Perspectives of circular RNAs in diabetic complications from biological markers to potential therapeutic targets (Review)

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Abstract. Chronic complications of diabetes increase mortality and disability of patients. It is crucial to find potential early biomarkers and provide novel therapeutic strategies for diabetic complications. Circular RNAs (circRNAs), covalently closed RNA molecules in eukaryotes, have high stability. Recent studies have confirmed that differentially expressed circRNAs have a vital role in diabetic complications. Certain circRNAs, such as circRNA ankyrin repeat domain 36, circRNA homeodomain-interacting protein kinase 3 (circHIPK3) and circRNA WD repeat domain 77, are associated with inflammation, endothelial cell apoptosis and smooth muscle cell proliferation, leading to vascular endothelial dysfunction and atherosclerosis. CircRNA LDL receptor related protein 6, circRNA actin related protein 2, circ_0000064, circ-0101383, circ_0123996, hsa_circ_0003928 and circ_0000285 mediate inflammation, apoptosis and autophagy of podocytes, mesangial cell hypertrophy and proliferation, as well as tubulointerstitial fibrosis, in diabetic nephropathy by regulating the expression of microRNAs and proteins. Circ_0005015, circRNA PWWP domain containing 2A, circRNA zinc finger protein 532, circRNA zinc finger protein 609, circRNA DNA methyltransferase 3β, circRNA collagen type I α2 chain and circHIPK3 widely affect multiple biological processes of diabetic retinopathy. Furthermore, circ_000203, circ_010567, circHIPK3, hsa_circ_0076631 and circRNA cerebellar degeneration-related protein 1 antisense are involved in the pathology of diabetic cardiomyopathy. CircHIPK3 is the most well-studied circRNA in the field of diabetic complications and is most likely to become a biological marker and therapeutic target for diabetic complications. The applications of circRNAs may be a promising treatment strategy for human diseases at the molecular level. The relationship between circRNAs and diabetic complications is summarized in the present study. Of note, circRNA-targeted therapy and the role of circRNAs as biomarkers may potentially be used in diabetic complications in the future.

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Contents

1. Introduction

Diabetes mellitus (DM) is one of the most common and costly chronic metabolic diseases. Compared with 451 million adults in 2017, it is estimated that there will be 693 million adults with DM worldwide by 2045 (1). Long-term hyperglycaemia may lead to macrovascular and microvascular complications, such as cerebro-cardiovascular diseases, diabetic nephropathy (DN), diabetic retinopathy (DR), diabetic neuropathy (DNP) and diabetic cardiomyopathy (DCM), leading to increased disability and mortality of diabetic patients (1,2). However, early diagnosis and precisely targeted treatment of diabetic complications may reduce the medical burden and improve prognosis.

Circular RNAs (circRNAs) are novel noncoding RNAs that were verified in viruses by electron microscopy. Recently, thousands of circRNAs in eukaryotes have been identified by high-throughput RNA sequencing (RNA-seq). Of note, they have differentially expressed tissue patterns (3,4) and may be detected non-invasively in blood samples (5). CircRNAs possess covalently closed circular structures and are difficult to degrade, suggesting that circRNAs exhibit higher stability than linear RNAs (6). In addition, circRNAs have been demonstrated to participate in the occurrence and development of various diseases (5,7-9), which is well known in the field of oncology (10,11). However, noteworthy results have become apparent from studies investigating diabetic complications (5), according to which circRNAs may be promising biomarkers

for diabetic complications. The circRNA_15698/microRNA (miR)-185/transforming growth factor (TGF)-β1 pathway has been found to participate in the pathogenesis of DN (12). In addition, circRNA homeodomain interacting protein kinase 3 (circHIPK3) has been demonstrated to be involved in DR and DCM both *in vivo* and *in vitro* (13,14). Furthermore, circRNA_000203 expression is upregulated in the myocardium of diabetic mice and caspase-1-associated circRNA (CACR) is enhanced in the serum of patients with DM and cardiomyocytes exposed to high glucose (HG) (15,16).

The present review summarizes recent studies about circRNAs and diabetic complications, highlighting the unmet needs and future research directions for diabetic complications. In doing so, the study aims to provide novel concepts of prevention and treatment for diabetic complications.

2. Characteristics of circRNAs

Regulation of circRNAs. CircRNAs are closed circular single-stranded RNA molecules formed by the variable cutting of pre-mRNA, without polyadenylation [poly(A)] and capping. According to the sequence type, circRNAs are divided into exon types, intron types and exon-intron complexes. CircRNAs are generated by back splicing, in which downstream splicing donor sites are covalently linked with upstream splice-acceptor sites (Fig. 1) (17). This high stability is attributed to covalently closed circular structures, which protect these molecules from exonuclease-mediated degradation compared to their linear mRNA counterparts (18). Furthermore, the high stability of circRNAs may allow them to be detected non-invasively in bodily fluids (10,19,20). Thus, circRNA may be a promising biomarker for various diseases.

There are three aspects in regulating circRNA biogenesis: i) Regulating circRNA-producing pre-mRNA-circRNAs may compete with linear splicing to exert gene regulation (21). ii) CircRNA degradation-circRNAs may be degraded by endonuclease activity *in vitro*, such as RNase H and RNase L (22). Furthermore, circRNA levels may be reduced through sponging miRNAs. For instance, circRNA circular cerebellar degeneration-related protein 1 antisense (circCDR1as) is degraded by sponging miR-671 via protein Argonaute 2 (23). In addition, circRNAs may be secreted into blood and urine in the form of exosomes, which are eliminated by the liver, kidney and reticuloendothelial system (20,24). iii) Controlling the back-splicing machinery (cis-regulatory elements and trans-acting factors). Usually, back-splicing events are facilitated by flanking inverted repeated Alu pairs (25). However, it is mammalian-wide interspersed repeats that regulate the circularization of circCDR1as (26).

Biological functions of circRNAs. The four main biological functions of circRNAs are as follows: Acting as miRNA sponges [competing endogenous (ceRNAs)] (18), RNA-binding protein (RBP) sponges, protein-coding in the cytoplasm and transcriptional regulation of host genes in the nucleus (Fig. 1). Individual circRNAs act as miRNA sponges or decoys, protecting target mRNAs from miRNA-dependent degradation. Thus, the target RNAs are more actively translated and bound by ribosomes (27) (Fig. 1). CircCDR1as and circRNA zinc finger protein 91 are the most likely circRNAs to act as miRNA sponges, since they contain a large number of conserved binding sites for miRNAs. CiRS-7, containing 63 target sites for miR-7, upregulates tumor genes through increasing miR-7 target mRNAs (8,28). In addition, circRNA zinc finger protein 91, with 24 binding sites for miR-23b-3p, participates in the differentiation of keratinocytes (29). More so, circRNA coiled-coil domain containing 66 contains a variety of miRNA binding sites, including miR-33b and miR-93, which target the MYC gene (30). Furthermore, circCHIPK3 regulates HG-induced human umbilical vein endothelial cell (HUVEC) apoptosis by sponging miR-124 and regulating retinal endothelial cell (EC) proliferation, viability, migration and tube formation in HG-induced human retinal vascular ECs (HRVECs) by decoying miR-30a-3p (13,31).

