52 can act as a positive regulator of Pol II to promote ankyrin repeat domain 52 (ANKRD52) gene transcription[66]. CircRNAs can also influence the translation of the cognate linear mRNA and thereby regulate protein expression. For example, circPABPN1 can interact with its cognate mRNA, PABPN1, to reduce the PABPN1 translational efficiency[59]. CircRNA EIciEIF3j could

160 combine with snRNPs and Pol II to promote its parent gene EIF3J transcription[67,68]. Recent
161 reports have shown that ci-mcm5 and ci-sirt7 can enhance the expression of RNA associated
162 with tumorigenesis[69]. In addition, circRNAs can inhibit mRNA maturation. For example,
163 circANRIL can bind the C-terminal lysine-rich domain of pescadillo (PES)1 thereby inhibiting
164 pre rRNA processing and impairing ribosome assembly and translation processes[60].
165

166 CircRNAs as competitors of linear splicing

167 A common pre-mRNA may produce many isoforms. The MBL gene contains sequences that
168 form a circRNA transcript having binding sites for MBL itself[49]. Subsequently, in an
169 autoregulatory manner, the circMBL influences the selective splicing of the MBL mRNA.
170 Further, MBL can interact with circMBL and its flanking introns to promote exon circularization.
171 In addition, the competition between canonical splicing and circRNA generation is evident from
172 the concomitant decrease in circRNA and increase in linear splicing.
173

174 CircRNAs as protein/peptide translators

175 Although classified as ncRNA, a recent report showed that circRNAs could be translated into
176 proteins. In fact, the human transcriptome seems to contain many circRNAs with coding
177 potential[61]. These have been associated in vivo with translating polysomes of *Drosophila*. Circ-
178 ZNF609 was able to translate a GFP protein during myogenesis because this special circRNA
179 contained an open reading frame at the start codon as the linear transcript[62]. However, because
180 these target circRNAs have no free 5' and 3' ends, this translation process occurred through
181 rolling circle amplification (RCA) mechanism, driven by internal ribosome entry sites (IRES)
182 and in a 5'-cap-independent manner. The circ-FBXW7 was found to encode functional proteins
183 in human U251 and U373 cell lines[63]. Initiation factor eIF4G2, YTHDF3, N6-meythyl adenosine
184 residues, as well as methyltransferase METTL3/14 were found to promote the translation
185 initiation of circRNAs[61]. Although the protein translation efficiency of target circRNAs is lower
186 in human and murine cells, the hepatitis D virus antigen (HDAg) encoded by circRNAs is exist

after infecting eukaryotic cells. In addition, mRNA modifications of m6A, Ψ, and m5C are important for circRNA translation process, and can alter the efficiency and fidelity of translation[64].

Recent report has shown that circRNAs can also promote protein-protein interactions. For example, CDK2 is a key marker of cell progression from G1 to S phase[70]. Circ-Foxo3 can interact with CDK2 via p21 to inhibit cell cycle progression. Recently, a circRNA was shown to decrease nuclear translocation of their co-localized proteins, ID1 and E2F1 and decrease the distribution of HIF1α[71], indicating that circRNA can also affect protein localization.

circRNAs as potential diagnostic and prognostic biomarkers of cardiovascular disease

Linear RNA molecules have been reported as potential biomarkers in several diseases, especially cancer[72]. However, circRNAs were identified to have more advantages than linear RNAs as biomarkers[73]. It is an ancient, evolutionarily conserved feature in humans and mice, as well as *Drosophila*. They are more abundant than expected and more stable than linear RNAs due to the covalently closed loop structures, which can resist RNA exonuclease and RNase R activity. In addition, the half-life of the stable ncRNAs is about 48 hours, which is much more than mRNAs that have a half-life of only 10 hours[74]. Moreover, they are located in cytoplasm, which can be easily acquired and examined.

