Molecular Therapy
Nucleic Acids
Review

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 noncoding 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 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]

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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.[42] 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 Ca^{2+} 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.[9,57] 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.[32,59,60]

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.[9]

Autophagy is elevated in ischemia and in the reperfusion phase of I/R injury.[57,61] 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 Ca^{2+} 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.[10–12,73] 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.[47,76,77] Accumulating studies have divided this healing process into three distinct but overlapping phases: the inflammatory, proliferation, and maturation phases.[43] 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

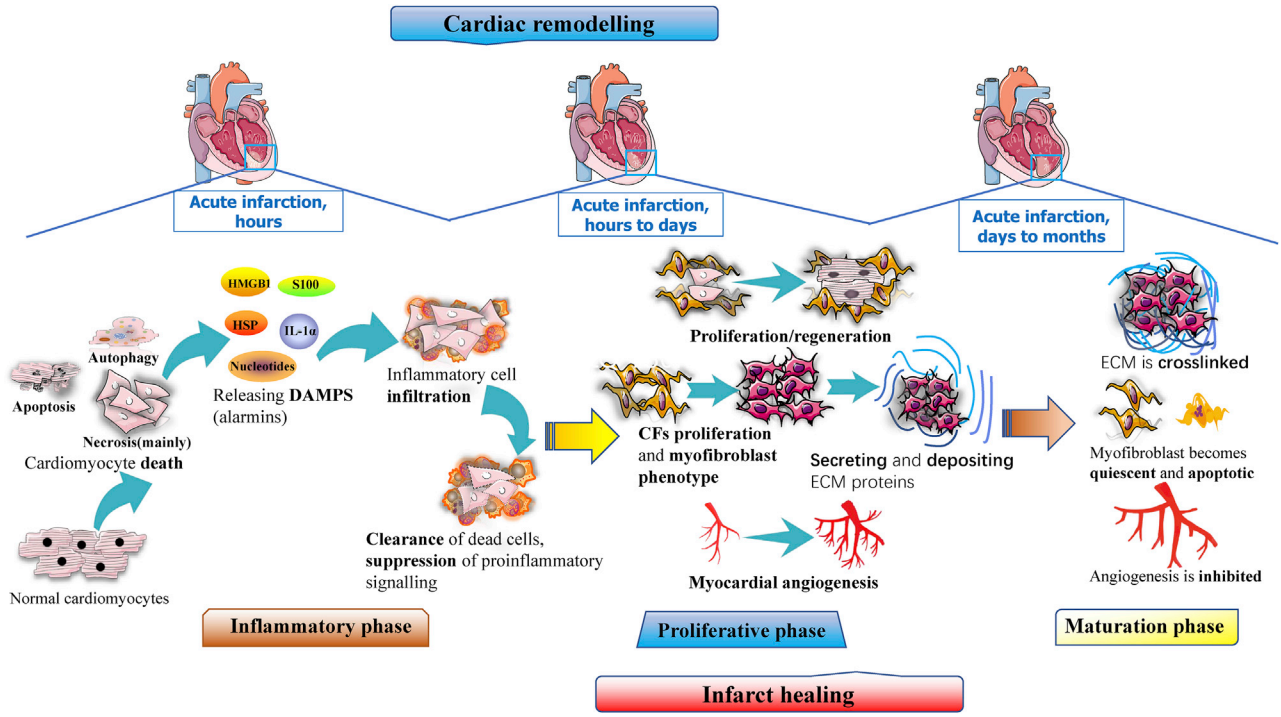

Figure 1. Three phases of post-MI infarct healing and cardiac remodeling

Inflammation phase: necrotic cells release alarmins, mainly damage-associated molecular patterns (DAMPs); activate innate immune pathways; and thereby mediate leukocyte activation and infiltration into inflamed tissues to clear the dead cells and matrix debris. Proliferation phase: CFs proliferate and transform into the synthetic and myofibroblast-like phenotype, damaged cardiomyocytes are repaired and proliferated, extracellular matrix (ECM) proteins are secreted and deposited, and myocardial angiogenesis is activated. Maturation phase: ECM proteins are crosslinked and decreased, myofibroblasts become quiescent and apoptotic, angiogenesis is suppressed, and vast-formed microvessels disappear.