CircRNAs acting as ceRNAs are more commonly studied in diabetic complications, but the other three biological functions of circRNAs are generally used in oncology. RBP sponges would block the process from mRNA to proteins, as RBPs are able to control the splicing stability and the translation of mRNAs. Therefore, circRNAs indirectly intervene in the post-transcriptional steps of mRNAs. In addition, circSMARCA5 regulates vascular endothelial growth factor (VEGF)A mRNA splicing in glioblastoma multiforme through the binding of serine and arginine rich splicing factor 1 (32). Abdelmohsen *et al* (33) found that RNA-binding protein human antigen R (HuR) directly interacted with autophagy related 16 like 1 (ATG16L1) mRNA via the 3'-UTR and enhanced ATG16L1 translation. Thus, circRNA poly(A) binding protein nuclear 1 (circPABPN1) was able to block HuR binding to Atg16l1 mRNA and decrease ATG16L1 protein production. The results suggest that the interaction between HuR and circPABPN1 modulated autophagy in the intestinal epithelium by altering ATG16L1 translation (34). As circRNAs lack the necessary elements for translation, such as the 5'Cap and Poly(A) tail, circRNAs may be translated either through the internal ribosomal entry site or after m6A RNA modification at 5'-UTR (35,36). CircRNA eukaryotic translation initiation factor 6 (EIF6) encodes a novel peptide called EIF6-224AA, which is responsible for the carcinogenic effect of circEIF6 in triple-negative breast cancer via stabilizing myosin heavy chain 9 and activating the Wnt/β-catenin pathway (37). Likewise, circRNAs also act as transcriptional regulators of host genes by binding to RNA polymerase II (12,38). More so, circular intronic RNA ankyrin repeat domain 52 (ANKRD52) was able to regulate its host gene, ANKRD52, in transcriptional elongation by influencing polymerase II (39).

3. CircRNAs and diabetic complications

Chronic diabetic complications increase disability and mortality in patients with DM. The availability of circRNA-seq and bioinformatics have indicated the critical role of circRNA in the pathogenetic mechanisms of diabetic complications and were reviewed below.

CircRNAs and diabetic macrovascular complications. Diabetic patients are more prone to suffering from life-limiting and life-threatening macrovascular events, such as cardio-cerebrovascular diseases (40). Of note, diabetic macrovascular

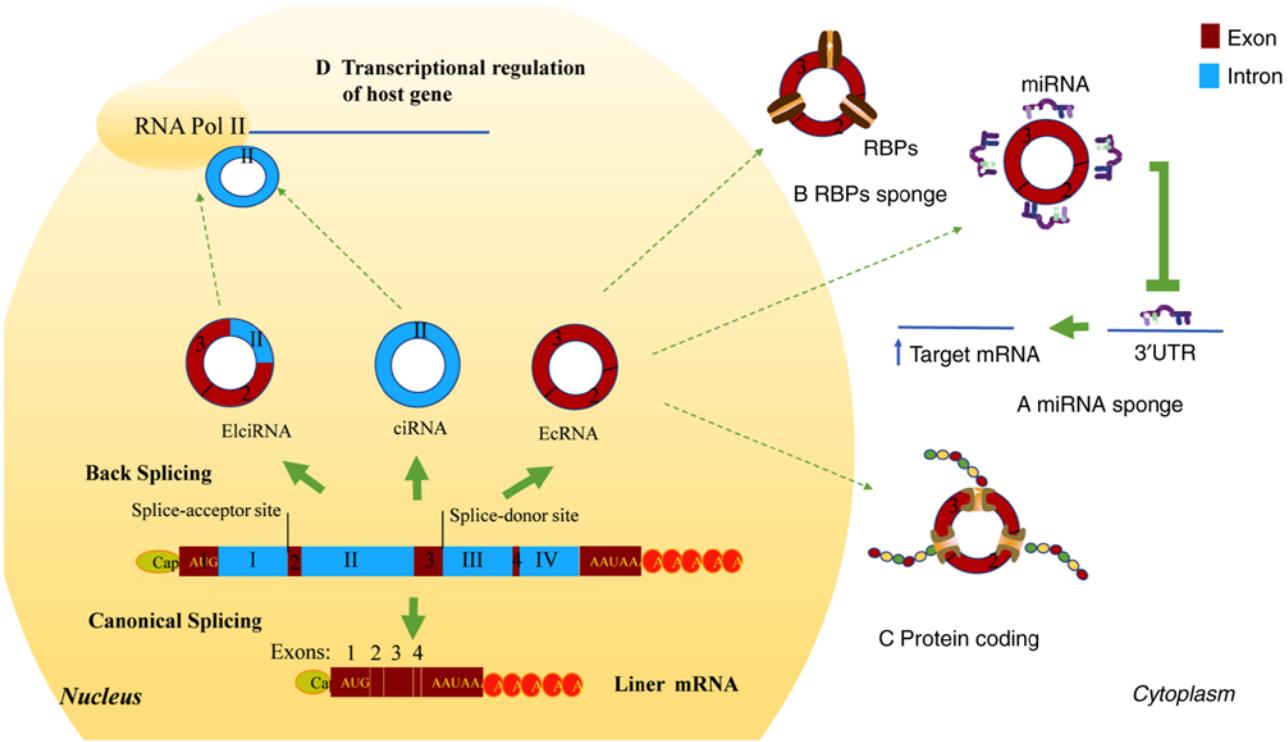

Figure 1. Biogenesis and biological functions of circRNAs. CircRNAs are generated by back splicing, in which downstream splice-donor sites are covalently linked with upstream splice-acceptor sites. CircRNAs are divided into EcRNA, ciRNA and ElciRNA. Functions of circRNAs include the following: (A) MiRNA sponge. Individual circRNAs act as miRNA sponges, protecting target mRNAs from miRNA-dependent degradation. The target RNA is, therefore, more actively translated. (B) RBP sponge. Individual circRNAs act as an RBP sponge, regulating their target mRNAs. (C) Protein coding. Certain circRNAs can be translated into proteins. (D) Transcriptional regulation of host gene. Certain circRNAs regulate the transcription of target genes by binding to RNA Pol II. CircRNAs, circular RNAs; ciRNA, intron circular RNA; EcRNA, exon circular RNA; ElciRNA, exon-intron complex circular RNA; miRNA, microRNA; RBPs, RNA-binding proteins; RNA Pol II, RNA polymerase II.

complications are closely related to the dysfunction of vascular ECs and proliferation of vascular smooth muscle cells (VSMCs), resulting in atherosclerosis under HG conditions. In two studies, as many as 95-214 differentially expressed circRNAs were found in HG-induced HUVECs by RNA-seq (41,42). Pan *et al* (43) also found that HG increased the expression of human circular RNA-0054633 (hsa_circ_0054633), while downregulation of hsa_circ_0054633 aggravated the HG-induced EC dysfunction. In addition, hsa_circ_0054633 has a protective effect against HG-induced ECs dysfunction through the miR-218/roundabout 1 and heme oxygenase-1 axes *in vitro* (43). The expression of circHIPK3 was decreased in HG-induced HUVECs and human aortic ECs from patients with type 2 DM (T2DM). Overexpression of circHIPK3 inhibited HG-induced apoptosis of ECs via the miR-124 axis (31). Likewise, the expression of circRNA CLIP-associating protein 2 (circCLASP2) was downregulated in HG-induced HUVECs. By contrast, upregulation of circCLASP2 inhibited apoptosis of HUVECs under HG conditions via miR-140-5p/F-box and the WD repeat domain-containing 7 axis (44). Therefore, upregulation of hsa_circ_0054633, circHIPK3 and circCLASP2 was able to alleviate HG-induced EC dysfunction.

