Emerging roles of circRNAs in the pathological process of myocardial infarction

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Myocardial infarction (MI) is defined as cardiomyocyte death in a clinical context consistent with ischemic insult. MI remains one of the leading causes of morbidity and mortality worldwide. Although there are a number of effective clinical methods for the diagnosis and treatment of MI, further investigation of novel biomarkers and molecular therapeutic targets is required. Circular RNAs (circRNAs), novel non-coding RNAs, have been reported to function mainly by acting as microRNA (miRNA) sponges or binding to RNA-binding proteins (RBPs). The circRNA-miRNA-mRNA (protein) regulatory pathway regulates gene expression and affects the pathological mechanisms of various diseases. Undoubtedly, a more comprehensive understanding of the relationship between MI and circRNA will lay the foundation for the development of circRNA-based diagnostic and therapeutic strategies for MI. Therefore, this review summarizes the pathophysiological process of MI and various approaches to measure circRNA levels in MI patients, tissues, and cells; highlights the significance of circRNAs in the regulation MI pathogenesis and development; and provides potential clinical insight for the diagnosis, prognosis, and treatment of MI.

INTRODUCTION
Myocardial infarction (MI) is a prevalent and frequently occurring cardiovascular disease (CVD) and includes acute MI (AMI) and chronic MI. AMI refers to sudden myocardial necrosis caused by a sudden loss of oxygen supply due to acute coronary artery occlusion or major epicardial vessel stenosis.1,2 After the acute phase, MI can develop into chronic MI. MI is a severe manifestation of coronary artery disease (CAD) or acute coronary syndrome (ACS) or ischemic heart disease (IHD) with very high morbidity and mortality.3 For example, the 30-day mortality rate of AMI is approximately 10% in Europe and North America.4,5

MI can be recognized by its specific clinical characteristics, including typical ischemia symptoms, increases in the levels of myocardial necrosis biomarkers, pathological Q waves on electrocardiogram (ECG), and imaging evidence of myocardium loss.6,7 Cardiac tissue damage resulting from MI usually triggers a wide range of intense pathological processes, including cardiomyocyte apoptosis,8 autophagy,9 inflammatory response,10 proliferation,11 angiogenesis,12 cardiomyocyte hypertrophy,13 fibrosis,14 cardiac healing or repair,15 and cardiac remodeling.16 Possibly culminating in heart failure (HF)17 or sudden cardiac death.

Circular RNAs (circRNAs) are single-stranded transcripts with a covalently closed-loop structure varying in lengths from dozens to thousands of base pairs.18 circRNAs are generated through back-splicing, in which the 5' and 3' termini are covalently connected by alternative splicing of exons from a single pre-mRNA.19,20 Various circRNA detection and characterization approaches have gradually revealed the biological features of circRNAs in recent decades: circRNAs are diverse, highly abundant, evolutionarily conserved, dynamically and specifically expressed, and they play crucial roles in different tissues.21–23 circRNAs are expressed in specific patterns in tissues, saliva, blood, and exosomes, and the methods used to detect circRNAs are simpler than those used to detect proteins.24,25 circRNAs, sometimes referred to as exon-only circRNAs, perform their physiological epigenetic functions and play non-negligible roles in various cellular processes in a variety of ways.26,27 circRNAs can function as microRNA (miRNA) sponges, RNA-binding protein (RBP) sequestering agents, or regulators of transcription and alternative splicing.18,28–30 Interestingly, some circRNAs can also be translated into functional proteins.31 Notably, a few circRNAs, such as circ_0060745, circ_0062389, circRNA ZNF292, and circ-Foxo3, might directly interact with proteins instead of sponging miRNAs via the circRNA/miRNA/protein axis during the process of MI.32–35 All of these biological properties and functions of circRNAs indicate that they have potential as sensitive and specific biomarkers and therapeutic targets for various diseases.36–38 Significantly, increasing evidence has shown that circRNA expression is changed in an MI mouse model and MI patients and that circRNAs may participate in the diverse pathophysiologic processes of MI, providing new diagnostic and therapeutic strategies for MI.28,39–41
PATHOPHYSIOLOGY OF MI

Common etiology of MI: Ischemia and I/R injury

Although the management of MI has improved over the past several decades, exploring the pathogenesis of MI is crucial. It is believed that MI damage is attributed to ischemia, reactive oxygen species (ROS), and other factors. 5 In the context of ischemia, cardiomyocytes exhibit a significant decrease in ATP production, a reduced intracellular pH, and inhibited activity of sodium pump, but they tend to be overloaded with intracellular sodium ions, inducing the lysis of organelles and plasma membranes, and extensive ischemia causes cardiac tissue damage and infarction. 43,44

Early and timely reperfusion, usually achieved by reopening of the occluded coronary artery, has widely been used in the clinic to protect the ischemic myocardium from MI. 45–47 However, in the early period after reperfusion, paradoxical and irreversible injury, such as further cardiomyocyte death due to altered Ca2+ handling and permeability transition pore opening, known as ischemia/reperfusion (I/R) injury, is induced by myocardial reperfusion itself. 48,49 Reperfusion-induced myocardial inflammation has been implicated as a secondary injury mechanism after I/R. 50 Overall, I/R, which acts as a double-edged sword, not only salvages the ischemic myocardium but also induces further aggravation of myocardial injury, 51 depending on the ischemic time and degree of reperfusion. 52,53 Generally, if myocardial reperfusion is carried out within 2 to 3 h after the onset of ischemia, the degree of myocardial salvage exceeds the damage from ROS, calcium loading, and inflammatory cells induced by reperfusion. 54–56

I/R injury induces and amplifies some myocardial injuries, such as myocardial apoptosis, autophagy, and inflammation, through multiple pathological mechanisms. 57–60 The oxidative stress theory, which posits that myocardial damage results from excessive production and accumulation of ROS and/or decreased antioxidant capacity of the organism during I/R, is a current hot topic. 58 Furthermore, cardiac damage due to MI has been widely studied in animal models of MI or I/R injury, generated by permanent ligation of the left anterior descending (LAD) coronary artery or LAD ligation followed by reperfusion (removing the ligation and restoring normal blood flow) after the ischemic period (30 to 60 min of ischemia), respectively. 35,36,60

MI AT THE CELLULAR AND MOLECULAR LEVEL: APOPTOSIS AND AUTOPHAGY

As mentioned above, MI manifests as ischemia or I/R-induced cardiomyocyte death, mainly apoptosis and autophagy. 9,61 The balance between apoptosis and autophagy has a direct link with cell survival and cardiac function in the MI heart and is a pivotal cellular and subcellular factor of MI. 62,63

Cardiomyocytes undergo apoptosis during ischemia or I/R injury, as shown by Saraste’s research 64 on myocardial samples from deceased AMI patients and Fliss’s research. 65 In addition, cardiomyocyte apoptosis has been found to occur in many areas in MI hearts, such as the ischemic region, the area near the ischemic border, and even areas distant from ischemic regions. 66,67 Therefore, it is suggested that apoptosis, the major determinant of infarct size, is of great significance in MI progression. 7

Autophagy is elevated in ischemia and in the reperfusion phase of I/R injury. 7,72 At a moderate level, cardiomyocyte autophagy can decrease the degree of apoptosis and act as an inflammatory suppressor, enhance the resistance of cardiomyocytes, and reduce the infarct size. 9,61,68 However, overactivation or dysregulation of autophagy may cause it to play a maladaptive role, increasing the infarct degree and leading to adverse cardiac remodeling. 9,69 In addition, mitophagy, a key mechanism of the degradation of mitochondria and the best understood pathways of selective autophagy, exerts a protective effect on cardiac function by coping with mitochondrial toxic conditions such as hypoxia and cytosolic Ca2+ overload. 70

MI AT THE TISSUE AND ORGAN LEVELS: INFARCT HEALING AND CARDIAC REMODELING

The pathophysiologic progression of MI at the tissue and organ levels basically involves two intertwined processes: infarct healing and cardiac remodeling. 15,71 Infarct healing occurs mostly in the infarct zone (IZ) through a series of processes. Cardiac remodeling takes place in the IZ as well as the noninfarct zone (NIZ) of the left ventricular (LV) wall; hence, it is often referred to as LV remodeling. Cardiac remodeling in the IZ is almost in sync and involves many pathological processes occurring simultaneously with infarct healing; thus, some authors consider infarct healing to be part of cardiac remodeling. 72 Simultaneous pathological events, such as inflammatory responses and angiogenesis, are reviewed and highlighted in the process of infarct healing. 16–12,73 Following MI, two types of fibrotic responses occur: 74 replacement fibrosis, which is involved in the proliferation phase because of its association with the activity of cardiac fibroblasts (CFs), 75 and reactive fibrosis, which mostly occurs during adverse remodeling in the NIZ (Figure 1).

In general, infarct healing refers to the process through with the IZ is replaced with capillary-rich granulation tissue, leaving a firm but noncontractile scar after MI. 67,76,77 Accumulating studies have divided this healing process into three distinct but overlapping phases: the inflammatory, proliferation, and maturation phases. 73 Timely resolutions and a proper balance among the above three phases are crucial for an appropriate wound-healing response. 78 These three phases, which are summarized in Figure 1, are characterized by dynamic and orchestrated variations in the numbers of inflammatory cells, mesenchymal cells such as CFs and myofibroblasts, extracellular matrix levels, and especially the angiogenesis response.

The response to an MI usually involves activation of progressive cardiac remodeling, which is simply defined as the geometric and functional changes in the LV. Cardiac remodeling involves a series
of initially adaptive and subsequently maladaptive alterations. Put simply, post-MI hearts have reduced contractility due to expansive IZ or scarring, and this contributes to compensatory hypertrophy of the viable NIZ; however, the hypertrophic myocardium generally decompensates and causes chamber dilation, eliciting cardiac dysfunction and even precipitating HF.79,80

The prevalence of HF caused by MI-induced cardiac remodeling is high, approximately 25%, as reported by epidemiological studies.17 Moreover, the infarct size, infarct healing, and ventricular wall stress are critical factors that determine the degree of cardiac remodeling.81,82 Cardiac remodeling after MI is often caused by the activation of compensatory mechanisms involving persistent pressure and volume overload, in combination with the activation of neurohormones and inflammatory mediators.83,84

In addition, adverse remodeling in the NIZ is closely linked with exaggerated reactive fibrosis, which could cause net accumulation of extracellular matrix proteins in the cardiac interstitium, thereby inducing adverse changes in cardiac geometry and function.14,75,85 Zhang et al.86 speculated that interstitial fibrosis can be enhanced by excessive ROS production and impairment of calcium homeostasis and lead to adverse cardiac remodeling.

**BIOLOGICAL FUNCTIONS AND CLINICAL SIGNIFICANCE OF circRNAs IN MI**

As mentioned above, the significant pathological events of MI mainly include cardiomyocyte apoptosis and autophagy, the inflammatory response, angiogenesis, and the proliferation of mesenchymal cells, cardiac remodeling, and myocardial fibrosis. circRNAs are highly stable and widely expressed, and their expression is tissue and time dependent. Many circRNAs that are dynamically expressed in the plasma/serum of MI patients, MI tissues, and cells have been found to significantly participate in the regulation of the above cellular processes and the pathophysiology of MI. Therefore, we summarized the differential expression, molecular mechanism, and important biological functions of circRNAs in MI, paying attention to the clinical significance of circRNAs in MI, such as their ability to serve as sensitive biomarkers and act as new therapeutic targets.

