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REVIEW

Circular RNA role in Atherosclerosis Development and Progression

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Abstract

Purpose of Review Circular RNAs (circRNAs), a distinct class of long noncoding RNAs characterized by covalently closed-loop structures, have emerged as pivotal regulators of gene expression. Their stability, abundance, and cell-type specificity make them increasingly relevant in cardiovascular disease pathogenesis and clinical management. Atherosclerosis, a chronic inflammatory disorder of the arterial wall, underlies many cardiovascular and cerebrovascular events, including myocardial infarction and stroke. This review provides a comprehensive analysis of circRNAs' influence on the development and progression of atherosclerotic plaques.

Recent Findings The role of circRNAs in atherogenesis, where they may function as atheroprotective or atherogenic factors by modulating endothelial and smooth muscle cell functions, macrophage activity, lipid metabolism, and inflammatory signaling, has recently emerged.

Summary This review explores both experimental and in vivo findings on the functions of specific circRNAs and their involvement in cellular autophagy, apoptosis, oxidative stress, and vascular remodeling. Additionally, the diagnostic potential of circulating circRNAs as biomarkers for plaque instability and rupture has been investigated. Understanding circRNA-mediated regulatory networks may open new avenues for precision diagnostics and targeted therapies in atherosclerotic cardiovascular disease.

Keywords CircRNA · Atherosclerosis · Atherosclerotic plaques · Vascular remodeling

Introduction

Atherosclerosis is a chronic, progressive inflammatory disease that affects the arterial wall, leading to the formation of atherosclerotic plaques. Despite significant advances in pharmacologic interventions and surgical techniques, the disease remains a primary etiological factor in coronary artery disease (CAD) and carotid artery disease, which underlie myocardial infarction and ischemic stroke, respectively—two of the leading causes of morbidity and mortality globally. Indeed, cerebro-cardiovascular events continue to represent a substantial public health burden and remain the leading cause of long-term disability [1].

Pathologically, atherosclerosis is characterized by the accumulation of lipids and inflammatory cells, such as macrophages and lymphocytes, within the intima of medium- and large-sized arteries, forming complex atherosclerotic plaques [2–4]. The emergence of high-throughput sequencing technologies has dramatically expanded our understanding of the transcriptomic landscape associated with various pathologies, including atherosclerosis [5–7]. Although initial efforts focused primarily on protein-coding genes, RNA sequencing (RNA-Seq) has enabled the identification of numerous noncoding RNAs (ncRNAs), revealing their involvement in diverse molecular pathways implicated in disease development and progression [8, 9].

NcRNAs, which are not translated into proteins, are typically classified according to transcript length into small noncoding RNAs typically formed by less than 200 nucleotides (e.g., microRNAs, or miRNAs) and long noncoding RNAs (lncRNAs), with more than 200 nucleotides. Circular RNAs

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(circRNAs), a subtype of lncRNAs, have gained increasing attention due to their unique structure and stability. Together with other ncRNAs, circRNAs have been extensively studied for their regulatory functions across various pathological contexts, including cardiovascular diseases (CVDs), as well as for their potential utility as disease biomarkers [10–12].

Currently, definitive identification of plaque vulnerability relies on histological analysis following surgical excision of the plaque. Although numerous studies have explored potential preoperative indicators—including clinical, biochemical, and radiological markers—that may correlate with features of plaque vulnerability [13–15], no individual biomarker has demonstrated sufficient sensitivity, specificity, or reliability for routine clinical use [16–19].

CircRNAs have emerged as key molecular players in this context, either promoting or inhibiting atherosclerotic processes depending on their functional roles. This review aims to synthesize current knowledge regarding the mechanistic roles of circRNAs in atherosclerosis, highlighting their atherogenic or atheroprotective properties. Only studies employing both in vitro and in vivo models were selected to ensure a robust interpretation of circRNA function in the pathophysiology of the disease [8, 20, 21]. Furthermore, we discuss recent findings on circulating circRNAs and their potential utility as biomarkers for assessing atherosclerotic plaque progression and instability.

Development and Progression of Atherosclerosis in Cardio-Cerebrovascular Diseases

The development of atherosclerotic lesions is influenced by an interplay of genetic predisposition, aging, and physiological conditions, alongside modifiable behavioral and lifestyle factors. These include systemic arterial hypertension, dyslipidemia, hyperglycemia, and tobacco smoking, all of which are established risk factors that accelerate the atherogenic process [22, 23].

While the morphological progression of atherosclerotic plaques has been well documented, the underlying molecular and cellular mechanisms remain incompletely understood. The prevailing hypothesis, often referred to as the"response-to-injury"theory, was first proposed by Virchow and later refined by Ross [24, 25]. According to this model, endothelial dysfunction initiates a cascade of pro-inflammatory signaling events, characterized by the upregulation of cytokines, chemokines, and enhanced oxidative stress. In this context, oxidized low-density lipoprotein (oxLDL) promotes the activation of endothelial cells (ECs), transforming them from a quiescent to a pro-adhesive phenotype [26, 27].

Activated ECs express chemokines such as *monocyte chemoattractant protein-1* (MCP-1), and adhesion molecules including *vascular cell adhesion molecule-1* (VCAM-1), *E-selectin*, and *P-selectin*, thereby facilitating leukocyte recruitment [28]. Oxidation of LDL is mediated by a variety of factors, including metal ions, enzymatic activity (e.g., lipoxygenase and myeloperoxidase), glycation reactions, and the reactive species peroxynitrite (ONOO −), which forms from endothelial-derived nitric oxide (NO) [29].

Once recruited into the subendothelial space, monocytes differentiate into macrophages and internalize oxLDL to become lipid-laden foam cells. These macrophages, in turn, produce a range of pro-inflammatory cytokines such as *interleukin-6* (IL-6), *tumor necrosis factor-alpha* (TNF-α), and *interleukin-1β* (IL-1β), along with growth factors and oxidative mediators that amplify local inflammation [16, 28]. Smooth muscle cells (SMCs), originating from the arterial media or resident within the intima, contribute to plaque formation through phenotypic switching and secretion of extracellular matrix proteins such as collagen, elastin, and proteoglycans. This activity forms a fibrous cap that overlays the lipid core, comprising foam cells, macrophages, T lymphocytes, and necrotic debris [3, 30].

A critical region of the plaque, known as the"shoulder,"is densely populated with inflammatory cells and prone to proteolytic degradation. Matrix metalloproteinases (MMPs), secreted by activated immune cells, particularly T lymphocytes, can weaken the fibrous cap, increasing susceptibility to rupture [31, 32]. The relative composition of the fibrous cap and lipid core is a crucial component determining the mechanical stability of the plaque, distinguishing vulnerable plaques from more stable ones [33].