However, various circRNAs have deleterious effects on blood vessels and may result in the dysfunction of ECs. Wei *et al* (45) reported that circVEGFC was upregulated in HG-induced HUVECs and able to sponge miR-338-3p; the latter targeted the 3'-UTR of hypoxia-inducible factor 1α (HIF-1α), thereby activating the transcription of VEGFA. Downregulation of circVEGFC was able to reduce HG-induced apoptosis of HUVECs and recover the proliferation through the miR-338-3p/HIF-1α/VEGFA axis (45). Similarly, downregulation of hsa_circ_0068087 inhibited HG-induced HUVEC inflammation by suppressing the Toll-like receptor (TLR)4/NF-κB/NOD-like receptor thermal protein domain associated protein 3 (NLRP3) pathway. However, the effects of inhibiting inflammation disappeared through downregulation of miR-197, suggesting that circ_0068087 functioned as a sponge of miR-197 (46) (Fig. 2). Chen *et al* (47) determined circRNA expression profiles in HG-induced VSMCs by circRNAs microarray analysis and verified that circWDR77 was upregulated in HG-induced VSMCs. Knockout of circWDR77 inhibited HG-induced VSMC proliferation and migration targeting miR-124/FGF-2. In their study, an RNA immunoprecipitation assay was used to validate the interaction between circRNA WD repeat domain 77 (circWDR77) and miR-124, suggesting that circWDR77 acted as a ceRNA of miR-124 (47) (Fig. 2). Recently, Zaiou (48) also indicated that these differentially expressed circRNAs in HG conditions among studies may act as vital contributors to the impairment of vascular ECs and the proliferation of VSMCs, therefore being involved in cardiovascular diseases. Fang *et al* (49) also found that circANKRD36 was markedly upregulated in the peripheral blood leucocytes of patients with T2DM and related to inflammatory factors, and speculated that circANKRD36 may serve as a biomarker for the development of inflammatory

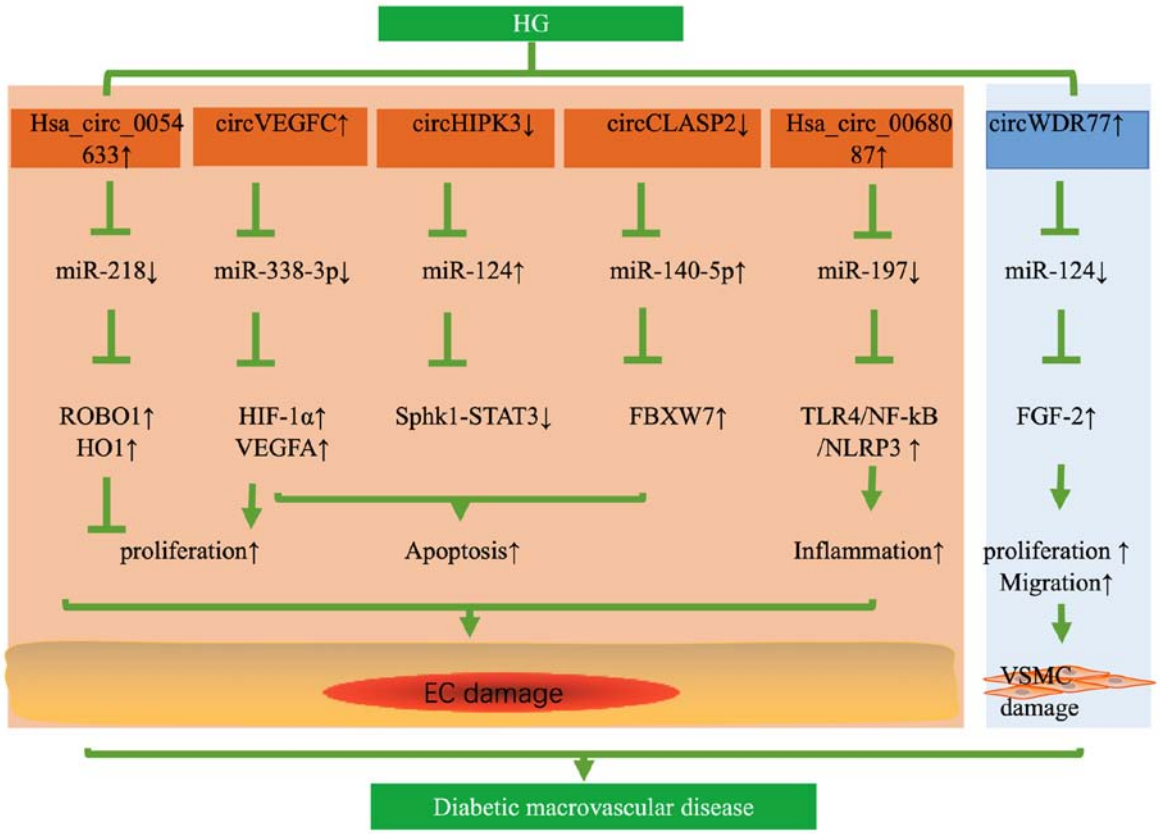

Figure 2. Related circRNAs in the pathogenesis of diabetic macrovascular diseases. The types of cell damage in studies of related circRNAs in diabetic macrovascular complications are EC damage and VSMC damage. HG conditions induce EC damage by enhancing proliferation, apoptosis and inflammation of EC. HG conditions induce VSMC damage by enhancing proliferation and migration. CircRNA, circular RNA; circCLASP2, circRNA CLIP-associating protein 2; circWDR77, circRNA WD repeat domain 77, hsa_circ_0013509; EC, endothelial cell; FBXW7, F-box and WD repeat domain-containing 7; FGF-2, fibroblast growth factor 2; HG, high glucose; HIF-1α, hypoxia-inducible factor-1α; HIPK3, homeodomain interacting protein kinase 3; ROBO1, roundabout homolog 1; HO1, recombinant heme oxygenase 1; NLRP3, NOD-like receptor thermal protein domain associated protein 3; Sphk1, sphingosine kinase 1; STAT3, signal transducer and activator of transcription 3; TLR4, toll-like receptor 4; VEGFCA, vascular endothelial growth factor A; VSM, vascular smooth muscle cell.

CVD among diabetic patients. Therefore, downregulation of circVEGFC, hsa_circ_0068087, circWDR77 may alleviate HG-induced dysfunction of ECs and VSMCs. Novel circRNAs have been found that may serve as therapeutic targets for cardio-cerebrovascular diseases and the prediction of inflammation in type 2 diabetes.