**circRNAs AND CARDIOMYOCYTE APOPTOSIS**

Cardiomyocyte apoptosis can be induced by I/R, hypoxia, or ischemia,9,42 and toxins such as doxorubicin (DOX),87 and these induction factors are used to construct a well-characterized mouse model of myocardial dysfunction, in which many circRNAs are implicated.85,89 Therefore, we divided the circRNAs that regulate cardiomyocyte apoptosis into the following four categories.
according to the different induction factors that change the expression of circRNAs (Figure 2).

First, three circRNAs were demonstrated to promote oxidation stress-induced cardiomyocyte apoptosis, which is a form of I/R-induced cardiomyocyte apoptosis. It was found that circHIPK2 can promote oxidation-induced cardiomyocyte apoptosis by sponging miRNA (miR)-485-5p and targeting ATG101.90 Li et al.91 revealed that similar to circHIPK2, circNCX1 functions as a powerful miR-133a-3p sponge to regulate cell death-inducing protein 1 (CDIP1) expression in cardiomyocytes, significantly enhancing oxidative stress-induced cardiomyocyte apoptosis. Additionally, circNFIIX was observed to act as a pro-apoptosis factor that markedly promotes oxidative stress-induced cell apoptosis.92 The circNFIIX/miR-125b-5p/Toll-like receptor 4 (TLR4) axis is suppressed by carvedilol, which thus reduces the progression of AMI and H$_2$O$_2$-induced cell dysfunction in vivo and in vitro.93 Notably, circNCX1 has been demonstrated to improve cardiac function in vivo in an I/R mouse model, and circNFIIX has shown to do so in a mouse model of ischemia.

Second, six circRNAs are overexpressed in cardiac tissues and cells subjected to I/R injury and facilitate general I/R-induced cardiomyocyte apoptosis in response to I/R, hypoxia/reoxygenation (H/R), or anoxia/reoxygenation (A/R). Mechanistically, circRNA MFACR positively regulates mitochondrial fission and I/R injury-induced cardiomyocyte apoptosis by directly sequestering miR-652-3p and consequently facilitating the translation and activity of mitochondrial 18 kDa protein (MTP18).94 In addition, circSAMD4A was found to promote H/R-induced apoptosis and cardiac injury by sponging miR-138-5p.95 Next, Gan et al.96 verified that knockdown of circRNA-101237 has an inhibitory effect on A/R-induced cardiomyocyte apoptotic death via modulation of the circRNA-101237/let-7a-5p/insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3) axis. circPostn depletion also inhibits H/R-induced myocardial apoptosis and injury in MI mice, as circPostn sponges miR-96-5p and positively regulates miR-96-5p-targeted Bcl2-interacting protein 3 (BNIP3) expression in human cardiomyocytes.97 Moreover, Luo et al.98 found that silencing circPVT1 inhibits H/R-induced cardiomyocyte apoptosis and alleviates MI and H/R injury by sponging miR-125b and miR-200a and suppressing miR-125b- and miR-200a-mediated apoptotic signaling. Zhang and Chen99 indicated that silencing circ_0062389 reduces H9c2 cell apoptosis by regulating the activity of the circ_0062389/transforming growth factor (TGF)-β1/Smad3 signaling pathway. Remarkably, circRNA MFACR and circPVT1 have the capacity to increase the infarct size in an I/R mouse model, whereas circSAMD4A and circPostn
exacerbate H/R-induced injury and dysfunction in vivo in an MI mouse model.

Third, abundant circRNAs are emerging as vital regulators of ischemia or hypoxia-induced cardiomyocyte apoptosis. The expression levels of ten circRNAs were shown to be significantly increased in MI mice or rats, and these circRNAs function as positive regulators of hypoxia-induced apoptosis. First, circDAPK1 was observed to induce cardiomyocyte apoptosis and aggravate injury.99 Specially, circDAPK1 sponges miR-7a and reduces the activity of miR-7a, thus suppressing the expression and function of poly (ADP-ribose) polymerase (PARP) and Sp1 transcription factor (SP1). Moreover, circ-0068655 can contribute to hypoxia-induced apoptotic death and impairment of cell migration via the circ-0068655/miR-498/protein serine/threonine kinase (PRKC) apoptosis WT1 transcription and impairment of cell migration via the circ-0068655/miR-498/protein serine/threonine kinase (PRKC) apoptosis WT1 transcription factor (WT1) regulator (PAWR) regulatory axis.100 Additionally, circ-0068655 may contribute to cardiomyocyte apoptosis and myocardial function under hypoxic insult.

Finally, the expression of a few circRNAs, such as circMACF1 and circSNRK, is downregulated in mouse models subjected to hypoxia or hypoxia/serum deprivation (H/SD). circMACF1 can downregulate miR-500b-5 expression by sponging it and then increase the expression of epithelial membrane protein 1 (EMP1), potently inhibiting cardiomyocyte apoptosis and ischemic heart injury.111 Overexpression of circSNRK suppresses primary cardiomyocyte apoptosis and increases the resistance of cardiomyocytes to H/SD by sponging and inhibiting miR-103-3p.112 Significantly, in a MI mouse model, circ-0106745, circRNA 010567, circRNA ACAP2, circROBO2, and circHIPK3 can promote MI development and cardiac dysfunction and increase the infarct size, whereas circ-Ttc3, circMACF1, and circSNRK play a protective role during MI and improve myocardial function under hypoxic insult in vivo.

circRNAs have been inserted into exosomes and transplanted into the ischemic hearts of rats. For instance, Li et al.113 performed a contrast experiment—downregulation of the expression of umbilical cord mesenchymal stem cell (UMSC)-derived exosome circRNA by designing small interfering (si)-circ-0001273—and the results revealed that exosomes delivery of circ-0001273 inhibits cardiomyocyte apoptosis and promotes myocardium repair and regeneration in vivo in MI model rats exposed to hypoxia.

Finally, DOX is toxic to cardiomyocytes and can induce cardiomyocyte apoptosis by modulating multiple signaling pathways.87 and the circRNAs mentioned below are pertinent to DOX-induced cardiomyopathy, such as cardiomyocyte apoptosis. Du et al.115 discovered that during DOX treatment, ectopic or overexpressed circ-Foxo3 promotes cardiac senescence and apoptosis by binding to ID1, E2F1, HIF1a, and FAK, thus restricting their translocation to the nucleus and inhibiting their functions. They also found that silencing endogenous circ-Foxo3 abrogates the cytotoxic effect of DOX in cardiomyocytes. Similarly, circArhgap12 was found to elevate the apoptotic cell rate and exacerbate oxidative stress injury by sponging miR-135a-5p and potentially targeting adenylate cyclase 1 (ADCY1). 115 Conversely, Ji’s group demonstrated that circPan3 suppresses DOX-induced apoptotic myocardial death, that circPan3 is regulated negatively by miR-31-5p, and that its maturation is impacted by decreased quaking (QKI) expression. Additionally, circAMOTL1 suppresses cardiomyocyte apoptosis and enhances cardiomyocyte proliferation and survival by interacting with pyruvate dehydrogenase kinase 1 (PDK1) and AKT1, thereby evoking AKT phosphorylation and promoting the nuclear translocation of AKT1 and PDK1.117 Importantly, in a mouse model of DOX-induced cardiomyopathy, circPan3, circAMOTL1, and silencing of circ-Foxo3 were demonstrated to have a powerful, protective impact on cardiac tissues and to alleviate cardiac impairment and DOX-induced cardiomyopathy in vivo.

circRNAs AND CARDIOMYOJECTE AUTOPHAGY

Recent studies have shown that cardiomyocyte autophagy, which has detrimental impacts on MI,118 is overwhelmingly induced by ischemia and cardiac I/R injury.65,118 The circRNAs that we summarized are
Some circRNAs aggravate cardiomyocyte autophagy and MI; for example, circHIPK2 worsens oxidative stress-induced autophagy, and circRNA_101237 facilitates A/R-induced autophagy. Zhou’s group discovered that circHIPK2 and ATG101 expression is upregulated in vitro in mouse cardiomyocytes treated with H2O2 and that circHIPK2 can promote autophagy in myocardial oxidative injury by sponging miR-485-5 and positively regulating the expression of ATG101. circRNA_101237 expression is markedly upregulated in response to A/R, and circRNA_101237 functions as a let-7a-5p sponge and increases the expression of its target protein IGF2BP3. Additionally, the researchers verified that circRNA_101237 can excessively activate A/R-induced autophagy, thereby promoting cardiomyocyte apoptosis by modulating the circRNA_101237/let-7a-5p/IGF2BP3 signaling axis.

However, unlike the above circRNAs, the circRNAs discussed below inhibit cardiomyocyte autophagy. Ren et al. observed that circRNA ZNF292 is significantly overexpressed in ischemic H9c2 cells subjected to oxygen glucose deprivation (OGD) and can inhibit apoptosis and autophagy by activating Wnt/b-catenin or the mechanistic target of rapamycin kinase (mTOR) signaling pathway through negatively target-
circRNA ACR alleviate I/R-induced autophagy, decrease the infarct size in the context of MI, and protect cardiac tissue in an I/R injury model in vivo.

circRNAs AND INFARCT HEALING AFTER MI

The post-MI infarct healing process involves three superbly orchestrated phases as shown in the above figure: the inflammatory phase, the proliferative phase, and the maturation phase. We found that numerous circRNAs are prominently involved in all three phases of infarct healing and thereby influence the progression of MI. Details are provided in the following paragraphs and Figure 4.

The following three circRNAs are positively associated with the inflammatory response during infarct healing. circSAMD4A not only dramatically enhances the H/R-induced inflammatory response but also stimulates H/R-induced apoptosis. Likewise, circ_0060745 participates in an intense inflammatory response and promotes hypoxia-induced cardiomyocyte apoptosis. Additionally, circJARID2 contributes to the inflammatory response and promotes apoptosis. In addition, Zhu et al. noticed that circMAT2B is highly expressed in ischemic H9c2 cells subjected to OGD. Knockdown of circMAT2B decreases ROS production and the expression of inflammatory factors, exerting a remarkable anti-inflammatory effect by upregulating miR-133 expression and thereby activating the PI3K/AKT and Raf/mitogen-activated protein (MAP) kinase-pathway.
extracellular regulated MAP kinase (ERK) kinase (MEK)/ERK pathways. Furthermore, circHelz expression is significantly upregulated in both MI mouse models and ischemic cardiomyocytes exposed to hypoxia, and when overexpressed, it sponges and inhibits miR-133a-3p and activates the NLRP3 inflammasome, thereby inducing a proinflammatory response and pyroptosis and exacerbating hypoxia-induced cardiomyocyte injury and cardiac dysfunction in vivo and in vitro.122

Many circRNAs are relevant to proliferation and intense angiogenic responses in MI tissues or cells. Four circRNAs serve as proliferative repair promoters. Zhang’s group123 verified that circCDYL expression was downregulated during MI and that circCDYL overexpression enhances myocardial regeneration and proliferation in vitro. Further investigations have revealed that circCDYL can sponge miR-4793-5p and increase APP protein levels. circSNRK has been found to promote post-MI cardiac regeneration in adults by positively modulating phosphorylation of glycogen synthase kinase 3β (GSK3β) and intracellular accumulation of β-catenin with the help of enhancive SNRK.112 Similarly, circFndc3b expression continuously decreases 6 weeks after MI. Moreover, overexpression of circFndc3b inhibits cardiomyocyte apoptosis and enhances the function of endothelial cells, the angiogenesis response, and cardiac repair via the circFndc3b/FUS RNA binding protein (FUS)/vascular endothelial growth factor (VEGF)-A signaling axis.124 In addition, the expression of circHIPK3 is increased in fetal and neonatal hearts compared with adult hearts, and this increase facilitates coronary artery endothelial cell proliferation, migration, and tube formation in angiogenesis by modulating the circHIPK3/miR-133a/connexin43 tissue growth factor (CTGF) axis and promoting cardiomyogenesis by regulating the stability of the Notch1 intracellular domain (N1ICD).125 Interestingly, Wang et al.126 found that exosomal circHIPK3 in hypoxia-exposed cardiomyocytes can accelerate the cell cycle and migration of cardiac endothelial cells and promote the angiogenesis response by sponging miR-29a.