For a long time, biomarkers have been used for early diagnosis and as indicators of the severity of abnormal processes as well as to predict treatment outcomes. An ideal biomarker must be sufficiently variable under normal and diseased condition for efficient diagnosis. At the same time, it must be easily acquired in blood or bodily fluids. Although many biomarkers have been used in clinical practices, there is still a need for biomarkers with more stability, sensitivity, and specificity.

CircRNAs occupy up to 1% of the total RNA and are differently (via specific isoforms) or specifically expressed in various types of cells [75], suggesting that their expression could be

associated with different conditions and consequently could serve as specific biomarkers. Furthermore, circRNAs are relatively abundant in different cells. Report shows that they show higher abundance in low-proliferating cells, such as in the brain, but are relatively lower in number in high-proliferating cells like liver. It is hypothesized that as the cells proliferate, circRNAs get divided into the daughter cells, resulting in a total lower abundance of the circRNAs in highly proliferating cells[76,77]. This is also evident in the highly proliferating tumour cells, which show lower levels of circRNAs than normal tissue[76].

Memczak et al. proved the existence of circRNAs in the blood[78]. However, it is more important to know if the amount of the circRNA in blood can be easily detected. Predictably, the abundance of circRNAs in blood is higher than those in tissues but lower than that in brain. Considering that the low amounts of circRNAs in liver have been suggested as biomarkers[79], we propose that the relatively more abundant blood circRNAs may efficiently serve as biomarkers. Recent research confirmed that circRNAs are also abundant in exosomes, which can be a potential new way to employ circRNAs diagnosis and prognosis[80]. Although exosomes contain some unwanted proteins, DNA, and RNA, these molecules can act as messenger shuttles between cells[81]. Therefore, exosomes are potential biomarkers, which have been used for diagnosing multiple diseases as well as assessing responses to drug treatments. Although circRNA is lower in high-proliferating cells, they are higher in the exosomes in such cells compared to normal exosomes. Additionally, it is possible that a larger number of exosomes shed from particular sites in the higher proliferating cells than in normal cells. Together, this suggests that detecting exosomes in such tissues may be more promising to diagnose disease.

Although the total amount of circRNAs is low in body fluids like blood, saliva, and gastric fluid and other tissues, it is still higher than linear RNAs. Multiple reports have proposed that circRNAs as disease biomarkers are superior to the corresponding mRNA and lncRNA in terms of abundance, stability, and specificity. The diagnosis and prediction of stage characteristics of a disease are also better with circRNAs than with mRNA and lncRNA. Additionally, it is more easy and convenient to detect circRNAs in blood samples, saliva, and gastric juice. Research

241 have identified 422 circRNAs in human cell-free saliva and these have been implicated in
242 intercellular signalling and inflammatory responses[82].

243 CVD has been reported to represent 31% of all global deaths. CVDs include coronary artery
244 disease (CAD), aneurysm, MI, and related diseases such as HF and pulmonary arterial
245 hypertension (PAH). Traditional treatment strategies such as controlling risk factors and early
246 treatment after diagnosis result in poor prognosis. Although various biomarkers for CVD have
247 been used for several years, the diagnosis and subsequent treatment often occurs very late as
248 these biomarkers reach significant detectable levels at later disease stages. BNP has been used
249 for the diagnosis of HF, cardiac TnT/I and CK-MB have been utilised for the diagnosis of MI,
250 and D-dimer for the diagnosis of aortic dissection. However, there is still a lack of biomarkers for
251 CAD and other CVDs. In recent years, ncRNAs such as miRNA and lncRNA have been
252 suggested as potential biomarkers for CVD. However, it is as yet unclear if circRNAs could be
253 potential new biomarkers for CVD. Therefore, we discuss below recent discoveries that illustrate
254 the potential of using circRNAs as diagnostic and prognostic CVD biomarkers.
255