The clinical significance of plaque composition is underscored by the observation that plaques with identical degrees of luminal narrowing may differ dramatically in their propensity to cause cardiovascular events [31, 32]. Vulnerable plaques, characterized by thin fibrous caps and large necrotic cores, are prone to rupture, exposing thrombogenic material to circulating blood and initiating thrombus formation [33–35]. Thrombosis following cap rupture accounts for a large portion of acute myocardial infarctions. An alternative, yet clinically relevant mechanism of plaque destabilization involves superficial erosion, defined by endothelial denudation in the absence of overt rupture, and is typically observed in plaques lacking extensive necrotic cores [36].

Following plaque rupture or erosion, the endogenous healing response includes fibrinolysis, mediated by endothelial-derived *tissue plasminogen activator* (t-PA) and *urokinase-type plasminogen activator* (u-PA), along with proteolytic enzymes from neutrophils and monocytes [34]. SMCs proliferate and deposit extracellular matrix, forming a fibrotic scar that may ultimately undergo calcification. Interestingly, microcalcifications within the plaque are associated with an increased risk of rupture, whereas extensive calcification tends to stabilize the lesion [34, 37].

Given this complex pathophysiology, comprehensive characterization of plaque composition—beyond mere

stenosis severity—is critical for risk stratification and guiding therapeutic interventions. Identifying molecular mediators that influence plaque vulnerability remains a central goal in translational cardiovascular research.

CircRNAs Biogenesis and Functions

Circular RNAs (circRNAs) were first described in 1976 by Sanger and colleagues, who identified covalently closed circular RNA molecules in plant viroids [38]. A decade later, circRNAs were also observed in the Hepatitis delta virus, highlighting their presence in mammalian systems [39]. However, the biological significance of circRNAs remained underappreciated for several years, as they were largely dismissed as byproducts of aberrant splicing due to their relatively low abundance.

Subsequent advances in high-throughput RNA sequencing technologies and bioinformatic tools have redefined circRNAs as widespread, stable, and functionally relevant components of the transcriptome. In eukaryotic cells, circRNAs are generated through a non-canonical back-splicing process, in which a downstream 3'splice donor is joined to an upstream 5'splice acceptor. This results in a closed-loop structure that lacks the 5'cap and 3'polyadenylated tail characteristic of linear messenger RNAs [40–42] (Fig. 1). The circular configuration imparts resistance to exonuclease-mediated degradation, such as RNase R digestion, thereby conferring superior stability compared to their linear counterparts [40, 42].

The biogenesis of circRNAs relies on the canonical spliceosomal machinery, although both cis-acting elements and trans-acting protein factors influence back-splicing efficiency [41, 42]. Based on their genomic origin, circRNAs are categorized into three main classes: exonic circRNAs (ecircRNAs), which consist exclusively of exonic sequences and are predominantly cytoplasmic; intronic circRNAs (ciRNAs), which are derived solely from introns and largely nuclear; and exon–intron circRNAs (EIciRNAs), which retain both exons and introns and are also nuclear-localized [9, 43, 44] (Fig. 1).

Back-splicing can occur through several proposed mechanisms. The lariat-driven circularization model, also known as exon-skipping, involves the formation

