



CircRNA–miRNA interactions in atherogenesis

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Abstract

Atherosclerosis is the major cause of coronary artery disease (CAD) which includes unstable angina, myocardial infarction, and heart failure. The onset of atherogenesis, a process of atherosclerotic lesion formation in the intima of arteries, is driven by lipid accumulation, a vicious cycle of reactive oxygen species (ROS)-induced oxidative stress and inflammatory reactions leading to endothelial cell (EC) dysfunction, vascular smooth muscle cell (VSMC) activation, and foam cell formation which further fuel plaque formation and destabilization. In recent years, there is a surge in the number of publications reporting the involvement of circular RNAs (circRNAs) in the pathogenesis of cardiovascular diseases, cancers, and metabolic syndromes. These studies have advanced our understanding on the biological functions of circRNAs. One of the most common mechanism of action of circRNAs reported is the sponging of microRNAs (miRNAs) by binding to the miRNAs response element (MRE), thereby indirectly increases the transcription of their target messenger RNAs (mRNAs). Individual networks of circRNA–miRNA–mRNA associated with atherogenesis have been extensively reported, however, there is a need to connect these findings for a complete overview. This review aims to provide an update on atherogenesis-related circRNAs and analyze the circRNA–miRNA–mRNA interactions in atherogenesis. The atherogenic mechanisms and clinical relevance of each atherogenesis-related circRNA were systematically discussed for better understanding of the knowledge gap in this area.

Keywords Atherogenesis · Atherosclerosis · Endothelial dysfunction · Circular RNA · microRNA

Introduction

Atherosclerotic cardiovascular diseases remain a leading cause of death globally. The presence of atherosclerotic plaque is the hallmark of coronary artery disease (CAD) [1, 2]. The development and progression of atherosclerosis involves cell adhesion, differentiation, migration, proliferation, and cell interaction with the extracellular matrix which are controlled by a complex network of cytokines and growth regulatory peptides [3–5]. The process of atherosclerotic lesion formation, atherogenesis, involves several sequential events and is initiated when low-density lipoproteins (LDLs) accumulate in the tunica intima and activate the endothelium. Apolipoprotein B100 containing-LDL transports high levels of circulating cholesterol through vascular endothelium,

which leads to retention of LDL within the blood vessel wall [6, 7]. Reactive oxidative species (ROS), myeloperoxidase, lipoperoxidase, and nicotinamide adenine dinucleotide phosphate (NADH/NADPH) induce lipid peroxidation on the trapped LDL and form oxidized LDL (ox-LDL) [8]. Ox-LDL induces endothelial activation by facilitating the production and secretion of inflammatory mediators (e.g., interleukin-8), adhesion molecules (e.g., vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and selectins), chemoattractants (e.g., platelets-activating factors), and monocyte chemoattractant protein-1 (MCP-1) that drive inflammation, recruitment, and adhesion of monocytes at the lesion site. Recruited monocytes transmigrate through intercellular junctions to the subendothelial space and differentiate into macrophages following the stimulation by macrophage-colony stimulating factor. Macrophages up-regulate scavenger receptors and mediate the internalization of modified or oxidized lipoproteins such as ox-LDLs, native lipoproteins, and anionic phospholipids and leads to the foam cell lesion formation [9, 10]. These triggers worsen inflammation in the vascular wall and attract more monocytes/macrophages to the subendothelial space in a vicious

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cycle. Inflammatory mediators from the endothelium and macrophages also convert the contractile phenotype of vascular smooth muscle cells (VSMC) into the active synthetic phenotype with increased proliferative and migration potentials. The accumulation of lipid-laden foam cells, as well as the parallel proliferation and migration of VSMC from tunica media to tunica intima, eventually lead to atherosclerotic plaque formation. Ox-LDL also induces matrix metalloproteinase-1 (MMP-1) and MMP-9 in vascular wall matrix causing degradation of extracellular collagen leading to weak and unstable atherosclerotic plaque.

Circular RNA (circRNA) is an emerging yet interesting class of non-coding RNA (ncRNA) in which a covalently closed circular loop is generated through the backsplicing of linear RNA. Although circRNA was first identified in the RNA viruses [11], it was regarded as an artifact of the splicing process for decades until a group of endogenous circRNAs was detected in the 1990s [12]. With the improvement in high throughput sequencing, biochemical analysis, and bioinformatic algorithms, circRNAs were later found to be ubiquitously expressed throughout the eukaryotic kingdom and recently, in viruses as well. Not only are circRNAs evolutionarily conserved across species, the expression of circRNAs is reported to be regulated in a tissue/organ or developmental stage-dependent manner with the majority being derived from protein-coding genes [13–15]. The abundance of circRNA is usually low, which is a hundred to a thousand times lower than the co-expressed linear RNA [16] and are predominantly localized in the cytoplasm [17–19]. Due to its circular structure that lacks 3' polyadenylation (poly-A) tail and 5' cap, circRNAs are immune to various cellular exonucleases. Although circRNAs are less abundant, they are more stable, some with a half-life of more than 48 h compared to its linear counterparts that have an average half-life of 10 h [17].

The roles played by circRNAs in human diseases such as cancers, diabetes, cardiovascular, and neurodegenerative diseases have been widely reported (reviewed by [20]). CircRNAs are expressed in vascular cells and are involved in the pathogenesis of cardiovascular diseases. In recent years, there is an increasing trend of published studies on the roles of various circRNAs in atherosclerosis. However, literatures detailing the interactions involving circRNAs, miRNAs, and mRNAs on the whole in driving atherosclerosis are limited. This review provides a summary of circRNAs involved in atherogenesis and categorized them according to their pro-atherogenic or atheroprotective effects for a clearer understanding of the biological functions of the various circRNAs identified thus far. We also attempted to investigate if there are common circRNA–miRNA–mRNA networks from these reports to provide a deeper understanding on the clinical significance of these micromanagers in cardiovascular diseases. In particular, we attempted to analyze the interactions of

circRNAs, miRNAs, and mRNAs that have been identified thus far via Cytoscape. A total of 12 circRNAs was identified and connected at a certain degree through their target miRNAs or mRNAs. This number is surprisingly low considering that numerous circRNAs and miRNAs have been reportedly involved in atherogenesis and cardiovascular diseases. Our analyses, hence, have highlighted the need for future studies to delineate a cohesive network of circRNA–miRNA–mRNA interactions to gain a more holistic view on the functional mechanisms of these micromanagers in the initiation and progression of atherosclerosis for better therapeutic design and development.

The endothelium

The endothelium is a monolayer of endothelial cells (EC) which forms the inner cellular lining of the blood vessels and lymphatic system in the body. The vascular endothelium acts as a barrier between circulating blood cells and the underlying tissues. It is selectively permeable and important in maintaining vascular homeostasis balance by regulating the exchange of nutrients, fluids, gases, and waste products between the surrounding cells or tissues and flowing blood. The endothelium controls the degree of vascular relaxation and constriction, detects and reacts to blood-borne signals and changes in hemodynamic forces. It regulates vascular tone by secreting vasodilators such as nitric oxide (NO) and prostacyclin (PGI₂) in response to hormones and physical stimulus such as blood flow-induced shear stress [21, 22]. EC also produces vasoconstrictors such as angiotensin II (Ang II), endothelin, superoxide, and thromboxane A₂ (TxA₂) [23].

In addition, the endothelium is important in maintaining the balance of anti-thrombotic and anti-coagulant activities via the Protein C anti-coagulant pathway and prevents thrombosis in order to ensure normal blood flow [24]. Upon endothelial inflammation or injury, the anti-coagulant pathway is suppressed where both plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) are secreted into the bloodstream [25]. Clot formation is allowed in an injured site but still maintains blood fluidity in the surrounding area [23]. Production of NO also prevents adhesion and activation of platelet and leukocyte to the vessel wall (Fig. 1) through both cyclic guanosine monophosphate (cGMP)-dependent and -independent (endothelial-platelet interaction) [26, 27] and ICAM-1-dependent (endothelial-leukocyte interaction) [28] mechanisms.

Endothelial dysfunction

Endothelial cell dysfunction is a key event in the initiation of atherosclerotic process and is characterized by the modification of the endothelial phenotype from a quiescent state

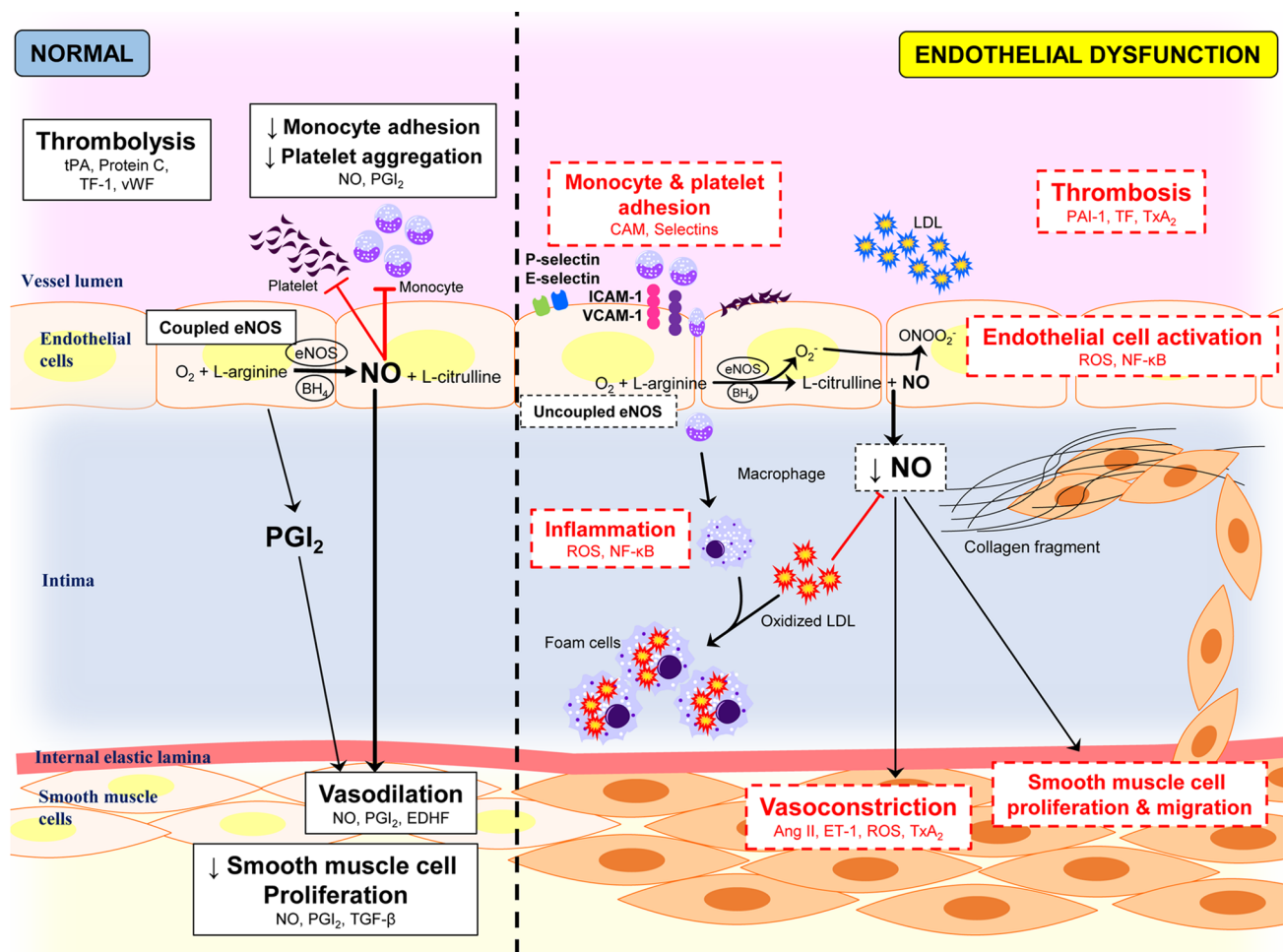


Fig. 1 The comparison of a normal and dysfunctional endothelium. Endothelial dysfunction is characterized by reduced nitric oxide (NO) bioavailability, increased reactive oxygen species (ROS), endothelin-1 (ET-1), and cell adhesion molecule (CAM) production and the imbalance of vasodilators and vasoconstrictors which favor a pro-inflammatory, proliferative and pro-coagulatory states that contribute to atherogenesis. *Ang II* Angiotensin II; *EDHF* endothelium-derived hyperpolarizing factor; *eNOS* endothelial nitric oxide syn-

thase; *ICAM-1* intercellular adhesion molecule-1; *LDL* low-density lipoprotein; *NO* nitric oxide; *NF- κB* nuclear factor-kappa B; *ONOO₂* peroxynitrite; *PGI₂* prostacyclin; *TGF- β* transforming growth factor β ; *TF-1* transcription factor 1; *tPA* tissue plasminogen activator; *TxA₂* thromboxane A2; *VCAM-1* vascular cell adhesion molecule; *vWF* von Willebrand factor. The figure was created with Motifolio Toolkit (Motifolio Inc, Ellicott City, MD)

(non-atherogenic/non-thrombogenic) to an activated state (atherogenic/thrombotic) in response to pathophysiological changes. The endothelium is incapable of maintaining vascular homeostasis balance in an activated state [29]. Dysfunctional endothelium is characterized by the reduction in production or availability of NO and/or an imbalance in the relative contribution of endothelium-derived relaxing factors (EDRF) (e.g., NO and PGI_2) and endothelium-derived contracting factors (EDCF) (e.g., TxA_2 , endothelin-1 (ET-1), and Ang II) [30] (Fig. 1). Endothelium in the quiescent state is mainly maintained by NO and laminar shear stress [31]. NO is generated from L-arginine via endothelial nitric oxide synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin (BH_4) and nicotinamide adenine dinucleotide phosphate (NADPH). Reduction of NO bioavailability

may be caused by reduction of eNOS expression, impaired eNOS activation, or NO inactivation by oxidative stress.