256 ## Coronary artery disease and atherosclerosis

257 CAD has been a heavy burden on social economy mainly due to late diagnosis and severe
258 complications. CAD and atherosclerosis are linked with endothelial injury and imbalance of lipid
259 metabolism. The important roles of miRNAs in atherosclerosis such as regulation of vascular
260 smooth muscle cell function, lipid homeostasis, and cytokine responsiveness has been described
261 thoroughly[83,84]. MI and HF are main results of CAD and lead to severe mortality. CircANRIL
262 can regulate cell functions related to atherosclerosis and plays a atheroprotective role. The
263 antisense ANRIL and the INK4/ARF genes are included in chromosome 9p21.3[85]. Patients with
264 high circANRIL expression develop less severe CAD. This circRNA can impair the process of
265 exonuclease-mediated pre-rRNA and ribosome biogenesis through binding PES1. Consequently,
266 this induces apoptosis and inhibition of proliferation of vascular smooth muscle cells and
267 macrophages, which can promote anti-atherogenic cell stablity and prevent degradation[60]. It also

268 can affect atherosclerosis by regulating INK4/ARF expression. Thus, stable circANRIL may act
269 as a potential biomarker and therapeutic target for atherosclerosis[86,87]

270 Pan et al. used circRNA microarray to detect the differentially expressed circRNAs between
271 three human CAD and three control plasma. They found hsa_circ_0006323, hsa_circ_0032970,
272 hsa_circ_0051172, hsa_circ_0054537, hsa_circ_0057576, hsa_circ_0068942, hsa_circ_0082824,
273 hsa_circ_0083357, and hsa_circ_0089378 to be differentially expressed with fold-change ≥ 1.5
274 and $P < 0.05$. These nine circRNAs act as an hsa-miR-130a-3p sponge to influence its target
275 mRNA, transient receptor potential cation channel subfamily M member 3 (TRPM3). TRPM3
276 regulates contractility and proliferation of vascular smooth muscle cells in coordination with
277 cholesterol, which plays an important role in CAD[88]. However, these results need to be verified
278 using larger sample size of CAD patients and normal individuals. Zhao et al. used the same
279 method to show differences between the peripheral blood of 12 patients with CAD and 12
280 normal individuals and found that hsa_circ_0124644 and hsa_circ_0082081 were significantly
281 associated with CAD. In addition, they found that hsa_circ_0124644 in blood could act as a
282 potential diagnostic biomarker for CAD with a specificity and sensitivity of 0.626 and 0.861,
283 respectively[89].
284

285 ## Myocardial fibrosis

286 Myocardial fibrosis is a disease of myocardial stiffness, which can reduce myocardial
287 shortening to induce diastole difficulty. Endothelial-to-mesenchymal transition (EndMT) is the
288 process of inducing normal endothelial cells into mesenchyma-like cells. Tissue fibrosis is
289 associated with fibroblast-specific protein-1 (FSP-1) expression and collagen deposition. The
290 profibrotic factor transforming growth factor β (TGF-β) is able to drive EndMT progression. It
291 has been shown that TGF-β1-mediated induction of α-SMA expression and concomitant loss of
292 VE-cadherin expression in aortic endothelial cells results in EndMT. Three EndMT-related
293 circRNAs, chr5:90817794|90827570, chr8:71336875|71337745, and chr6:22033342|22038870,
294 were found to be significantly upregulated in TGF-β1-treated rat coronary artery endothelial cells

(CAEC). These three circRNAs may be potential biomarkers of EndMT-induced myocardial fibrosis[90].

Zhou et al. found 24 up-regulated circRNAs and 19 down-regulated circRNAs with fold change > 3 and $P < 0.05$ between diabetic db/db mice and db/m control mice. They selected one of the markedly increased circRNAs (circRNA_010567) for further bioinformatics analysis and found it to contain binding sites for miR-141, which is predicted to regulate TGF-β1 function. Further, results suggested that circRNA_010567 could directly target miR-141 and regulate TGF-β1 expression, which can mediate resection-associated fibrosis[91]. These results imply that circRNA_010567 may play a key role in myocardial fibrosis, and thus provide a novel insight into cardiopathy pathogenesis and act as a potential diagnostic biomarker.