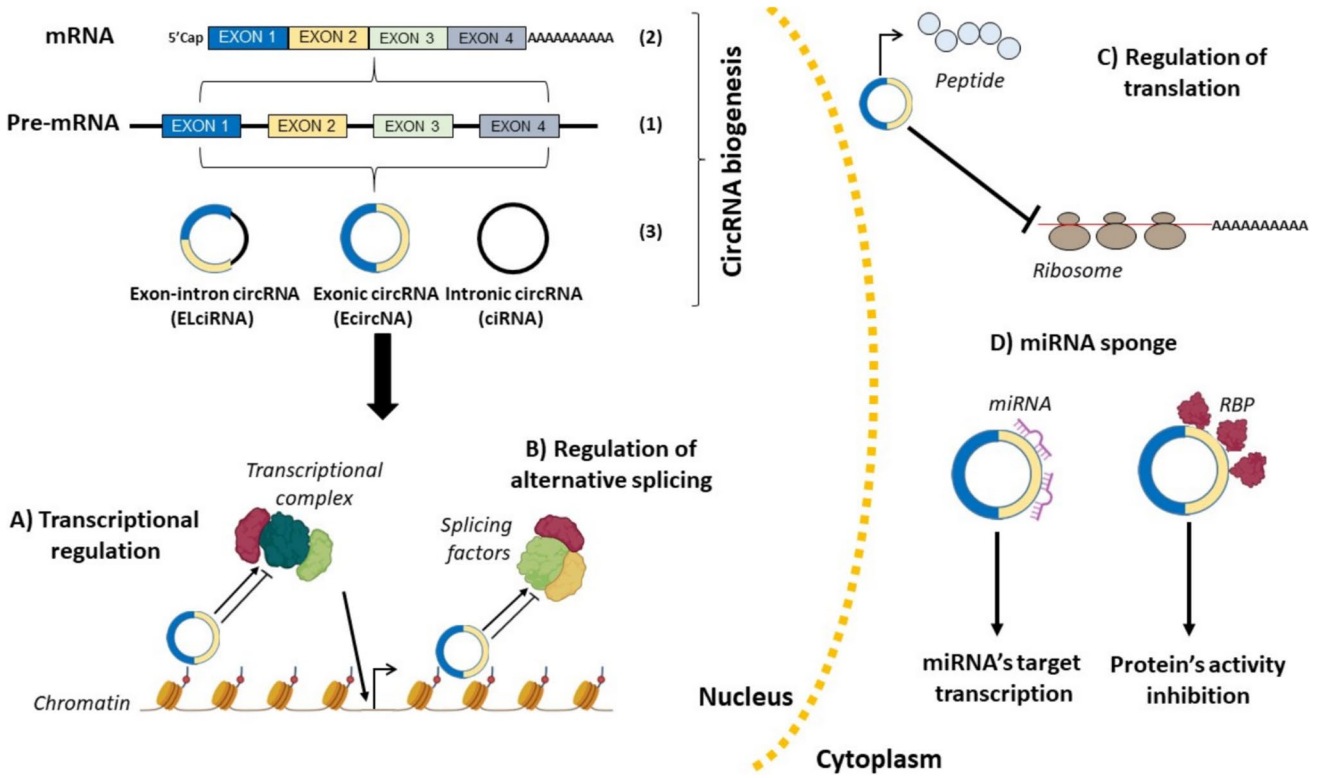

Fig. 1 CircRNA biogenesis and functions of circRNAs. A pre-mRNA containing four exons and three introns (1) is processed in a mRNA containing a 5′ cap and a poly-adenosine tail (2). Some pre-mRNAs are backspliced (3) to form exonic circRNAs (ecircRNAs), which consist of exonic sequences and are predominantly cytoplasmic; intronic circRNAs (ciRNAs), which are derived from introns and largely nuclear; and exon–intron circRNAs (EIciRNAs), which retain both exons and introns and are also nuclear-localized. CircRNAs are regulatory molecules that can function within the nucleus, or in the cytoplasm. In the nucleus, circRNAs are involved in the regulation of transcription and RNA splicing (**A** and **B**). In the cytoplasm, they influence translational control by mechanisms such as blocking ribosome binding to target RNAs, or, in some cases, they can themselves be translated into small peptides (**C**). Additionally, circRNAs can act as molecular sponges, sequestering microRNAs or proteins and preventing them from exerting their regulatory functions (**D**)

of lariat structures containing skipped exons, which are then circularized (Fig. 1). Alternatively, intron-pairing-driven circularization relies on complementary sequences within flanking introns that bring splice sites into proximity. RNA-binding protein (RBP)-mediated circularization represents a third mechanism, whereby trans-acting factors such as quaking (QKI) or muscleblind (MBL) facilitate back-splice site juxtaposition by bridging flanking intronic regions [45]. Some researchers consider RBP-driven circularization a subtype of intron-pairing-driven circularization, given its dependence on sequence complementarity and spatial proximity.

The evolutionary conservation of circRNAs has been widely studied because function and conservation are often linked [46]. However, circRNAs, like other long non-coding RNAs, are generally less conserved than mRNAs. Instead of focusing on long, conserved sequences, a better approach might be to look for shorter, conserved regions, such as binding sites for miRNAs or RNA-binding proteins (RBPs) [47]. Additionally, the conservation of circRNA's secondary and 3D structures could provide important clues about their conserved functions.

Functionally, circRNAs act through diverse mechanisms, depending on their subcellular localization and molecular composition. One of the most extensively studied functions of cytoplasmic circRNAs is their role as miRNA sponges [48, 49]. Many circRNAs harbor multiple miRNA response elements (MREs), enabling them to sequester specific miRNAs and thereby prevent them from downregulating their mRNA targets. In this context, circRNAs act as competing endogenous RNAs (ceRNAs), modulating gene expression post-transcriptionally [50–54]. The Cerebellar degeneration-related protein 1 antisense RNA (CDR1as), the product of back-splicing of a CDR1 gene exon, was the first identified and the best-known circRNA interacting with miRNAs. It is highly expressed in the brain, involved in cerebrovascular diseases [55, 56] and in cancer tissues [57]. CDR1as, also called ciRS-7, contains over 70 conventional binding sites for hsa-miR-7 [52]. By blocking the function of hsa-miR-7, CDR1as can induce the transcription of multiple key target genes. In various cancers, aberrant expression of CDR1as often leads to the inhibition of hsa-miR-7. This inhibition can result in the translation of miRNA targets into proteins, contributing to disease progression [57]. However, the "circRNA-miRNA sponge" hypothesis should be viewed critically [58]. The expression levels of most circRNAs (apart from a few, including CDR1as) are generally low, while miRNAs might be more abundant [58]. Therefore, studies investigating the sponge function of circRNAs would benefit from robust stoichiometric quantification.

In addition to miRNA sponging, circRNAs can interact with RBPs, affecting RNA stability, transport, and translation. These interactions may influence the circRNA stability or modulate the expression and function of bound proteins [59, 60]. Nuclear-localized circRNAs, particularly EIciRNAs and ciRNAs, have been shown to interact with transcriptional machinery, such as RNA polymerase II, and influence gene expression at the transcriptional level [61]. An example is circFOXO3, hosted by the Forkhead box O3 (FOXO3) gene. It has been shown to repress the cell cycle by interacting with both p21 and cyclin-dependent kinase 2 (CDK2), forming a ternary complex [62]. Wu et al. [63] reported that tropomyosin-4 (TMP4) and gamma-actin (ACTG) bind with Yes-associated protein circular RNA (circYap) in cardiac cells and mouse heart tissues, increasing the interaction of TPM4 with ACTG and inhibiting actin polymerization, which induces cardiac fibrosis.

In addition, some circRNAs can compete with linear splicing, thereby regulating gene expression. In particular, circMBNL adversely affects canonical splicing by competition with pre-*muscleblind protein* (MBL) mRNA splicing [64].

In 2017, two research groups independently reported that circRNAs can be translated in *Drosophila melanogaster* heads and mammalian muscle cells [64, 65]. Legnini et al. [65] demonstrated that circZNF609, derived from the zinc finger protein 609 gene, contains a 753-nucleotide open reading frame (ORF) and is translated into a protein in murine and human skeletal muscle, as confirmed by polysome profiling and Western blot analysis. Inhibiting this circRNA significantly reduced myoblast proliferation [65].

CircRNAs represent a highly versatile and functionally diverse class of regulatory RNAs. Their inherent stability, tissue-specific expression, and involvement in key signaling networks underscore their potential as therapeutic targets and diagnostic biomarkers in atherosclerosis and other pathologies.

circRNAs Detection

The identification and quantification of circular RNAs have been significantly facilitated by high-throughput sequencing technologies, particularly RNA sequencing (RNA-Seq) [66, 67]. CircRNAs are expressed in a tissue- and cell-specific manner and have also been detected in various biofluids, making them attractive candidates for diagnostic biomarker discovery. However, accurate circRNA detection presents several methodological challenges, primarily due to their non-linear structure.

Unlike linear transcripts, circRNAs are identified by back-splicing junctions, which connect a downstream 5'splice site

to an upstream 3'splice site. These junctions typically yield a limited number of sequencing reads, necessitating greater sequencing depth and longer read lengths to improve the likelihood of detection [66, 68]. Standard polyadenylated (poly-A) library preparations used in RNA-Seq preferentially enrich for mature linear mRNAs, thereby depleting circRNAs. Ribosomal RNA-depleted libraries are commonly employed to address this, as they retain non-polyadenylated RNAs, including circRNAs. Additionally, RNase R digestion, which selectively degrades linear RNAs while preserving circular forms, is often used to enrich circRNA content [48, 66].