Nitric oxide is an important vasodilator for balancing the vasodilation and vasoconstriction in the endothelium and diminished NO bioavailability is one of the major causes leading to vasoconstriction resulting in the lack of VSMC relaxation. NO diffuses to the VSMC and stimulates guanylate cyclase and leads to cGMP-mediated cell relaxation and subsequently vasodilation [32, 33]. Activation of cGMP-dependent protein kinase (cGK) by NO in VSMC inhibits VSMC proliferation, migration, and fibrotic gene expression [34]. Reduced NO bioavailability will lead to the proliferation and migration of VSMC and thus accelerates vascular hypertrophy and finally culminates in endothelial dysfunction [35–37]. In addition, reduction in NO bioavailability

also inhibits platelet aggregation and contributes to thrombosis [38]. Upon vascular injury, inflammation, or mechanical perturbation, decreased NO level promotes the secretion of selectin family proteins (P-selectin, E-selectin, and L-selectin) which recruit and induce both platelet and leukocyte adhesion to endothelial surface before transmigrating to damaged tissue site from the blood vessels [39]. These endothelial modifications promote inflammation within the vessel wall, setting the stage for the initiation, and progression of an atherosclerotic lesion.

Endothelial activation by oxidative stress and inflammation

Apart from endothelium-dependent vasodilation impairment, EC dysfunction is also characterized by “endothelial activation” which comprises a milieu of pro-inflammatory, proliferative, and pro-coagulatory activations that favor all phases in atherogenesis [40, 41]. EC activation is characterized by the increment of the expressions of cell-surface adhesion molecules, such as ICAM-1, VCAM-1, and endothelial leukocyte adhesion molecule (E-selectin) which convert quiescent EC to migratory and proliferative phenotypes, and differentiates into an angiogenic phenotype [7]. Pro-inflammatory cytokines such as tumor necrosis factor α (TNF- α) and interleukin-6 (IL-6) activate EC and promote the recruitment and attachment of circulating leukocytes to the vessel wall [42].

The vascular endothelium is a major target of oxidative stress. Typical atherosclerotic risk factors such as aging, diabetes mellitus, hypertension, hyperglycemia, hypercholesterolemia, and smoking have been linked to the increased production of ROS in cells [43, 44]. Oxidative stress refers to the imbalance between ROS production and the efficacy of antioxidant system in the body. ROS such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), nitric oxide (NO \cdot), and peroxynitrite (ONOO $^-$) and extrinsic factors such as environmental pollution, UV light, radiation or high-glucose and high-fat food intake, induce oxidative stress and tissue damage through glycoxidation and advanced glycation end-products (AGE) formation [45]. This can trigger the production of oxidants which reduce the level of antioxidants and lead to an imbalance in the redox potential of cells. ROS activated-NADPH oxidase reduces O_2 to O_2^- and leads to the production of ONOO $^-$. Highly reactive ONOO $^-$ inactivates eNOS, reduces NO bioavailability, and leads to endothelial dysfunction [45]. In addition, ox-LDL increases synthesis of caveolin-1, which binds to calmodulin to inhibit eNOS activity and thus reduces the production of NO [46]. The disruption of redox balance in EC under oxidative stress results in the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant responsive

element (ARE) pathway which targets multiple ARE-regulated antioxidants, regulates the expression of antioxidative enzymes, such as heme oxygenase-1 (HO-1) [47]. Nrf2 plays an important role in atherosclerosis resistance by targeting multiple ARE-regulated antioxidants.

Plasma and macrophage content of ox-LDL in coronary plaques are well associated with the severity of acute coronary syndrome. Formation of ox-LDL is mediated by ROS in the subendothelial space and serves as a major trigger of atherogenesis. Binding of ox-LDL to the lectin-like oxidized LDL receptor-1 (LOX-1) activates nuclear factor-kappa B (NF- κ B) in EC [48]. Activation of NF- κ B induces the production of pro-inflammatory cytokines (e.g., TNF- α and IL-6), chemotactic factors, and adhesion molecules which then leads to inflammation and infiltration of immune cells at lesion sites, whereas NF- κ B in monocytes facilitates inflammatory gene expression and converts macrophages into foam cells [49]. Phagocytosis of ox-LDL by macrophages via scavenger receptor amplifies and retains ox-LDL. Accumulative formation of ox-LDL in the atherosclerotic lesions acts as potent stimulus for ROS production within the vascular wall and further propagates oxidative stress at atherosclerotic plaques producing inflammation in a vicious cycle [50].

VSMC activation

Progression of atherosclerotic plaque formation continues with the accumulation of lipid and lipid-engorged cells. Proliferation, migration, and synthesis of collagen by VSMC facilitate the evolution of fatty streak toward a complex lesion. The activities of VSMC are stimulated by the continuous release of cytokines such as MCP-1 by activated EC and T cells [51]. Expansion of the atherosclerotic lesion within coronary arteries results in lumen obstruction and hence reduces blood flow which drive angina in clinical condition. Other than macrophages, T cells also localize within the lesions and play a role in the growth of atherosclerotic plaque. Activated T cells secrete interferon (IFN)- γ , a pro-inflammatory cytokines which limits the synthesis of new collagen required for fibrous cap preservation [52] and secretion of transforming growth factor β (TGF- β) restricts the inflammation and VSMC proliferation [53]. Accumulation of ox-LDL causes apoptosis of macrophages and VSMC, forming the necrotic core of the advancing atheroma [54], whereas inhibition of lipoprotein-associated phospholipase A_2 reduces macrophage death [55, 56]. The death of macrophage foam cells leads to lipid spillage, further promoting inflammation [57], while VSMC death further reduces collagen synthesis and promotes fibrous cap thinning [58]. The thinning of the fibrous cap is enhanced by the overexpression of MMPs, interstitial collagenases, and gelatinases, which

degrade supportive collagen [59]. MMP overexpression and activation within the plaque are mediated by IL-1 β , TNF- α , ox-LDL, and CD40L. Once the fibrous cap is weakened, the plaque is vulnerable to rupture, precipitating acute thrombotic complications.

Circular RNAs

CircRNAs are generated when an upstream splice acceptor is covalently joined to a downstream splice donor and can be classified into 3 major subtypes based on their compositions, which are exonic circRNA (ecircRNA), intronic circRNA (ciRNA), and exon–intron circRNA (EIciRNA) [60]. Nevertheless, circRNA can also be derived from the intron of transfer RNA (tricRNA), intergenic region, and anti-sense RNAs. To date, three models have been proposed for circRNA biogenesis mechanisms which are RNA-binding protein (RBP)-mediated circularization, intron pairing-driven circularization, and lariat-driven circularization (Fig. 2). During RBP-mediated circularization, RBPs bind to specific sequence motifs on the upstream and downstream introns flanking the exons of a linear pre-mRNA in a *trans* manner, followed by dimerization of the RBPs to facilitate backsplicing. For example, Quaking (QKI) can bring the circle-forming exons into close proximity through binding to available

intronic QKI binding motifs [13] while immune factors NF90/NF110 [61] and RNA editing enzyme, ADAR1 [62], both can regulate the expression of circRNAs through stabilizing and destabilizing the intronic RNA pairing step of the circularization process, respectively. On the other hand, the presence of inverted complementary sequences in flanking intronic regions (e.g., *Alu* repeats) may promote alternative circularization through intron pairing-driven circularization model, resulting in the generation of various circRNAs [17]. For example, the presence of ~400 bp long inverted repeats flanking the mouse *Sry* gene leads to the formation of the circ*Sry* in HEK 293 EBNA cells [63]. In humans, Ivanoc et al. revealed that 88% of human circRNAs contain complementary *Alu* elements (~300 nt long) in their flanking introns which could facilitate exon circularization [64]. In some cases, relatively short (30–40 nt) inverted repeats are sufficient for intron pairing-driven circularization [65]. The third circRNA biogenesis model, lariat-driven circularization involves an exon-skipping event during pre-mRNA transcription. The process begins with canonical splicing, resulting in the production of linear RNA with skipped exons within a long intron lariat. Subsequently, internal splicing facilitates the removal of flanking intronic sequence, allowing the generation of ecircRNA [66]. Whereas retention of the intronic sequences results in the production of EIciRNAs, having a consensus motif containing a 7 nt GU-rich

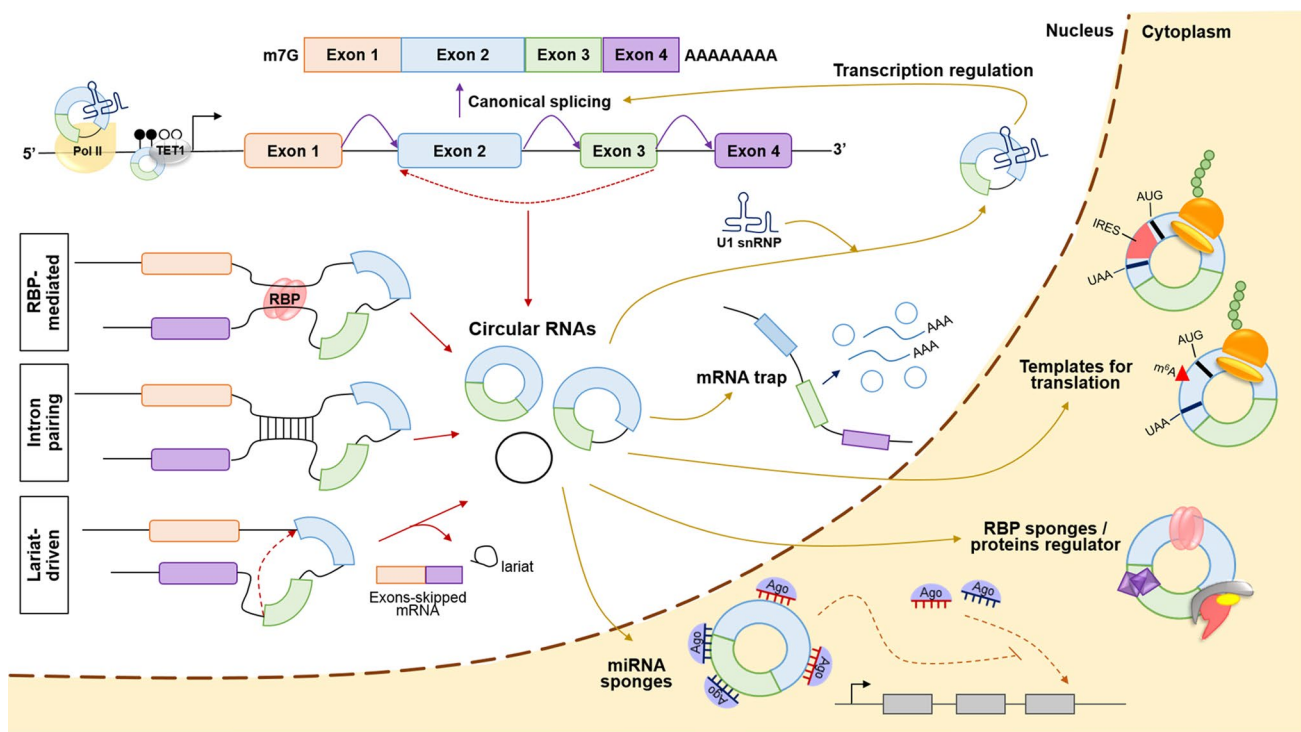


Fig. 2 Biogenesis and functions of circRNAs. CircRNAs can be derived through RNA-binding proteins (RBP)-mediated circularization, intron pairing and lariat-driven circularization. Their roles

include sponging of miRNAs and RBP, as regulators of parental gene through *cis* or *trans*-actions, as modulators of protein–protein interactions, and as templates for protein translation

element near the 5' splice site and an 11 nt C-rich element close to the branchpoint site would facilitate the generation of ciRNAs [67].