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.[88,89] Therefore, we divided the circRNAs that regulate cardiomyocyte apoptosis into the following four categories

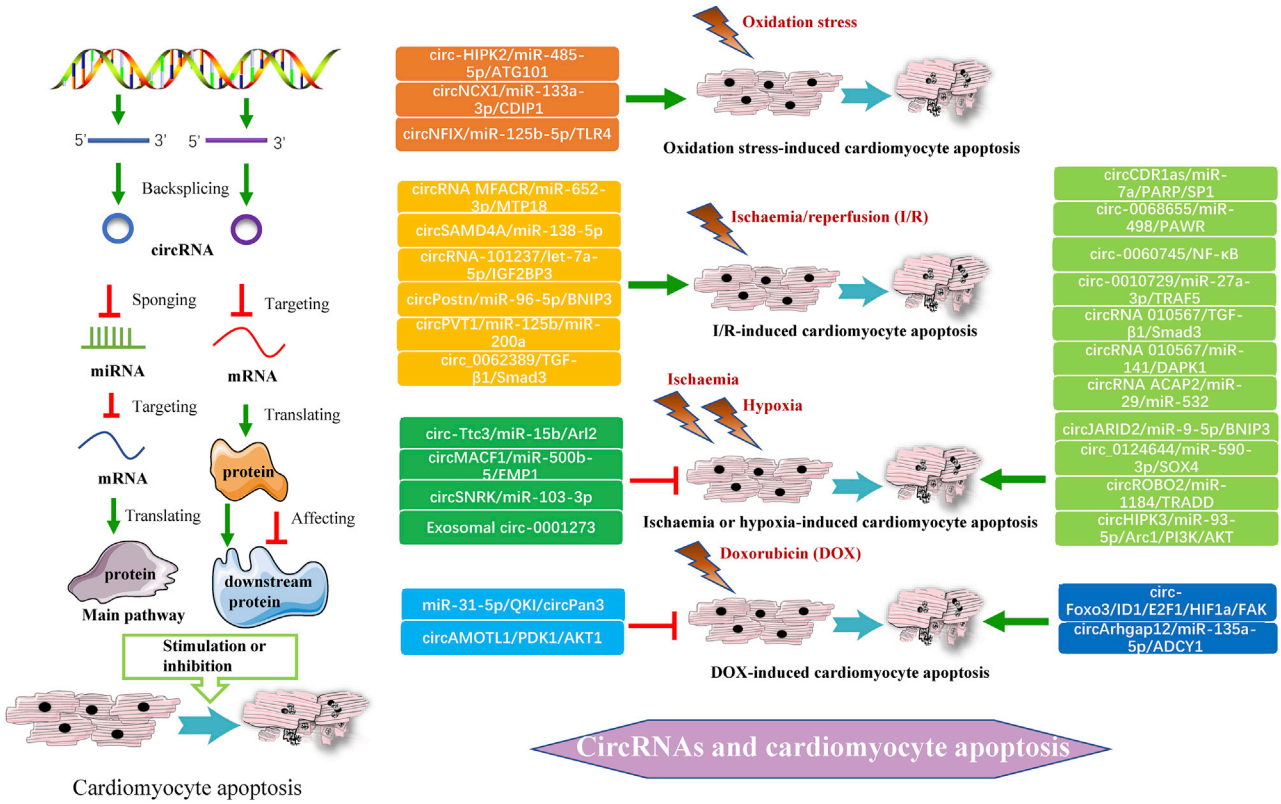

Figure 2. General pathways and specific regulatory axes of circRNAs in various cardiomyocyte apoptosis

circRNAs stimulate or inhibit MI-associated cardiomyocyte apoptosis via the circRNA/miRNA/protein axis or by directly regulating expression of protein. A wide range of circRNAs regulates four categories of cardiomyocyte apoptosis, and their expressions are changed by related induction factors.

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, circNFIX was observed to act as a pro-apoptosis factor that markedly promotes oxidative stress-induced cell apoptosis.[92] The circNFIX/miR-125b-5p/Toll-like receptor 4 (TLR4) axis is suppressed by carvedilol, which thus reduces the progression of AMI and H_2O_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 circNFIX 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 Chen[33] 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, circCDR1as was observed to induce cardiomyocyte apoptosis and aggravate injury.[99] Specially, circCDR1as 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 factor (WT1) regulator (PAWR) regulatory axis.[100] Additionally, Zhai's group[32] demonstrated that enhanced expression of circ-0060745 aggravates cell apoptosis by decreasing Bcl-2 expression and increasing Bax expression and that knockdown of circ_0060745 suppresses hypoxia-induced cardiomyocyte apoptosis through inhibition of nuclear factor κB (NF-κB) activation. Moreover, researchers found that circ-0010729 sponges miR-27a-3p, evokes tumor necrosis factor receptor (TNFR)-associated factor 5 (TRAF5) expression, and then aggravates hypoxia-induced cardiomyocyte apoptosis and injury.[101] In addition, circRNA 010567 was identified to significantly promote cardiomyocyte apoptosis and exacerbate MI via modulation of the circRNA 010567/TGF-β1/Smad3 signaling pathway and circRNA 010567/miR-141/death-associated protein kinase 1 (DAPK1) axis.[102,103]