CircRNAs are not yet systematically annotated in widely used genomic reference databases such as Ensembl or RefSeq. Nonetheless, multiple circRNA-specific databases have been developed to facilitate circRNA analysis. One of the most commonly utilized platforms is CircBase, which aggregates circRNA annotations from several large-scale studies and enables circRNA identification by gene symbol, genomic coordinates, or specific identifiers (e.g., hsa_circ_0000010) [69]. Another valuable resource is CircInteractome, which incorporates TargetScan-based predictions of miRNA and RBP interactions and supports the design of divergent primers and small interfering RNAs (siRNAs) for experimental applications [70].

CircAtlas is another useful database that compiles circRNA annotations from 1,070 RNA-Seq datasets derived from 19 normal tissues across six species. It offers extensive information on genomic localization, host gene identity, mature circRNA sequences, expression levels, cross-species conservation, and interaction networks involving miRNAs and RBPs [71]. Additionally, ENCORI/StarBase provides integrative analyses of interactions between ncRNAs, mRNAs, and RBPs using over 700 CLIP-Seq datasets and numerous RNA-RNA interaction datasets [72].

Despite recent advances, technical limitations such as variability in library preparation, limited read coverage of back-splice junctions, and the need for standardized annotation frameworks continue to pose obstacles to the comprehensive cataloging of circRNAs. Nonetheless, circRNA-specific databases and enrichment protocols provide a strong foundation for exploring the biological and clinical relevance of these unique noncoding transcripts.

The back-splicing activity frequently generates multiple distinct circRNAs from a gene locus via back-splicing, and the resulting mature circRNA can exert a unique function according to the combination of exons. Unfortunately, names currently given to circRNAs are often ambiguous, hampering the transcript identification [73]. To overcome this limitation, in this review the names of circRNAs, whenever possible, are indicated with the prefix "circ" followed by the host gene symbol and exon information, according to recently published guidelines [73].

Atheroprotective CircRNAs

Mounting evidence supports the involvement of circRNAs in the regulation of vascular inflammation, endothelial dysfunction, and smooth muscle cell phenotype—key processes in atherogenesis. Certain circRNAs exert protective effects, mitigating plaque formation, promoting cellular homeostasis, and dampening inflammatory responses. This section highlights the most well-characterized atheroprotective circRNAs, based on combined in vitro and in vivo studies.

CircANRIL(5–7)

Genome-wide association studies (GWAS) have consistently identified the chromosome 9p21 (Chr9p21) locus as robustly associated with CAD [74]. This locus transcribes two key noncoding RNAs: the linear antisense noncoding RNA at the INK4 locus (ANRIL) and its circular counterpart, circANRIL(5–7) [75–77]. This locus hosts several circRNA transcripts originating from different splicing activities and containing various combinations of exons [74]. Notably, these two forms appear to exert opposing effects; while linear ANRIL promotes atherosclerosis, circANRIL(5–7) confers protection against CAD development [78, 79].

Mechanistically, circANRIL(5–7) has been shown to bind pescadillo homolog 1 (PES1), a component of the 60S preribosomal subunit. This disrupts pre-rRNA maturation and ribosome biogenesis, leading to p53 activation. This effect induced apoptosis in vascular smooth muscle cells (VSMCs) and macrophages, and inhibits their proliferation, limiting foam cell accumulation and lesion formation [79].

In an ex vivo study, Shi et al. [78] discovered that circANRIL overexpression in endothelial cells (ECs) isolated from the intima of rats with vitamin D- and high-fat diet-induced vascular calcification [80] attenuated oxidative stress, inflammatory cytokine secretion (IL-6, TNF-α), and cell damage, supporting its atheroprotective role [78].

These findings are summarized in Fig. 2.

circHIPK3(2)

One of the most extensively studied circRNAs, circHIPK3(2), has been implicated in various cardiovascular pathologies [9, 81]. Wei and colleagues investigated the role of circHipk3(2) in atherosclerosis using both $ApoE^{-/-}$ mouse models and human ECs exposed to oxLDL [82]. In both systems, circHipk3(2) expression was significantly reduced, coinciding with impaired autophagy—a protective response against oxidative damage and inflammation in vascular cells [83, 84].

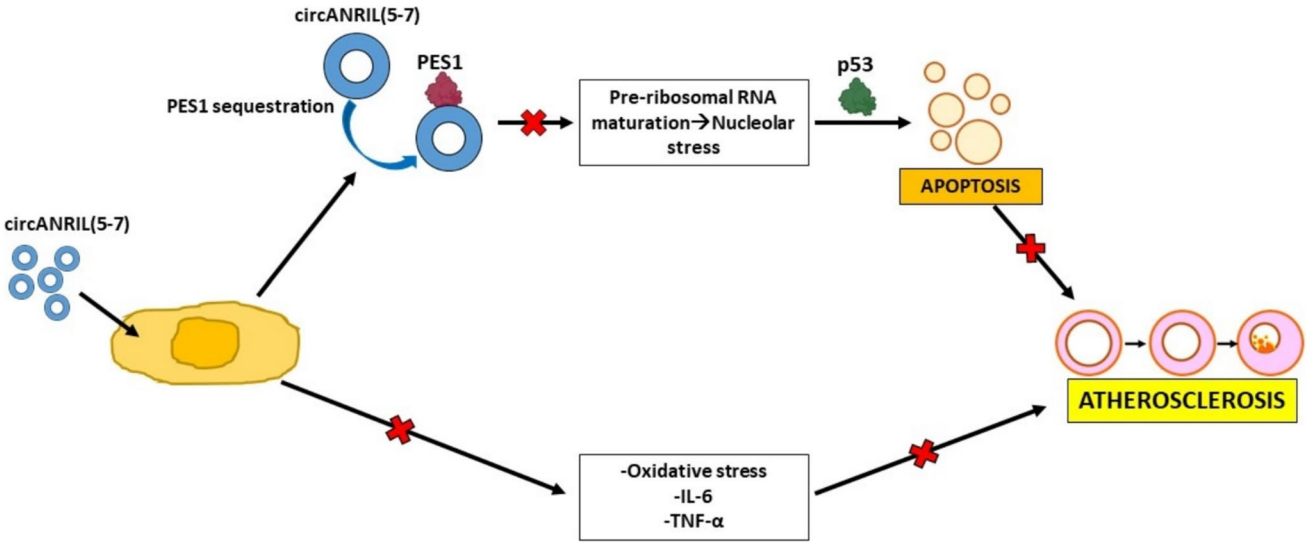

Fig. 2 circANRIL(5–7) protective effects on atherosclerosis. Forced expression of circANRIL(5–7) in human vascular smooth muscle cells or macrophages [79] and endothelial cells isolated from the intima of rats with vitamin D- and high-fat diet-induced vascular calcification [78], blunted the atherosclerotic process by inhibiting apoptosis [79] or inflammation and oxidative stress [78]

Restoration of circHIPK3(2) levels reversed oxLDL-induced autophagy inhibition, mediated by sponging hsa-miR-190b and de-repressing its target, autophagy-related protein 7 (ATG7). These findings highlight the circHIPK3/miR-190b/ATG7 axis as a crucial modulator of vascular autophagy and cell survival during atherosclerotic stress [82].