However, four other circRNAs inhibit proliferation and angiogenesis during MI. The circPVT1/miR-125b/miR-200a axis was found to suppress cell viability and proliferation in MI tissues and H/R-exposed cardiomyocytes.98 Du et al.35 demonstrated that circ-Foxo3 expression is elevated in mouse CFs and that ectopic expression of circ-Foxo3 arrests the function of CDK2 and enhances p21 activity to suppress cell-cycle progression as well as CF proliferation by forming the circ-Foxo3-p21-CDK2 ternary complex. Additionally, when ectopically or excessively expressed, circFASTKD1 can directly sponge miR-106a and then increase the expression of large tumor suppressor kinases 1 and 2 (LATS1/2), suppressing the angiogenesis of vascular endothelial cells.127 circFASTKD1 was also demonstrated to have the highest expression in human cardiac microvascular endothelial cells (HCMEC). Furthermore, loss of circNFIX enhances cardiomyocyte proliferation and angiogenesis. The regulatory mechanism involves circNFIX interacting with Y-box binding protein 1 (Ybx1) and promoting its degradation, and the circNFIX/miR-214/Gsk3β pathway regulates the release of VEGF.128 Notably, in vivo in an adult MI mouse model, circSNRK, circFndc3b, and silencing circPVT1 were found to contribute to cardioprotection and inhibit MI progression such as by decreasing the infarct size, and circHIPK3 and downregulation of circFASTKD1 expression promote

Figure 4. The regulatory roles of circRNAs in post-MI infarct healing
Many circRNAs are prominently involved in various events during infarct healing, such as inflammation, myocardial angiogenesis, CF proliferation, and regeneration of cardiomyocytes.
angiogenesis and cardiac regeneration, thus alleviating hypoxia-induced cardiac dysfunction.

circRNAs AND CARDIAC REMODELING AFTER MI

Cardiac remodeling, which involves extensive alterations to ventricular geometry caused by a series of profound cellular and molecular changes in both the IZ and NIZ, is relevant because it is related to a higher incidence of arrhythmias, an adverse prognosis, and increased mortality in AMI patients.129 Several research groups have investigated the role of circRNAs in cardiac remodeling. In rat models of MI, circ-Ttc3 expression in the ventricular myocardium is notably increased at the 5th week post-MI, and circ-Ttc3 prevents cardiomyocytes from ischemia-related apoptosis and adverse remodeling by directly sponging miR-15b and regulating Arl2.110 Similarly, overexpression of circCDR1as can also protect the heart from infarct-related LV remodeling and improve ventricular function, and two natural compounds, bufalin and lycorine, exert their effects by increasing circCDR1as expression.130 Conversely, upregulation of circPostn expression was found to facilitate MI-induced cardiac remodeling and dysfunction via modulation of circPostn/miR-96-5p/BNIP3.97 Furthermore, the above two circRNAs have cardioprotective effects and can prevent adverse remodeling, whereas circPostn negatively affects post-MI cardiac remodeling and function in vivo.

Cardiac fibrosis, divided into replacement and reactive fibrosis, is a common pathological manifestation of most post-MI cardiac remodeling.15,74 Some circRNAs have shown a remarkable capacity to regulate cardiac fibrosis (Figure 5). For instance, when its expression is elevated, circRNA_000203 can promote cardiac fibrosis by sponging miR-26b-5p and abolishing its effect, thus upregulating the expression of collagen type I alpha 2 chain (Col1a2) and CTGF in Ang-II-treated CFs.131 Additionally, circPAN3 expression is markedly increased in the IZ of MI rat hearts, and circPAN3 promotes autophagy-activated fibrosis.132 circPAN3 can facilitate CF proliferation and migration and induce excessive autophagy by modulating the circPAN3/miR-221/FoxO3/ATG7 axis in CFs stimulated by TGF-β1. Moreover, circUbe3a, which is transported into CFs from M2 macrophages (M2Ms) through small extracellular vesicles (SEVs), was also verified to sponge miR-138-5p and indirectly upregulate the expression of the target protein RhoC to accelerate proliferation, migration, and myofibroblastic transformation of CFs.133 In contrast, circNFIB alleviates fibroblast proliferation, and its expression is

![Figure 5. Many circRNAs have a remarkable capacity to regulate cardiac fibrosis](image-url)

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significantly decreased in post-MI mouse hearts and TGF-β-treated primary adult CFs. Mechanistically, circNFIB can directly sponge miR-433 and consequently promote the expression of target genes such as AZIN1 and JNK1 and inhibit their downstream signaling pathways, including the p38, ERK, and Smad3 pathways. Similarly, circ_LAS1L expression is downregulated in AMI patients and activated CFs. Circ_LAS1L directly binds to and sponges miR-125b, increases the expression of secreted frizzled-related protein 5 (SFRP5), and inhibits the expression of alpha-smooth muscle actin (SMA) and collagen I and III. Ultimately, the above mentioned circ_LAS1L/miR-125b/SFRP5 pathway leads to the inhibition of CF activation, growth, proliferation, and migration.

In addition to acting as regulators of cardiac regeneration and remodeling, some of the circRNAs mentioned above also have a significant influence on cardiac fibrosis; for example, circ_0060745, circRNA_010567, and circHIPK3 aggravate cardiac fibrosis, whereas circCDR1as has a moderate beneficial effect in terms of reducing fibrosis. It was shown that in an MI model in vivo, circPAN3, circUbe3a, circ_0060745, and circHIPK3 facilitate cardiac fibrosis, whereas circNFIIB, circRNA_010567, and circCDR1as inhibit cardiac fibrosis.

CircRNAs AS DIAGNOSTIC AND PROGNOSTIC BIOMARKERS FOR MI

Thousands of circRNAs have been reproducibly detected in human peripheral whole blood, and the activity of hundreds of coding genes can be revealed and quantified by evaluating circRNA expression in human blood. CircRNAs exist extensively in cardiac tissue; sequencing data have revealed the presence of more than 15,000 circRNAs in the human heart, and an analysis by Tan et al. identified 1664 circRNAs specifically expressed in cardiac tissue. Moreover, it has been demonstrated that the expression of some circRNAs is specifically changed in AMI patients. Yin et al. identified 650 circRNAs, including 535 upregulated circRNAs and 115 downregulated circRNAs, that were differentially expressed in AMI patients compared with control subjects. Myocardial expression of circRNAs is very high in AMI patients, and due to the stability and conserved nature of circRNAs, they are likely to be robust and sensitive biomarkers for MI. The circRNAs that are discussed below are listed in Table 1.

| Related process or special significance | Name | Dysregulation | Function | Ref. |
|----------------------------------------|------|---------------|----------|-----|
| Apoptosis                              | circNFIX | upregulated (early) downregulated (subsequently) | auxiliary diagnostic biomarker for AMI | 92,93,141 |
|                                        | circRNA MFACR | upregulated | evidence for the clinical prognosis of MI | 94 |
|                                        | circROBO2 | upregulated | | 106 |
|                                        | circSNRK | Downregulated (continuously; follow-up of 7 days post-MI) | biomarker for clinical prognosis after MI | 112 |
|                                        | circSLC8A1 | upregulated | auxiliary diagnostic biomarker for IHD-like AMI | 141 |
| Angiogenesis                           | circFASTKD1 | unclear | prognostic biomarker in MI patients | 127 |
| Porcine post-MI heart tissue           | circCDR1as | upregulated | prognostic biomarker for the AMI | 99,130 |
|                                        | circ-RCAN2 | downregulated | prognostic biomarker for the AMI | 143 |
|                                        | circPostn | upregulated | precision diagnostic biomarker in MI patients | 97 |
| Plasma samples                         | circRNA_081881 | downregulated | precision diagnostic biomarker in MI patients | 142 |
|                                        | hsa_circ_0124644 (introducing hsa_circ_009896) | upregulated | diagnostic biomarker of CAD patients, including AMI | 107,146 |
| Post-MI risk classification            | circRNA MICRA | downregulated | prognostic biomarker for post-MI patients | 144,145 |
|                                        | circPAN3 | upregulated | potential prognostic biomarker for cardiac fibrosis during MI | 132 |
| Cardiac fibrosis                       | circ_LAS1L | Downregulated | diagnostic biomarker in AMI patients and cardiac fibrosis | 135 |
|                                        | circRNA_000203, circRNA_010567 | upregulated | biomarkers for cardiac fibrosis | 136,147 |
| DOE-induced cardiomyopathy            | circ-Foxo3 | upregulated | biomarker for DOX-induced MI | 95 |
|                                        | circRNAs derived from Ttn, Strn3, and Fhod3 | downregulated | possible biomarkers of DOX-induced cardiotoxicity | 99 |

Cui et al. speculated that circNFIX is a promising biomarker for MI because it acts as a proapoptotic factor for cardiomyocyte apoptosis and because its expression is downregulated in H9c2 cells under...
oxidative stress and in ischemic heart tissues. Similarly, the apoptosis-related circRNA MFACR could be a sensitive biomarker for MI, as it regulates miR-652-3p and then facilitates the activity of MTP18. circROBO2, which increases its expression in a mouse MI model and may be a potential pathogenic factor, provides evidence for the clinical prognosis of AMI. Additionally, the expression of circSNRK was found to continuously downregulate throughout the follow-up of 7 days after MI and locate mainly in the cytoplasm of cardiomyocytes in rat MI hearts, suggesting circSNRK has diagnostic value in the context of MI. Tian et al. demonstrated that circSLC8A1 and circNFIX can also serve as auxiliary diagnostic markers for sudden cardiac death caused by sudden IHD, such as AMI.