CircRNAs and diabetic microvascular complications. Microvascular complications of DM comprise DN, DR and DNP. DN is a leading cause of end-stage renal disease worldwide. However, the current treatment for DN is insufficient. DR and, notably, DNP severely affect the life quality of patients with DM. Thus, there is an urgent need to further explore ideal therapies for diabetic microvascular complications. As such, circRNAs can regulate the occurrence and development of diseases, and it is reported that there is a significant relationship between microangiopathy and circRNAs (50).

CircRNAs and DN. The underlying mechanisms of DN include oxidative stress, inflammatory cell recruitment and infiltration, mesangial cell hypertrophy, tubulointerstitial fibrosis, as well as podocyte loss and apoptosis (12,51-57). Recently, the roles of circRNAs in the pathogenesis of DN have received increasing attention. Evidence for the roles of circRNAs in DN is mainly derived from mesangial cells (MCs), tubular epithelial cells (TECs) and podocytes (Fig. 3). Hu *et al* (12) revealed that circRNA_15698, a sponge of miR-185, was upregulated in the kidney cortex of db/db mice and HG-treated MCs. Likewise, knockdown of circRNA_15698 alleviated extracellular matrix (ECM) accumulation by inhibiting the expression of transforming growth factor-β 1 (TGF-β1) protein *in vivo* and *in vitro*. The results suggested that the CircRNA_15698/miR-185/TGF-β1 axis has a role in diabetic renal fibrosis (12). Mou *et al* (58) confirmed 18 upregulated circRNAs and 22 downregulated circRNAs in the DN kidney from db/db mice using circRNA-seq. Furthermore, circ_0000491 levels were significantly augmented in DN mice and HG-induced mouse MCs. In addition, circ-0000491 sponged miR-101b and activated TGFβR1, leading to ECM accumulation (58). Chen *et al* (52) also demonstrated that circRNA LDL receptor related protein 6 (circLRP6) was increased in HG-treated mouse mesangial SV40-Mes13 cells. Knockdown of circLRP6 mitigated HG-induced proliferation, oxidative stress, inflammation and ECM in MCs via upregulating miR-205 and repressing the high mobility group box 1 and TLR4/NF-κB pathway. Similarly, Ge *et al* (54) found that circ_0000064 was upregulated in HG-induced mouse MCs and knockdown of circ_0000064 inhibited cell proliferation and fibrosis through upregulating miR-143.

Accumulating research has demonstrated that circRNAs are associated with tubular epithelial cell damage in

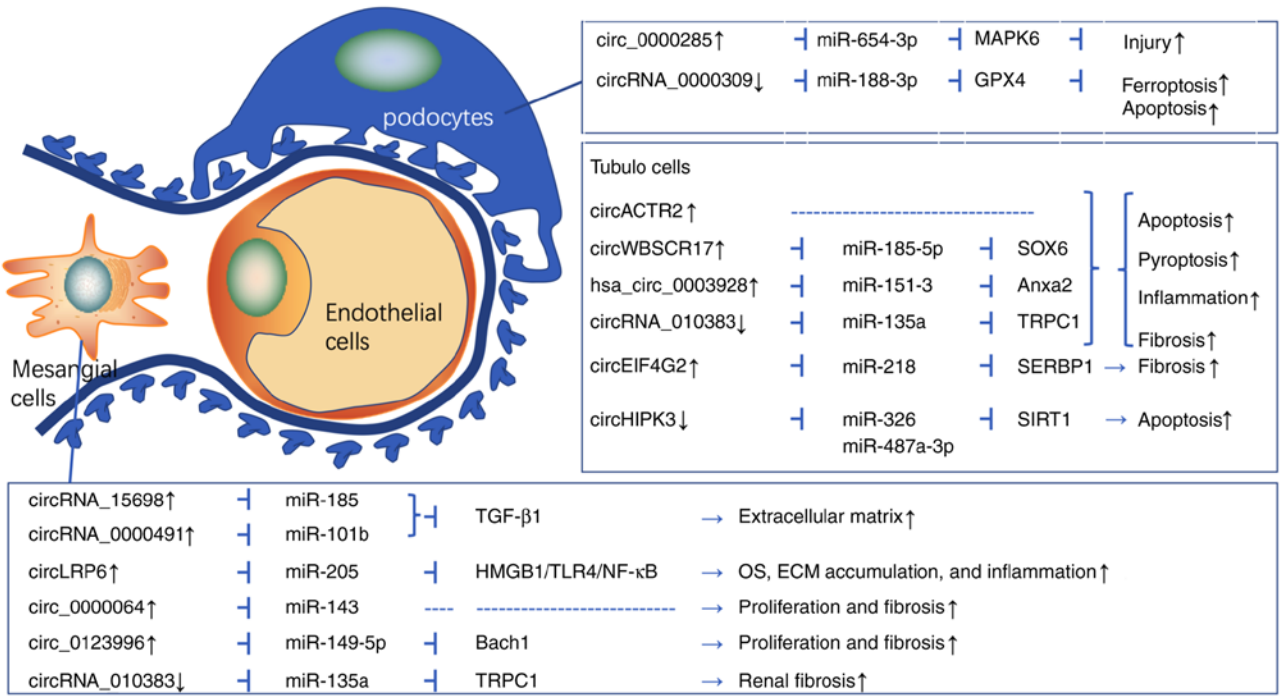

Figure 3. CircRNAs involved in DN and their mechanisms. The pathophysiology of DN includes mesangial cells, tubulointerstitial cells (not shown in the schematic diagram) and podocyte dysfunction. CircRNAs related to DN are shown in the figure according to different types of cell damage. Anxa2, annexin A2; Bach1, BTB and CNC homology 1; circRNA, circular RNA; circACTR2, circRNA actin related protein 2; circEIF4G2, circRNA eukaryotic translation initiation factor 4 gamma 2; circHIPK3, circRNA homeodomain interacting protein kinase 3; circLRP6, circRNA LDL receptor related protein 6; circWBSCR17, circRNA Williams-Beuren syndrome chromosome region 17; DN, diabetic nephropathy; ECM, extracellular matrix; GPX4, glutathione peroxidase 4; HMGB1, high mobility group box 1; MAPK6, mitogen-activated protein kinase 6; miR, microRNA; OS, oxidative stress; SERBP1, SERPINE1 mRNA binding protein 1; SIRT1, sirtuin 1; SOX6, transcription factor SOX 6; TGF-β1, transforming growth factor-β 1; TLR4, toll-like receptor 4; TRPC1, transient receptor potential cation channel subfamily C member 1.