Of note, besides this atheroprotective effect of circHIPK3(2), a detrimental activity of the circRNA mediated by necroptosis has also been reported [85]. Discrepancies in the circRNAs effects may be due to isoform-specificity and/or cell-context and they will be further discussed later, in the "Atherogenic circRNAs" section.

circSIRT1(2–7)

SIRT1, a class III histone deacetylase, exerts pleiotropic protective effects in CVDs through its regulatory influence on inflammation, metabolism, and endothelial function [86, 87]. Among the multiple circular transcripts derived from the SIRT1 gene, a circRNA formed by circularization of exon-2 to exon-7, circSIRT1(2–7), was highly expressed in VSMCs [88].

Using a rat model of carotid artery injury, Kong et al. reported a time-dependent decline in circSIRT1(2–7) expression paralleling increased neointimal formation. In human renal arterial tissues, reduced circSIRT1(2–7) levels correlated with elevated MCP-1, VCAM-1, and ICAM-1 [88]. Functional assays confirmed that circSIRT1(2–7) overexpression suppressed inflammatory gene expression by sequestering miR-132/212 and enhancing SIRT1 mRNA activity, ultimately inhibiting the NF-κB signaling pathway (Fig. 3). AAV-mediated delivery of circSIRT1(2–7) to injured arteries significantly reduced intimal hyperplasia, highlighting its therapeutic potential [88].

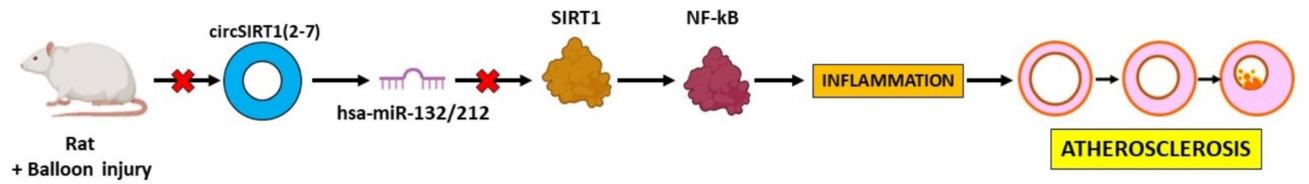

Fig. 3 circSIRT1(2–7) protective effects on atherosclerosis. circSirt1(2–7) expression is decreased in the carotid arteries and plasma of rat after balloon injury paralleling increased neointimal formation. In vitro experiments, in human renal arterial tissues, indicated that reduced level of circSirt1(2–7) corresponded to increased hsa-miR-132/212 inhibitory activity on SIRT1 transcription, with increased expression of NF-kB that exacerbates atherosclerosis

circSmoc1-1(4–7)

Vascular calcification is a hallmark of advanced atherosclerosis. In phosphate-induced calcification models using primary rat VSMCs, Ryu et al. identified circSmoc1-1(4–7) as significantly downregulated [89]. This circRNA originates from exons 4 to 7 of the SMOC1 gene and functions as a sponge for miR-874-3p, which targets ADAM19, a metalloproteinase involved in ECM remodeling.

Loss of circSmoc1-1(4–7) enhanced phosphate-induced calcium accumulation, while its overexpression suppressed calcification both in vitro and in vivo, including in vitamin D3-induced and ApoE$^{-/-}$ mouse models [89]. These data position circSmoc1-1(4–7) as a protective modulator against vascular calcification and associated plaque destabilization.

circSQSTM1(3–8)

Atorvastatin, a widely used lipid-lowering agent, also exerts anti-inflammatory and antioxidative effects. Mo et al. [90] revealed that atorvastatin treatment improved endothelial function. Chen et al., found that atorvastatin upregulated circSQSTM1(3–8) in human ECs by downregulating YTHDF2, an m6 A-binding protein involved in circRNA degradation [91]. CircSQSTM1(3–8) modulated endothelial inflammation by sponging hsa-miR-23b-3p and increasing SIRT1 expression [92]. In addition to its cytoplasmic function, circSQSTM1(3–8) also enhanced FOXO1 mRNA export and expression through its interaction with eIF4 A3. In vivo, AAV-mediated overexpression of circSQSTM1 in ApoE$^{-/-}$ rats reduced plaque area and oxidative stress markers, providing strong support for its atheroprotective function [92].

circFOXO1(2)

Yu et al. identified circFOXO1(2) (hsa_circ_0030042) as significantly downregulated in peripheral blood mononuclear cells (PBMCs) from patients with CAD [93]. OxLDL-induced suppression of this circRNA in ECs was associated with excessive autophagy and increased apoptosis. Restoration of circFOXO1(2) reduced autophagosome formation and improved EC viability by antagonizing eIF4 A3-mediated upregulation of FOXO1 and downstream autophagy mediators such as Beclin1 and LC3B [93].

In ApoE$^{-/-}$ mice, overexpression of circFOXO1(2) decreased markers of inflammation and plaque vulnerability, suggesting that excessive autophagy can be detrimental in this context. Thus, circFOXO1(2) is a negative regulator of pathologic autophagy and vascular injury.