Emerging evidence reveals that circRNAs have different biological functions (Fig. 2), but the mechanism behind most of these functions remains unclear. MicroRNA (miRNA) sponge is the most reported role of circRNAs in many studies. MiRNAs can regulate protein production through transcriptional repression of messenger RNAs (mRNAs). With the miRNA response elements (MRE) on the circRNA sequence, circRNA are able to sponge and sequester away the miRNAs, indirectly regulating the miRNA-target genes that form part of the complex competing endogenous RNA (ceRNA) network [68]. A prominent example is the cytoplasmic CDR1 antisense (CDR1as), also known as ciRS-7 which contains miR-7 and miR-671 binding sites [69–71]. MiR-7 is a well-known regulatory molecule that functions as a tumor suppressor by repressing the expression of multiple oncogenes, hence, binding of miR-7 on ciRS-7 promotes tumorigenesis. The perfect complementarity binding of miR-671 on ciRS-7 on the other hand, will cause the slicing of ciRS-7. CircRNAs could also be a risk factor in disease development as well, for example circ*TMEM45A* is a reported oncogenic circRNA that promotes hepatocellular carcinoma through the circ*TMEM45A*/miR-665/IGF2 axis [72]. It is possible for circRNA to act as an RBP sponge or RBP assembly platform that may have a role in disease development [73]. Mapping of RBPs onto human circRNAs by Dudekula et al. indicated that many circRNAs could bind different RBPs for example, EIF4A3, HuR, and FMRP either at the body or backsplice junctions [74]. Binding of HuR to circ*PABPN1* (previously known as hsa_circ_0031288) suppressed the translation of PABPN1 by preventing the binding of HuR to linear *PABPN1* mRNA [75]. In addition, Fang and team reported a nuclear-retained circ*FAT1(e2)* acts as a tumor suppressor that specifically interacts with an RBP, namely YBX1 to prevent tumorigenesis in gastric carcinoma [76].

Other than being sponges, circRNA could regulate the expression of parental gene through *cis*- or *trans*-actions. For instance, nuclear-retained circ*EIF3J* and circ*PAIP2* regulate transcription of its parental genes by binding to U1 snRNP through RNA-RNA interaction. Such complexes will further interact with the Pol II transcription complex at the promoters of their parental genes to enhance gene expression [77]. In addition, Chen et al. demonstrated that a circRNA, circ*FECRI* derived from *FLII* gene promotes breast cancer metastasis through epigenetic regulation mechanism [78]. Circ*FECRI* binds to its parental gene in *cis* manner and recruits TET1 demethylase to induce DNA demethylation of *FLII* promoter. Concurrently, circ*FECRI* also interacts with DNMT1 promoter in a *trans* manner that results in *DNMT1* silencing.

CircRNAs can also act as an mRNA trap, in which formation of circRNA competes with the linear mRNA splicing process. A good example of the mRNA trap is circ*Mbl*, which contains MBL binding sites on its flanking introns, thus it is strongly and specifically bound by MBL proteins [79]. When the MBL protein is in excess, it binds to the *Mbl* pre-mRNA and favors the biosynthesis of circ*Mbl* through backsplicing, in turn reduces the production of the parental mRNA.

On the other hand, circRNAs are able to bind, store, sort, and sequester proteins to particular subcellular locations, and act as dynamic scaffolding molecules that modulate protein–protein interactions. Circ*FOXO3*, is a circRNA that is negatively correlated to cancer by regulating protein in cells. Circ*FOXO3* interacts with cyclin-dependent kinase 2 (CDK2) and CDK inhibitor p21, forming a ternary complex. Such complex enhances the inhibitory effect of p21 on CDK2 preventing the formation of cyclin E/CDK2 complex which is essential for G1-S transition [80]. Apart from acting as a scaffold, circ*FOXO3* is also able to alter subcellular localization of proteins by sequestration. Circ*FOXO3* sequesters several proteins that are involved in cell survival (e.g., ID1 and E2F1) and stress response (e.g., FAK and HIF-1 α) pathways to co-localize in the cytoplasm [81]. Sequestration by circ*FOXO3* prevents nuclear translocation of ID-1, E2F, and HIF-1 α , as well as mitochondrial translocation of FAK, which in turn leads to cellular senescence.

Based on its circular structure, circRNA was initially presumed to be untranslated. By performing ribosome footprinting, a subgroup of circRNAs was found to be translated into protein due to its association with translating ribosomes. CircRNA translation initiation is cap-independent and mediated by IRESs and/or m⁶A motifs [82, 83]. Two of the best examples are the muscle-enriched circ*ZNF609* and liver cancer-associated circ *β -catenin*. Both of them contain an open reading frame spanning from the start codon that is similar with their linear mRNA and terminating at the stop codon that was created upon circularization [84, 85]. Translated circ*ZNF609* was reported to be involved in controlling the proliferation of myoblast [84], and positively associated with rhabdomyosarcoma [86]. On the other hand, a small peptide translated from circ *β -catenin* acts as a decoy for GSK3 β -induced β -catenin phosphorylation and degradation to protect full-length β -catenin, resulting in Wnt- β -catenin pathway activation [85].

Due to the involvement of circRNAs in modulating signaling pathways that play important roles in human diseases, circRNAs are being extensively studied today to identify clinically relevant circRNAs as potential pharmacological targets and disease markers. The roles of circRNAs have been summarized by Verduci et al. [20] and here we present an update of circRNAs involved in endothelial dysfunction and vascular injury leading to atherosclerosis and other

vascular-related diseases and complications (Tables 1, 2, 3, Fig. 3).

CircRNAs that promote endothelial injury

Common cellular models of oxidative stress-induced vascular endothelium injury include treatment of EC lines such as human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs) with ox-LDL or high glucose (HG). Ox-LDL contributes to the pathogenesis of atherosclerosis and atherosclerotic plaque rupture by promoting lipid accumulation, pro-inflammatory responses and apoptosis of EC and VSMC as documented in vivo [87]. In vitro, ox-LDL induces HUVECs proliferation at low concentrations (5 to 10 $\mu\text{g}/\text{mL}$) and apoptosis at higher concentrations (10 to 300 $\mu\text{g}/\text{mL}$) and is mediated by O_2^- formation, with NADPH oxidase as the major source for O_2^- [88]. In many of the studies reviewed, EC injury is induced using high concentrations of ox-LDL, and then EC health were measured by determining proliferation, migration, tube formation, and apoptosis in vitro. EC dysfunction protective or worsening effect of circRNAs is based on whether the overexpression or knockdown of a particular circRNA worsens or improves EC injury by these measures. On the other hand, hyperglycemic condition produces advanced glycation end products that initiate oxidative reactions and induce vascular hyperpermeability. Using these in vitro models, a number of circRNAs were found upregulated in oxidative stress-induced EC injury (Fig. 3, Table 1).

Circ_USP36 (circ_0003204)

Circ_USP36 (chr17:76,798,405–76,800,060) is derived from exon 16 and 17 regions within ubiquitin specific peptidase 36 (USP36) locus. The expression of circ_USP36 is reported to be elevated in HUVECs exposed to ox-LDL, whereby, it aggravates ox-LDL-induced EC injury [89, 90]. Its downstream target, miR-942-5p regulates histone deacetylase 9 (HDAC9) which promotes aortic calcification in mice and human aortic smooth muscle contractility [91]. Increased HDAC9 is associated with MMP1 and MMP2 expression in pro-inflammatory macrophages of human carotid plaque [92]. It was also shown that HDAC9 mediates ox-LDL-induced apoptosis and inflammatory reactions [90, 93].

MiR-942-5p also targets *HMGB1*, which stimulates pro-inflammatory cytokine synthesis of IL-1 β , IL-6, and TNF- α in human monocytes and macrophages [94]. In addition to miR-942-5p, circ_USP36 also sponges miR-330-5p which regulates *TLR4* [95]. TLR4 activates NF- κ B-mediated inflammatory response [96] and has been

associated with the initiation and progression of atherosclerotic disease [97].

Circ_USP36 targets miR-98-5p [98] which has an endothelial protective role. MiR-98-5p has been shown to protect brain endothelium and improves neurological outcomes in mouse ischemia/reperfusion stroke model [99] and inhibits HUVECs apoptosis by targeting *caspase-3*, *MAPK6*, *LOX-1* [100–102]. Moreover, miR-98-5p also directly targets *VCAM-1*, an adhesion molecule that is highly expressed in atherosclerosis and *HMGB1*, a DNA binding protein that produces pro-inflammatory response [103]. In addition, circ_USP36 also mediates ox-LDL-induced cell cycle arrest, apoptosis, and inflammatory response in HUVECs by targeting miR-20a-5p which directly binds to Rho-associated coiled-coil kinase 2 (*ROCK2*). The inhibitory effects were reversed upon circ_USP36 knockdown, supporting its role in mediating EC dysfunction and inflammatory response [104].

Circ_0004104

Circ_0004104 was found significantly up-regulated in CAD patients compared to healthy controls [108]. Circ_0004104 overexpression resulted in increased pro-atherosclerotic genes expression such as indoleamine 2,3-dioxygenase 1 (*IDO1*), *MMP8*, soluble CD40 and decreased anti-atherosclerotic gene expression, apolipoprotein AI (*ApoA I*) in THP-1-derived macrophages [108]. In ox-LDL-induced EC injury model, circ_0004104 level was also up-regulated together with increased oxidative stress and released of pro-inflammatory mediators TNF- α and IL-1 β . These effects were mediated by sponging miR-328-3p and in turn increased *TRIM14* [109]. *TRIM14* has been shown to promote endothelial activation by activating NF- κ B signaling [110]. Indeed, another study has shown that miR-328-3p by targeting *FOXO4* can protect HUVECs against ox-LDL induced injury. The suppression of miR-328-3p led to the overexpression of *FOXO4* and decreased cell viability, migration and invasion and induced apoptosis and inflammation [111]. It was also reported that miR-328-3p is also a target of circHIPK3 where it drives the pathogenesis of pulmonary arterial hypertension via the miR-328-3p-*STAT3* axis [112].

Circ_0124644

Circ_0124644 was found significantly up-regulated in the peripheral blood of CAD patients compared to healthy subjects and appears to have relatively high specificity and sensitivity in diagnosing CAD [113]. Subsequently, its pro-atherogenic role was demonstrated by mediating EC injury through miR-149-5p/*PAPP-A* axis [114]. The

Table 1 CircRNAs in endothelial injury

No.	CircRNA	Study model	Inducing factor	Expression	Pathways	Effects	References
1	Circ_ <i>USP36</i> (hsa_circ_0003204)	HUVECS	Ox-LDL	Up	miR-98-5p/ <i>VCAM1</i> axis	Promotes apoptosis, Increases leukocyte adhesion	[98]
		HUVECS	Ox-LDL	Up	miR-942-5p/ <i>HDAC9</i>	Increases oxidative stress and inflammatory cytokines	[90]
		THP cells	Ox-LDL	Up	miR-330-5p/ <i>TLR4</i>	Promotes apoptosis, oxidative stress, and inflammatory response via NF-κB signaling	[95]
		HUVECS	Ox-LDL	Up	miR-98-5p/ <i>caspase-3, MAPK6, LOX-1</i>	Promotes apoptosis	[100–102]
		HUVECS	Ox-LDL	Up	miR-20a-5p/ <i>ROCK2</i>	Promotes apoptosis, increases oxidative stress, and inflammatory cytokines	[104]
2	Circ_0004104	HUVECS	Ox-LDL	Up	miR-328-3p/ <i>TRIM14</i>	Promotes apoptosis, inflammation, and oxidative stress	[109]
3	Circ_0124644	HUVECS	Ox-LDL	Up	miR-149-5p/ <i>PAPP-A</i> axis	Promotes apoptosis and inhibits proliferation	[114]
4	Circ_ <i>RELL1</i> (circ_0002194)	HUVECS	Ox-LDL	Up	miR-6873-3p/ <i>MyD88/NF-κB</i>	Promotes inflammation	[119]
5	Circ_ <i>NOL12</i>	HUVECS	Ox-LDL	Up	miR-6873-3p/ <i>FRS2</i>	Promotes inflammation, apoptosis, and oxidative stress	[120]
6	Circ_ <i>BPTF</i> (circ_0045462)	HUVECS	High glucose	Up	miR-384/ <i>LIN28B</i>	Promotes apoptosis, inflammation, and oxidative stress	[123]
7	Circ_0068087	HUVECS	High glucose	Up	miR-197/ <i>TLR4/NF-κB/NLRP3</i>	Promotes inflammation	[131]
			Ox-LDL	Up	miR-186-5p/ <i>ROBO1</i>	Promotes apoptosis, inflammation, and oxidative stress	[133]
8	Circ_ <i>VEGFC</i> (circ_0071465)	HUVECS	High glucose	Up	miR-338-3p/ <i>HIF-1α/VEGFA</i>	Promotes apoptosis	[135]
9	CZNF609	HUVECS	High glucose	Up	miR-615-5p/ <i>MEF2A</i>	Inhibits proliferation, tube formation, migration, promotes apoptosis and oxidative stress	[141]
		Mouse diabetic retinopathy	High glucose	Up		Increases retinal vascular leakage, capillary degeneration	
		Mouse retinopathy	Oxygen-induced retinopathy	Down		Decreases retinal vessel loss	
10	Circ_ <i>AFF1</i>	HUVECS and human brain endothelial cells (HBEC-5i)	CoCl ₂ induced hypoxic conditions	Up	miR-516b/ <i>SAV1/YAP1</i> axis	Inhibits tube formation, promotes apoptosis	[144]
11	Circ_0010729	HUVECS	Hypoxia	Up	miR-186/ <i>HIF-1α</i>	Inhibits proliferation and migration	[149]