Tang et al. reported a new target circRNA_000203, which was significantly upregulated in diabetic myocardium. This circRNA can act as a sponge for miR-26b-5p to restrain its downstream targets Col1a2 and CTGF, thus promoting pro-fibrosis effects[92]. Although circRNA_000203 may play important role in myocardial fibrosis, its efficacy as a biomarker needs further investigation.

Cardiomyopathy

Cardiomyopathy is a disease with primary abnormalities in the structure and function of the heart. Dilated cardiomyopathy is a morphological subtype of this disease. Siede et al. reported increased expression of three circRNAs SLC8A1, CHD7, and ATXN10 relative to their host gene expression and decreased circDNA6JC in patients with dilated cardiomyopathy[93]. Xie et al. found that Foxo3 is the protective agent in Ganoderma spore oil against cardiomyopathy[94]. In addition, circAmotl1 was associated with cardiac dysfunction, wherein it could promote cardiac repair through binding AKT1 and PDK1 to accelerate the function of cardioprotective nuclear translocation of pAKT. This may provide a way to detect cardiac dysfunction before HF. circAmotl1 was also shown to be important for ameliorating the negative effects of Dox on the heart, such as resistant fibrosis, apoptosis, and hypertrophy. Thus, circAmotl1 could have

322 therapeutic potential in the treatment of cardiomyopathy[95]. Khan reported that RBM20-
323 dependent TTN circRNAs play important roles in dilated cardiomyopathy[96]. However, there are
324 only few studies on the role of circRNAs in cardiomyopathy.

325

326 Heart Failure

327 Heart failure (HF) is a disease that is often a secondary effect caused by another disease. It is
328 also a final outcome of some CVDs such as CAD, vascular disease, and MI. Therefore, it is
329 challenging to find an ideal biomarker for HF. HF leads to a decrease in systolic and/or diastolic
330 function[97]. Traditional standard biochemical biomarkers as BNP had been used to distinguish HF
331 from other conditions[98]. However, studies have shown that ncRNAs are more sensitive than
332 traditional biomarkers such as BNP or cardiac troponins. Up till now, there are more than 50
333 miRNAs and 3 lncRNAs described as biomarkers of HF.

334 Salgado-Somoza et al. reported use of the circRNA, myocardial infarction-associated circular
335 RNA (MICRA), to predict the risk in MI patients. Using blood samples from 472 acute MI
336 patients, they found the expression levels of MICRA were lower in patients with ejection
337 fraction (EF) $\leq$ 40% compared with patients with EF > 41%. Patients with lower levels of
338 MICRA were also at high risk of decreased EF (0.78 [0.64 – 0.95]). They further identified that
339 MICRA was present in 86% of the samples and bootstrap internal validation indicated that it
340 could act as an optimal HF predictive biomarker, similar to the traditional markers (Nt-proBNP,
341 creatine phosphokinase, CPK)[99]. Wang et al. reported that a special heart-related circRNA
342 (HRCR) may play an important role in HF by acting as an miR-223 sponge to increase the
343 expression of ARC (apoptosis repressor with CARD domain)[100]; however, this requires further
344 investigation.

345

346 Myocardial injury

347 Myocardial injury and apoptosis are associated with HF and MI as well as reperfusion injury.

Increase in apoptosis augments MI and HF, whereas reduced apoptosis protects the heart. Kun Wang found the circRNA, MFACR, could increase mitochondrial fission and apoptosis as well as cardiomyocyte cell death through downregulation of the ceRNA, miR-652-3p, and promotion of MTP18 translation. In this study, MFACR was shown to act as a sponge to inhibit the activity of miR-652-3p, which directly targets MTP18 to inhibit mitochondrial fission and apoptosis. Thus, MFACR may act as a biomarker to predict apoptosis in the heart and may serve as a potential therapeutic target for treatment[101].