Atherogenic circRNAs

Numerous circRNAs have been identified as promoters of atherogenesis through mechanisms such as enhancing inflammation, vascular remodeling, and cellular necroptosis. Unlike their protective counterparts, these atherogenic circRNAs are often upregulated during disease progression and can destabilize plaque composition or promote intimal thickening.

circDcbld1

Microarray profiling of the rat common carotid artery post-balloon injury revealed elevated levels of circDcbld1 in animals exhibiting intimal hyperplasia relative to sham-operated controls [94]. Fluorescence in situ hybridization (FISH) localized circDcbld1 to the cytoplasm, with predominant expression in the medial and neointimal layers, especially within VSMCs [94]. Functional studies showed that silencing circDcbld1 promoted the expression of contractile markers such as α-SMA, SM-MHC, and calponin, while reducing VSMC migratory capacity. Furthermore, hsa-miR-145-3p was experimentally validated as a circDcbld1-interacting microRNA. The inhibition of this miRNA reversed the anti-migratory effect of circDcbld1 knockdown. Neuropilin-1 (Nrp1), a known target of hsa-miR-145-3p involved in cell migration [95, 96], was downregulated following circDcbld1 silencing. In vivo, circDcbld1 knockdown mitigated neointimal hyperplasia, underscoring its potential as a therapeutic target in vascular remodeling [94].

CircEsyt2(2–6)

CircEsyt2(2–6) upregulation was identified in aortic plaques from ApoE$^{-/-}$ mice subjected to an HFD for three months [97]. FISH and immunofluorescence localized CircEsyt2(2–6) predominantly in the cytoplasm, with pronounced expression in VSMCs, ECs, fibroblasts, and macrophages. Notably, CircEsyt2(2–6) was more abundant in human coronary artery plaques from patients with severe CAD than in those with mild disease, underlying the translational relevance of the animal model results [97]. Wire injury-induced expression of CircEsyt2(2–6) was associated with increased neointima formation and cell proliferation, while silencing reduced these effects and increased apoptosis. In vitro, CircEsyt2(2–6) knockdown in human aortic and mouse VSMCs curtailed proliferation and migration while promoting apoptosis and the expression of differentiation markers, such as α-SMA, calponin,

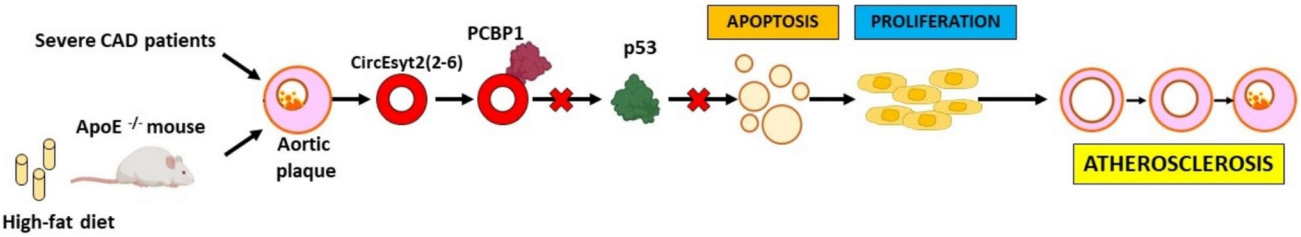

Fig. 4 CircEsyt2(2–6) atherogenic effects. Increased expression of CircEsyt2(2–6) was observed in both aortic plaque of CAD severe patients and of ApoE$^{-/-}$ mouse fed with HFD. The mechanisms supporting the effects of CircEsyt2(2–6) in aortic plaques are mediated by circRNA binding to PCBP1 that mitigate p53 activity with an increase of cell proliferation and intima hyperplasia

and Myh11 [97]. Mechanistically, CircEsyt2(2–6) sequestered polyC-binding protein 1 (PCBP1), which regulates p53 expression [98], inhibiting its nuclear translocation [92–94]. This sequestration inhibited PCBP1-dependent splicing of p53β, which normally enhances full-length p53 activity and induces apoptosis [99]. Thus, CircEsyt2(2–6) appears to promote VSMC remodeling by disrupting apoptotic signaling pathways [97]. These findings are summarized in Fig. 4.

CircHIPK3(2)

Advanced bioinformatics analysis has allowed Li et al. to identify circHIPK3(2) as significantly upregulated in human atherosclerotic arteries [85]. RT-qPCR confirmed this upregulation, which was especially marked in vulnerable plaques and localized near the necrotic core, as shown by FISH [85]. Silencing of circHIPK3(2) reduced hydrogen peroxide-induced necroptosis in VSMCs, while overexpression increased necroptosis, as indicated by elevated p-RIPK3 and p-MLKL levels. circHIPK3(2) was found to interact with dynamin-related protein 1 (DRP1), a regulator of mitochondrial dynamics [100], promoting mitochondrial fission and necroptosis [85]. In ApoE$^{-/-}$ mice, AAV-mediated circHIPK3(2) knockdown reduced plaque area and thickness, along with necroptosis in plaque regions, suggesting a role in promoting plaque instability [85]. This atherogenic role of circHIPK3(2) contrasts with the pro-autophagic, protective action [82], reported above in the "Atherotective circRNAs" section. This discrepancy can be attributed to the different cell-systems involved: VSMCs in the study of Li et al. [85] and endothelial cells in the case of the atheroprotective effect identified by Wei et al. [82], and also to the two different targets involved. Indeed, in VSMCs, circHIPK3(2) regulates DRP1 activity that induces necroptosis [85], which is detrimental for the arterial integrity, while in endothelial cells the circRNA reduction converged on the hsa-miR-190b/ATG7 pathway [82], which reduces blood vessel inflammation (Fig. 5).

CircLONP2(8–11)

Atherosclerotic lesions often localize to arterial branches and curvatures, where disturbed blood flow induces endothelial shear stress [101, 102]. Wang et al. identified circLONP2(8–11) as a flow-sensitive circRNA [103]. Laminar shear stress (LSS) suppressed its expression in ECs, while oscillatory shear stress (OSS) upregulated it. circLONP2(8–11) knockdown mitigated OSS-induced expression of VCAM-1 and ICAM-1 and enhanced anti-inflammatory NRF2 and HO-1 expression. These effects were mediated through the miR-200a-3p/KEAP1/NRF2 pathway [103]. In a partial carotid ligation model in ApoE$^{-/-}$ mice, circLONP2(8–11) overexpression exacerbated plaque development and inflammation. Furthermore, circLONP2 regulated oxidative stress and inflammation via a miR-200a-3p/YAP1/EZH2 axis, highlighting species-specific differences in its regulatory mechanisms [103]. Although clinical validation remains limited, this study marks circLONP2(8–11) as the first circRNA implicated in flow-mediated vascular remodeling.

circLrp6 (4–7)

CircLrp6(4–7) was identified through transcriptomic analyses of IL-1α and PDGF-stimulated human VSMCs and RNA-Seq data from mouse aorta [104, 105]. It was the only circRNA enriched for conserved binding sites of miRNAs relevant to VSMC biology, particularly hsa-miR-145-5p [106]. CircLrp6(4–7) co-localized with hsa-miR-145-5p in cytoplasmic P-bodies and modulated its activity, as evidenced by luciferase reporter assays targeting the Integrin β 8 (Itgβ8) 3'UTR [105]. CircLrp6(4–7) silencing impaired VSMC proliferation, migration, and differentiation. However, its expression remained unchanged in various murine vascular disease models and human aneurysmal tissues. In vivo knockdown slightly decreased neointimal formation in ApoE$^{-/-}$ mice with carotid stents, suggesting a modest functional role in vascular pathology [105].