Table 1 (continued)

No.	CircRNA	Study model	Inducing factor	Expression	Pathways	Effects	References
12	Circ_ADAM9	Endothelial progenitor cells (EPC) Diabetic mice	High glucose	Up	miR-20a-5p	Promotes autophagy apoptosis	[153]
13	CZBTB44 (circ_0002484)	Choroid-retinal endothelial RF/6A cells Choroidal neovascularization (CNV) mouse model	Laser-induced CNV in mice	Up	miR-578-VEGFA/VCAM1	Promotes pathological neovascularization	[155]
14	CircHECW2	Human brain microvascular endothelial cells (HBMECs) Mice	LPS	Up	miR-30d/ATG-5	Promotes endothelial-mesenchymal transition (EndMT)	[158]

role of miR-149-5p in CAD is also well established clinically, whereby, its plasma level is down-regulated in CAD patients [115, 116]. MiR-149-5p is also shown to reduce the production of pro-inflammatory mediators, including NF- κ B, TNF- α , and IL-6 in macrophages [117]. The role of miR-149-5p in vascular injury was first identified in HG-induced endothelial dysfunction by targeting TNF- α , resulting in decreased levels of ET-1, vWF, and ICAM-1 and increased level of NO and eNOS expression and reduced apoptosis [118].

Circ_RELL1 (circ_0002194) and circ_NOL12

In ox-LDL-induced EC inflammation, circ_RELL1 was significantly up-regulated compared to the untreated cells, whereas, knockdown of circ_RELL1 reduced the expression of adhesion molecules ICAM1 and VCAM1. Circ_RELL1 also sponges miR-6873-3p, resulting in the up-regulation of its target gene, *MyD88* (myeloid differentiation primary response 88) which activates NF- κ B and promotes inflammatory response [119]. MiR-6873-3p is also a target of circ_NOL12, which is derived from nucleolar protein 12 (*NOL12*) gene. Circ_NOL12 expression was increased following ox-LDL induced HUVECs injury. Overexpression of circ_NOL12 inhibited miR-6873-3p expression in HUVECs and decreased HUVECs viability, oxidative stress and inflammatory response [120]. Under the regulation of circ_NOL12, the effects of miR-6873-3p, which are mediated by targeting fibroblast growth factor receptor substrate 2 (*FRS2*) regulate cell proliferation via MAPK signaling [121]. In addition, the miR-6873-3p/*FRS2* axis is also modulated by lncANRIL in the progression of atherosclerosis [122]. Collectively,

these studies revealed that several circRNAs can target the same miRNA and produce various outcomes depending on the target mRNA.

Circ_BPTF (circ_0045462)

EC dysfunction is also a diabetes-related vascular complication. Hence, a HG-induced vascular inflammatory cellular model is used to study endothelial dysfunction. Circ_BPTF is up-regulated in HG-induced oxidative stress in HUVECs, but its knockdown improves cell viability and suppresses cell apoptosis, the release of pro-inflammatory cytokines and oxidative stress. Circ_BPTF mediates these effects by sponging miR-384, which in turns up-regulates *LIN28B*. MiR-384 has been reported to play a protective role on EC [123]. In the middle cerebral artery occlusion mouse model, overexpression of miR-384 increases the proliferation and angiogenesis of endothelial progenitor cells (EPC) [124]. MiR-384 has also been shown to be protective against retinal neovascularization caused by ischemia in microvascular impairment of diabetic retinopathy mice model [125]. The effects of miR-384 is mediated through targeting Lin-28 homolog B (*LIN28B*), Delta-like ligand 4 (*DLL4*), and hexokinase 2 (*HK2*). *LIN28B* up-regulation was previously shown to induce angiogenesis in human retinal EC and promotes diabetic retinopathy progression [126]. *DLL4* is an endothelium-specific Notch ligand that is involved in vascular development and angiogenesis [127] and a high expression of *DLL4* is associated with activation of the Notch signaling pathway and suppression of EC growth [128]. *HK2* is a key enzyme of glucose metabolism [129] and decreased *HK2* expression in coronary EC of type 2 diabetes mice leads to an increase in mitochondrial calcium

Table 2 CircRNAs protective of endothelial dysfunction

No.	CircRNA	Study model	Inducing factor	Expression	Pathways	Effects	References
1	Circ <i>HIPK3</i>	HUVECS	Ox-LDL	Down	miR-124/ <i>STAT3</i> / SphK1	Inhibits apoptosis	[160]
		HUVECS	Ox-LDL	Down	miR-190b/ <i>ATG7</i>	Promotes autophagy	[161]
		HAECs	N/A	Overexpression vector	mir-133a/ <i>CTGF</i>	Promotes proliferation, tube formation, and migration	[162]
		Cardiac micro- vascular EC (CMVECs)	H ₂ O ₂	Internalization of exosomal circH- IPK3	miR-29a/ <i>IGF-1</i>	Inhibits apopto- sis and oxidative stress	[163]
		Diabetic retina and retinal endothelial cells	Streptozotocin- induced diabetic mice	Up	miR-30a-3p/ <i>VEGF-</i> <i>c</i> , <i>FZDA</i> and <i>WNT2</i>	Promotes prolif- eration, tube formation, and migration	[164]
2	Circ <i>ANRIL</i>	Pulmonary artery endothe- lial cells (hPAECs)	Platelet-derived growth factor	Up	miR-328-3p/ <i>STAT3</i>	Promotes prolif- eration, tube formation, and migration	[112]
		Human VSMCs, macrophages	CAD patients	Up-regulated	<i>PES1</i>	Promotes apoptosis	[170]
		Rat EC	High fat diet-treated rats	Overexpression vector	N/A	Inhibits oxidative stress and inflam- mation, reduces endothelial injury	[172]
3	Circ <i>DNMT3B</i>	Rat coronary arteries	High fat diet-treated rats	Overexpression vector	N/A	Promotes endothe- lial apoptosis and inflammation	[173]
		Human retinal microvascular endothelial cells (HRM- VECs)	High glucose	Down	miR-20b-5p/ <i>BAMBI</i>	Inhibits prolif- eration, migration, and tube forma- tion	[174]
4	Circ <i>DLGAP4</i>	HUVECs C57 mice	Ischemia/reperfu- sion (I/R) injury	Down	miR-143/ <i>HECTD1</i>	Inhibits endothe- lial mesenchy- mal transition (EndMT)	[255]
5	Circ_0058092	Endothelial progenitor cells (EPC)	High glucose	Down	miR-217/ <i>FOXO3</i>	Protects EPC against HG- induced damage by preserving cell survival, prolif- eration, migration, and angiogenic differentiation	[185]
6	Circ_0003575	Aortic EC	Apo-E knocked down mice	Down	<i>FOXO3</i> , <i>FOXO4</i>	Promotes pro- liferation and angiogenesis	[190]
7	Circ <i>RSF1</i> (circ_0000345)	HAECs	High glucose	Down	miR-758/ <i>CCND2</i> axis	Promotes prolif- eration, tube formation, migra- tion, and inhibits apoptosis	[192]
		HUVECs	Ox-LDL	Down	miR-135b- 5p/ <i>HDAC1</i>	Inhibits EC apopto- sis and inflam- mation	[195]

Table 2 (continued)

No.	CircRNA	Study model	Inducing factor	Expression	Pathways	Effects	References
8	Circ_ <i>CLASP2</i> (circ_0064772)	HUVECs	High glucose	Down	miR-140-5p/ <i>FBXW7</i>	Promotes proliferation and inhibits apoptosis	[198]
9	Circ_0054633	HUVECs	High glucose	Up	miR-218/ <i>ROBO1</i> and <i>HO-1</i>	Promotes proliferation, migration, tube formation, inhibits apoptosis	[203]

Table 3 CircRNAs that regulate VSMC and foam cell formation

No.	CircRNA	Study model	Inducing factor	Expression	Pathways	Effects	References
1	Circ_ <i>NRG-1</i>	Mouse aortic smooth muscle cells (MASMC)	N/A	Down	miR-193b-5p/ <i>NRG-1</i>	Inhibits apoptosis	[210]
2	Circ_0010283	VSMC	ox-LDL	Up	miR-370-3p/ <i>HMGB1</i>	Promotes VSMCs viability and migration	[212]
3	Circ_ <i>CHFR</i> (circ_0029589)	VSMC	ox-LDL	Up	miR-370/ <i>FOXO1</i>	Promotes proliferation, migration, and inflammation of VSMC	[214]
					miR-214-3p <i>Wnt3</i> /β-catenin	Promotes migration and inflammation of VSMC	[215]
4	Circ_ <i>SIRT1</i> (circ_0093887)	VSMC	TNF-α or PDGF-BB	Up	miR-132/212/ <i>SIRT1</i>	Inhibits inflammatory phenotypic switching of VSMCs	[217]
5	Circ_0020397	Human umbilical artery smooth muscle cells (HUASMC)	N/A	Up	miR-138/ <i>VEGFR2</i>	Promotes proliferation and migration of VSMCs	[221]
6	Circ_0044073	Human vascular smooth muscle cells (HUVSMC) and HUVEC	LPS	Up	miR-107/ <i>JAK-STAT</i>	Promotes proliferation, migration, and inflammation of VSMCs	[225]
7	Circ_ <i>PTPRA</i> (circ_102984)	VSMCs	ox-LDL	Up	miR-636/ <i>SP1</i>	Promotes proliferation and inhibits apoptosis	[230]
8	Circ_ <i>DENND1B</i>	RAW264.7 mouse monocytes	ox-LDL	Down	miR-17-5p/ <i>ABCA1</i>	Promotes cholesterol efflux	[233]
9	Circ_ <i>TM7SF3</i>	THP-1-derived macrophages	ox-LDL	Up	miR-206/ <i>ASPH</i>	Promotes oxidative stress	[236]

level and excess ROS production, whereas, overexpression of HK2 restores endothelial function [130].