circRNA ACAP2 was found to sponge miR-29 and miR-532 to facilitate cardiomyocyte apoptosis after MI.[104,105] Moreover, circJARID2 sponges miR-9-5p and indirectly upregulates BNIP3 expression, promoting hypoxia-induced cardiomyocyte apoptosis and cell injury.[106] Additionally, circ_0124644 acts as a target gene of miR-590-3p and has a positive effect on SRY-box transcription factor 4 (SOX4). Silencing of circ_0124644 attenuates hypoxia-induced cardiomyocyte injury, such as by suppressing apoptosis and oxidative stress and inhibiting AMI progression through the circ_0124644/miR-590-3p/SOX4 axis.[107] In addition, it was demonstrated that knockdown of circROBO2 alleviates hypoxia-induced cardiomyocyte injury and apoptosis and improves cardiac dysfunction by modulating the circROBO2/miR-1184/TNFR1-associated death domain protein (TRADD) axis.[108] Finally, Wu et al.[109] found the circHIPK3 silencing can upregulate miR-93-5p expression and abrogate the activation of the Rac1/phosphatidylinositol 3-kinase (PI3K)/serine/threonine kinase (Akt) pathway, inhibiting hypoxia-induced cardiomyocyte apoptosis and improving myocardial function after MI. In contrast, Cai et al.[110] found that circ-Ttc3 is overexpressed in MI tissues and cells but has an inhibitory effect on hypoxia-induced cardiomyocyte apoptosis. Mechanistically, circ-Ttc3 could serve as an endogenous miR-15b sponge and had a positive influence on ADP ribosylation factor like GTPase 2 (Arl2) targets, suppressing hypoxia-induced ATP depletion and cardiomyocyte apoptosis.

Conversely, 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, CDR1as, circ_0060745, 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.[35,114] 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[116] 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 CARDIOMYOCYTE AUTOPHAGY

Recent studies have shown that cardiomyocyte autophagy, which has detrimental impacts on MI,[10] is overwhelmingly induced by ischemia and cardiac I/R injury.[65,118] The circRNAs that we summarized are

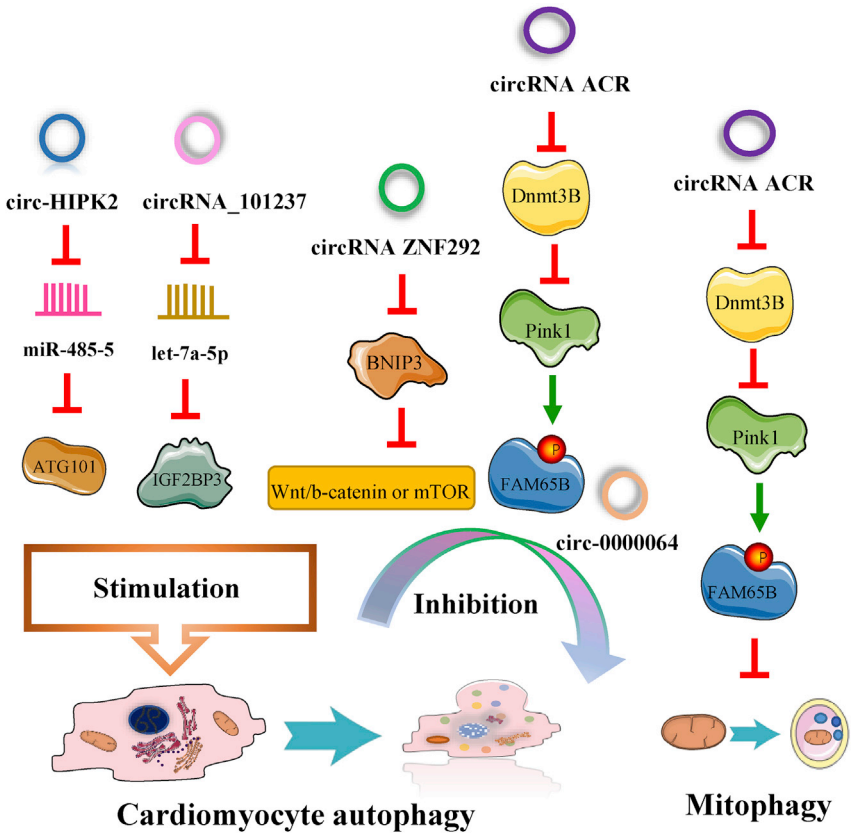

Figure 3. circRNAs are capable of regulating MI-associated cardiomyocyte autophagy and mitophagy

capable of regulating MI and influencing cardiac function by modulating cardiomyocyte autophagy (Figure 3).