Myocardial infarction

MI is a catastrophic result caused by ischemic heart disease and can lead to heart tissue damage and mortality. Despite revascularization as a valuable method to stabilize the crisis, selective biomarkers are required to assess the risk and therapeutic response after the infarction[102]. Acute MI poses sizeable morbidity and mortality risks. Hsa-miR-122-5p levels that are different in the plasma of CVD patients was proposed as an early prognostic biomarker of acute MI[103].

Through circRNA microarray analysis in mouse hearts with autophagy, Zhou et al.[104] found that mmu_circRNA_006636 (ACR) was significantly decreased after I/R injury. ACR was found to regulate Pink1 expression through inhibition of DNA methylation of Pink1 by binding to DNMT3B. Further, after verifying the functional role of Pink1 in autophagy, they identified the downstream target of Pink1 as FAM65B. Thus, ACR can repress autophagy and MI by targeting Pink1-mediated phosphorylation of FAM65B, and could act as potential therapeutic target and biomarker for ischemia/reperfusion and MI.

Geng et al. found circRNA CDR1AS could act as an miR-7a sponge to regulate expression of its target gene (PARP and SP1) and interfere with its protective role in MI injury[105]. In addition, apoptosis-related circRNA (MFACR) could regulate miR-652-3p to promote the progress of MTP18 which is relevant for MI[101]. Vausort et al. reported that MICRA was highly expressed in peripheral blood of MI patients[106]. However, their potential value act as a biomarker for MI

remains to be further investigated. Deng et al found circRNA_081881 was differentially expressed in plasma samples of acute MI patients. This report shows that the downregulated circRNA_081881 can sequester miR-548 through competitive binding sites to reduce PPARγ (a heart-protective factor) expression, which is reduced in the plasma of acute patients with MI[107].

Hypertension

Hypertension has become a major risk factor for the development of CVD and is highly prevalent across the world, even in young people. It has been a major contributor to morbidity and mortality and is considered as a socioeconomic burden. The diagnosis and treatment are influenced by many factors and there is a lack of useful biomolecules for clinical prediction and diagnosis.

Bao et al. detected differences in the expression of some circRNAs between five pairs of newly diagnosed essential hypertension (EH) and non-EH whole blood samples. They found that hsa_circ_0037911 levels in EH group were significantly higher than in controls group ($t = 2.834$, $P = 0.005$). In addition, they proved that the target hsa_circ_0037911 was an effective predictor of EH by using the ROC curve to investigate its diagnostic value (AUC = 0.627; $P = 0.002$). Their study also showed that there was a direct correlation between hsa_circ_0037911 and sc[illegible], which indicates that this RNA plays an important role in the pathogenesis of essential hypertension[108]. Another study reported that 46 circRNAs were significantly upregulated and 13 downregulated (FC > 2.0 and $P < 0.05$) between human hypertensive plasma and normal plasma. The downregulation of hsa-circ-0005870 was verified to be significantly downregulated in hypertensive patients. Bioinformatics analysis also indicated that hsa-circ-0005870 may represent a novel biomarker for the diagnosis of hypertension[109].

Aneurysm disease

Aneurysm disease especially aortic dissection is a life-threatening condition with a lethality rate

401 of 1 to 2% per hour after onset of symptoms in untreated patients. The treatment is complex and
402 the prognosis is poor, especially Stanford A aortic dissection. However, there is no available
403 biomarker that can reveal this disease before acute onset. Therefore, predictive diagnosis and
404 treatment is vital to improve the survival rate and to prevent severe complications.