Circ_0068087

HG condition also up-regulates circ_0068087 in HUVECs and knockdown of circ_0068087 suppresses the secretion

of pro-inflammatory mediators, TNF-α, IL-6, IL-1β, and MCP-1 [131]. Circ_0068087 promotes HG-induced TLR4/NF-κB/NLRP3 inflammasome-mediated inflammation and EC dysfunction by suppressing miR-197 which directly targets *TLR4*. MiR-197 has been reported to be a prognostic marker for CAD patients [132]. Circ_0068087 also promotes ox-LDL-induced oxidative injury in HUVECs by sponging miR-186-5p [133]. MiR-186-5p exerts protective effects

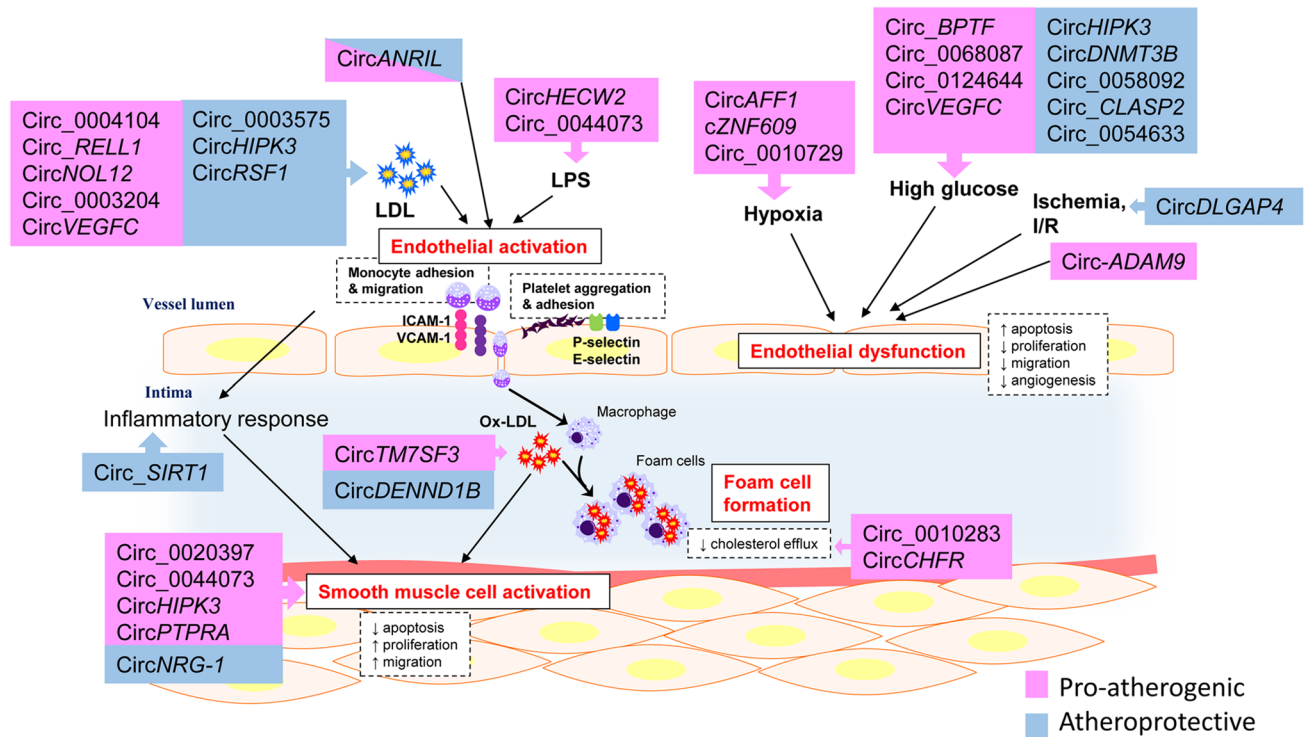


Fig. 3 Pro-atherogenic and atheroprotective circRNAs involved in atherosclerosis initiation and progression. *ICAM-1* intercellular adhesion molecule-1; *I/R* ischemic/reperfusion; *LDL* low-density lipoprotein; *LPS* lipopolysaccharide; *ox-LDL* oxidized low-density lipoprotein; *VCAM-1* vascular cell adhesion molecule-1. The figure was created with Motifolio Toolkit (Motifolio Inc, Ellicott City, MD)

from ox-LDL-induced endothelial dysfunction by suppressing its target Roundabout guidance receptor 1 (*ROBO1*), a receptor of slit guidance ligand 1 (*SLIT1*) and *SLIT2*. *SLIT2*-*ROBO* signaling plays a role in vascular injury where it can exert either pro-angiogenic or anti-angiogenic effects in EC, depending on the cell types [134].

CircVEGFC (circ_0071465)

In a HG-induced endothelial injury model, Wei et al. [135] showed that circVEGFC was up-regulated, concomitant with decreased proliferative ability induced by HG exposure. Whereas the knockdown of circVEGFC attenuated apoptosis and increased the survival of HUVECs. CircVEGFC inhibits EC proliferation by sponging miR-338-3p which targets the transcription factor, hypoxia-inducible factor 1 alpha (*HIF-1α*). Up-regulation of *HIF-1α* then leads to increased transcription of vascular endothelial growth factor A (*VEGFA*), an angiogenesis stimulating factor. However, it is unclear how *VEGFA* contributed to apoptosis in this study [135] since *VEGFA* overexpression when regulated by miR-320 in HG-treated HUVECs has been shown to enhance protein kinase C (PKC) and receptor for advanced

glycation endproducts (*RAGE*) protein levels which subsequently promotes proliferation and angiogenesis but suppresses HUVECs apoptosis instead [136].

Contrary to the study by Wei et al. (2020), another study has observed an increase of miR-338-3p following ox-LDL-induced endothelial injury concomitant with decreased cell viability and increased apoptosis. These inhibitory effects were reversed following the inhibition of miR-338-3p [137].

It was shown that bone morphogenetic protein (*BMP*) and activin membrane-bound inhibitor (*BAMBI*) is a direct target of miR-338-3p [137]. *BAMBI* regulates cell proliferation and differentiation by stably associating with TGFβ family receptors and represses TGFβ/Smad signaling [138, 139]. As discussed earlier, TGFβ signaling plays a protective role in vascular injury, whereby it is rapidly activated following vascular injury to stimulate proliferation and migration of EC and VSMC for vascular repair [105–107]. Hence, down-regulation of miR-338p may also increase *BAMBI* which in turn represses TGFβ signaling activation, thereby decreasing EC survival. Collectively, these studies revealed that circRNA-miRNA regulations in different EC injury models may produce different outcomes.

CZNF609

With regards to clinical relevance, *cZNF609* levels in peripheral blood leukocytes and plasma of CAD and hypertensive patients were found decreased compared to healthy volunteers, concomitant with the up-regulation of circulating levels of miR-615-5p [140, 141]. In HG-treated HUVECs, *cZNF609* expression was increased. Silencing of *cZNF609* increased the cell viability and reduced oxidative stress or hypoxic stress-induced apoptosis in HUVECs. By contrast, overexpression of *cZNF609* worsen oxidative stress or hypoxic stress-induced HUVEC apoptosis. In vivo, *cZNF609* knockdown in both diabetic retinopathy and oxygen-induced retinopathy mouse model also decreased retinal vascular leakage and pathological angiogenesis, respectively. *CZNF609* causes in vitro EC and in vivo vascular dysfunction by sponging miR-615-5p, which in turn increases its target, myocyte enhancer factor 2A (*MEF2A*). *MEF2A* is a transcription factor that is highly expressed in the endothelium of coronary arteries and has been implicated with the pathogenesis of CAD and myocardial infarction (MI) [142]. Overexpression of miR-615-5p could decrease retina vascular leakage and capillary degeneration in diabetic retinopathy and also pathological angiogenesis in oxygen-induced retinopathy mouse model. Moreover, *MEF2A* overexpression could also rescue *cZNF609* silencing-mediated effects on HUVEC migration, tube formation, and apoptosis [141]. These comprehensive clinical, in vivo and in vitro findings strongly support the pro-atherosclerotic and abnormal angiogenic roles of *cZNF609* in the pathogenesis of diabetes, hypertension, and CAD.

CircAFF1

EC dysfunction can also be induced by hypoxia, which is an important contributor of ischemic injury [143]. Using a hypoxic cellular model, Wang et al. [144] showed that *circAFF1* was up-regulated in HUVECs and human brain endothelial cells (HBEC-5i) treated with CoCl_2 , an inducer of hypoxia and also in sera of patients with subarachnoid hemorrhage. The viability of EC in the hypoxic condition overall was further affected by the overexpression of *circAFF1* which resulted in apoptosis, impaired wound healing, and impaired tube formation [144]. These effects were mediated by suppression of its target, miR-516b, whereby, miR-516b overexpression could attenuate EC suppression induced by *circAFF1*. MiR-516b targets Salvador family protein 1 (*SAVI*), which leads to the down-regulation of *SAV1* and reduces the phosphorylation of Yes-associated protein 1 (*YAP1*). *SAV1* and *YAP1* are components of Hippo signaling involved in the regulation of cell proliferation. Phosphorylation of *YAP1* by *SAV1* will prevent its

entry into the nucleus and transcription of relevant genes important for cell viability [145, 146].

Circ_0010729

The knockdown of *circ_0010729* has been previously shown to have a protective role from cardiomyocyte injury by several studies [147, 148]. The role of *circ_0010729* on EC dysfunction has also been reported. It was shown that hypoxia-induced HUVECs injury up-regulates *circ_0010729* expression, concomitant with proliferation and migration inhibition and the up-regulation of hypoxia-inducible factor, *HIF-1 α* [149]. HIFs are transcription factors that respond to low oxygen tension in tissue and induce genes that promote angiogenesis, energy metabolism and cell survival, which then promote EC migration, growth, and differentiation [150]. However, the EC inhibitory effects caused the hypoxic condition were reversed by the knockdown of *circ_0010729*. It was further shown that *circ_0010729* targets miR-186, resulting in the increased expression of *HIF-1 α* [149]. These findings, hence suggest that *circ_0010729* drives EC dysfunction caused by hypoxia and its knockdown plays a protective role.

Circ_ADAM9

Endothelial progenitor cells (EPC) which are immature EC are vital for wound healing. These cells differentiate into mature EC, proliferate, and form new blood vessels at the injured site [151]. These cells also secrete angiogenic factors to stimulate angiogenesis. EPC are also vulnerable to oxidative stress caused by hyperglycemia, resulting in EPC apoptosis and suppression of angiogenesis [152]. In diabetic EPC or EPC subjected to HG condition, these cells undergo apoptosis and angiogenic suppression. Overexpression of *circ_ADAM9* has been shown to promote EPC apoptosis under HG treatment, whereas its knockdown significantly suppressed apoptosis [153]. Furthermore, down-regulation of *circ_ADAM9* also promotes angiogenesis in the hind limbs of diabetic mice. The apoptosis promotion effect was mediated through sponging miR-20a-5p, which has apoptosis and autophagy suppressive actions by directly targeting *PTEN* and *ATG7*. Suppression of the PI3K/AKT/mTOR pathway promotes autophagy, whereas activation of PI3K/AKT/mTOR inhibits autophagy by regulating *ATG7*. *PTEN* negatively regulates PI3K/AKT/mTOR pathway [154]. Hence, its increased expression following the binding of miR-20a-5p by *circ_ADAM9*, leads to increased autophagy and apoptosis [153]. As discussed earlier, miR-20a-5p is also a target of *circ_0003204* (*circ_USP36*), which mediates ox-LDL-induced HUVECs injury via *ROCK2* [104].

CZBTB44 (circ_0002484)

CZBTB44 is reported to be up-regulated in a laser-induced choroidal neovascularization (CNV) mouse model and in choroid-retinal endothelial RF/6A cells subjected to CoCl_2 -induced hypoxia stress [155]. Abnormal angiogenesis and increased vessel sprouting occurred concurrently with the overexpression of *cZBTB44* in hypoxia, but knockdown of *cZBTB44* decreased choroidal sprouting assay *ex vivo*, reduced CNV lesion area in laser-induced CNV in mouse and also inhibited the viability, proliferation, migration, and tube formation of RF/6A cells under basal and hypoxic conditions. The effects of *cZBTB44* were mediated through the sponging of miR-578 which targets *VEGFA* and *VCAM1*. Inhibition of miR-578 leads to increased *VEGFA* and *VCAM1* expression which promotes abnormal angiogenesis, whereas overexpression of miR-578 mimicked *cZBTB44* knockdown-mediated anti-angiogenic effects *in vivo* and *in vitro* [155]. These findings suggest that *cZBTB44*-miR-578-*VEGFA/VCAM1* crosstalk promotes pathological neovascularization by regulating EC function.

CircHECW2

Endothelial to mesenchymal transition (EndMT) is also known to drive vascular inflammation and the progression of atherosclerotic plaque [156, 157]. EndMT is a process by which EC dedifferentiate into mesenchymal phenotype and lose their expression markers and functions. Acquisition of the mesenchymal phenotype enables these cells to migrate into underlying tissues and compromise vascular integrity. A genome-wide bioinformatic analysis has identified that *circHECW2*, which is derived from exon 12 and exon 13 of the *HECW2* gene can sponge miR-30d and drives the EndMT process [158]. Lipopolysaccharide (LPS) stimulation induced EndMT, as characterized by increased expression of mesenchymal markers *COL1A2/Collagen I* and *ACTA2/α-SMA* (alpha-actin-2/alpha-smooth muscle actin) and decreased expressions of endothelial tight junction proteins (TJP-1, occludin, and claudin 5). Yang et al. showed that miR-30d was down-regulated and *ATG5* up-regulated in LPS-stimulated human brain microvascular endothelial cells (HBMECs) and in mice administered with LPS [158]. Subsequent experiments revealed that miR-30d regulates *ATG5* in the EndMT process. These effects were confirmed with the knockdown of *circHECW2* which resulted in the inhibition of EndMT *in vitro* and *in vivo*. The decrease of *ATG5* caused by *circHECW2* knockdown was reversed by silencing of miR-30d.

