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REVIEW ARTICLE

Monocytic microRNAs—Novel targets in atherosclerosis therapy

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Abstract

Atherosclerosis is a chronic proinflammatory disease of the vascular wall resulting in narrowing of arteries due to plaque formation, thereby causing reduced blood supply that is the leading cause for diverse end-organ damage with high mortality rates. Monocytes/macrophages, activated by elevated circulating lipoproteins, are significantly involved in the formation and development of atherosclerotic plaques. The imbalance between proinflammatory and anti-inflammatory macrophages, arising from dysregulated macrophage polarization, appears to be a driving force in this process. Proatherosclerotic processes acting on monocytes/macrophages include accumulation of cholesterol in macrophages leading to foam cell formation, as well as dysfunctional efferocytosis, all of which contribute to the formation of unstable plaques. In recent years, microRNAs (miRs) were identified as factors that could modulate monocyte/macrophage function and may therefore interfere with the atherosclerotic process. In this review, we present effects of monocyte/macrophage-derived miRs on atherosclerotic processes in order to reveal new treatment options using miRmimics or antagomiRs.

KEYWORDS
atherosclerosis, inflammation, macrophages, microRNA, monocytes

1 | INTRODUCTION

Atherosclerosis is a global health problem, affecting more than 200 million people worldwide (Vaduganathan et al., 2022). It provokes end-organ damage and is associated with elevated mortality rates. Ischaemic heart disease and stroke, which are the leading causes of death worldwide, are the result of ongoing atherosclerotic processes (Giugliano et al., 2020).

Atherosclerosis is a systemic chronic disease of the artery wall, involving endothelial cells, vascular smooth muscle cells (VSMCs), and inflammatory cells. Among these, monocytes/macrophages are considered to play a major role in the atherosclerotic process.

Furthermore, atherosclerosis is initiated by increased levels of cholesterol-rich low-density lipoprotein (LDL-C) or triglyceride-rich lipoproteins that penetrate the endothelial cell layer, preferentially at sites where blood flow is disturbed (Zhang et al., 2018). Upon modification of LDLs, mainly due to oxidation, endothelial cells are activated to initiate leucocyte attraction. By expression of selectins, weak adhesion of leukocytes to endothelial cells is initiated, while firm adhesion is mediated by expression of **integrins** (vascular cell adhesion molecule 1 [VCAM-1] and intercellular adhesion molecule

Abbreviations: ApoE, apolipoprotein E; EVs, extracellular vesicles; LDL-C, cholesterol-rich low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; LRP1, LDL receptor-related protein; oxLDL, oxidized LDL; VSMCs, vascular smooth muscle cells.

1 [ICAM-1]) (Langer & Chavakis, 2009; Makó et al., 2010). Expression of these adhesion molecules on the endothelial cell surface and the release of chemokines, such as monocytes chemotactic protein 1 (**MCP-1**) (Lin et al., 2014b), recruit circulating monocytes and initiate their penetration into the vessel wall and differentiation into macrophages (Shapouri-Moghaddam et al., 2018). Pattern recognition receptors on macrophages, such as Toll-like receptors (**TLR**s) and **scavenger receptor**, like CD36 (**cluster of differentiation** 36), mediate the internalization of oxidized LDL (oxLDL). This results in foam cell formation (Kzhyshkowska et al., 2012) and triggers NFκB (nuclear factor kappa-light-chain-enhancer of activated B-cells)-dependent expression of chemokines, such as CXCL1 (C-X-C-motiv ligand 1), resulting in further recruitment of monocytes (Stewart et al., 2010). In nonpathological conditions, macrophages are able to externalize the lipids again via cholesterol transporters, such as scavenger receptor B1 (SR-B1) or ATP-binding cassette subfamily A member 1 (**ABCA1**), thereby counteracting foam cell formation. SR-B1 is the physiological high-density lipid protein (HDL) receptor and exerts protective functions via removal of excess cholesterol from peripheral tissues (Graham, 2023). However, the proinflammatory microenvironment of atherosclerotic lesions impairs the cholesterol efflux system and promotes foam cell accumulation and plaque formation (Maitra et al., 2009). Cholesterol uptake via scavenger or **TLR**s activates the transcription factor NFκB to promote the transcription of **NLRP3** (NACHT, LRR, and PYD domains-containing protein 3). Additional stress signals in the cells, such as enhanced production of reactive oxygen species (ROS), trigger the activation of the NLRP3 inflammasome in macrophages (Lu et al., 2022). Its activation results in caspase-1 maturation and release of inflammatory cytokines, interleukin-1β (**IL-1β**) and IL-18, promoting the amplification of the chronic proinflammatory situation (Sheedy et al., 2013). In addition, growth factors released by foam cells and activated endothelial cells promote proliferation of VSMCs, which contribute to the formation of a fibrous cap and the stabilization of the plaque (Badimon et al., 2012). Furthermore, VSMCs can transdifferentiate into macrophage-like cells, take up lipids via scavenger receptors, and contribute to foam cell formation (Wang et al., 2019). Within the plaque, a necrotic core is formed due to apoptotic cell death. Apoptotic cells lose their phagocytic receptors, such as LDL receptor-related protein (LRP1), resulting in decreased clearance of apoptotic cells by phagocytes, a process known as efferocytosis (Linton et al., 2016). As a result, dead cells accumulate, which increases chronic inflammation and leads to plaque growth. Defective efferocytosis of dying cells is a critical event in the formation of vulnerable atherosclerotic plaques. Prevailing inflammation and oxidation products lead to death of VSMCs and thinning of the fibrous cap. Furthermore, macrophage-derived matrix metalloproteinases (**MMP**s) degrade extracellular matrix components, like collagen, thereby contributing to cap thinning (Lin et al., 2014a). These processes make the cap unstable and susceptible to rupture. The thickness of the cap correlates with its vulnerability (Jebari-Benslaiman et al., 2022). Finally, plaque rupture triggers a coagulation process involving the complement system, platelet activation, and entrapment of erythrocytes, which leads to red thrombus formation. However, other plaques accumulate more matrix, smooth muscle cells, and less lipids. Such plaques are prone to erosion provoking white thrombus formation (Libby, 2021). Finally, thrombus formation leads to arterial volume narrowing and a severe reduction in blood flow. This ultimately leads to end-organ damage, which is associated with a high mortality rate or may result in severe patient disability.