405 Zou reported 106 downregulated and 156 upregulated circRNA between 3 normal patients and
406 3 thoracic aortic dissection (TAD) patients. They found hsa_circRNA_101238 to be upregulated
407 with a fold-change ≥ 1.5, $P < 0.05$ and co-expression network revealed that only this target
408 circRNA interacted with the three altered miRNAs (hsa-miR-320a, hsa-miR-320b, and hsa-miR-
409 320c). The circRNA-miRNA-mRNA network predicted miRNA targets of
410 hsa_circRNA_101238 were hsa-miR-320b, hsa-miR-320a, hsa-miR-138-5p, hsa-miR-593-5p,
411 and hsa-miR-320c. Through target gene prediction and luciferase assays, they found
412 hsa_circRNA_101238 acted as an miR-320a sponge through inhibiting the expression of hsa-
413 miR-320a to increase MMP9 expression, which may be involved in the pathogenesis of TAD.
414 Thus, hsa_circRNA_101238 may be a potential biomarker for TAD [110]. However, the
415 function and mechanism of the circRNA in TAD needs further experimental evidence.

416

417 ## Pathological hypertrophy

418 Cardiomyopathies are myocardium diseases with morphological and functional abnormalities
419 and can be classified as primary or intrinsic cardiomyopathies. Pathological diagnosis of
420 cardiomyopathies requires invasive and potentially dangerous tests. Therefore, many patients
421 with cardiomyopathies usually opt for biochemical tests that can help diagnosis[111].

422 Meng et al reported differentially expressed circRNA in cardiac hypertrophy cells cultured in
423 presence of high and normal levels of D-glucose. Five circRNAs, namely ciRNA261, ciRNA26,
424 circRNA1191, circRNA4251, and circRNA6913, were found to be significantly differentially
425 expressed ($P < 0.05$ and fold change > 2 or < 0.5) and had more than 60 target miRNAs, which
426 implied that these circRNAs may play important roles in cardiac hypertrophy and potentially
427 serve as biomarkers[112].

Wang reported that heart-related circRNA (HRCR) could act as an endogenous miR-223 sponge and inhibit the activity of miR-223. In addition, they also reported that miR-223 are functionally related to hypertrophy through ARC. Thus, HRCR inhibits miR-223 activity, resulting in increased expression of its downstream target ARC and inhibition of cardiac hypertrophy and heart failure in mice. Thus, HRCR may be a useful biomarker for diagnosis and prognosis of cardiac hypertrophy and HF[100].

Cardiac senescence

Cardiac senescence accompanies ageing and may decrease heart function. Chen et al. reported that aging significantly affects the cardiac muscle. By using high throughput RNA sequencing, they found 21 up-regulated circRNA and 1 down-regulated gene in cardiac muscle during aging. Within the network, circRNA005698 was found to be associated with 7 miRNAs. Further, they found circRNAs including circRNA005698 might play a key role in regulating pro-coagulation process during aging. Thus, circRNA005698 can act as a biomarker for cardiac senescence[113].

Du also found that circFoxo3 is relevant for cell senescence in doxorubicin-induced mouse cardiomyopathy. This circRNA could interact with senescence-related proteins (ID1 and E2F1) and stress-related proteins (HIF1a and FAK) in cytoplasm, leading to the inhibition of the anti-stress and anti-senescent roles of these proteins and consequently promoting cardiac senescence. The circFoxo3 is not only found in aged heart of mice but also in humans[70].

Conclusion

In summary, circRNAs are a class of ncRNAs that mainly regulates gene expression. Increasing evidence indicate that circRNAs are abundantly found in saliva, exosomes, and clinical standard blood samples, which make them promising biomarkers for diagnosis and outcome prediction. They are more stable and sensitive as well as specific than standard biomarkers. In CVD, secreted lncRNAs have been described as biomarkers of several conditions

including MI, cardiac failure, and atrial fibrillation. Hopefully in the future, the use of circRNAs as biomarkers will become routine in clinical practice.