In contrast to the above circRNAs, circFASTKD1 was suggested to exert a suppressive influence on angiogenesis during MI and had the potential to be a novel prognostic biomarker in MI patients. In addition, Cheng et al. observed that the expression of circPostn is markedly upregulated in plasma samples of MI patients, the MI mouse model, and the I/R-treated cell model, and they speculated that circPostn expression is closely associated with MI. Conversely, circRNA_081881 expression is dramatically decreased in plasma samples from AMI patients, suggesting that circRNA_081881 levels in the plasma can be measured to precisely diagnose AMI. Downregulation of circRNA_081881 expression can reduce PPAR levels because circRNA_081881 acts as a competing endogenous RNA of miR-548, abrogating the protective effect of PPAR in AMI. Moreover, Mester-Tonczar et al. revealed that circCDR1as is expressed in pig hearts and that its value as a biomarker is obvious and could be further validated in vivo in the hearts of several porcine MI models. They also demonstrated that the expression of circ-RCAN2 is significantly downregulated in vivo in reperfused infarcted porcine hearts, whereas circRNA expression exhibits an obvious ischemia time-dependent increase in vitro in hypoxic porcine cardiac progenitor cells. The different expression patterns of circ-RCAN2 between MI models and hypoxia-exposed cardiomyocytes imply that it has diagnostic significance for MI. Additionally, the expression of circRNA MICRA was reported to be lower in MI patients than in healthy people, and circRNA MICRA could serve as a novel prognostic biomarker for post-MI patients, predicting LV outcome and improving risk classification. In addition, researchers suggested that the biomarker circRNA MICRA may assist in fine tuning risk stratification after MI and circRNA_010567 are implicated in cardiac fibrosis and lead to increased levels of profibrotic proteins by sponging their corresponding miRNAs, which may also be possible biomarkers for cardiac fibrosis.

In a previous study, circ-Foxo3 was found to be related to DOX-induced senescence and to promote cardiac senescence by modulating cytoprotective proteins in different cellular compartments, suggesting it could be a good biomarker for DOX-induced MI. It was observed that circRNAs derived from Ttn, Strn3, and Fhod3 can be regulated by DOX treatment, of course, whether these circRNAs can serve as biomarkers for DOX-induced MI remains to be elucidated. Finally, it is worth noting that circRNA_081881, circRNA MICRA, and circ_LAS1L expression levels are decreased in the blood samples of patients with AMI and that hsa_circ_0124644 expression is upregulated in the peripheral blood of CAD patients.

Unfortunately, the sensitivity and reliability of most circRNAs as biomarkers for MI are currently less than satisfactory. Schulte et al.’s comparative analysis revealed that the detectability of circRNAs in plasma is insufficient, although some circRNAs are abundant and readily detectable in cardiac tissue. Moreover, they concluded that cardiac circRNAs are less sensitive cardiac biomarkers than some miRNAs or protein biomarkers, such as cMyC and cardiac troponin I (cTnI).

Moreover, detection of circRNAs in blood or exosomes is more expensive, laborious, and time consuming than existing protein analysis methods. However, there is great interest in using circRNAs as diagnostic and prognostic biomarkers for MI, and Lin’s group demonstrated that the regulation of circRNA-miRNA networks is closely related to the pathological process of MI. In addition, Schulte et al. revealed that combined protein/non-coding RNA (ncRNA) biomarker approaches can integrate the different characteristics of various biomarkers and be more comprehensive and valid, suggesting that circRNAs may be useful in multibiomarker combinations.

circRNA-BASED APPROACHES AS POTENTIAL THERAPEUTIC STRATEGIES FOR MI

Various pieces of evidence have indicated that numerous endogenous ncRNAs, which are being or will be assessed as new therapeutic tools and in clinical trials, have great potential for the treatment of CVD, including MI. RNA interference (RNAi) approaches, which include the use of siRNAs, organ-targeted RNAi using on viral vectors, and modulation of long ncRNAs (lncRNAs) and miRNAs are particularly advanced. Innovatively, circRNAs, a newly identified and studied ncRNA that has high cytoplasmic stability, can be knocked down by RNAi (such as with siRNAs), and functions mainly by sponging miRNAs and indirectly upregulating the expression of miRNA-targeted proteins, have been employed to specifically target and regulate the various aforementioned pathophysiological processes of MI. Therefore, blocking or mimicking specific circRNA/miRNA/protein axes through regulation of...
| Effects                      | Name            | Expression in MI                | Regulatory pathway                                                                 | Function                                                                                                                                   | Ref. |
|-----------------------------|-----------------|--------------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|------|
| Anti-apoptotic effects      | circRNA MFACR   | upregulated (A/R treatment)    | circRNA MFACR/miR-652-3p/ MTP18                                                    | inhibition of circRNA MFACR alleviates mitochondrial fission and cardiomyocyte apoptosis and improves I/R injury-induced MI in vivo and in vitro | 94   |
|                             | circ_0062389    | upregulated (H/R treatment)    | circ_0062389/TGF-β1/Smad3 (upregulates both TGF-β1 and Smad3)                    | inhibition of circ_0062389 alleviates H/R-induced cardiomyocyte apoptosis in vitro                                                      | 33   |
|                             | circ_0068655    | upregulated (hypoxia treatment) | circ_0068655/miR-498/PAWR                                                        | inhibition of circ_0068655 alleviates hypoxia-induced cardiomyocyte apoptosis and improves I/R injury-induced MI in vivo and in vitro | 100  |
|                             | circ_0010729    | upregulated (hypoxia treatment) | circ_0010729/miR-27a-3p/TRAF5                                                     | inhibition of circ_0010729 alleviates hypoxia-induced myocardial apoptosis and injury in vivo                                              | 101  |
|                             | circRNA ACAP2   | unregulated (hypoxia treatment) | sponge miR-29 and miR-532                                                          | inhibition of circRNA ACAP2 alleviates hypoxia-induced cardiomyocyte apoptosis and MI in vivo and in vitro                                   | 104,105 |
|                             | circ_0124644    | unregulated (hypoxia treatment) | circ_0124644/miR-590-3p/SOX4                                                      | inhibition of circ_0124644 alleviates hypoxia-induced cardiomyocyte injury in vivo                                                       | 107  |
|                             | circROBO2       | unregulated (hypoxia treatment) | circROBO2/miR-1184/TRAADD                                                         | inhibition of circROBO2 alleviates hypoxia-induced cardiomyocyte injury and apoptosis in vivo and in vivo                                    | 108  |
|                             | circMACF1       | downregulated (hypoxia treatment) | circMACF1/miR-500b-5p/EMP1                                                        | circMACF1 suppresses hypoxia-induced cardiomyocyte apoptosis and improves the progression of AMI in vivo and in vitro                        | 111  |
|                             | circArhgap12    | upregulated (DOX treatment)    | circArhgap12/miR-135a-5p/ADCY1                                                    | inhibition of circArhgap12 alleviates cardiomyocyte apoptosis and oxidative stress injury in vivo                                           | 115  |
| Anti-autophagic effects     | circRNA ACR     | downregulated (A/R treatment)  | circRNA ACR/Pink1/FAM65B (binds to Dnmt3B, relieves DNA methylation of Pink1, and phosphorylates FAM65B) | circRNA ACR suppresses I/R-induced autophagy and mitophagy and reduces MI sizes in vivo and in vitro                                         | 120  |
| Anti-inflammatory effects    | circHelz        | upregulated (hypoxia treatment) | circHelz/miR-133a-3p/NLRP3                                                         | circHelz promotes hypoxia-induced cardiac inflammatory response and cardiomyocyte pyroptosis in vivo and in vitro                             | 122  |
| Proliferative and proangiogenic effects | circCDYL    | downregulated (hypoxia treatment) | circCDYL/miR-4793-5p/APP                                                           | circCDYL promotes the proliferation of cardiomyocytes and angiogenesis after MI in vivo                                                  | 123  |
|                             | circFASTKD1     | highest (HCMECs)               | circFASTKD1/miR-106a/LATS1/2/ YAP (increases LATS1/2 and then suppresses YAP signaling pathway) | inhibition of circFASTKD1 promotes angiogenesis and ameliorates MI in vivo and in vitro                                                    | 127  |

(Continued on next page)
circRNAs might result in effective inhibition of myocardial I/R injury, myocardial cell death, and adverse cardiac remodeling. Thus, when properly packaged and delivered, circRNAs represent novel long-acting therapeutic targets and attractive tools for improving MI-induced cardiac dysfunction and preventing MI development.\textsuperscript{63,125,165-167} In the following paragraphs and Tables 2 and 3, we review published studies connecting circRNAs with MI and summarize the wide range of circRNAs that may be potential therapeutic targets for MI pathogenesis (Tables 2 and 3), emphasizing the more promising circRNAs that exert multiple therapeutic effects via various regulatory mechanisms in multiple processes of MI (Table 3).

There are a large number of circRNAs that may serve as targets for the treatment of MI. For example, circRNA MFACR, circ_0062389, circ_0068655, circ_0010729, circRNA ACAP2, circ_0124644, circROBO2, circMACF1, and circArhgap12 may exert potential anti-apoptotic effects to inhibit AMI progression.\textsuperscript{33,94,100,101,104,105,107,108,111,116} In addition, circRNA ACR may have an effective anti-inflammatory effect.\textsuperscript{120} During infarct healing, circHelz can exert anti-inflammatory effects.\textsuperscript{122} circCDYL and circFASTKD1 are likely to contribute to proliferation and angiogenesis,\textsuperscript{123,127} and circUbe3a can exert anti-fibrotic effects.\textsuperscript{133} Moreover, circRNA_000203, circNFIB, and circ_LAS1L can have vital anti-apoptotic, anti-inflammatory effects via the modulation of the circRNA_000203/miR-26b-5p/Col1a2/CTGF increases both Col1a2 and CTGF regulatory pathway.\textsuperscript{131} circUbe3a derived from M2M-SEVs circUbe3a/miR-138-5p/RhoC inhibition of circRNA_000203 promotes cardiac fibrosis in vitro function.\textsuperscript{133} circNFIB downregulated (CFs with TGF-\beta treatment) circNFIB/miR-433 (promotes AZIN1 and JNK1 but inhibits p38 and ERK enzyme and Smad3 signaling pathway) circNFIB/miR-125b-5p/TLR4 axis,\textsuperscript{93} and circNFIB alleviates cardiomyocyte proliferation and angiogenesis by regulating the circNFIX/miR-214/Gsk3b axis and binding with Ybx1.\textsuperscript{125} Silencing of circ-Foxo3 has a protective effect against cardiac senescence and DOX-induced cardiomyopathy, as circ-Foxo3 binds to ID1, E2F1, HIF1a, and FAK,\textsuperscript{114} and disruption of the circ-Foxo3-p21-CDK2 ternary complex can promote cell-cycle progression as well as CF proliferation.\textsuperscript{35}