Endothelial dysfunction protective circRNAs

CircHIPK3

CircHIPK3 is abundantly expressed in various tissues and it promotes EC growth by regulating its target miRNAs: miR-124, miR-193, miR-379, and miR-654 [159]. Although its role may be conflicting, several studies have reported that it plays protective roles in endothelial dysfunction (Table 2). Overexpression of *circHIPK3* (i) inhibits HG-induced cell death and apoptosis in HG-treated HUVECs by sponging miR-124 which up-regulates pro-survival targets such as *SphK1* and *STAT3* [160]; (ii) promotes autophagy of ox-LDL-stimulated HUVECs and inhibits lipid accumulation by targeting miR-190b which in turn targets *ATG7*, a driver of autophagy [161]; (iii) promotes human coronary aortic artery EC proliferation and angiogenesis after myocardial infarction by suppressing its target miR-133a and promotes connective tissue growth factor (*CTGF*) expression, which is associated with angiogenesis [162]. Moreover, exosomal *circHIPK3* released from hypoxic cardiomyocytes has been shown to protect cardiac microvascular EC (CMVECs) following internalization by inhibiting miR-29a, thereby increasing IGF-1 expression that modulates oxidative damage and protects CMVECs from oxidative stress [163].

Nevertheless, the pro-angiogenic activity of *circHIPK3* can be deleterious in pulmonary arterial hypertension and diabetic retinopathy. The stimulatory effects of *circHIPK3* on human pulmonary artery endothelial cells (hPAECs) proliferation, migration, and angiogenesis, however, can promote the pathogenesis of pulmonary arterial hypertension. These effects are mediated via miR-328-3p-*STAT3* axis [112]. The pro-angiogenic effect of *circHIPK3* is also detrimental for diabetic retinopathy, whereby it is up-regulated in diabetic retinas and retinal EC stimulated with HG. Silencing *circHIPK3* decreased retinal acellular capillaries, vascular leakage, and inflammation and alleviated retinal vascular dysfunction. The abnormal EC growth was found mediated by the suppression of miR-30a-3p which led to increased *VEGF-c*, *FZDA* and *WNT2* expression [164].

Although *circHIPK3* in general has a protective role in EC dysfunction, a study has shown that *circHIPK3* can enhance the proliferative ability of VSMC, leading to the conversion to synthetic active VSMC phenotype that is pro-atherosclerotic. It was shown that knockdown of *circHIPK3* up-regulates miR-637 expression which then decreases its target, *CDK6* levels [165]. Whereas overexpression of *circHIPK3* increases *CDK6* levels and subsequently promotes the proliferation of VSMC that accelerates the progression of atherosclerosis. Hence, *circHIPK3* modulates different outcomes in cell types and vascular diseases [165].

CircANRIL

Antisense noncoding RNA in the INK4 locus (*ANRIL*) is transcribed on chromosome 9p21.3 locus, which has been identified by GWAS and robustly replicated as a genetic risk factor for CAD [166, 167]. According to NCBI, three *ANRIL* transcripts were annotated, respectively, as NR_003529 (the 3,857-bp full-length *ANRIL* transcript), DQ485454 (2,659-bp transcript), and EU741058 (short 688-bp transcript). *ANRIL* regulates glucose and fatty acid metabolism [168]. Up-regulation of the circulatory form, *circANRIL* is associated with lower risk of CAD development [169, 170], whereas its linear counterpart, *linANRIL* (NR_003529 and EU741058) is associated with increased risk for CAD [171]. In the modulation of atherosclerosis, *circANRIL* was shown to increase apoptosis and decrease viability of VSMC and macrophages by inhibiting pescadillo homolog 1 (*PES1*), a 60S pre-ribosomal assembly factor vital for ribosome biogenesis. Impairment of pre-ribosomal RNA processing and ribosome biogenesis lead to nucleolar stress and activation of p53 which in turn, promotes apoptosis and inhibits proliferation of VSMC and macrophages [170]. Similarly, overexpression of *circANRIL* in EC isolated from atherosclerotic rats also resulted in reduced oxidative stress and inflammatory mediators released [172], further supporting the protective role of *circANRIL* in atherosclerosis. On the contrary, Song et al. showed that overexpression of *circANRIL* increases serum lipids level, exacerbates endothelial inflammation by increasing IL-1, IL-6, MMP-9, and CRP levels and induces endothelial apoptosis in coronary arteries that can cause vessel regression in rat atherosclerotic model [173]. Whereas opposite effects were observed in rats injected with *circANRIL* inhibitor. Therefore, *circANRIL*'s role in atherosclerosis may also be dependent on its expression level. It is also worth noting that miRNAs regulated by *circANRIL* in atherosclerosis have yet to be reported.

CircDNMT3B

CircDNMT3B and *BAMBI* expressions were found down-regulated in retinal EC of diabetic retinopathy patients and diabetic rats [174]. *BAMBI* is a target of miR-20b-5p and *circDNMT3B* has been shown to sponge miR-20b-5p. In human retinal microvascular endothelial cells (HRMECs) treated with HG as a mimic of diabetic condition, it was shown that up-regulation of miR-20b-5p promotes abnormal proliferation, migration, and tube formation of HRMECs, all of which contributes to diabetic retinopathy. MiR-20b-5p induced excessive EC proliferation, migration, and tube formation under HG conditions was mediated through the suppression of *BAMBI*. In diabetic nephropathy, *BAMBI* ensures endothelial stability by reducing capillary growth and migration [175–177]. These results show that *circDNMT3B* can

control abnormal EC growth and migration that can contribute to vascular dysfunction.

CircDLGAP4

Endothelial dysfunction also contributes to ischemic heart disease. Conversion of quiescent EC to the migratory and angiogenic phenotype could result in abnormal angiogenesis as seen in late stages of ischemic/reperfusion (I/R) injury [178]. Bai et al. reported that *circDLGAP4* levels were significantly decreased in the plasma of acute ischemic stroke patients and in a mouse stroke model [179]. In a subsequent study, Chen et al. showed that *circDLGAP4* was also down-regulated in HUVECs following I/R-induced injury, whereas its overexpression attenuated the I/R-induced increase in EC migration [180]. Increased endothelial migration contributes to the progression of atherosclerotic lesion formation [9, 181]. EndMT was also attenuated as evidenced by reduced expression of mesenchymal markers following microinjection of *circDLGAP4* into mice with cerebral ischemia-induced injury [179]. In both studies, the expression of miR-143 which is a target of *circDLGAP4* were up-regulated in I/R-induced injury in HUVECs and mice with cerebral ischemia-induced injury. It was further shown that miR-143 mediates endothelial dysfunction and EndMT by inhibiting HECT domain E3 ubiquitin protein ligase 1 (*HECTD1*). The role of *HECTD1* in EndMT and metastasis in tumorigenesis has been previously reported [182]. MiR-143 was also previously implicated in EndMT [183] and also up-regulated in clinical atherosclerotic plaque samples [180]. In HUVECs, overexpression of miR-143 suppresses glycolysis by directly targeting hexokinase 2 (*HK2*), a key enzyme in glucose metabolism, and leads to endothelial dysfunction [184]. Collectively, these findings suggest the protective role of *circDLGAP4* in endothelial dysfunction.

Circ_0058092

EPC are important for wound healing as they differentiate into mature EC and proliferate to promote new blood vessels formation of the injury site [151]. CircRNA has also been shown to affect EPC. For example, *circ_0058092* was down-regulated in type 2 diabetic patients. A further study has revealed that the expression of *circ_0058092* is decreased in EPC subjected to hyperglycemic condition, whereas *circ_0058092* overexpression preserved EPC survival, proliferation, migration, and angiogenic differentiation as well as decreased oxidative stress and inflammatory response [185]. These protective effects were mediated through increased FOXO3 expression as overexpression of *circ_0058092* sponges miR-217 which targets *FOXO3* [185]. Although this study has shown the EPC protective effects of increased FOXO3 expression in HG-induced EPC injury

[180], other studies have also shown that overexpression of *FOXO3* could suppress human EPC proliferation by modulating cell cycle regulators [186]. Knockdown of *FOXO3* gene could also protect EC in *FOXO*-knocked out mice from vascular dysfunction and atherosclerosis [187] and increases HUVECs sprouting and migration [188]. These findings suggest the pleiotropic role of *FOXO3* in endothelial health depending on the study system or the injury inducer used.

Circ_0003575

Circ_0003575 was reportedly up-regulated in ox-LDL-induced endothelial damage and its silencing promoted the proliferation and angiogenesis ability of HUVECs [189]. Subsequently, Shang et al. demonstrated using aortic EC isolated from an atherosclerosis in vivo model, ApoE deficient mouse, that overexpression of circ_0003575 suppresses *FOXO3* and *FOXO4* protein expressions [190]. However, ectopic expression of miR-148a-3p impaired the circ_0003575-mediated up-regulation of *FOXO4* and *FOXO3* [190]. As discussed above, knockdown of *FOXO* is protective of EC dysfunction [187, 188]. Shang et al. also showed that overexpression of miR-148a-3p, a regulator of lipid metabolism [191] enhances EC proliferation and inhibits apoptosis [190]. However, in this study, there was no direct evidence of circ_0003575 sponging miR-148a-3p, suggesting that circ_0003575 and miR-148a-3p interaction may involve other regulators.

CircRSF1 (circ_0000345)

In sera of patients with atherosclerosis, the expression of circRSF1 was reportedly down-regulated together with the up-regulation of miR-758 [192]. This observation was further demonstrated in ox-LDL-treated HAECs, whereby overexpression of circRSF1 and silencing miR-758 improved HAECs viability, tube formation, and migration while apoptosis was attenuated. It was demonstrated that circRSF1 positively regulates cyclin D2 (*CCND2*) expression by sponging miR-758 [192]. *CCND2* complexes with CDK4 or CDK6 and initiates cell cycle progression from G1 to S phase by phosphorylating retinoblastoma protein [193]. It has been reported that *CCND2* is also a target of miR-98. In a HG-induced EC injury, miR-98 expression was found down-regulated while *CCND2* was also up-regulated [194]. This further supports the role of *CCND2* in regulating EC growth under stress conditions. In ox-LDL treated HUVECs, the expression of circRSF1 was similarly down-regulated, whereas its target, miR-135b-5p was up-regulated [195]. MiR-135b-5p induced EC apoptosis and inflammation by regulating its target gene, histone deacetylase 1 (*HDAC1*), which has been reported to increase VCAM-1 expression, promoting the early events of atherogenesis [196]. Overexpression of circRSF1 rescued the inhibitory effects of EC

[195]. Collectively, these findings support the protective role of circRSF1 in endothelial dysfunction by regulating miR-758 and miR-135b-5p.

Circ_CLASP2 (circ_0064772)

CircRNA CLIP-associating protein 2 (circ_CLASP2) expression was initially found decreased in HUVECs exposed to HG condition in a circRNA profiling study using RNA sequencing [197]. Subsequent mechanistic study revealed that circ_CLASP2 overexpression promotes proliferation and inhibits apoptosis in HUVECs under HG conditions by interacting with miR-140-5p which directly targets *FBXW7* [198]. *FBXW7* is an important regulator of endothelial barrier and angiogenesis [199, 200]. MiR-140-5p is up-regulated in HUVECs under HG conditions and knockdown of miR-140-5p promoted proliferation, inhibited apoptosis, and increased VEGF expression. An earlier study has shown that miR-140-5p expression is increased in mice with atherosclerosis, whereby its up-regulation increases oxidative stress and ROS levels by suppressing the protein expression of *Nrf2*, sirtuin 2 (*Sirt2*), Kelch-like enoyl-CoA hydratase-associated protein 1 (*Keap1*), and heme oxygenase 1 (*HO-1*) in HUVECs. By contrast, down-regulation of miR-140-5p decreased oxidative stress and ROS levels [201].

Circ_0054633

Circ_0054633, first found up-regulated in the peripheral blood of type 2 diabetes mellitus patients [202] has been subsequently validated to play a protective role in HG-induced endothelial dysfunction [203]. Under HG-induced HUVECs injury, circ_0054633 was up-regulated and the down-regulation of circ_0054633 further suppressed EC proliferation, increased apoptosis, migration, and tube formation [203]. The down-regulation of circ_0054633 has led to increased expression of its target, miR-218 which then decreased *ROBO1* and *VEGF* expression levels, which are vital angiogenic drivers for vascular injury. Furthermore, decreased circ_0054633 also resulted in the reduction of *HO-1*, which is another target of miR-218 [203]. *HO-1* is an anti-oxidant and cytoprotective enzyme vital for protection from oxidative stress and inflammation [204]. Hence, these findings are supportive of the protective role of circ_0054633 in EC dysfunction.