Limitations

To be able to use circRNAs as routine biomarkers, there are some limitations or factors that need to be addressed. First, several methodological factors including sample collection and processing, as well as assay performance and ncRNA quantification can influence the quality of the resulting data and needs to be improved. Second, analysis of case studies can be limited by the sample size due to lack of statistical power. In addition, sex, age, and cardiovascular risk factors may introduce bias in limited samples. Therefore, large cohorts and multicentre studies are necessary to interpret data and form conclusions. Third, patients with CVD are likely to take anticoagulant drugs such as aspirin and clopidogrel before blood collection, which can alter circulating ncRNA concentrations and thereby their quantification. Fourth, primer design and normalization in quantitative real-time polymerase chain reaction can influence the outcome of biomarker studies. Therefore, it is necessary to standardize such procedures to eliminate technical and analytical variability.

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Figure legend:

Figure 1. The proposed models of circRNA formation

a. Direct lariat-driven cyclization. Exon splicing generates a lariat structure. The 3' splice donor site of exon 1 covalently links to the 5' splice acceptor of exon 4. Circular intronic RNA forms after removal of intronic sequence.

b. Intron-pairing-driven circularization. Direct base-pairing of the introns flanking inverted repeats or ALU elements forms a circular structure. The introns are removed or retained to form ecircRNA or EIciRNA.

c. The circular intronic RNAs are generated from lariat introns that can escape debranching. nt GU-rich sequences near exon 1 (yellow box) and 11 nt C-rich sequences near exon 2 (blue box) form the ciRNAs due to avoiding the debranching and become a stable circRNA.

d. RNA binding proteins (RBPs) driven circularization: circRNA is formed through RBPs (Y-shape) and introns are removed.

Figure 2. The circ101238/miR/target gene regulatory network

Contributions

W.T.W, B.L, H.L.P , M.X.H, and Y.W wrote the paper. Z.C.Z, D.L, T.C.W, R.H.X, and K.X.L checked the References. All authors reviewed the final manuscript.

Additional Information

Competing financial interests: The authors declare no competing financial and/or non-financial interests in relation to the work described.

Abbreviation

noncoding RNAs (ncRNAs); microRNAs (miRNAs) ;small nuclear RNA (snRNAs); piwi-interacting RNA (piRNAs); small interfering RNA (siRNAs) ; long noncoding RNAs (lncRNAs); cardiovascular disease (CVD); circular RNAs (circRNAs) ; major adverse cardiovascular events (MACE) ; myocardial infarction (MI); beta-natriuretic peptide (BNP); heart failure (HF); RNA sequencing (RNA-Seq) ; RNA-binding proteins (RBP); Quaking (QKI) ; Muscleblind (MBL) ; adenosine deaminases acting on RNAs (ADAR1); heterogeneous nuclear ribonucleoprotein (hnRNP); serine-arginine (SR); fragile mental retardation protein (FMRP); Argonaute 2 (AGO2); polyadenylate binding protein 1(PABPN1); human antigen R (HuR); ANRIL (antisense non-coding RNA in the INK4 locus) ; ankyrin repeat domain 52(ANKRD52); polymerase (Pol); Pescadillo (PES); internal ribosome entry site (IRES); rolling circle amplification (RCA); hepatitis D virus antigen (HDAg) ; coronary artery disease(CAD); pulmonary arterial hypertension (PAH); troponin T/I (TnT/I); transient receptor potential cation channel subfamily M member 3 (TRPM3); Endothelial-to-mesenchymal transition (EndMT); fibroblast-specific protein-1 (FSP-1); transforming growth factor β (TGF-β) ; coronary artery endothelial cells (CAEC);creatine phosphokinase, CPK; heart-related circRNA (HRCR); ARC (apoptosis repressor with CARD domain); myocardial infarction-associated circular RNA (MICRA);

742 essential hypertension (EH); thoracic aortic dissection (TAD)