DN (Fig. 3). Wen *et al* (59) explored the circRNA expression profiles and found that circRNA circular RNA actin related protein 2 (circACTR2) was upregulated in glucose-stressed HK-2 cells and mediated inflammation and pyroptosis. Knockdown of circACTR2 prevented HG-induced pyroptosis, inflammation and fibrosis of TECs, suggesting that circACTR2 has a vital role in the pathogenesis of DN (59). Analogously, circRNA Williams-Beuren syndrome chromosome region 17 (circ_0080425) was increased in kidney tissues from streptozotocin (STZ)-induced diabetic mice and HG-induced human kidney tubular cells and aggravated inflammatory responses and kidney fibrosis by targeting the miR-185-5p/transcription factor SOX 6 axis (56). CircRNA eukaryotic translation initiation factor 4 gamma 2 (circEIF4G2) was increased in db/db mice and HG-induced NRK-52E cells. By contrast, the downregulation of circEIF4G2 mitigated renal fibrosis in DN by sponging miR-218 (60). Recent evidence also suggested that circRNAs are involved in the pathological processes of podocytes injury and apoptosis in DN (Fig. 3). Yao *et al* (55) found that circ_0000285 was increased in kidney tissues of mouse models of DN and podocytes exposed to HG, leading to inflammation and podocyte injury through sponging miR-654-3p/mitogen-activated protein kinase 6.

Although most circRNAs have negative effects on DN, certain circRNAs have protective effects against DN. CircHIPK3 was decreased in HG-induced HK-2 cells, while upregulation of circHIPK3 could reverse the HG-induced HK-2 cell proliferation and apoptosis via miR-326/miR-487a-3p/sirtuin 1 (61). However, further *in vivo* experiments on circHIPK3 and DN are still needed. Research has also found that circRNA-010383 was downregulated in the diabetic kidneys, HG-induced MCs and TECs. Overexpression of circRNA_010383 in the kidney inhibited renal fibrosis and proteinuria in db/db mice via miRNA-135a/transient receptor potential cation channel subfamily C member 1 (51). Mmu_circRNA_0000309 showed a sharp decrease in DN mice and a remarkable recovery in Germacrone-challenged DN mice. Furthermore, mmu_circRNA_0000309 knockdown inhibited the protective effect of Germacrone via ferroptosis-depended mitochondrial damage and podocyte apoptosis by regulating miR-188-3p/glutathione peroxidase 4 axes both *in vivo* and *in vitro* (62). Therefore, upregulation of circHIPK3, circRNA-010383 and mmu_circRNA_0000309 could alleviate the dysfunction of TECs, MCs and podocytes, respectively.

CircRNAs in patients with DN are increasingly becoming the subject of ongoing research. Wang *et al* (53) reported that circ_0123996 was consistently increased in kidney tissues from patients with DN and mouse models of DN, as well as in HG-exposed MCs. Inhibition of circ_0123996 reduced cell proliferation and fibrosis in MCs via sponging miR-149-5p and inducing Bach1 expression. A recent study also indicated that hsa_circ_0003928 was upregulated, while miR-151-3p was downregulated in the serum from patients with DN and HG-induced HK-2 cells. Furthermore, downregulation of hsa_circ_0003928 repressed HG-induced cell apoptosis

and inflammation via miR-151-3p/annexin A2 *in vitro* (57). Therefore, these studies hint at circRNAs having an essential role in the pathogenesis of DN by acting as a sponge for miRNAs and may be a novel therapeutic target for DN.

CircRNAs and DR. Proliferative DR is a major cause of sustained blindness, which greatly impacts the life of patients with DM. Consequently, changes in the interaction among retinal cells due to diabetes result in severe vascular damage, loss of the blood-retinal barrier and impaired neuronal function (63). Of note, circRNAs regulate certain important physiological and pathological processes. Numerous studies have demonstrated differentially expressed circRNAs in serum, vitreous humour and retinas from patients with DR or in the retinas of db/db mice and human retinal pericytes induced by HG (64-68). Representative circRNAs in DR are listed in Table I.

Gu *et al* (64) found that 30 circRNAs were upregulated in the serum of patients with T2DM with DR compared with those with T2DM without DR and healthy volunteers. A total of 7 circRNAs (hsa_circRNA_063981, hsa_circRNA_404457, hsa_circRNA_100750, hsa_circRNA_406918, hsa_circRNA_104387, hsa_circRNA_103410 and hsa_circRNA_100192) were verified by reverse transcription-quantitative PCR. Another study showed that 356 circRNAs were upregulated and 173 circRNAs were downregulated in the retinas of diabetic patients (69). Furthermore, they also verified that circ_0005015 was significantly upregulated in the vitreous, plasma and fibrovascular membranes from patients with DR (69). Of note, 122 upregulated and 9 downregulated circRNAs were found in vitreous humour samples of patients with proliferative DR (67).

The crosstalk between pericytes and ECs is vital for microvascular homeostasis and remodeling. First, with regard to HG-induced human retinal pericytes, Liu *et al* (65) found that circRNA PWWP domain containing 2A (cPWWP2A, circ_0000254) has high homology in gene sequence with humans. Likewise, cPWWP2A was upregulated in the retinas of STZ-induced diabetic mice *in vivo* and HG-induced human retinal pericytes *in vitro*, and pericyte-derived cPWWP2A was able to affect pericyte coverage and vascular integrality by acting on miR-579 and its target genes. This study revealed that cPWWP2A-mediated signaling has a vital role in retinal microvascular dysfunction (65). In addition, overexpression of circRNA zinc finger protein (ZNF)532 was verified in human retinal pericytes treated with HG and in the vitreous of diabetic patients with macular edema, proliferative DR or neovascularization. Knockdown of cZNF532 exacerbated pericyte damage and retinal vascular dysfunction via the miR-29a-3p/neuron glial antigen 2-lysyl oxidase like 2-cyclin-dependent protein kinase 2 network (66). In another study, Wu *et al* (68) proved that hsa_circ_0001953 was upregulated in the serum of patients with proliferative DR and revealed that hsa_circ_0001953 was a potential diagnostic biomarker for proliferative DR. As such, there were consistent changes in the expression of certain circRNAs in serum of patients with DR and in the retinas of diabetic animal models, as well as in HG-induced human retinal pericytes. Therefore, these findings imply that serum circRNAs may be used as a biomarker to diagnose DR.