Some circRNAs aggravate cardiomyocyte autophagy and MI; for example, circHIPK2 worsens oxidative stress-induced autophagy, and circRNA_101237 facilitates A/R-induced autophagy.[90,96] Zhou's group[90] discovered that circHIPK2 and ATG101 expression is upregulated *in vitro* in mouse cardiomyocytes treated with H_2O_2 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.[96] 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.[34] 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 targeting BNIP3. circRNA ZNF292 could ameliorate OGD-stimulated IHD, including MI. Coincidentally, circ-0000064 levels are increased by salidroside (Sal) pretreatment in the hearts of rats subjected to I/R injury, and circ-0000064 can ameliorate I/R-induced MI by inhibiting apoptosis and autophagy.[119] Sal treatment attenuates I/R-induced autophagy and protects cardiac function during MI by upregulating circ-0000064 expression. Moreover, circRNA ACR protects cardiac function by inhibiting A/R-induced autophagy and mitophagy. circRNA ACR expression is significantly downregulated in the hearts of mice subjected to I/R injury, and circRNA ACR can reduce the number of autophagic vacuoles and inhibit I/R-induced mitophagy in anoxic cardiomyocytes.[120] Mechanistically, circRNA ACR (autophagy-related circRNA) upregulates the expression of its downstream target phosphatase and tensin homolog-induced putative kinase 1 (Pink1) and suppresses the DNA methylation of Pink1 by interacting with DNA methyltransferase 3B (Dnmt3B). Then, Pink1 targets family with sequence similarity 65 member B (FAM65B) and phosphorylates it at S46. Importantly, circ-0000064 and 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.[43] 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.[95] Likewise, circ_0060745 participates in an intense inflammatory response and promotes hypoxia-induced cardiomyocyte apoptosis.[32] Additionally, circJARID2 contributes to the inflammatory response and promotes apoptosis.[106] In addition, Zhu et al.[121] 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) kinse-

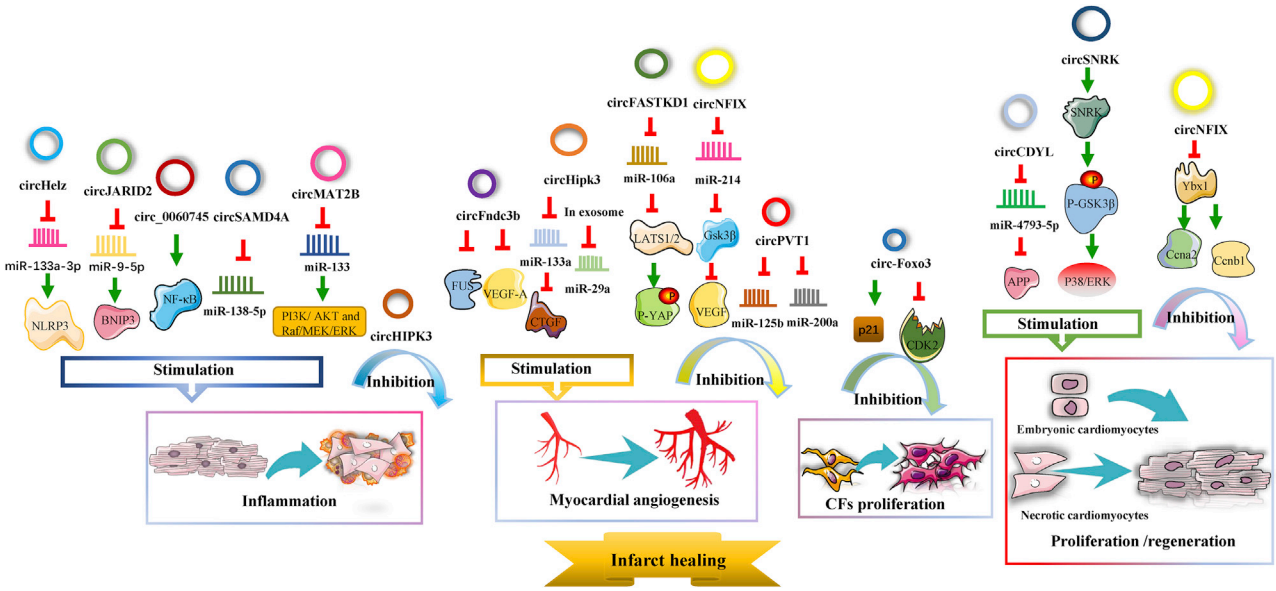

Figure 4. The regulatory roles of circRNAs in post-MI infarct healing

Many circRNAs are prominently involved in various events during infarct healing, such as inflammation, myocardial angiogenesis, CF proliferation, and regeneration of cardiomyocytes.