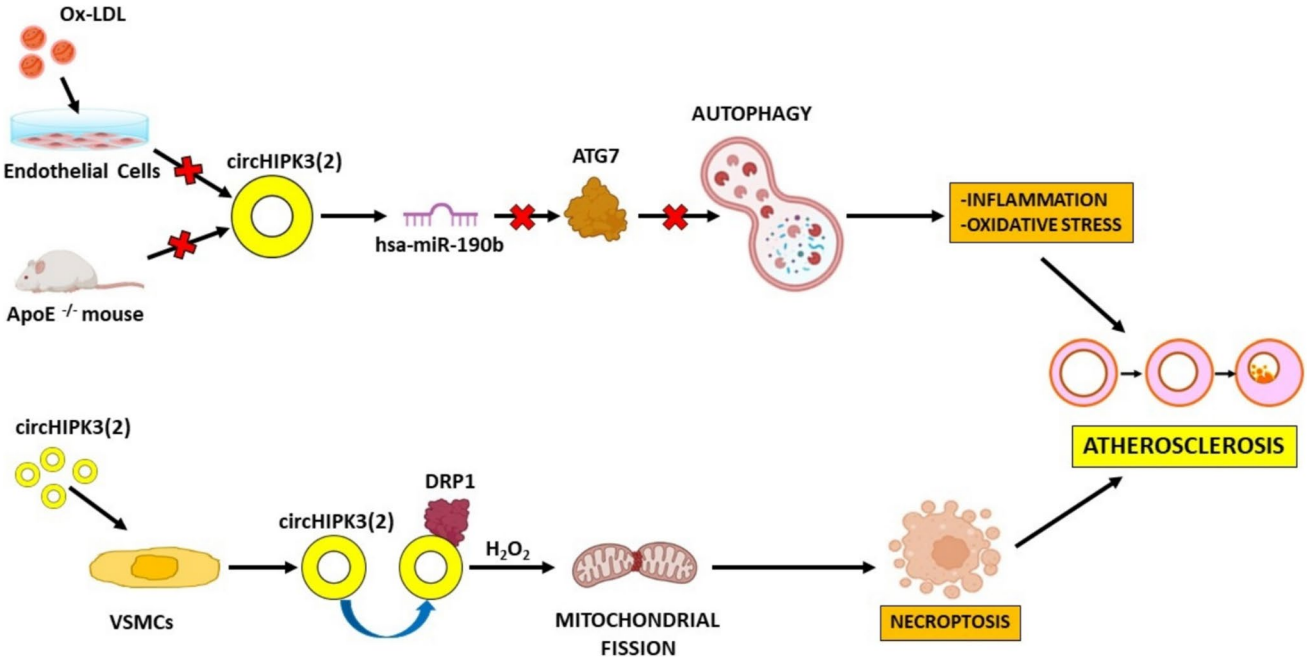

Fig. 5 CircHIPK3(2) effects. Apparently conflicting results on the role of circHIPK3(2) in atherosclerosis. The upper panel illustrates the decrease of circHIPK3(2) induced by a pro-atherosclerotic environment, leading to autophagy depression and atherosclerosis [82]. The lower panel, shows that the atherogenic process was exacerbated by circHIPK3(2) over-expression through the necroptosis induction [85]. Of note, in vitro experiments were conducted in different vascular cell types, endothelial cells (top) and VSMCs (bottom)

circSOD2(3–5)

PDGF-BB stimulation is a well-known inducer of SMC proliferation [107, 108]. Mei et al. [109] performed RNA sequencing on PDGF-BB-stimulated human aortic SMCs and identified circSOD2(3–5), derived from exon 3, 4 and 5 of the SOD2 gene, as significantly upregulated. Silencing circSOD2(3–5) reduced PDGF-BB-induced proliferation, while overexpression had the opposite effect. CircSOD2 exerted its function via sponging hsa-miR-206, thereby increasing NOTCH3 expression [109]. This pathway activated cyclin D1/CDK4/6 transcription, enhancing proliferation [110]. In a rat carotid balloon injury model, circSod2(3–5) was upregulated in neointimal tissue. Silencing the circRNA reduced neointimal formation, VSMC proliferation, and macrophage infiltration, while promoting apoptosis. These findings implicate circSOD2(3–5) as a mediator of neointimal hyperplasia through the miR-206/NOTCH3 axis [109].

circUHRF2(2)

Zhang et al. [111] identified a circRNA derived from exon 2 of UHRF2 (hsa_circ_00086296), as upregulated in human carotid plaques. Oxidized LDL (ox-LDL) stimulation of ECs elevated its expression. Viral overexpression of circUHRF2(2) exacerbated ox-LDL-induced EC migration, angiogenesis, and inflammation. FISH indicated colocalization with EIF4 A3, which was shown to regulate its transcription. The circRNA functioned via a sponge effect on hsa-miR-576-3p, thereby upregulating IFIT1, a promoter of pro-inflammatory macrophage polarization [112]. In $ApoE^{-/-}$ mice fed with a high-fat diet, both circUhrf2(2) and Ifit1 were increased, whereas miR-576-3p was suppressed. Knockout of the circRNA reduced lesion size, inflammation, and plaque area, highlighting its pathogenic role and therapeutic potential through modulation of the circUHRF2(2)/miR-576-3p/IFIT1 axis [111].

Circulating circRNAs as Biomarkers of Plaque Instability

CAD and carotid artery disease share similar histopathological mechanisms, including lipid accumulation, inflammation, and fibrous cap disruption. Consequently, identifying circulating biomarkers predictive of plaque instability has the potential to enhance diagnosis and risk stratification for both conditions. Traditionally, high-risk or rupture-prone plaques—typically characterized by thin-cap fibroatheroma, large necrotic cores, and heightened inflammation—are detected using invasive imaging modalities such as intravascular ultrasound (IVUS) and optical coherence tomography [113, 114].