Moreover, circPostn and circ-Ttc3 may be applied for anti-apoptotic and anti-remodeling effects.\textsuperscript{97,110} circNCX1, circPAN3, and circRNA 010567 are capable of exerting anti-apoptotic and anti-fibrotic effects.\textsuperscript{91,102,103,116,132,147} circPAN3 suppresses cardiomyocyte apoptosis, but the regulatory pathway involved is unclear, miR-31-5p downregulates circPAN3 expression, and QKI expression has a positive impact on the maturation of circPAN3.\textsuperscript{116} Furthermore, knockdown of circPAN3 can promote CF proliferation and migration and autophagy-activated myocardial fibrosis by regulating the circPAN3/miR-221/FoxO3/ATG7 axis.\textsuperscript{132} circRNA 010567 siRNA can reduce cardiomyocyte apoptosis via the circRNA 010567/TGF-\beta1/Smad3 signaling pathway\textsuperscript{102} and the circRNA 010567/miR-141/DAPK1 axis,\textsuperscript{103} whereas silencing of circRNA 010567 can contribute to alleviation of myocardial fibrosis through the circRNA 010567/miR-141/TGF-\beta1 axis.\textsuperscript{147} Additionally, circ_0060745 exerts anti-apoptotic, anti-fibrotic, and anti-inflammatory effects via the modulation of the circRNA 000203/miR-26b-5p/Col1a2/CTGF increases both Col1a2 and CTGF regulatory pathway.\textsuperscript{131} circUbe3a derived from M2M-SEVs circUbe3a/miR-138-5p/RhoC inhibition of circRNA_000203 promotes cardiac fibrosis in vitro function.\textsuperscript{133} circNFIB downregulated (CFs with TGF-\beta treatment) circNFIB/miR-433 (promotes AZIN1 and JNK1 but inhibits p38 and ERK enzyme and Smad3 signaling pathway) circNFIB/miR-125b-5p/TLR4 axis,\textsuperscript{93} and circNFIB alleviates cardiomyocyte proliferation and angiogenesis by regulating the circNFIX/miR-214/Gsk3b axis and binding with Ybx1.\textsuperscript{125} Silencing of circ-Foxo3 has a protective effect against cardiac senescence and DOX-induced cardiomyopathy, as circ-Foxo3 binds to ID1, E2F1, HIF1a, and FAK,\textsuperscript{114} and disruption of the circ-Foxo3-p21-CDK2 ternary complex can promote cell-cycle progression as well as CF proliferation.\textsuperscript{35}

Unlike the abovementioned circRNAs, which exert therapeutic effects by affecting only one process involved in MI (Table 2), many circRNAs affect multiple processes involved in MI and may exert therapeutic effects through various regulatory mechanisms simultaneously (Table 3). For instance, circRNA ZNF292, circ_0000064, circHIPK2, and circRNA_101237 could be employed for their anti-apoptotic and anti-angiogenic roles.\textsuperscript{37,90,96,116} circSAMD4A, circARID2, and circMAT2B may have important anti-apoptotic and anti-angiogenic roles.\textsuperscript{95,106,121} In addition, circ_001273 probably exerts anti-apoptotic and reparative effects,\textsuperscript{113} and circSNRK, circNFIX, circFndc3b, circPVT1, circ-Foxo3, and circAMOTL1 can have vital anti-apoptotic, proliferative, and proangiogenic effects.\textsuperscript{35,92,96,113,114,117,126,131} The details are as follows: circSNRK was found to suppress cardiomyocyte apoptosis by sponging miR-103-3p and to promote cardiac regeneration and angiogenesis by regulating the circSNRK/GSK3b/\beta-catenin axis when it is overexpressed.\textsuperscript{112} Knocking down circNFIX inhibits oxidative stress-induced H9c2 cell apoptosis, but the signaling pathway involved is unclear.\textsuperscript{92} Carvedilol diminishes H$_2$O$_2$-induced damage to CFs via the circNFIX/miR-125b-5p/TLR4 axis,\textsuperscript{93} and circNFIX alleviates cardiomyocyte proliferation and angiogenesis by regulating the circNFIX/miR-214/Gsk3b axis and binding with Ybx1.\textsuperscript{125} Silencing of circ-Foxo3 has a protective effect against cardiac senescence and DOX-induced cardiomyopathy, as circ-Foxo3 binds to ID1, E2F1, HIF1a, and FAK,\textsuperscript{114} and disruption of the circ-Foxo3-p21-CDK2 ternary complex can promote cell-cycle progression as well as CF proliferation.\textsuperscript{35}
Table 3. circRNAs exert therapeutic effects through various regulatory mechanisms in multiple processes of MI

| Effects                                           | Name               | Expression in MI                                      | Regulatory pathways                                                                                       | Function                                                                                   | Ref.  |
|---------------------------------------------------|--------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|-------|
| Anti-apoptotic and anti-autophagic effects         | circRNA ZNF292     | upregulated (OGD treatment)                          | circRNA ZNF292/BNIP3 (inhibits BNIP3 and activates Wnt/β-catenin and mTOR signaling pathways)            | circRNA ZNF292 suppresses ischemic H9c2 cell apoptosis and autophagy and alleviates OGD-induced injury in vitro | 34    |
|                                                   | circ-0000064       | upregulated (Sal pretreatment)                       | unclear                                                                                                   | circ-0000064 suppresses cardiomyocyte autophagy and apoptosis and improves myocardial I/R injury in vivo and in vitro | 119   |
|                                                   | circHIPK2          | upregulated (H2O2 treatment)                         | circHIPK2/miR-485-5p/ATG101                                                                               | inhibition of circHIPK2 suppresses cardiomyocyte autophagy to alleviate myocardial apoptosis and oxidative-induced injury in vitro | 90    |
|                                                   | circRNA_101237     | upregulated (A/R treatment)                          | circRNA_101237/let-7a-5p/IGF2BP3 axis (sponge let-7a-5p and upregulates IGF2BP3)                         | inhibition of circRNA_101237 suppresses cardiomyocyte autophagy and apoptosis and improves A/R injury in vitro and in vitro | 95    |
| Anti-apoptotic and anti-inflammatory effects       | circSAMD4A         | upregulated (H/R treatment)                          | circSAMD4A/miR-138-5p (then possibly through the SIRT1-PGC-1α pathway)                                   | inhibition of circSAMD4A alleviates H/R-induced cardiomyocyte apoptosis and inflammatory response in vivo and in vitro | 106   |
|                                                   | circJARID2         | upregulated (hypoxia treatment)                      | circJARID2/miR-9-5p/BNIP3                                                                                 | inhibition of circJARID2 alleviates hypoxia-induced cardiomyocyte apoptosis and inflammatory response in vitro | 121   |
|                                                   | circMAT2B          | upregulate (OGD treatment)                           | circMAT2B/miR-133/PDK3/AKT/Ral/MEK/ERK                                                                   | circMAT2B knockdown reduces ROS production, hypoxia-induced apoptosis, and release of inflammatory factors in vitro |       |
| Anti-apoptotic and regenerative effects            | circ-0001273       | downregulated (post-MI hearts)                      | circ-0001273 in exosomes                                                                                 | circ-0001273 suppresses ischemia-induced cardiomyocyte apoptosis and promotes myocardial repair and regeneration after MI in vitro | 113   |
|                                                   | circSNRK           | downregulated (post-MI hearts)                      | sponge miR-103-3p (anti-apoptotic) and circSNRK/GSK3β/β-catenin (proliferative)                          | circSNRK suppresses H/SD-induced cardiomyocyte apoptosis and promotes myocardial repair and proliferation after MI in vivo and in vitro | 112   |
| Anti-apoptotic and proliferative and proangiogenic effects | circNFX            | downregulated (H2O2 treatment) and upregulated (adult hearts) | unclear (anti-apoptotic) and circNFX/miR-214/Gsk3β and interacts with Ybx1 (proliferative and proangiogenic) | inhibition of circNFX suppresses oxidative stress-induced apoptosis in vitro and enhances cardiomyocyte proliferation and angiogenesis in vivo and in vitro | 92,93,128 |
|                                                   | circFndc3b         | downregulated (post-MI hearts)                      | circFndc3b/FUS/VEGF (downregulates FUS but upregulates VEGF)                                              | circFndc3b suppresses cardiomyocyte and endothelial cell apoptosis and promotes angiogenesis response and cardiac repair in vivo and in vitro | 124   |
|                                                   | circPVT1           | at high levels (MI tissue and H/R model)            | circPVT1/miR-125b/miR-200a                                                                               | inhibition of circPVT1 promotes cell viability and proliferation and suppresses H/R-induced cardiomyocyte apoptosis and enlargement of IZ in vivo and in vitro | 98    |
|                                                   | circ-Foxo3         | upregulated (cell cycle was arrested; older hearts)  | circ-Foxo3-p21-CDK2 ternary complex (proliferative) and binding to ID1, E2F1, HIF1α, and FAK (anti-apoptotic) | inhibition of circ-Foxo3 promotes cell proliferation in vitro, and circ-Foxo3 suppresses cardiomyocyte apoptosis and exerts the anti-senescent and anti-stress roles in vivo and in vitro | 35,114 |
|                                                   | circAMOTL1         | downregulated (adult hearts)                        | binds to PDK1 and AKT1 and leads to phosphorylation and nuclear translocation of AKT1                    | circAMOTL1 suppresses cardiomyocyte apoptosis, protects DOX-induced cardiomyopathy, and enhances | 117   |

(Continued on next page)
| Effects | Name | Expression in MI | Regulatory pathways | Function | Ref. |
|---|---|---|---|---|---|
| Anti-apoptotic and anti-remodeling effects | circPostn | upregulated (H/R model) | circPostn/miR-96-5p/BNIp3 | inhibition of circPostn | 97 |
| | circ-Ttc3 | upregulated (hypoxia model) | circ-Ttc3/miR-15b-Arl2 | suppress hypoxia-induced ATP depletion and cardiomyocyte apoptosis and enlargement of IZ and cardiac dysfunction | 110 |
| | circNCX1 | abundant | circNCX1/miR-133a-3p/Cdip1 | inhibition of circNCX1 | 91 |
| Anti-apoptotic and anti-fibrotic effects | circPAN3 | downregulated (DOX treatment) and upregulated (MI hearts) | miR-31-5p directly inhibits QKI and downregulates circPAN3 (anti-apoptotic); circPAN3/miR-221/FostO3/Ai7 (anti-fibrotic) | circPAN3 suppresses DOX-induced cardiomyocyte apoptosis, and inhibition of circPAN3 alleviates autophagy-activated fibrosis after MI in vivo and in vitro | 116,132 |
| | circRNA 010567 | downregulated (hypoxia treatment) and upregulated (CF with fibrotic phenotype) | upregulates both TGF-β1 and Smad3; circRNA 010567/miR-141/DAKP1 (anti-apoptotic); circRNA 010567/miR-141/TGF-β1 (anti-fibrotic) | inhibition of circRNA 010567 suppresses cardiomyocyte apoptosis, alleviates cardiac fibrosis, and improves MI degree in vivo and in vitro | 102,103,147 |
| Anti-apoptotic, anti-fibrotic, and anti-inflammatory effects | circ_0060745 | upregulated (hypoxia treatment) | activates NF-κB pathway | inhibition of circ_0060745 suppresses hypoxia-induced cardiomyocyte apoptosis, alleviates myocardial fibrosis and inflammation, and limits MI size in vivo and in vitro | 32 |
| Anti-apoptotic, anti-fibrotic, and anti-remodeling effects | circCDR1as | upregulated (MI mice hearts) and exists (IZ of porcine hearts) | circCDR1as/miR-7a/PARP/SP1 (anti-apoptotic) and sponge miR-7 (anti-remodeling) | inhibition of circCDR1as suppresses hypoxia-induced cardiomyocyte apoptosis and MI injury in vivo and in vitro, and circCDR1as alleviates myocardial fibrosis, protects MI hearts from LV remodeling, and improves post-MI cardiac function | 99,130 |
| Anti-apoptotic, anti-inflammatory, proangiogenic, and anti-fibrotic effects | circHIPK3 | upregulated (fetal, neonatal, and post-MI hearts) in exosomes | circHIPK3/miR-93-5p/Rac1/Pi3K/Akt (anti-apoptotic); circHIPK3/miR-133a/CTGFs, circHIPK3/Notch1, and circHIPK3/miR-29a/VEGFA (proangiogenic); and circHIPK3/miR-17-3p/Adcy6 (anti-fibrotic) | circHIPK3 promotes angiogenesis and cardiac-regenerative repair and alleviates hypoxia-induced cardiac dysfunction in vivo and in vitro, and inhibition of circHIPK3 suppresses cardiomyocyte apoptosis, alleviates inflammation and cardiac fibrosis, and improves post-MI cardiac function | 109,125,126,130,148 |

upregulated by bufalin and lycorine, alleviates LV remodeling and cardiac fibrosis by sponging miR-7.130 circHIPK3 may be an ideal anti-apoptotic, anti-inflammatory, proangiogenic, and anti-fibrotic candidate, as silencing of circHIPK3 suppresses cardiomyocyte apoptosis via the circHIPK3/miR-93-5p/Rac1/Pi3K/Akt axis.109 circHIPK3 promotes myocardial angiogenesis by modulating the circHIPK3/miR-133a/CTGF axis,125 the stability of N1ICD, and the circHIPK3/miR-29a/VEGFA axis126 and circHIPK3, the expression of which is upregulated by adrenaline, is beneficial to the heart in the short term, but a reduction in its expression can maintain post-MI cardiac function in the long term through regulation of myocardial fibrosis via the circHIPK3/miR-17-3p/Adcy6 axis.136,168