extracellular regulated MAP kinase (ERK) kinase (MEK)/ERK pathways.[121] 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 group[123] 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 3 β (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/connective 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

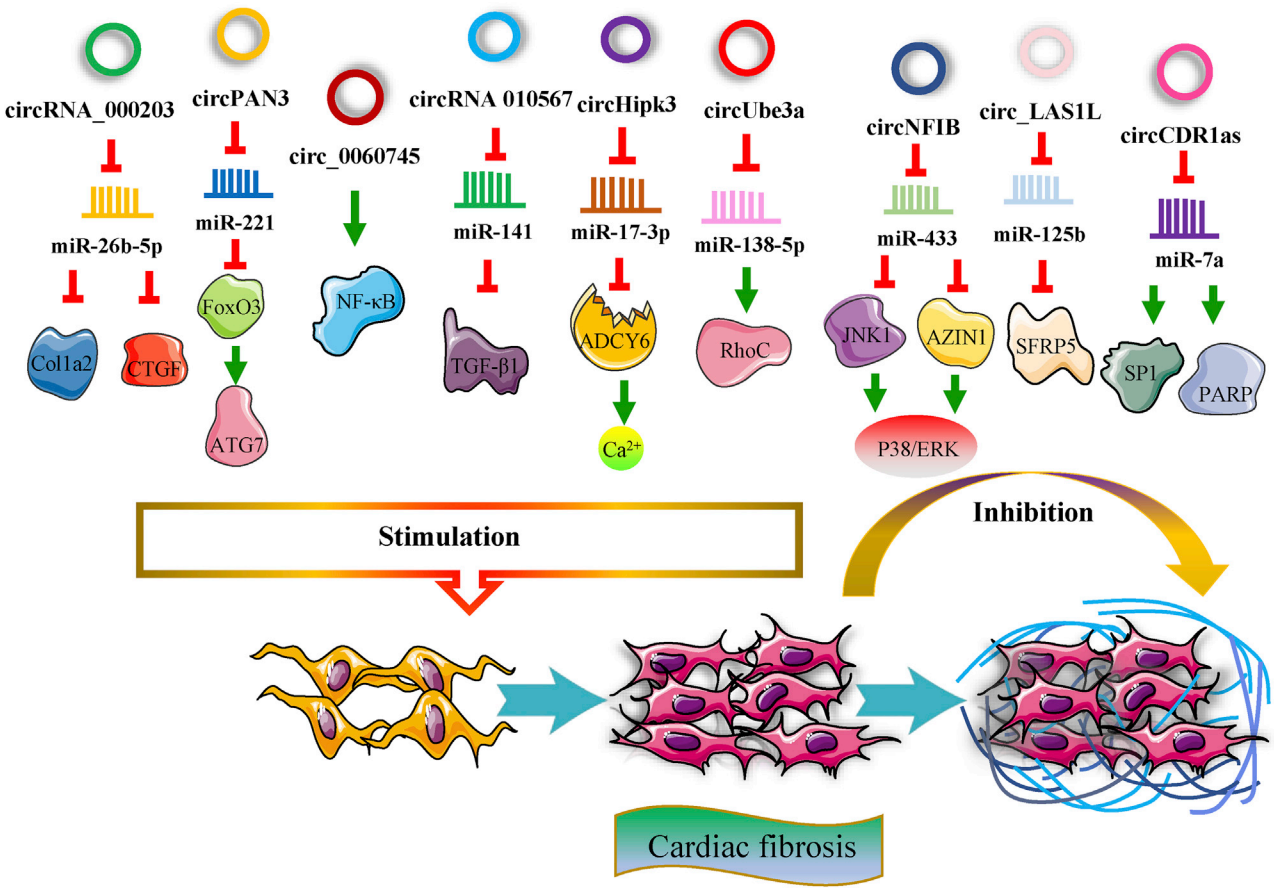

Figure 5. Many circRNAs have a remarkable capacity to regulate cardiac fibrosis

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

Table 1. circRNAs as diagnostic and prognostic biomarkers for MI

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	biomarker for MI	94
	circROBO2	upregulated	evidence for the clinical prognosis of MI	108
	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
Plasma samples	circPostn	upregulated	precision diagnostic biomarker in MI patients	97
	circRNA_081881	downregulated	precision diagnostic biomarker in AMI 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
Cardiac fibrosis	circPAN3	upregulated	potential prognostic biomarker for cardiac fibrosis during MI	132
	circ_LAS1L	downregulated	diagnostic biomarker in AMI patients and cardiac fibrosis	135
	circRNA_000203, circRNA_010567	upregulated	biomarkers for cardiac fibrosis	131,147
DOX-induced cardiomyopathy	circ-Foxo3	upregulated	biomarker for DOX-induced MI	35
	circRNAs derived from Ttn, Strn3, and Fhod3	downregulated	possible biomarkers of DOX-induced cardiotoxicity	89

significantly decreased in post-MI mouse hearts and TGF-β-treated primary adult CFs.[134] 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 in activated CFs.[135] 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,[32,102,126,136] whereas circCDR1as has a moderate beneficial effect in terms of reducing fibrosis.[99,130] It was shown that in an MI model *in vivo*, circPAN3, circUbe3a, circ_0060745, and circHIPK3 facilitate cardiac fibrosis, whereas circNFIB, 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.[137] 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.[138] 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.[139] 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.[140] The circRNAs that are discussed below are listed in Table 1.

Cui et al.[92] 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.[94] 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.[108] 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.[112] Tian et al.[141] 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.[127] In addition, Cheng et al.[97] 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.[142] Downregulation of circRNA_081881 expression can reduce PPARc levels because circRNA_081881 acts as a competing endogenous RNA of miR-548, abrogating the protective effect of PPARc in AMI.[142] Moreover, Mester-Tonczar et al.[130] 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.[143] 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.[144,145]