However, these techniques are costly and not always feasible in routine clinical settings, underscoring the need for reliable, minimally invasive biomarkers. While inflammatory proteins (e.g., hsCRP, IL-6, TNFα) and lipids (e.g., LDL-C, HDL-C) have been evaluated for this purpose [115], recent studies have focused on noncoding RNAs, particularly circulating microRNAs, long noncoding RNAs (lncRNAs), and circRNAs [116–118]. Compared to other RNA classes, circRNAs offer increased stability in plasma due to their resistance to exonuclease-mediated degradation, making them promising candidates for liquid biopsy applications.

circHIPK3(2)/hsa- miR-221 Ratio

In a study by Bazan et al. [119], miRNA levels were measured in carotid plaques of patients undergoing carotid endarterectomy, stratified according to severity. Hsa-miR-221 and −222 were significantly reduced in the plaque shoulder of patients with acute neurological events (urgent group) [119]. Since miR-221/222 target p27 Kip1, a cell cycle inhibitor in VSMCs, their downregulation may contribute to plaque instability. Building on these findings, the same research group assessed serum levels of hsa-miR-221, hsa-miR-222, and circHIPK3(2) (hsa_circ_0000284), which harbors binding sites for these miRNAs [120]. While hsa-miR-222 was undetectable in serum, hsa-miR-221 levels were reduced in the urgent group. Although circHIPK3(2) expression remained unchanged, the circRNA/miRNA ratio was significantly elevated in the urgent group and uncovered a strong discriminative power in receiver operating characteristic (ROC) analysis. Validation in a larger cohort confirmed that this ratio correlated with recent carotid plaque rupture, indicating its potential as a predictive biomarker [120].

circVIRMA(3–4)

Wen et al. [121] explored the expression of circRNAs in serum-derived exosomes from patients with unstable and stable carotid plaques, as determined by magnetic resonance imaging. Microarray analysis revealed 75 differentially expressed circRNAs, with circVIRMA (hsa_circ_0006896), derived from exons 3 to 4 of the VIRMA gene, showing a robust upregulation in patients bearing unstable plaques. Functional assays indicated that exosomes from these patients promoted EC proliferation and migration through the DNMT1/SOCS3/JNK/STAT3 signaling pathway. Moreover, circVIRMA(3–4) levels correlated positively with LDL-C, triglycerides, and CRP in unstable-plaque patients, and negatively with albumin, suggesting its involvement in atherogenic processes. Despite a small sample size of the study precluding multivariate and ROC analyses, the findings underscore the potential diagnostic value of exosomal circRNAs [121].

circKRT14(3–4)

Yan et al. [122] employed circRNA microarray analysis to identify four circRNAs, including circKRT14(3–4) (hsa_circ_0043621), that were upregulated in patients with carotid artery plaques compared to matched controls. circKRT14(3–4) was further validated by RT-qPCR and shown to modulate NLRP3 inflammasome-related miRNAs. Its regulatory role was confirmed via luciferase assays, Western blotting, and cell migration and proliferation studies. Clinical validation in a cohort comprising carotid artery atherosclerotic patients, individuals with increased carotid intima-media thickness (IMT), and healthy controls revealed significantly elevated expression of circKRT14(3–4) in the first two groups. ROC analysis indicated its ability to discriminate pathological cases from healthy individuals. Additionally, expression levels were positively correlated with plasma NLRP3, IL-1β, and IL-18, and the circRNA was identified as an independent predictor of carotid plaque and IMT, suggesting its utility as a biomarker for early atherosclerosis [122].

Conclusion

CircRNAs are emerging as key molecular players in the pathophysiology of atherosclerosis. Their tissue-specific expression, structural stability, and ability to modulate transcriptional and post-transcriptional processes confer unique regulatory properties. A wide range of circRNAs have been shown to exert either protective or deleterious effects in vascular cells, influencing endothelial function, smooth muscle phenotype, inflammation, lipid accumulation, oxidative stress, and cell death pathways such as apoptosis, autophagy, and necroptosis.

Experimental studies have revealed the mechanistic underpinnings of numerous circRNAs in atherosclerosis progression and plaque destabilization. Moreover, discovering circulating circRNAs with diagnostic potential offers promising avenues for early risk prediction and personalized treatment of cerebrovascular and cardiovascular diseases.

Despite these advances, several challenges remain. The lack of standardized detection pipelines, circRNA annotation in reference databases, and translational studies in human populations are notable gaps. Additionally, isoform-specific and cell-context-dependent functions necessitate further research to define the precise role of each circRNA in different disease stages and vascular territories. Future efforts combining transcriptomics, single-cell analysis, and clinical studies will be essential to realize the full potential of circRNAs as therapeutic targets and biomarkers in atherosclerosis.

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  The authors reveal cell-type-specific and atherosclerosis-related changes in gene expression, as well as localized alterations in cell–cell communication.

Abbreviations *AAV*: Adeno-Associated Virus; *ApoE*$^{-}$*/*$^{-}$: Apolipoprotein E Knockout; *CAD*: Coronary Artery Disease; *ceRNAs*: Competing Endogenous RNAs; *CVDs*: Cardiovascular Diseases; *circRNAs*: Circular RNAs; *CLIP-Seq*: Crosslinking and Immunoprecipitation Sequencing; *CRP*: C-Reactive Protein; *ECs*: Endothelial Cells; *FISH*: Fluorescence In Situ Hybridization; *HFD*: High-Fat Diet; *hsCRP*: High-Sensitivity C-Reactive Protein; *IMT*: Intima-Media Thickness; *IVUS*: Intravascular Ultrasound; *lncRNAs*: Long Noncoding RNAs; *LSS*: Laminar Shear Stress; *miRNAs*: MicroRNAs; *MMPs*: Matrix Metalloproteinases; *ncRNAs*: Noncoding RNAs; *NO*: Nitric Oxide; *ONOO*$^{-}$: Peroxynitrite; *OSS*: Oscillatory Shear Stress; *PBMCs*: Peripheral Blood Mononuclear Cells; *P-bodies*: Processing Bodies; *qPCR/RT-qPCR*: Quantitative Polymerase Chain Reaction/Reverse Transcription Quantitative PCR; *RBP*: RNA-Binding Protein; *RNA-Seq*: RNA Sequencing; *ROC*: Receiver Operating Characteristic; *siRNAs*: Small Interfering RNAs; *SMCs*: Smooth Muscle Cells; *UTR*: Untranslated Region

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Declarations

Conflict of interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent No animal or human subjects by the authors were used in this study

Figures Originality All the Figures are original and created by the Authors..

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