CircRNAs that regulate the functions of VSMC

Physiologically, VSMC assumes a quiescent contractile state which performs contractile function to regulate blood flow and blood vessel diameter by vasoconstriction and

vasodilation [205]. The maintenance of VSMC in the contractile phenotype is dependent on the interaction between EC and VSMC. However, in response to inflammatory responses from EC and macrophages, VSMC is converted to the active synthetic phenotype with increased proliferation and migration capacity but decreased contractility [206]. Active synthetic VSMC plays a key role in pathological vascular remodeling, which is a pathophysiological process that contributes to atherosclerosis and hypertension. Recent literature has discussed the role of proliferation of VSMC, which can be argued as reparative or detrimental depending on the stages of atherosclerosis [58, 207]. Increased VSMC proliferation is observed following vascular injury and during early atherogenesis [208], suggesting a reparative role. Whereas human VSMC derived from both aged vessels and advanced atherosclerotic plaques showed reduced proliferation [209]. This suggests that in the later stage of atherosclerosis, enhancement of proliferation could be beneficial for plaque stability since plaque rupture commonly occurs in areas with reduced VSMC, increased VSMC apoptosis, and increased macrophages. VSMC apoptosis, hence, could drive atherogenesis and progression of established lesions. The roles of circRNAs in regulating VSMC in the various events leading to atherosclerosis has also begun to unfold and are discussed below (Fig. 3, Table 3).

CircNRG-1

CircNRG-1 has been shown to inhibit apoptosis of mouse aortic smooth muscle cells (MASMCs) by regulating miR-193b-5p/NRG-1 [210]. Neuregulin-1 (NRG-1), a member of the epidermal growth factor (EGF) family, is expressed in vascular EC and VSMC. It has been shown that NRG-1 expression can be decreased by Ang II, an effector peptide of the renin–angiotensin–aldosterone-system (RAAS) involved in the regulation of blood pressure and fluid balance. Ang II is also known for its role in regulating VSMC proliferation or apoptosis in the early stages of vascular remodeling [211]. Sun et al. showed that Ang II inhibits apoptosis of MASMCs by decreasing both NRG-1 and circNRG-1 levels [210]. Whereas, overexpression of NRG-1 reversed the inhibitory action of Ang II on MASMC apoptosis. Luciferase reporter assays revealed that circNRG-1 up-regulates NRG-1 expression by acting as miR-193b-5p sponge. MiR-193b-5p has been shown to target the 3'-UTR of NRG-1 and suppresses NRG-1 expression in MASMCs. VSMC apoptosis is implicated in vascular remodeling and these findings suggest that circNRG-1/miR-193b-5p/NRG-1 axis is modulated by Ang II in apoptosis resistance of VSMC [210].

Circ_0010283

In an ox-LDL treated VSMC, circ_0010283 and HMGB1 were found significantly up-regulated, whereas the expression of miR-370-3p was down-regulated [212]. Ox-LDL promotes VSMC proliferation and migration and knockdown of circ_0010283 suppresses VSMC viability and migration, as evidenced by the suppression of cell cycle regulator cyclin D1 and proliferating cell nuclear antigen, and migration-associated proteins MMP2 and MMP9 [212]. Hence, circ_0010283 promotes the viability and migration of ox-LDL-induced VSMC. Circ_0010283 was shown to sponge miR-370-3p which targets HMGB1. The inhibition of miR-370-3p reversed the circ_0010283 silencing-mediated inhibitory effects on VSMC viability and migration. Additionally, the miR-370-3p-mediated suppressive effects on cell viability and migration were rescued by overexpression of HMGB1. Hence, circ_0010283 mediated cell viability and migration via a miR-370-3p/HMGB1 axis in ox-LDL-induced VSMC. HMGB1 has been reported to accelerate foam cell formation and cholesterol accumulation in VSMC [213] and is involved in pro-inflammatory response [103].

Circ_CHFR (circ_0029589)

The checkpoint with forkhead-associated and ring-finger domains (circ_CHFR), was identified by microarray profiling of VSMC exposed to ox-LDL treatment [214]. A subsequent study also showed that circ_CHFR is up-regulated in serum of atherosclerosis patients and in ox-LDL-stimulated VSMC [215]. In both studies, knockdown of circ_CHFR suppressed the ox-LDL-induced promotion of cell growth, migration, and inflammation in VSMC. Yang et al. showed that circ_CHFR increases the expression of FOXO1 by sponging miR-370 [214]. In addition, miR-370 has also been reported as a target of circ_0010283 [212]. These studies revealed that both circ_CHFR and circ_USP36 indirectly increase the expression of FOXO1 by suppressing miR-370 and promoting apoptosis of VSMC and EC. In addition to miR-370, circ_CHFR also targets miR-214-3p and increases the expression of Wnt3 [215]. On the contrary, knockdown of miR-214-3p and overexpression of Wnt3 attenuated circ_CHFR induced cell migration and inflammation triggered by ox-LDL exposure in VSMC [215]. Previously, it has been shown that Wnt3a produces pro-migratory effects on EC [216]. Collectively, these studies show the existence of an indirect network of common circRNAs-miRNAs-mRNA in regulating the active synthetic VSMC phenotype that contributes to atherogenesis.

Circ_ *SIRT1* (circ_0093887)

Circ_ *SIRT1* which is derived from the *SIRT1* host gene, is expressed in VSMC and is enriched in response to vascular injury induced by TNF- α or platelet-derived growth factor-BB (PDGF-BB) [217]. Its expression is decreased in the plasma of CAD patients as well as in human renal arterial neointima, concomitant with the increased expression of pro-inflammatory cytokine MCP-1 and adhesion molecules VCAM-1 and ICAM-1. In contrast, overexpression of circ_ *SIRT1* decreased TNF- α -induced expression of VCAM-1, ICAM-1, and MCP-1 levels in VSMC, by inhibiting the nuclear translocation of NF- κ B p65, which is vital for the transcription of pro-inflammatory cytokines and adhesion molecules [217]. These findings show that circ_ *SIRT1* inhibits inflammatory phenotypic switching of VSMC. Kong et al. further showed that circ_ *SIRT1* directly binds to miR-132/212 which targets *SIRT1* resulting in enhanced SIRT1 activity [217]. This then increases deacetylation and inactivation of the nuclear NF- κ B p65 and prevents inflammatory phenotype switching of VSMC. Other studies have also shown the protective effects of enhanced SIRT1 activity by inhibiting VSMC proliferation and migration that underlie neointima formation [218], vascular endothelial dysfunction, and cardiovascular diseases [219].

Circ_0020397

Neointimal thickening occurs in response to vascular injury in atherosclerosis [220]. It is characterized by VSMC proliferation and migration from the media to the neointima. Circ_0020397 has been shown to modulate VSMC proliferation and migration via the miR-138/*VEGFR2* axis [221]. *VEGFR2* is the principal receptor of VEGFA which enhances proliferation, migration, and survival of ECs [222]. Studies have shown that *VEGFR2* also plays an important role in regulating VSMC proliferation [223, 224]. The expression of circ_0020397 was found significantly decreased in the arterial smooth muscle cells isolated from intracranial aneurysm patients, in association with increased expression of miR-138 and decreased the protein expression of *VEGFR2* [221]. It was reported that overexpression of circ_0020397 in human umbilical artery smooth muscle cells (HUASMCs) promotes cell proliferation, suppresses miR-138 expression but down-regulates *VEGFR2* expression. MiR-138 was also shown to negatively regulate *VEGFR2* by binding with the 3'UTR of *VEGFR2* mRNA. In contrast, overexpression of miR-138 induces HUASMC apoptosis [221].

Circ_0044073

Circ_0044073 was found up-regulated in blood cells of atherosclerosis patients, in association with the down-regulation of

miR-107 expression and up-regulation of the levels of JAK1 and STAT3 [225]. In vitro validation revealed that under the stimulation of LPS, the migration and invasion potentials of human vascular smooth muscle cells (HUASMC) and HUVECs increased together with a significant increase of circ_0044073 expression. Circ_0044073 increases the proliferative and invasive potentials of these cells by sponging miR-107, which targets *JAK1* [225]. The JAK/STAT signaling pathway is known to promote vascular cell inflammation, proliferation, migration, and adhesion [226]. In agreement to this, overexpression of circ_0044073 activated the JAK/STAT signaling pathway along with up-regulation of Bcl-2, c-myc, IL-1 β , IL-6, and TNF- α level [225]. Contrary to the effects seen with the overexpression of circ_0044073, transfection with mimic miR-107 inhibited the proliferation of HUASMC and HUVECs. MiR-107 is linked to insulin sensitivity and inflammation [227] and our previous study has also shown that overexpression of miR-107 promotes EC senescence and inhibits tube formation by increasing MTORC1 activity [228]. Collectively, these findings are supportive of the role of circ_0044073 in promoting the pro-atherosclerotic VSMC and EC phenotype.

CircPTPRA (circ_102984)

CircPTPRA was reported as a tumor suppressor that represses EndMT and metastasis in non-small cell lung carcinomas (NSCLC) [229]. However, up-regulation of circPTPRA and down-regulation of miR-636 level were reported in plasma of patients with atherosclerosis compared to healthy individuals. Inhibition of miR-636 via the direct sponging by circPTPRA increased SP1 and further promoted cell proliferation and repressed apoptosis in VSMC [230]. Inhibition of circPTPRA or *SP1* in VSMC promoted G0/G1 cell cycle arrest with down-regulation of Cyclin D1 and cyclin E protein levels.

CircRNAs that modulate foam cell formation and progression

Circ_0092317/circ_0003546 and circ_0028198/circ_0092317

Foam cell formation is a hallmark of atherosclerosis progression. Foam cell lesions are cholesterol ester-enriched macrophages while some of them are macrophage-like cells originating from VSMC. The formation of foam cells is determined by the combination effect of lipid uptake, cholesterol esterification, and cholesterol efflux. Bioinformatic analysis revealed the impact of circRNAs in the cholesterol transport process in THP-1 macrophages. Circ_0092317/circ_0003546 and circ_0028198/circ_0092317 possibly sponged miR-326 and miR-543, respectively, in reducing

the cholesterol efflux in macrophages by targeting aspartate beta-hydroxylase (*ASPH*) and phosphodiesterase 3B (*PDE3B*) [231, 232]. *ASPH* regulates cellular response to calcium ions, while *PDE3B* plays important roles in the hydrolysis of cyclic adenosine monophosphate (cAMP) and prohibits the activation of cAMP-dependent protein kinases which phosphorylates *ABCA1*.

Circ*DENND1B*

High density lipoprotein (HDL) is important in removing cholesterol from cells and tissues involved in the early stages of atherosclerosis back to the liver for excretion. Down-regulation of circ*DENND1B* was reported in ox-LDL-induced monocytes. Circ*DENND1B* produces anti-atherosclerotic effect by regulating adenosine triphosphate (ATP)-binding cassette transporter A1 (*ABCA1*) through sponging miR-17-5p and hence promotes cholesterol efflux in foam [233]. *ABCA1* is an important receptor involved in the initiation of cholesterol efflux to HDL in macrophage foam cells. Pro-inflammatory cytokines such as IL-1 β , IFN- γ , and TNF- α activate macrophages by regulating *ABCA1* expression and drive foam cell formation. Increased circulating miR-17-5p was reported in plasma of CAD patients [234]. MiR-17-5p binds directly to *ABCA1* mRNA and down-regulation of miR-17-5p significantly up-regulated *ABCA1* in RAW264.7 mouse macrophage cells accompanied with decreased inflammatory cytokines production and inhibition of lipid accumulation [235].

Circ*TM7SF3*

CircRNA transmembrane 7 superfamily member 3 (circ*TM7SF3*) was significantly up-regulated in the plasma of atherosclerosis patients and ox-LDL-induced THP-1-derived macrophages compared to their corresponding counterparts [236]. Silencing of circ*TM7SF3* partly attenuated the production of inflammatory cytokines such as TNF- α and IL-6 and oxidative stress in ox-LDL-induced human monocyte-derived macrophages (hMDMs) and THP-1 derived macrophages. The macrophage cell injury was induced through circ*TM7SF3*/miR-206/*ASPH* signal axis. The role of miR-206 in atherosclerosis has been revealed in a previous study, whereby, decreased level of miR-206 in atherosclerosis tissue samples and overexpression of miR-206 markedly reduced VSMC relative survival rate [237].