circ_0124644 was demonstrated to be a diagnostic biomarker for CAD, including MI, because it is easily detected in peripheral blood with high specificity and sensitivity and because its expression is upregulated in serum samples from AMI patients, suggesting that its diagnostic value would be greater when combined with circ_0098964.[107,146] Li et al.[132] proposed that the circPAN3/miR-221/Foxo3/ATG7 axis, a strong predictor of cardiac fibrosis during MI, may serve as a sensitive biomarker for cardiac fibrosis during MI. circ_LAS1L expression is downregulated in AMI patients and activated CFs, suggesting its diagnostic value.[135] Similarly, the Tang group[131] and Zhou and Yu[147] investigated whether circRNA_000203 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.[35] It was observed that circRNAs derived from Ttn, Strn3, and Fhod3 can be regulated by DOX treatment;[89] 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.[107,135,142,145,146]

Unfortunately, the sensitivity and reliability of most circRNAs as biomarkers for MI are currently less than satisfactory. Schulte et al.'s comparative analysis[148] 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.[149] However, there is great interest in using circRNAs as diagnostic and prognostic biomarkers for MI, and Lin's group[150] demonstrated that the regulation of circRNA-miRNA networks is closely related to the pathological process of MI. In addition, Schulte et al.[148] 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.[151–154] RNA interference (RNAi) approaches, which include the use of siRNAs,[155,156] organ-targeted RNAi using on viral vectors,[157,158] and modulation of long ncRNAs (lncRNAs)[159,160] and miRNAs,[161,162] 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.[145,163,164] Therefore, blocking or mimicking specific circRNA/miRNA/protein axes through regulation of

Table 2. circRNAs exert therapeutic effects by affecting only one process involved in MI

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 impaired cell migration *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 vitro*	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 vitro*	107
	circROBO2	unregulated (hypoxia treatment)	circROBO2/miR-1184/TRADD	inhibition of circROBO2 alleviates hypoxia-induced cardiomyocyte injury and apoptosis *in vivo* and *in vitro*	108
	circMACF1	downregulated (hypoxia treatment)	circMACF1/miR-500b-5p/EMP1	circMACF1 suppresses hypoxia-induced cardiomyocyte apoptosis and injury 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 vitro*	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 vitro*	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*)