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Figure 1

The circ101238/miR/target gene regulatory network

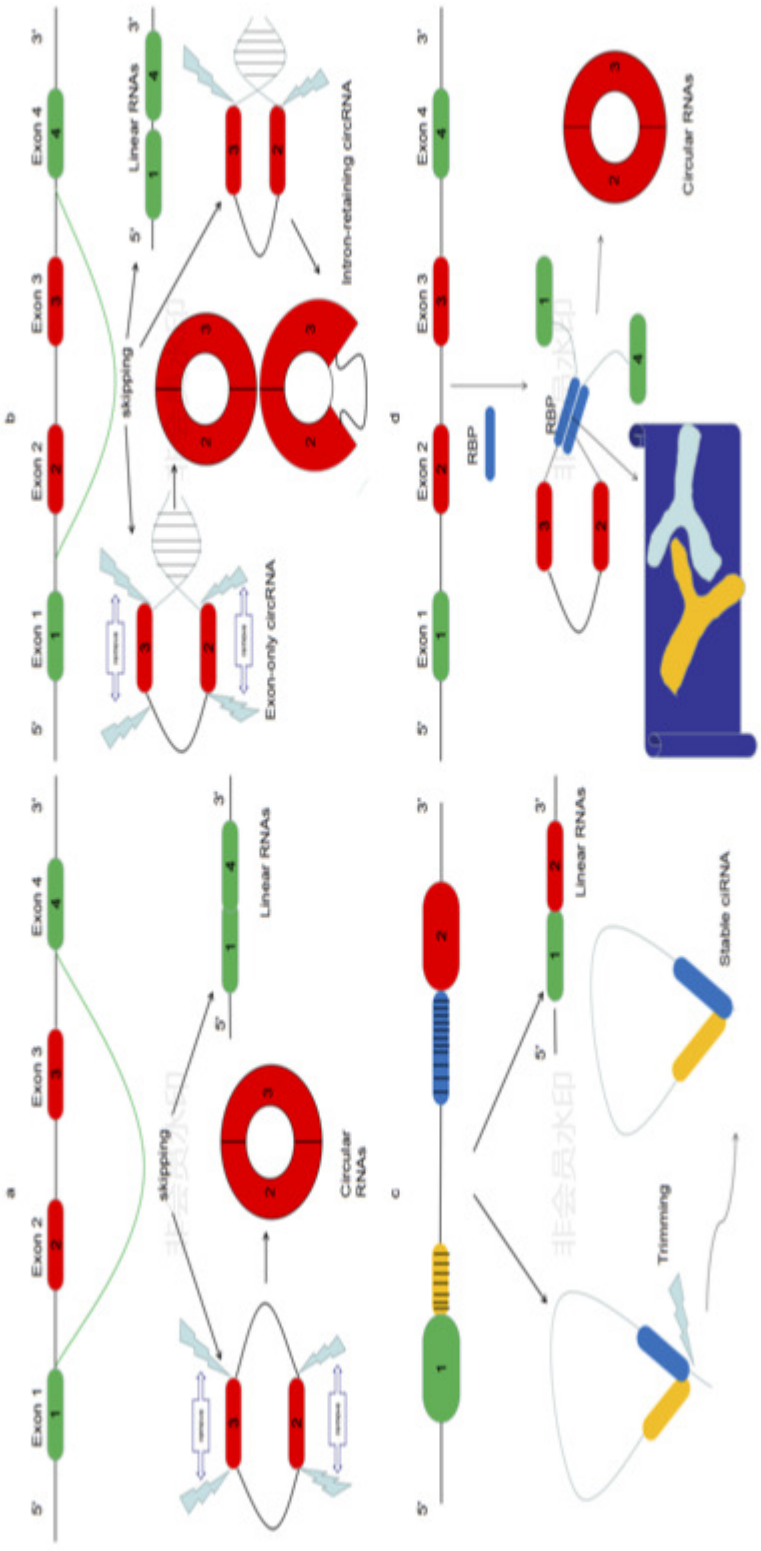

Figure 2

The proposed models of circRNA formation