Furthermore, more detailed studies have been conducted on HG-induced ECs and circRNAs. A study indicated that circHIPK3 was significantly upregulated in diabetic retinas and retinal ECs following stressors related to DM, and circHIPK3 acted as a ceRNA of miR-30a-3p. Knocking down circHIPK3 was able to reduce retinal EC proliferation, viability, migration and tube formation *in vitro* and ameliorate hyperglycemia-induced retinal acellular capillaries, vascular leakage and inflammation *in vivo* (13). Another investigation suggested that circZNF609 was upregulated in HG-treated HUVECs and retinas from STZ-induced diabetic mice, in which silencing of circZNF609 alleviated endothelial dysfunction in HUVECs and retinal vessel loss and pathological angiogenesis *in vivo* by targeting the miR-615-5p/myocyte-specific enhancer factor 2A axis (70). Zou *et al* (71) found that circRNA collagen type I α2 chain (circCOL1A2, hsa_circ_0081108) was upregulated in HG-induced HRMECs. CircCOL1A2 knockdown inhibited HG-induced migration, proliferation, angiogenesis and vascular permeability of HRMECs *in vitro* and suppressed angiogenesis *in vivo* through regulating the miR-29b/VEGF axis. Endothelial tip cell specialization is vital for angiogenesis. CircMET was increased in STZ-induced mice and DR patients' retinas. CircMET silencing decreased the expression of tip cell-enriched genes (CXCR4, CD34 and VEGFA) in HRVECs (72). Another study found that circFTO was upregulated in HG-treated retinal vascular ECs. In addition, circFTO knockdown reversed angiogenesis and impaired the blood-retinal barrier in HG-induced retinal vascular ECs via miR-128-3p/thioredoxin (73). However, Zhu *et al* (74) demonstrated that circDNMT3B was downregulated in HRMECs under HG conditions and retinas from STZ-induced diabetic rats. CircDNMT3B overexpression reduced the retinal acellular capillary number and alleviated visual damage in STZ-induced diabetic rats, hinting at circDNMT3B regulating diabetic retinal vascular dysfunction via miR-20b-5p/target gene. In another approach, Ye *et al* (75) found that exosomal circEhmt1 released from hypoxia-stimulated pericytes protected endotheliocytes from HG-induced injury by downregulating the NFIA/NLRP3 pathway. In addition to pericytes and ECs, studies on circRNAs and HG-induced retinal pigment epithelial cells have also appeared in recent years. Upregulated circRNA_0084043 and hsa_circ_0041795 participate in DR via sponging miR-140-3p and miR-646, subsequently increasing TGFα and VEGFC expression in retinal pigment epithelial cells (76,77). Therefore, these data suggest that these differentially expressed circRNAs have an important role in the pathogenesis of DR and may be potential targets to control DR.

CircRNAs and DNP. Neuropathic pain is one of the most common diabetic complications in the clinic, but the aetiology of DNP has remained to be fully elucidated. Two studies suggested that circRNAs contribute to the development of DNP (78,79). One of the studies showed that the expression of circHIPK3 in the serum of patients with T2DM was significantly higher than that in the control group. Furthermore, upregulated circHIPK3 was positively associated with grade neuropathic pain in T2DM patients. Knockout of circHIPK3 was able to relieve neuropathic pain and inhibit neuroinflammation in STZ-induced diabetic rats (78). Likewise, circHIPK3 interacted with miR-124 and negatively regulated its expression (78). The study implies that intrathecal circHIPK3 short hairpin RNA may be used to treat DNP in the future. Zhang *et al* (79) also

Table I. Representative circRNAs investigated in diabetic retinopathy.

CircRNAs	Source	Regulation; function; target	Approach	Year	(Refs.)
Circ_0005015	Retinas of diabetic patients; vitreous samples, plasma and fibrovascular membranes of patients with DR	Up; Diagnostic biomarker for diabetic retinopathy, regulates retinal endothelial cell function; Sponging miR-519d-3p	Circular RNA microarray; RT-qPCR	2017	(69)
circRNA cPWWP2A (circ_0000254)	Retinas of db/db mice; human retinal pericytes	Up; Directly regulates pericyte biology, indirectly regulates EC biology via exosomes carrying cPWWP2A; cPWWP2A, miR-579 and angiopoietin 1/occludin/SIRT1 in pericytes	CircRNA microarray; RT-qPCR	2019	(65)
circ_0047814 (circZNF532)	T1DM mice; Human retinal pericytes (ACBRI-183) and HRVECs (ACBRI-181)	Up; Regulates pericyte function *in vitro*, regulates pericyte function and vascular integrity *in vivo*; miR-29a-3p, miR-29a-3p-NG2/LOXL2/CDK2	CircRNA microarray; RT-qPCR	2020	(66)
hsa_circ_0001953	Human blood of patients with proliferative DR	Up; Biomarker	High-throughput sequencing, RT-qPCR	2020	(68)
circHIPK3	T1DM mice, HRVECs (ACBRI-181) and human retinal pericytes (ACBRI-183)	Up; Regulates retinal endothelial cell proliferation, viability, migration and tube formation *in vitro*, and retinal acellular capillaries, vascular leakage and inflammation *in vivo*; circHIPK3/miR-30a-3p/vascular endothelial growth factor-C/FZD4/WNT2	RT-qPCR; northern blots	2017	(13)
cZNF609	HG-treated HUVECs; retinas of T1DM mice	Up; Regulates endothelial cell function in HUVECs; regulates retinal vessel loss and pathological angiogenesis *in vivo*; miR-615-5p/MEF2A	RT-qPCR	2017	(70)
circRNA DMNT3B (hsa_circ_0059802)	HRMECs; diabetic rats; epiretinal membranes of patients with DR	Down; Downregulated circDNMT3B alleviates retinal vascular dysfunction; miR-20b-5p/BAMBI	RT-qPCR	2019	(74)
circRNA_0084043	ARPE-19 cells treated with HG	Up; Loss of circRNA_0084043; protects HG-induced ARPE-19 cell injury; miR-140-3p/TGFA	RT-qPCR	2020	(76)
circCOL1A2 (hsa_circ_0081108)	HRMECs; STZ-induced DR in mice	Up; CircCOL1A2 knockdown inhibited HG-induced proliferation, migration, angiogenesis and vascular permeability of HRMECs *in vitro*; suppresses angiogenesis *in vivo*; miR-29b/VEGF expression	RT-qPCR and western blotting	2020	(71)
hsa_circ_0041795	HG-treated human retinal pigment epithelial cells (ARPE 19)	Up; Regulates cell proliferation and apoptosis; miR-646/VEGFC	RT-qPCR	2020	(77)
circMET	Retinas of STZ mice; retinas of patients with DR	Up; Pathological angiogenesis and inhibits tip cell specialization *in vivo*, endothelial migration and sprouting *in vitro*; circMET/IGF2BP2/NRARP or ESM1 complex	RT-qPCR	2022	(72)

Table I. Continued.

CircRNAs	Source	Regulation; function; target	Approach	Year	(Refs.)
circEhmt1	Hypoxia-stimulated pericytes	Up; Protects endotheliocytes from HG-induced injury; downregulation of NFIA/NLRP3 pathway	Circular RNA microarray of exosomal RNAs, RT-qPCR	2021	(75)
circFTO (circ_0005941)	HG-treated retinal vascular endothelial cells	Up; Viability and angiogenesis of RVECs and impairment of the BRB; miR-128-3p/thioredoxin	RT-qPCR	2021	(73)