Table 2. *Continued*

Effects	Name	Expression in MI	Regulatory pathway	Function	Ref.
Anti-fibrotic effects	circRNA_000203	upregulated (CF with fibrotic phenotype)	circRNA_000203/miR-26b-5p/Col1a2/CTGF (increases both Col1a2 and CTGF)	inhibition of circRNA_000203 promotes cardiac fibrosis *in vitro*	131
	circUbe3a	derived from M2M-SEVs	circUbe3a/miR-138-5p/RhoC	circUbe3a promotes CF proliferation, migration, myofibroblastic transformation, and cardiac fibrosis *in vivo* and *in vitro*	133
	circNFIB	downregulated (CFs with TGF-β treatment)	circNFIB/miR-433 (promotes AZIN1 and JNK1 but inhibits p38 and ERK enzyme and Smad3 signaling pathway)	circNFIB alleviates CF proliferation and cardiac fibrosis *in vivo* and *in vitro*	134
	circ_LAS1L	downregulated (AMI patients, CFs with TGF-β1 treatment)	circ_LAS1L/miR-125b/SFRP5 and then inhibits alpha-SMA and collagen I and III	circ_LAS1L suppresses activation, growth, and migration of CFs *in vitro*	135

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.[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.[33,94,100,101,104,105,107,108,111,116] In addition, circRNA ACR may have an effective anti-autophagic effect.[120] During infarct healing, circHelz can exert anti-inflammatory effects,[122] circCDYL and circFASTKD1 are likely to contribute to proliferation and proangiogensis,[123,127] and circUbe3a can exert anti-fibrotic effects.[133] Moreover, circRNA_000203, circNFIB, and circ_LAS1L can have anti-fibrotic effects.[131,134,135]

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-autophagic effects,[37,90,96,119] and circSAMD4A, circJARID2, and circMAT2B may have important anti-apoptotic and anti-inflammatory roles.[95,106,121] In addition, circ-0001273 probably exerts anti-apoptotic and reparative effects,[113] and circSNRK, circNFIX, circFndc3b, circPVT1, circ-Foxo3, and circAMOTL1 can have vital anti-apoptotic, proliferative, and proangiogenic effects.[35,92,98,112,114,117,124,128] 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/GSK3β/β-catenin axis when it is overexpressed.[112] Knocking down circNFIX inhibits oxidative stress-induced H9c2 cell apoptosis, but the signaling pathway involved is unclear.[92] Carvedilol diminishes H_2O_2-induced damage to CFs via the circNFIX/miR-125b-5p/TLR4 axis,[93] and circNFIX alleviates cardiomyocyte proliferation and angiogenesis by regulating the circNFIX/miR-214/Gsk3β axis and binding with Ybx1.[128] 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,[114] and disruption of the circ-Foxo3-p21-CDK2 ternary complex can promote cell-cycle progression as well as CF proliferation.[35]

Moreover, circPostn and circ-Ttc3 may be applied for anti-apoptotic and anti-remodeling effects.[97,110] circNCX1, circPAN3, and circRNA 010567 are capable of exerting anti-apoptotic and anti-fibrotic effects.[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.[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.[132] circRNA 010567 siRNA can reduce cardiomyocyte apoptosis via the circRNA 010567/TGF-β1/Smad3 signaling pathway[102] and the circRNA 010567/miR-141/DAPK1 axis,[103] whereas silencing of circRNA 010567 can contribute to alleviation of myocardial fibrosis through the circRNA 010567/miR-141/TGF-β1 axis.[147] Additionally, circ_0060745 exerts anti-apoptotic, anti-fibrotic, and anti-inflammatory effects via the modulation of the circ_0060745/NF-κB pathway.[32] Furthermore, circCDR1as is a potential therapeutic target due to its anti-apoptotic, anti-fibrotic, and anti-remodeling effects, as inhibition of circCDR1as suppresses cardiomyocyte apoptosis via modulation of the circCDR1as/miR-7a/PARP/SP1 axis,[99] and circCDR1as, being

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 (H_2O_2 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*	96
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*	95
	circJARID2	upregulated (hypoxia treatment)	circJARID2/miR-9-5p/BNIP3	inhibition of circJARID2 alleviates hypoxia-induced cardiomyocyte apoptosis and inflammatory response *in vitro*	106
	circMAT2B	upregulate (OGD treatment)	circMAT2B/miR-133/PI3K/AKT/Raf/MEK/ERK	circMAT2B knockdown reduces ROS production, hypoxia-induced apoptosis, and release of inflammatory factors *in vitro*	121
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
Anti-apoptotic and proliferative and proangiogenic effects	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
	circNFIX	downregulated (H_2O_2 treatment) and upregulated (adult hearts)	unclear (anti-apoptotic) and circNFIX/miR-214/Gsk3β and interacts with Ybx1(proliferative and proangiogenic)	inhibition of circNFIX 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, HIF1a, 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*)