In addition, Gallet et al.169 proposed that cardiosphere-derived, cell-secreted exosomes are potential cell-free agents for the treatment for MI, and some circRNAs, such as circ-0001273 and circHIPK3, are derived from exosomes and have the potential to be applied for exosome-associated treatment of MI.113,126 However, although having good potential according to extensive basic research, there have been an insufficient number of clinical trials testing the application of circRNAs for MI treatment, and some issues, such as the use of appropriate preclinical animal models, the identification of drug-delivery systems that specifically target MI tissues and cells, the heterogeneity of different populations, and the in vivo safety and possible long-lasting effects of circRNA mimics or inhibitors, need to be addressed.
STRATEGIES FOR MEASURING circRNA LEVELS IN THE CONTEXT OF MI

A variety of approaches and strategies have been developed to detect, characterize, enrich, and verify the presence of circRNAs and reveal the interaction between circRNAs and miRNAs in MI patients, in vivo in MI animal models, and in vitro in cardiomyocytes exposed to MI-related conditions. In recent years, next-generation RNA sequencing and microarray technology have been widely used for the identification of new circRNA species, profiling of circRNA expression, and analysis of the correlation between epigenetics and genetic phenotype. For example, Lin et al. detected a total of 266 circRNAs (121 upregulated and 145 downregulated) that were differentially expressed in peripheral blood between patients in the control group and patients with ACS. Wu et al. used Arraystar microarray analysis to identify 63 circRNAs with distinct expression patterns (29 upregulated and 34 downregulated) between mice in the MI-induced HF and sham groups.

The accuracy and comprehensiveness of circRNA identification strategies are determined by the rigor and reliability of the algorithm; however, there is little overlap in the results of the identification of various algorithms; thus choosing appropriate algorithms and combination of different bioinformatics tools should be used to address these biases. A variety of bioinformatics tools and algorithms have been developed for the prediction and identification of circRNAs, such as circRNA finder, find-circ, CIRI, MapSplice, CIR-C explorer, and Acfs. For instance, by using bioinformatic tools, such as Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses, Yu et al. identified dysregulated circRNAs (70 downregulated and 30 upregulated) in coronary heart disease (CHD) patients compared to controls and predicted 12 circRNAs that might act as circRNA-miRNA sponges to regulate their CHD-related parental genes.

RNase R, a member of the E. coli RNR superfamily, can degrade most linear RNA molecules (e.g., miRNAs) in the 3′–5′ direction but has no effect on circRNAs with closed loop structures; therefore, RNase R treatment is used to increase the relative concentration of circRNAs, also known as circRNA enrichment. For example, RNase R treatment, polyadenylation, and the poly(A)+ RNA depletion (RPAD) method are used to isolate circRNAs, thus enabling systematic identification and characterization of highly enriched, highly pure populations of circRNAs. Then, two approaches can be employed to verify and validate circRNAs: reverse transcription-polymerase chain reaction (RT-PCR) and Northern blotting.

cDNA for RT-PCR is gotten from RNase R-digested RNA through reverse transcription and then amplified with divergent primers and convergent primers. Subsequent agarose gel electrophoresis reveals that the divergent primer generates a band, but the convergent primer does not in samples treated with RNase R, whereas both divergent and convergent primers produce bands in samples not treated with RNase R. This proves that circRNAs are present and resistant to digestion by RNase R; therefore, RT-PCR can serve as a feasible quantitative estimation of circRNA abundance. In addition, online resources such as CircInteractome can be employed to design divergent primers that span the circRNA junction for quantitative real-time PCR (qRT-PCR) analysis of circRNA. Northern blotting can be conducted by using circRNA- and mRNA-specific probes, which specifically hybridize with circRNAs and linear mRNAs. Only a circRNA band is visible in samples treated with RNase R, as mRNAs are digested, whereas both circRNA bands and mRNA bands can be seen in samples not treated with RNase R. This indicates that Northern blotting can be used to convincingly verify the circular configuration of putative circRNAs.

Furthermore, some approaches, such as dual-luciferase reporter assay and fluorescence in situ hybridization (FISH) coupled with high-resolution microscopy, are widely applied to analyze the abundance, subcellular localization, and binding sites of circRNAs, and circRNA-miRNA interactions in cells and tissues. The circRNA-miRNA interaction can be predicted with the help of Arraystar’s proprietary miRNA target-prediction software Interaction. In addition, the RNA pull-down assay and RNA immunoprecipitation (RIP) assay followed by circRNA sequencing are practical strategies for predicting circRNA-protein interactions.

CONCLUSIONS

MI is an ischemic and life-threatening emergency endangering human life. There are a series of physiological and pathological processes involved in the progression of MI, such as cardiomyocyte apoptosis and autophagy, myocardial I/R, post-MI infarct healing, and cardiac remodeling. Recently, novel diagnostic and therapeutic approaches for MI have attracted much attention, and numerous candidate targets, especially circRNAs, have been investigated and developed. A growing body of evidence has verified that circRNAs function by binding to miRNAs or RBPs and that circRNAs prominently participate in and regulate the pathogenesis of MI and the pathophysiological processes and events of MI mainly through different circRNA/miRNA/protein axes. In this review, we discussed the pathogenesis and pathophysiology of MI and the biological functions of circRNAs in the process of MI, emphasizing the clinical application of circRNAs in MI, including their diagnostic and therapeutic values and approaches for measuring circRNAs in the context of MI (Figure 6).

The advancement of novel bioinformatic approaches (such as microarray analysis and algorithms), coupled with biochemical enrichment strategies (such as RNase R treatment and RPAD), deep sequencing, and gene-editing strategies (such as CRISPR-Cas9 technologies), will help allow comprehensive analysis of the involvement of circRNAs in the regulation of MI. In conclusion, the future holds promising application prospects for circRNAs serving as specific biomarkers and therapeutic targets for MI. However, unlike that of coding RNAs, lncRNAs and miRNAs, our current understanding of the biology of circRNAs is far from complete, and application of circRNAs in the clinic is still very difficult and requires much more research. For example, circRNAs have multiple cellular target sites and regulatory mechanisms and may induce unwanted adverse influences and off-
target effects in other cells or tissues. Moreover, there are individual differences in the expression and function of circRNAs, which are affected by inter- and intra-individual factors such as ethnicity, gender, diet, and activity. Additionally, the safety and efficacy of circRNA-based therapeutic approaches, including of the vectors used in some gene therapies, are unknown and need to be thoroughly evaluated.

There are some suggestions and directions related to circRNAs and MI in future research and clinical trials. First, MI is known to involve large areas of the myocardium, including cardiomyocytes, CFs, cardiac inflammatory cells, and endothelial cells; hence, the exact mechanism by which circRNAs in various kinds of cells participate in the development of MI deserves additional attention. Second, there is a pressing need to perform a large number of in vivo experiments, such as targeting circRNAs at specific sites and observing their long-term effects, to improve the diagnostic and therapeutic specificity of circRNA blockers and mimics for MI. Third, circRNA detection technology needs to be improved, such as by making high-throughput sequencing and molecular bioinformatics approaches faster and more reliable. Fourth, the safety and effectiveness of multiple circRNAs need to be further monitored and evaluated in numerous preclinical trials. Finally, through advancing circRNA detection technology and attaining a comprehensive understanding of the pathogenesis of MI, we believe that the novel circRNA-based approaches will someday be widely applied for the monitoring, diagnosis, treatment, and prognostication of MI, thereby dramatically alleviating the high morbidity and mortality of MI and benefiting all of mankind.

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AUTHOR CONTRIBUTIONS
Z.-J.W., Y.-C.W., and H.-W.L. searched the literature. Y.-F.Z. and H.X. provided inspiration and guidance for writing. Z.-J.W. wrote the manuscript and prepared all of the figures and tables. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

REFERENCES
1. Agewall, S., Beltrame, J.F., Reynolds, H.R., Niessner, A., Rosano, G., Caforio, A.L., De Caterina, R., Zimarino, M., Roffi, M., Kjeldsen, K., et al.; WG on Cardiovascular Pharmacotherapy (2017). ESC working group position paper on myocardial infarction with non-obstructive coronary arteries. Eur. Heart J. 38, 143–153.
2. Reed, G.W., Rossi, J.E., and Cannon, C.P. (2017). Acute myocardial infarction. Lancet 389, 197–210.

3. Smolowitz, N.R., Mahajan, A.M., Roe, M.T., Hellkamp, A.S., Chiswell, K., Gulati, M., and Reynolds, H.R. (2017). Mortality of Myocardial Infarction by Sex, Age, and Obstructive Coronary Artery Disease Status in the ACTION Registry-GWTG (Acute Coronary Treatment and Intervention Outcomes Network Registry-Get With the Guidelines). Circ. Cardiovasc. Qual. Outcomes 10, e003443.

4. Townsend, N., Wilson, L., Bhatnagar, P., Wickramasinghe, K., Rayner, M., and Nichols, M. (2016). Cardiovascular disease in Europe: epidemiological update 2016. Eur. Heart J. 37, 3232–3245.

5. Benjamin, E.J., Virani, S.S., Callaway, C.W., Chamberlain, A.M., Chang, A.R., Cheng, S., Chuve, S.E., Cushman, M., Delling, F.N., Deo, R., et al.; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee (2018). Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. Circulation 137, e66–e492.

6. Thygesen, K., Alpert, J.S., Jaffe, A.S., Simoons, M.L., Chaitman, B.R., White, H.D., Thygesen, K., Alpert, J.S., White, H.D., Jaffe, A.S., et al.; Writing Group on the Joint ESC/ACC/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction; ESC Committee for Practice Guidelines (CPG) (2012). Third universal definition of myocardial infarction. Eur. Heart J. 33, 2551–2567.