CircRNA–miRNA–mRNA interactions

To date, most circRNA studies on atherogenesis have focused on a single circRNA, as shown in Tables 1, 2, 3. We attempted to identify common circRNA–miRNA–mRNA axis involved in atherogenesis using Cytoscape 3.8.2. Cytoscape is an open-source bioinformatic platform that allows visualization of interaction between biomolecules [238]. After removing unconnected clusters, we identified 12 circRNAs (circ*HIPK3*, circ_0004104, circ-*ADAM9*, circ-*USP36*, circ_0068087, circ_0010283, circ-*CHFR*, circ_0054633, circ*VEGFC*, circ_0010729, circ-*RELL1*, and circ-*NOL12*) that have demonstrated some degree of connectivity, either through miRNAs or their target mRNAs (Fig. 4). We noted three clusters of circRNAs that target a common miRNA and exert either pro-atherogenic or athero-protective effects. The first cluster consists of circ_0010283 and circ-*CHFR* which can sponge miR-370, thereby up-regulating targets of miR-370 such as *HMGB1* and *FOXO1*, resulting in endothelial inflammation and injury as well as VSMC activation, all of which favor atherogenesis. The second cluster includes circ-*RELL1* and circ-*NOL12* which target miR-6878-3p and produces pro-atherogenic effects such as induction of oxidative stress, EC inflammation, and apoptosis. In contrast, circ*HIPK3* and circ_0004104 both act on miR-328-3p which targets *STAT3* and *TRIM14*, respectively, but produce opposing effects. Modulation of *STAT3* is protective of EC health, whereas modulation of *TRIM14* promotes EC apoptosis and inflammation. Nevertheless, other studies have identified that circ*HIPK3* engages several other miRNAs to exert an overall protective effect from EC dysfunction.

It is well established that a single miRNA can have multiple target mRNAs. In this regard, both miR-338-3p and miR-186, which can be sponged by circ*VEGFC* and circ_0010729, respectively, thereby, increasing *HIF1* and promote EC apoptosis, inhibit growth and migration. Likewise, both circ*HIPK3* and circ-*ADAM9* also modulate *ATG7* by targeting miR-190b and miR-20a-5p, respectively, and promote autophagy in EC and EPC. MiR-197 and miR-330-5p similarly target *TLR4* and activate NF- κ B pathway in promoting EC activation, whereby, miR-197 and miR-330-5p can be sponged by circ-*USP36* and circ_0068087, respectively. On the other hand, targeting of *ROBO1* by miR-186-5p and miR-218 produce opposing effects on EC health. MiR-186-5p and miR-218 are regulated by circ_0068087 and circ_0054633, respectively. In this scenario, opposing effects were observed likely because of the difference in the two EC injury models used in their studies, i.e., ox-LDL and HG-induced EC injury. Collectively, these findings revealed that while there is accumulating evidence of the role of numerous

circRNAs in atherogenesis, identification of common circRNA–miRNA–mRNA networks in atherosclerosis remains limited. Identification of cohesive networks is warranted to gain deeper understanding to the complexity of circRNA–miRNA roles in atherogenesis as combination use of these small RNAs have great potential as diagnostic and preventive strategies.

Clinical applicability of circRNAs

The greater aim would be to develop clinical diagnostic and prognostic circRNA biomarkers as well as therapeutics for human diseases including CAD. Although the regulatory functions of circRNAs on disease development and progression are yet to be fully elucidated, the reported stability along with the cell-type, tissue, and developmental stage specificity of circRNAs [15] present an immense potential for circRNAs to be developed into useful diagnostic and prognostic biomarkers. Notably, circRNAs were reported to be stably detected in blood cells of whole blood or circulating cell-free components such as exosomes in plasma, serum, and saliva, making it ideal for liquid biopsy that is easily accessible yet non-invasive in clinical setting [239]. Many circRNAs have been identified as biomarkers for

human diseases especially cancers, cardiovascular diseases, neurodegenerative diseases, and diabetes [20, 240].

In addition, effort to understand the mechanism of actions of disease-associated circRNAs will provide new treatment avenues such as circRNA-based therapy. Possible concepts for therapeutic modulation of disease-associated circRNAs include overexpressing native protective circRNAs or depleting endogenous disease-promoting circRNAs. The overview of pro-atherogenic or atheroprotective circRNAs in EC, VSMC, and macrophages which was extensively discussed in the current review highlights the potential of circRNAs as therapeutic target in inhibiting the initiation or delaying the progression of atherogenesis. Furthermore, the use of evolutionarily optimized artificial circRNAs as inhibitor of miRNA activity could be made and introduced into the patients to absorb one or multiple specific over-expressed disease-linked miRNAs, respectively, making them as disease suppressors. The engineered circRNAs were reported to possess higher stability and efficiency in action compared to their typical linear miRNA sponges as showed in human diseases other than CAD [241, 242]. Such concept can be extended to miRNA and RNA-binding protein sponges necessary for the development and progression of CAD. However, effective targeting of circRNAs and reduction of off-target effects and activation of innate immune system [243,

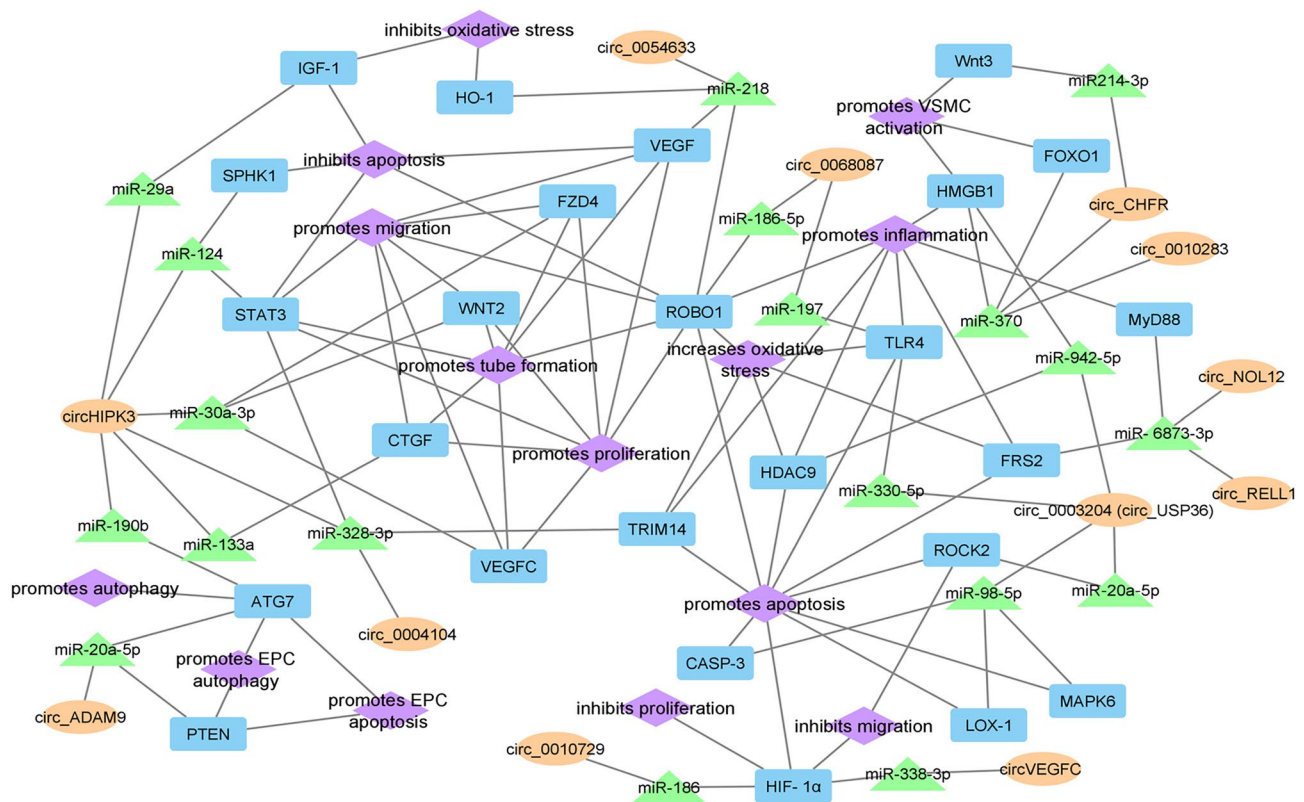


Fig. 4 CircRNA–miRNA–mRNA regulatory network in atherogenesis. The network consisting of 12 circRNAs, 19 miRNAs and their respective miRNA-targeted genes was generated by Cytoscape 3.8.2

[244] warrant the development of effective and safe tools for delivering and silencing circRNAs in vivo. Generation of a large amount and delivery of synthetic circRNAs is challenging. Nanoparticles and exosome-based delivery methods seem feasible but the safety aspect must be addressed. Similarly, the safety aspect of the use of CRISPR and siRNA technology for silencing circRNA must be evaluated. Lastly, questions remain on whether targeting circRNAs could produce the expected and long-lasting clinical outcome in human and this awaits further studies.

Concluding remarks

The pathophysiological roles of miRNAs of EC, VSMC, and macrophage in atherosclerosis have been well established in vitro, in vivo, and in clinical studies (reviewed in [245–247]). Notably are also groups of miRNAs identified as potential diagnostic markers for CAD and acute myocardial infarction based on their detections in PBMCs or plasma or serum samples in patients (reviewed by [248]). Some of the well-established anti-atherosclerotic miRNAs include miR-126, miR-24, and miR146a, whereas miR-92a, miR-30, miR-33, and miR-342-5p are pro-atherosclerotic. We noted that miR-370 (regulated by circ_ *USP36* in THP cells; regulated by circ_0010283 and circ_ *CHFR* in VSMC), miR-20a-5p (regulated by circ_ *USP36* and circ_ *ADAM9*) and miR-197 (regulated by circ_0068087) have been reported as miRs involved in CAD [247]. However, many other well-defined miRNAs involved in atherosclerosis are not among those regulated by the circRNAs identified from our literature review.

The vascular EC are enriched with miR-126, which is responsive to oxidative and hyperlipidemic stress and maintains the proliferative capacity, survival of EC, reduces lesions formation and hence an overall protective effect on atherosclerosis [249]. Yet, circRNA(s) that may regulate miR-126 has yet to be identified in HUVECs or other common EC subjected to oxidative and hyperlipidemic stress. Similarly, circRNA that may regulate miR-92a whose expression is increased in EC exposed to ox-LDL to reduce NF- κ B activity and promote the expression of eNOS has yet to be identified by studies listed in Table 1. One possible reason is that miR-92a is a mechanosensitive atheromiR whose expression is dependent on shear stress or flow but was not recapitulated in in vitro EC injury models used in many circRNA studies. In VSMCs, circRNAs that modulate the actions of miR-26a, miR-490-3p, and miR-143/145 which promote VSMC proliferation, migration, and acquisition of contractile phenotype also remain to be identified [249–251]. It can be argued that the EC injury models used are dissimilar but it is also observed that clinical samples used for the detection

of circRNAs expression were also less well-defined than those used in studies involving miRNAs. In vitro models may not accurately reflect in vivo angiogenesis as there is lack of cell–cell contact/signaling, shear stress, or even clearance of apoptotic cells seen in vivo.

Notably, the circRNA studies reporting a miRNA sponging model during atherogenesis only have a single to, at most, a few miRNA binding sites. This implies that the endogenous circRNA must be expressed at a high level and/or localized to the same location as its targeted miRNA to sufficiently exert a significant sponging effect to perturb the expression of the miRNA targets [252]. This is due to the fact that most circRNAs are present in relatively low abundance compared to their cognate linear counterpart and miRNA target derepression is only detected at a high threshold of alternative miRNA binding sites available [253, 254]. However, most atherogenesis circRNA studies to date, did not address the stoichiometric quantifications, e.g., copy number measurement, between the endogenous circRNA, and its targeted miRNA. Caution needs to be applied on interpreting results of forced overexpression of circRNAs, a method that is commonly used for functional validation of the particular circRNA as the increased fold change in multitudes of hundreds or thousands folds may not be physiological relevant. In order to prove that the circRNA act as a miRNA sponge, it is important to demonstrate the overexpression of circRNA with mutated miRNA binding site could abolish the sponging effect, whereas the effect of circRNA knockdown can be rescued with the overexpression of the respective circRNA [252].

Lastly, many of the studies have thus far focused on a simplified model, which delineate the effects of individual circRNAs, their downstream targets and outcomes on EC health and atherogenesis. Undeniably, these studies have advanced our understanding on the circRNA–miRNA–mRNA axis as drivers of atherosclerosis. Nevertheless, the fact that a single circRNA can regulate several miRNAs and similarly, a single mRNA can be regulated by the simultaneous coordinated actions of a number of different miRNAs cannot be overlooked. There is a need for future studies to establish a complete network of circRNAs and miRNAs to gain a deeper understanding on the concerted simultaneous actions of these micromanagers in the development and progression of atherosclerosis to enable the design of diagnostic and preventive strategies for early intervention. It is evident now that the role of circRNAs as regulators of atherogenesis and cardiovascular diseases are gaining importance but there remains the need to identify true “atherocircs” that are clinically relevant as diagnostics, prognostics, or therapeutic targets.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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