Table 3. *Continued*

Effects	Name	Expression in MI	Regulatory pathways	Function	Ref.
				cardiomyocyte proliferation and survival *in vivo* and *in vitro*	
Anti-apoptotic and anti-remodeling effects	circPostn	upregulated (H/R model)	circPostn/miR-96-5p/BNIP3	inhibition of circPostn suppresses H/R-induced cardiomyocyte apoptosis and injury and post-MI remodeling *in vivo* and *in vitro*	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 *in vivo* and *in vitro*	110
Anti-apoptotic and anti-fibrotic effects	circNCX1	abundant	circNCX1/miR-133a-3p/CDIP1	inhibition of circNCfX1 suppresses cardiomyocyte apoptosis and improves myocardial fibrosis and I/R injury *in vivo* and *in vitro*	91
	circPAN3	downregulated (DOX treatment) and upregulated (MI hearts)	miR-31-5p directly inhibits QKI and downregulates circPAN3 (anti-apoptotic); circPAN3/miR-221/FoxO3/ATG7 (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/DAPK1 (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 *in vivo* and *in vitro*	99,130
Anti-apoptotic, anti-inflammatory, proangiogenic, and anti-fibrotic effects	circHIPK3	upregulated (fetal, neonatal, and post-MI hearts) in exosomes	circHPK3/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 *in vivo* and *in vitro*	109,125,126,136,168

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 circHPK3/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 axis,[126] 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.[170–172] For example, Lin et al.[150] 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.[173] 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.[174,175] 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, CIRCexplorer, and Acfs.[176,177] For instance, by using bioinformatic tools, such as Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses, Yu et al.[178] 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.[112,179] 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.[180] 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.[120] 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.[181] 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.[182]

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.[133,183] In addition, the RNA pull-down assay and RNA immunoprecipitation (RIP) assay followed by circRNA sequencing are practical strategies for predicting circRNA-protein interactions.[184]

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-

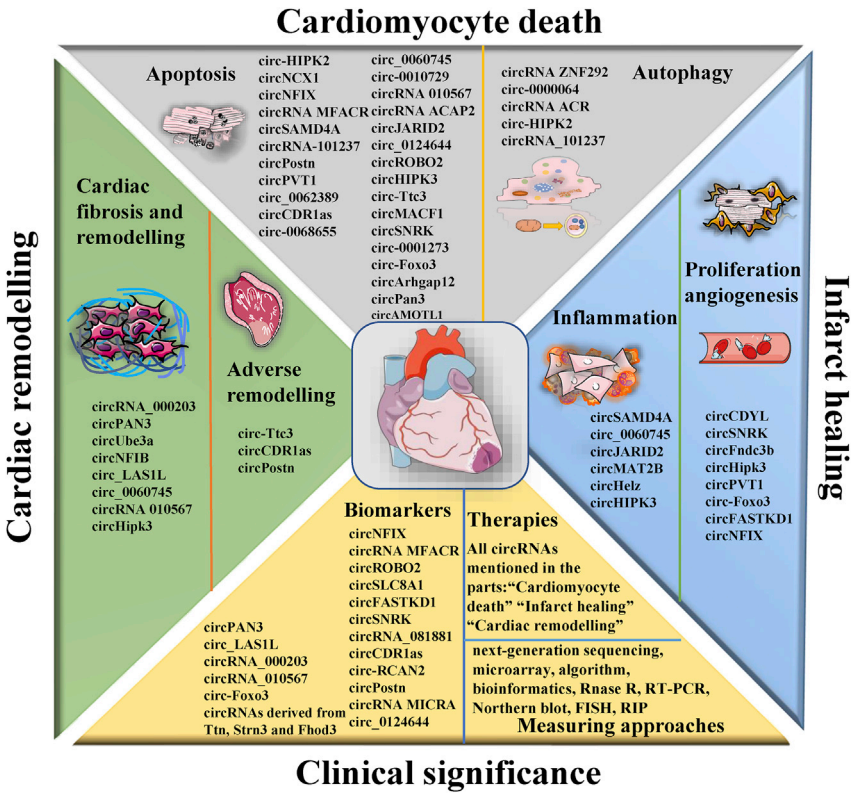

Figure 6. The biological functions and clinical significances of circRNAs for MI

circRNAs, which can be measured by various approaches in MI patients, tissues, and cells, significantly participate in regulation of the cellular processes and the pathophysiology of MI and have excellent biological functions and clinical significances for MI. Considerable circRNAs are involved in cardiomyocyte apoptosis and autophagy, inflammation, proliferation and angiogenesis, cardiac remodeling, and fibrosis, providing novel diagnostic and treatment strategies for MI.

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.

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