BAMBI, BMP and activin membrane-bound inhibitor; BRB, blood-retinal barrier; circRNA, circular RNA; circCOL1A2, circRNA collagen type I α2 chain; circEhmt1, circRNA euchromatic histone lysine methyltransferase 1; circHIPK3, circRNA homeodomain interacting protein kinase 3; circMET, circRNA MET proto-oncogene, receptor tyrosine kinase; circPWWP2A, circRNA PWWP domain containing 2A; circRNA DMNT3B, circRNA DNA methyltransferase 3β; circZNF532, circRNA zinc finger protein 532; CDK2, cyclin-dependent protein kinase 2; cZNF609, circular RNA zinc finger protein 609; DR, diabetic retinopathy; ESM1, endothelial-specific molecule 1; FZD4, frizzled-4; HG, high glucose; HRMECs, human retinal microvascular endothelial cells; HRVECs, human retinal vascular endothelial cells; HUVECs, human umbilical vein endothelial cell; IGF2BP2, insulin-like growth factor 2 mRNA binding protein 2; LOXL2, lysyl oxidase like 2; MEF2A, myocyte-specific enhancer factor 2A; NFIA, nuclear factor I-A; NG2, neuron glial antigen 2; NLRP3, NOD-like receptor thermal protein domain associated protein 3; NRARP, notch-regulated ankyrin repeat protein; RT-qPCR, reverse-transcription quantitative PCR; RVECs, retinal vascular endothelial cells; SIRT1, sirtuin 1; STZ, streptozotocin; T1DM, type 1 of diabetes mellitus; TGFA, transforming growth factor α; VEGFC, vascular endothelial growth factor C; WNT2, Wnt family member 2; Up, upregulated; Down, downregulated.

observed the expression profile of circRNAs in dorsal root ganglia from DM mice by high-throughput RNA-seq, in which 15 differentially expressed circRNAs and 133 differentially expressed mRNAs were identified. A total of 11 circRNAs and 14 mRNAs had a marked correlation and circRNA-Atp9b was validated to be observably upregulated in dorsal root ganglia of DM mice (79). Liu *et al* (80) performed circRNA sequencing on sural nerves in patients with diabetic peripheral neuropathy, and 11 differentially expressed circRNAs were verified and circ_0002538 was downregulated. The authors found that overexpression of circ_0002538 improved the function of the sciatic nerve *in vivo*. Indeed, further studies are necessary to explore the mechanisms by which circRNAs are involved in DNP.

CircRNAs and DCM. DCM is a specific cardiovascular complication in patients with DM; it first manifests as diastolic dysfunction and eventually progresses to refractory heart failure (HF). Before the emergence of systolic dysfunction and clinical HF, DCM is initially characterized by cardiomyocyte apoptosis, hypertrophy, myocardial fibrosis and remodeling. It has been reported that circRNAs potentially act on cardioprotective processes by sponging miRNAs in DCM (14,15,16,81,82). Representative circRNA dysregulation mechanisms in DCM are presented in Fig. 4.

Through microarray analysis and RNA-seq, differentially expressed circRNAs were detected in heart tissues of diabetic animal models, such as 45 circRNAs up-regulated by >2-fold and 31 circRNAs downregulated by >2-fold in db/db mice (15). Dong *et al* (82) also found that 58 circRNAs were significantly differentially expressed in the myocardium at an early stage of DCM, including 29 upregulated circRNAs and 29 downregulated circRNAs. Of note, cardiac fibrosis is a critical event in the pathogenesis of DCM. This study revealed that circ_000203 was upregulated in myocardium from db/db mice and overexpression of circRNA_000203 increased the expression of COL1A2, COL3A1 and α-smooth muscle actin (α-SMA) in mouse cardiac fibroblasts (CFs) via sponging miR-26b-5p, and COL1A2 and connective tissue growth factor were the target genes of miR-26b-5p (15). Zhou and Yu (81) found 24 upregulated circRNAs and 19 downregulated circRNAs in the myocardium of db/db mice. Particularly, circ_010567 was markedly increased and circ-010567 silencing was able to reduce fibrosis-associated protein resection via upregulating miR-141 and inhibiting the TGF-β1 pathway in Angiotensin II (AngII)-treated CFs (81). The limitation of this *in vitro* study was that AngII instead of HG was used to induce CFs, which does not wholly reflect the pathogenesis of diabetic myocardial fibrosis. Wang *et al* (14) also found that circHIPK3 was upregulated in a DCM model of STZ-induced diabetic mice. CircHIPK3 knockdown was able to ameliorate myocardial fibrosis and improve cardiac function *in vivo*, while decreasing the proliferation of CFs treated with AngII via miR-29b-3p/Col1a1-Col3a1 *in vitro*. Pyroptosis is a newly discovered form of programmed cell death and has an important role in the progression of DCM alongside apoptosis. Of note, Yang *et al* (16) found that hsa_circ_0076631, also known as CACR, was increased in HG-induced AC16 cells and the serum of diabetic patients. Knockdown of CACR alleviated myocardial pyroptosis and inflammation via targeting miR-214-3p/caspase-1 in HG-induced AC16 cells. Only diabetic models were used in these studies, but it was not verified whether these mice also had DCM. It was reported that circRNA CDR1as was upregulated in DCM hearts in STZ-induced diabetic mice, which promoted cardiomyocyte apoptosis through activating the MST1-Hippo pathway *in vivo* and in HG-treated primary cardiomyocytes. Knocking down CDR1as was able to inhibit cardiomyocyte apoptosis in DCM. Thus, inhibition of CDR1as may become a potential therapeutic strategy for DCM (83).