7. Armstrong, P.W. (2008). Defining myocardial infarction: a work in progress: Ischaemic heart disease. Heart 94, 1076–1079.

8. Krijnen, P.A., Nijmeijer, R., Meijer, C.J., Visser, C.A., Hack, C.E., and Nissen, H.W. (2002). Apoptosis in myocardial ischemia and infarction. J. Clin. Pathol. 55, 801–811.

9. Xu, D., Zhang, K., and Hu, P. (2019). The Role of Autophagy in Acute Myocardial Infarction. Front. Pharmacol. 10, 551.

10. Frangogiannis, N.G. (2014). The inflammatory response in myocardial injury, repair, and remodelling. Nat. Rev. Cardiol. 11, 255–265.

11. Malliaras, K., Zhang, Y., Seinfeld, J., Galang, G., Tsiou, E., Cheng, K., Sun, B., Aminzadeh, M., and Marban, E. (2013). Cardiomyocyte proliferation and progenitor cell recruitment underlie therapeutic regeneration after myocardial infarction in the adult mouse heart. EMBO Mol. Med. 5, 191–209.

12. Badimon, L., and Borrell, M. (2018). Microvascular Recovery by Angiogenesis After Myocardial Infarction. Curr. Pharm. Des. 24, 2967–2973.

13. Nakamura, M., and Sadoshima, J. (2018). Mechanisms of physiological and pathological cardiac hypertrophy. Nat. Rev. Cardiol. 15, 387–407.

14. Talman, V., and Ruskouho, H. (2016). Cardiac fibrosis in myocardial infarction-from repair and remodeling to regeneration. Cell Tissue Res. 365, 563–581.

15. Broughton, K.M., Wang, B.J., Firouzi, F., Khalafalla, F., Dimmeler, S., Fernandez-Aviles, F., and Sussman, M.A. (2018). Mechanisms of Cardiac Repair and Regeneration. Circ. Res. 122, 1151–1163.

16. Schirone, L., Forte, M., Palmero, S., Yee, D., Nocella, C., Angelini, F., Pagano, F., Schiavon, S., Bordin, A., Carruccio, A., et al. (2017). A Review of the Molecular Mechanisms Underlying the Development and Progression of Cardiac Remodeling. Oxid. Med. Cell. Longev. 2017, 3920195.

17. Minicucci, M.F., Azevedo, P.S., Polegato, B.F., Paiva, S.A., and Zornoff, L.A. (2011). Heart failure after myocardial infarction: clinical implications and treatment. Clin. Cardiol. 34, 410–414.

18. Memczak, S., Jens, M., Elefnioti, A., Torti, F., Krueger, J., Rybak, A., Maier, L., Mackowiak, S.D., Gregersen, L.H., Munschauer, M., et al. (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495, 333–338.

19. Ebbesen, K.K., Hansen, T.B., and Kjems, J. (2017). Insights into circular RNA biology. RNA Biol. 14, 1035–1045.

20. Pfafendorf, C., and Preußler, C. (2019). Establishing essential quality criteria for the validation of circular RNAs as biomarkers. Biomol. Detect. Quantif. 17, 100085.

21. Jeck, W.R., and Sharpless, N.E. (2014). Detecting and characterizing circular RNAs. Nat. Biotechnol. 32, 453–461.

22. Xu, T., Wu, J., Han, P., Zhao, Z., and Song, X. (2017). Circular RNA expression profiles and features in human tissues: a study using RNA-seq data. BMC Genomics 18 (Suppl 6), 680.
47. O’Gara, P.T., Kushner, F.G., Ascheim, D.D., Casey, D.E., Jr., Chung, M.K., de Lemos, J.A., Ettinger, S.M., Fang, J.C., Fesmire, F.M., Franklin, B.A., et al.; CF/AHA Task Force (2013). 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 127, 529–555.

48. Hausenloy, D.J., and Yellon, D.M. (2013). Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J. Clin. Invest. 123, 92–100.

49. Hausenloy, D.J., Barrabes, J.A., Botker, H.E., Davidson, S.M., Di Lisa, F., Downey, J., Engstrom, T., Ferdinandy, P., Carbrera-Fuentes, H.A., Heusch, G., et al. (2016). Ischaemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery. Basic Res. Cardiol. 111, 76.

50. Neri, M., Finocchi, V., Di Paolo, M., Pomara, C., Riezzo, I., Turillazzi, E., and Cerretani, D. (2015). Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. Curr. Vasc. Pharmacol. 13, 26–36.

51. Hausenloy, D.J., Botker, H.E., Engstrom, T., Erlinge, D., Heusch, G., Ibanez, B., Klener, R.A., Ovize, M., Yellon, D.M., and Garcia-Dorado, D. (2017). Targeting reperfusion injury in patients with ST-segment elevation myocardial infarction: trials and tribulations. Eur. Heart J. 38, 935–941.

52. Kalogeris, T., Baines, C.P., Krenz, M., and Korthuis, R.J. (2016). Ischemia/ Reperfusion. Compr. Physiol. 7, 113–170.

53. Heusch, G. (2020). Myocardial ischaemia-reperfusion injury and cardioprotection in perspective. Nat. Rev. Cardiol. 17, 773–789.

54. DeVon, H.A., Hogan, N., Ochs, A.L., and Shapiro, M. (2010). Time to treatment for acute coronary syndromes: the cost of inaction. J. Cardiovasc. Nurs. 25, 110–114.

55. Deleon, K.Y., de Castro Brás, L.E., Lange, R.A., and Lindsey, M.L. (2012). Extracellular matrix proteomics in cardiac ischemia/reperfusion: the search is on. Circulation 125, 746–748.

56. Burke, A.P., and Virmani, R. (2007). Pathophysiology of acute myocardial infarction. Med. Clin. North Am. 91, 553–572, ix.

57. Ma, S., Wang, Y., Chen, Y., and Cao, F. (2015). The role of the autophagy in myocardial ischemia/reperfusion injury. Biochim. Biophys. Acta 1852, 271–276.

58. Cadenas, S. (2018). ROS and redox signaling in myocardial ischemia-reperfusion: a murine model. Am. J. Physiol. Cell Physiol. 315, C271–C276.

59. Michael, L.H., Entman, M.L., Hartley, C.J., Youker, K.A., Zhu, J., Hall, S.R., Cigola, E., and Anversa, P. (1996). Acute myocardial infarction in humans is an adaptive response to hemodynamic stress. J. Clin. Invest. 107, 1782–1793.

60. Bravo-San Pedro, J.M., Kroemer, G., and Galluzzo, L. (2017). Autophagy and Mitophagy in Cardiovascular Disease. Circ. Res. 120, 1812–1824.

61. Yan, L., Vatner, D.E., Kim, S.J., Ge, H., Masurekar, M., Massover, W.H., Yang, G., Levine, B., Rothermel, B.A., and Hill, J.A. (2007). Cardiac autophagy is a mal-adaptive response to hemodynamic stress. J. Clin. Invest. 117, 3881–3893.

62. Wang, X., Guo, Z., Ding, Z., and Mehta, J.L. (2018). Inflammation, Autophagy, and Mitophagy in Cardiovascular Disease. Circ. Res. 120, 1812–1824.

63. Buja, L.M., and Vela, D. (2008). Cardiomyocyte death and renewal in the normal myocardium. Proc. Natl. Acad. Sci. USA 105, 1072–1077.

64. Saraste, A., Pulkki, K., Kallajoki, M., Henriksen, K., Parvinen, M., and Vepsäläinen, K.M. (1997). Apoptosis in human acute myocardial infarction. Circulation 95, 320–323.

65. Fliss, I., and Gattinger, D. (1996). Apoptosis in ischaemic and reperfused rat myocardium. Circ. Res. 79, 949–956.

66. Kayastha, J., Cheng, W., Reiss, K., Clark, W.A., Sonnenblick, E.H., Krajewski, S., Reed, J.C., Olivetti, G., and Anversa, P. (1996). Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. Lab. Invest. 74, 86–107.

67. Olivetti, G., Quaini, F., Sala, R., Lagrasta, C., Corradi, D., Bonacini, E., Gambert, S.R., Cigola, E., and Anversa, P. (1996). Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. J. Mol. Cell. Cardiol. 28, 2005–2016.
Sponging miR-485-5p and Targeting ATG101. J. Cardiovasc. Pharmacol. 76, 427–436.

91. Li, M., Ding, W., Tariq, M.A., Chang, W., Zhang, X., Xu, W., Hou, L., Wang, Y., and Wang, J. (2018). A circular transcript of nci1 gene mediates ischemic myocardial injury by targeting miR-133a-3p. Theranostics 8, 5855–5869.

92. Cui, X., Dong, Y., Li, M., Wang, X., Jiang, M., Yang, W., Liu, G., Sun, S., and Xu, W. (2020). A circular RNA from NFIX facilitates oxidative stress-induced H9c2 cells apoptosis. In Vitro Cell. Dev. Biol. Anim. 56, 715–722.

93. Wang, X., Sun, Q., and Hu, W. (2021). Carvedilol protects against the H2O2-induced cell damages in rat myoblasts by regulating the circ_NFIX/miR-125b-5p/TLR4 signal axis. J. Cardiovasc. Pharmacol. , Published online June 25, 2021. https://doi.org/10.1097/JFC.0000000000001095.

94. Wang, K., Gan, T.Y., Li, N., Liu, C.Y., Zhou, L.Y., Gao, J.N., Chen, Y.N., Ponnsamy, M., Zhang, Y.H., and Li, P.F. (2017). Circular RNA mediates cardiomyocyte death via miRNA-dependent upregulation of MTP18 expression. Cell Death Differ. 24, 1111–1120.

95. Hu, X., Ma, R., Cao, J., Du, X., Cai, X., and Fan, Y. (2020). CircSAMD4A aggravates H/R-induced cardiomyocyte apoptosis and inflammatory response by sponging miR-138-5p. J. Cell. Mol. Med. , Published online November 21, 2020. https://doi.org/10.1111/jcmm.16093.

96. Gan, J., Yuan, J., Liu, Y., Lu, Z., Xue, Y., Shi, L., and Zeng, H. (2020). Circular RNA_101237 mediates anoxia/reoxygenization injury by targeting let-7a-5p/IGF2BP3 in cardiomyocytes. Int. J. Mol. Med. 45, 451–460.

97. Cheng, N., Wang, M.Y., Wu, Y.B., Cui, H.M., Wei, S.X., Liu, B., and Wang, R. (2021). Circular RNA POSTN Promotes Myocardial Infarction-Induced Myocardial Injury and Cardiac Remodeling by Regulating miR-96-5p/BNP3 Axis. Front. Cell. Dev. Biol. 8, 618574.

98. Luo, C., Ling, G.X., Lei, B.F., Feng, X., Xie, X.Y., Fang, C., Li, Y.G., Cai, X.W., and Zheng, B.S. (2021). Circular RNA PVT1 silencing prevents ischemia-reperfusion injury in rat by targeting microRNA-125b and microRNA-200a. J. Mol. Cell. Cardiol. 159, 80–90.

99. Geng, H.H., Li, R., Su, Y.M., Xiao, J., Pan, M., Cai, X.X., and Ji, X.P. (2016). The Circular RNA Cdr1as Promotes Myocardial Infarction by Mediating the Regulation of miR-7a on Its Target Genes Expression. PLoS ONE 11, e0157733.

100. Chai, Q., Zheng, M., Wang, L., Wei, M., Yin, Y., Ma, F., Li, X., Zhang, H., and Liu, G. (2020). Circ_0068655 Promotes Cardiomyocyte Apoptosis via miR-498/PAWR Axis. Tissue Eng. Regen. Med. 17, 659–670.

101. Lei, D., Wang, Y., Zhang, L., and Wang, Z. (2020). Circ_0010729 regulates hypoxia-induced cardiac injury by activating TRAF5 via sponging miR-27a-3p. Life Sci. 262, 118511.

102. Bai, M., Pan, C.L., Jiang, G.X., and Zhang, Y.M. (2020). CircRNA_001567 mediates myocardial infarction rats through inhibiting TGF-β1. Eur. Rev. Med. Pharmacol. Sci. 24, 369–375.

103. Zhao, Q., Li, W., Pan, W., and Wang, Z. (2021). CircRNA_001567 plays a significant role in myocardial infarction via the regulation of the miRNA-141/DAPK1 axis. J. Thorac. Dis. 13, 2447–2459.

104. Liu, X., Wang, M., Li, Q., Liu, W., Song, Q., and Jiang, H. (2020). CircRNA ACAP2 induces myocardial apoptosis after myocardial infarction by sponging miR-29. Minerva Med. , Published online May 13, 2020. https://doi.org/10.23736/S0026-2472.20.06600-8.

105. Zhang, J., Tang, Y., Zhang, J., Wang, J., He, J., Zhang, Z., and Liu, F. (2021). CircRNA ACAP2 is overexpressed in myocardial infarction and promotes the maturation of miR-352 to induce the apoptosis of cardiomyocytes. J. Cardiovasc. Pharmacol. 78, 247–252.

106. Cai, X., Li, B., Yang, W., Zhu, H., Zhang, P., Jiang, P., Yang, X., Sun, J., Hong, L., and Shao, L. (2021). CircJARID2 Regulates Hypoxia-Induced Injury in H9c2 Cells by Affecting miR-9-5p-Mediated BNIP3. J. Cardiovasc. Pharmacol. 78, e77–e85.

107. Tan, J., Pan, W., Chen, H., Du, Y., Jiang, P., Zeng, D., Wu, J., and Peng, K. (2021). Circ_0124644 Serves as a ceRNA for miR-590-3p to Promote Hypoxia-Induced Cardiomyocytes Injury via Regulating SOX4. Front. Genet. 12, 667724.
Cardiomyocytes Regulate Cardiac Angiogenesis after Myocardial Infarction. Oxid. Med. Cell. Longev. 2020, 8418407.

127. Gao, W.Q., Hu, X.M., Zhang, Q., Yang, L., Lv, X.Z., Chen, S., Wu, P., Duan, D.W., Lang, Y.H., Ning, M., et al. (2020). Downregulation of circFASTKD1 ameliorates myocardial infarction by promoting angiogenesis. Aging (Albany NY) 13, 3588–3604.

128. Huang, S., Li, X., Zheng, H., Si, X., Li, B., Wei, G., Li, C., Chen, Y., Chen, Y., Liao, W., et al. (2019). Loss of Super-Enhancer-Regulated circRNA Nxf1 Induces Cardiac Regeneration After Myocardial Infarction in Adult Mice. Circulation 139, 2857–2876.

129. St John Sutton, M., Lee, D., Rouleau, J.L., Goldman, S., Plappert, T., Braunwald, E., and Pfeffer, M.A. (2003). Left ventricular remodeling and ventricular arrhythmias after myocardial infarction. Circulation 107, 2577–2582.

130. Mester-Tonczar, J., Winkler, J., Einzinger, P., Stagsted, L.V.W., Kjems, J. (2019). Novel Identiﬁcation of Cardiac-Fibrosis-Associated Genes by Derepressing Targets of miR-26b-5p, Col1a2 and CTGF, in cardiac ﬁbroblasts. Sci. Rep. 7, 40342.

131. Deng, Y.Y., Zhang, W., She, J., Zhang, L., Chen, T., Zhou, J., and Yuan, Z. (2016). miR-125b/SFRP5 pathway. Cell Biochem. Funct. 34, 450–456.

132. Li, F., Long, T.Y., Bi, S.S., Sheikh, S.A., and Zhang, C.L. (2020). circPAN3 exerts a profibrotic role via sponging miR-221 through FoxO3/ATG7-activated autophagy in a rat model of myocardial infarction. Life Sci. 257, 118015.

133. Sun, L.Y., Zhao, J.C., Ge, X.M., Zhang, H., Wang, C.M., and Bie, Z.D. (2020). CircLAS1L regulates cardiac ﬁbroblast activation, growth, and migration through miR-125b/Smad5 pathway. Cell Biochem. Funct. 38, 443–450.

134. Wang, Y., Li, C., Zhao, R., Qiu, Z., Shen, C., Wang, Z., Liu, W., Zhang, W., Ge, J., and Shi, B. (2021). CircSbea from M2 macrophage-derived extracellular vesicles mediates myocardial ﬁbrosis after acute myocardial infarction. Theranostics 11, 6315–6333.

135. Shi, B. (2021). CircUbe3a from M2 macrophage-derived small extracellular vesicles promotes myocardial ﬁbrosis via suppressing miR-141 by targeting TGF-β1. J. Biomed. Biophys. Res. Commun. 487, 769–775.

136. Schulte, C., Barwari, T., Joshi, A., Theofilatos, K., Zampetaki, A., Barallobre-Barreiro, J., Singh, B., Sörensen, N.A., Neumann, J.T., Zeller, T., et al. (2019). Comparative Analysis of Circulating Noncoding RNAs Versus Protein Biomarkers in the Detection of Myocardial Injury. Circ. Res. 125, 328–340.

137. Zhang, Z., Yang, T., and Xiao, J. (2018). Circular RNAs: Promising Biomarkers for Human Diseases. EBioMedicine 34, 267–274.

138. Lin, F., Yang, Y., Guo, Q., Xie, M., Sun, S., Wang, X., Li, D., Zhang, G., Li, M., Wang, J., and Zhao, G. (2020). Analysis of the Molecular Mechanism of Acute Coronary Syndrome Based on circRNA-miRNA Network Regulation. Evid. Based Complement. Alternat. Med.

139. Ho, P.Y., and Yu, A.M. (2016). Bioengineering of noncoding RNAs for research and therapeutic applications. Wiley Interdiscip. Rev. RNA 7, 186–197.

140. Lim, G.B. (2019). MicroRNA-directed cardiac repair after myocardial infarction in pigs. Nat. Rev. Cardiol. 16, 454–455.

141. Gao, J., Chen, X., Wei, P., Wang, Y., Li, P., and Shao, K. (2021). Regulation of pyroptosis in cardiovascular pathologies: Role of noncoding RNAs. Mol. Ther. Nucleic Acids 5, 220–236.

142. Majumdar, M.D., Koller, I.E., Heidt, T., Leuschner, F., Treulove, I., Sena, B.F., Horvath, R., Iwamoto, Y., Dutta, P., Woytikiewicz, G., et al. (2013). Monocyte-directed RNAi targeting CCR2 improves infarct healing in atherosclerosis-prone mice. Circulation 127, 2038–2046.

143. Hausecker, D., and Kay, M.A. (2015). RNA interference. Drugging RNAi. Science 347, 1069–1070.

144. Hulot, J.S., Ishikawa, K., and Hajjar, R.J. (2016). Gene therapy for the treatment of heart failure: promise postponed. Eur. Heart J. 37, 1651–1658.

145. Suckau, L., Fechner, H., Chemaly, E., Krohn, S., Hadri, L., Kockskämper, J., Westermann, D., Busing, E., Ly, H., Wang, X., et al. (2009). Long-term cardiac-targeted RNA interference for the treatment of heart failure restores cardiac function and reduces pathological hypertrophy. Circulation 119, 1241–1252.

146. Boon, R.A., Jaé, N., Holdt, L., and Dimmeler, S. (2016). Long Noncoding RNAs: From Clinical Genetics to Therapeutic Targets? J. Am. Coll. Cardiol. 67, 1214–1226.

147. Oumzain, S., Micheletti, R., Beckmann, T., Schroen, B., Alexaniam, M., Pezzuto, L., Crippa, S., Nemir, M., Sarre, A., Johnson, R., et al. (2015). Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. Eur. Heart J. 36, 353–368.

148. Boon, R.A., and Dimmeler, S. (2015). MicroRNAs in myocardial infarction. Nat. Rev. Cardiol. 12, 135–142.

149. Gao, J., Chen, X., Shan, C., Wang, Y., Li, P., and Shao, K. (2020). Antisense therapy in cardiovascular diseases: role of noncoding RNAs. Mol. Ther. Nucleic Acids 23, 101–118.

150. Kristensen, L.S., Andersen, M.S., Stagsted, L.V.W., Ebbesen, K.K., Hansen, T.B., and Kjems, J. (2019). The circular RNA-miRNA-mRNA axes in cardiovascular diseases. Life Sci. 233, 274–281.
Riquelme, J.A., Chavez, M.N., Mondaca-Ruff, D., Bustamante, M., Vicencio, J.M., Quest, A.F., and Lavandero, S. (2016). Therapeutic targeting of autophagy in myocardial infarction and heart failure. Expert Rev. Cardiovasc. Ther. 14, 1007–1019.

Haider, H.Kh., Akbar, S.A., and Ashraf, M. (2009). Angiomyogenesis for myocardial repair. Antioxid. Redox Signal. 11, 1929–1944.

Kirkton, R.D., and Bursac, N. (2012). Genetic engineering of somatic cells to study and improve cardiac function. Europace 14 (Suppl 5), v40–v49.

Fan, S., Hu, K., Zhang, D., and Liu, F. (2020). Interference of circRNA HIPK3 alleviates cardiac dysfunction in lipopolysaccharide-induced mice models and apoptosis in H9C2 cardiomyocytes. Ann. Transl. Med. 8, 201–211.

López-Jiménez, E., Rojas, A.M., and Andrés-León, E. (2018). RNA sequencing and Prediction Tools for Circular RNAs Analysis. Adv. Exp. Med. Biol. 1087, 17–33.

Li, S., Teng, S., Xu, J., Su, G., Zhang, Y., Zhao, J., Zhang, S., Wang, H., Qin, W., Lu, Z.J., et al. (2019). Microarray is an efficient tool for circRNA profiling. Brief. Bioinform. 20, 1420–1433.

Hao, X.D., Liu, Y., Li, B.W., Wu, W., and Zhao, X.W. (2020). Exome sequencing analysis identifies novel homoygous mutation in ABCA4 in a Chinese family with Stargardt disease. Int. J. Ophthalmol. 13, 671–676.

Wu, H.J., Zhang, C.Y., Zhang, S., Chang, M., and Wang, H.Y. (2016). Microarray Expression Profile of Circular RNAs in Heart Tissue of Mice with Myocardial Infarction-Induced Heart Failure. Cell. Physiol. Biochem. 39, 205–216.