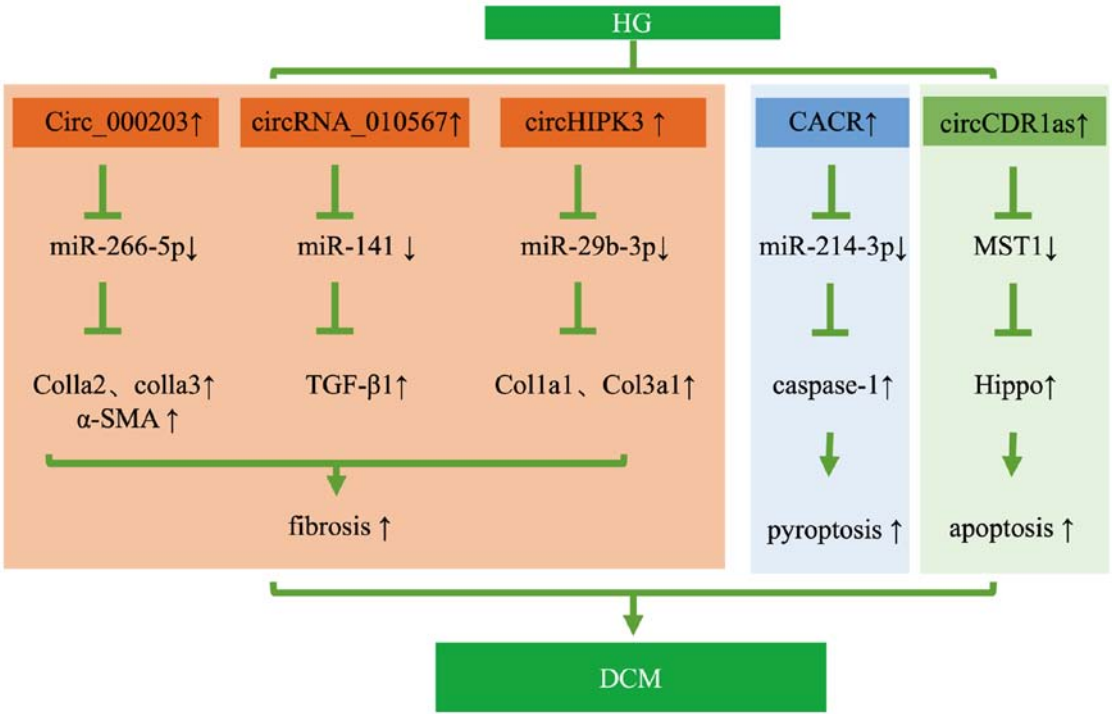

Figure 4. CircRNAs involved in DCM. The expression of circ_000203, circRNA_010567 and circHIPK3 was upregulated under HG conditions, which promoted the progression of DCM by aggravating myocardial fibrosis. Circ_0076631 (CACR) was increased in the HG environment, which accelerated the progression of DCM by enhancing cardiomyocyte pyroptosis. CircCDR1as was upregulated in DCM hearts of streptozotocin-induced diabetic mice, which promoted cardiomyocyte apoptosis. CACR, caspase-1-associated circRNA, circ_0076631; circRNA, circular RNA; circCDR1as, circular RNA cerebellar degeneration-related protein 1 antisense; circHIPK3, circRNA homeodomain interacting protein kinase 3; COL1A1, collagen type I α1 chain; DCM, diabetic cardiomyopathy; HG, high glucose; miR, microRNA; MST1, mammalian sterile 20-like kinase 1; TGF-β1, transforming growth factor-β 1; α-SMA, α-smooth muscle actin.

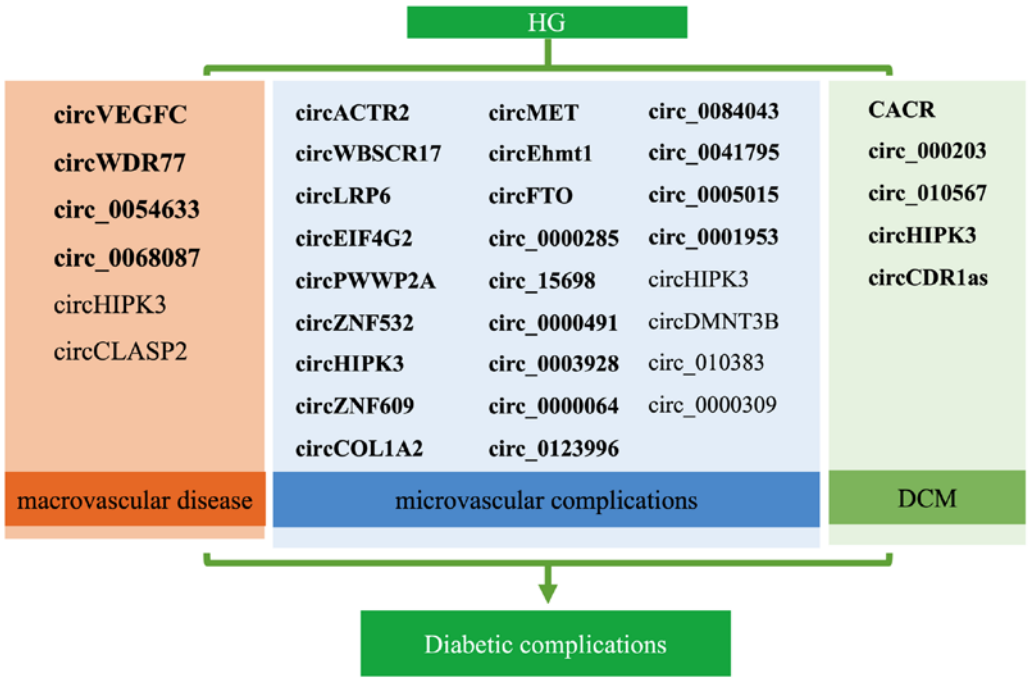

Figure 5. CircRNAs involved in diabetic complications. Bold font means upregulation, not bold means downregulation. CACR, caspase-1-associated circRNA, circ_0076631; circRNA, circular RNA; circACTR2, circRNA actin related protein 2; circCOL1A2, circRNA collagen type I α2 chain, circ_0081108; circCDR1as, circRNA cerebellar degeneration-related protein 1 antisense; circCLASP2, circRNA CLIP-associating protein 2; circDMNT3B, circRNA DNA methyltransferase 3 beta; circEhmt1, circRNA euchromatic histone lysine methyltransferase 1; circEIF4G2, circRNA eukaryotic translation initiation factor 4 gamma 2; circFTO, circRNA FTO α-ketoglutarate dependent dioxygenase; circHIPK3, circRNA homeodomain interacting protein kinase 3; circLRP6, circRNA LDL receptor related protein 6; circMET, circRNA MET proto-oncogene, receptor tyrosine kinase; circPWWP2A, circRNA PWWP domain containing 2A, circ_0000254; circVEGFC, circRNA vascular endothelial growth factor C; circWBSCR17, circRNA Williams-Beuren syndrome chromosome region 17, circ_0080425; circWDR77, circRNA WD repeat domain 77, hsa_circ_0013509; circZNF532, circRNA zinc finger protein 532, circ_0047814; circZNF609, circRNA zinc finger protein 609; HG, high glucose; DCM, diabetic cardiomyopathy.

The roles of circRNAs in ischemic heart disease have also been reported. CircRNA sodium/calcium exchanger 1 (circNCX1) was increased in the myocardial ischemia-reperfusion mouse model and silencing of circNCX1 in the heart attenuated myocardial fibrosis via action on miR-133a-3p (84). In addition, downregulation of circRNA actin α2 alleviated myocardial fibrosis via targeting miR-548f-5p and suppressing α-SMA expression in human aortic smooth muscle cells of coronary artery diseases (85). Furthermore, CircHIPK3 increased the expression of fibrosis-associated genes, such as COL1A2, COL3A1 and α-SMA, via sponging miR-29b-3p in AngII-induced mouse myocardium (86). Furthermore, circRNA nuclear factor I B (circNFIB) was decreased in cardiac fibrosis *in vivo* and *in vitro*, and upregulation of circNFIB attenuated cardiac fibrosis by directly targeting miR-433 in a mouse myocardial infarction model (87). Thus, overexpression or inhibition of circRNA may regulate gene expression and potentially become an effective method to improve cardiac function.

4. Conclusions

Increasing evidence indicates that certain circRNAs have crucial roles in the pathogenesis of diabetic complications through ceRNA mechanisms, acting as miRNA sponges. Due to their abundance and stability, circRNAs may serve as useful biomarkers or therapeutic targets for diabetic complications. An overview of circRNAs involved in diabetic complications is provided in Fig. 5. CircHIPK3 is the most well-studied circRNA in the field of diabetes and is most likely to become a biological marker and therapeutic target for diabetes and its complications. The present review indicated that the number of upregulated circRNAs was higher than that of downregulated circRNAs in diabetic complications both *in vivo* and *in vitro* (Fig. 5), implying that exploring the mechanisms of promoting DNA repair is important. Although circRNA-miRNA-mRNA axis mechanisms have been predicted in current studies, circRNAs as drugs may cause side effects or resistance. Despite these limitations, circRNAs have immense potential as therapeutic targets and stable biomarkers for diabetic complications.

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Authors' contributions

LY and JD searched and selected the literature, wrote the original draft, and contributed equally to this work. HZ conceived the study, and reviewed and edited the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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