2 | MACROPHAGE POLARIZATION

M1 macrophages, activated by stimuli like interferony, lipopolysaccharides (**LPS**), or oxLDL, are responsible for the release of proinflammatory cytokines like tumour necrosis factor alpha (TNFα) or IL-1β, as well as the release of oxygen radicals. This creates a sustained proinflammatory, detrimental environment in the plaque (Murray, 2017; Wu et al., 2023). In contrast, M2 macrophages act in an anti-inflammatory and antioxidative manner and contribute to tissue repair, thereby counteracting M1 macrophages. Interleukins, like IL-4 or IL-13, or glucocorticoids, stimulate M2 polarization. These cells secrete anti-inflammatory cytokines like IL-10 or IL-12, as well as profibrotic factors like collagen or fibronectin to promote tissue repair (Mushenkova et al., 2022). The fibrotic fibres serve to cover the necrotic core resulting in plaque stabilization. Furthermore, M2 macrophages exert high phagocytic activity for clearance of apoptotic cell debris (Xie et al., 2022), which helps to reduce the necrotic core size and thus to stabilize the plaque. Some M2 subtypes express high levels of the cholesterol transporter ABCA1 and can thereby enhance cholesterol efflux and thus reduce foam cell formation. All these functions of M2 macrophages counteract M1 cells and increase plaque stability.

In early stages of atherosclerosis, M2 macrophages prevail in the initial stable plaque. However, as the disease progresses, the number of M1 exceeds M2 macrophages and contributes to the formation of unstable, rupture-prone plaques. Thus, maintenance of a low M1/M2 ratio is a sign for stable plaque building and prevents adverse atherosclerotic progression. M1/M2 polarization is a dynamic process. M1 or M2 macrophages can originate from monocytes. However, M1 can also differentiate into M2 macrophages and vice versa. Promoting M2 polarization at the expense of M1 macrophages is an interesting therapeutic target for the treatment of atherosclerosis.

3 | MONOCYTIC microRNAs

miRs are small noncoding RNAs of about 20 nucleotides. They are known as regulators of diverse cellular processes. miRs, together with a member of the argonaute (AGO) protein family, form the ribonucleoprotein complex known as RNA-induced silencing complex (RISC). The mature RISC binds target mRNAs mainly at the 3′ untranslated region (3′ UTR), but also at the 5′ UTR or in open reading frames, and silences their expression by slicing them or recruiting proteins that mediate translational repression and/or destabilization. The binding of miRs to UTRs and coding regions have silencing effects on gene expression

while miR interaction with promoter regions has been reported to enhance transcription (Iwakawa & Tomari, 2022; O'Brien et al., 2018). Changes in miR expression patterns have been detected in blood samples (in serum, extracellular vesicles [EVs], as well as in blood cells) but also in plaques from atherosclerotic patients (Rafiei et al., 2021; Wang et al., 2021; Zhang et al., 2016). They can be used as biomarkers for atherosclerosis progression (Parahuleva et al., 2017, 2018), but they are also directly involved in atherosclerotic processes. miR expression changes have been found in all cell types that participate in atherosclerotic events, like endothelial and VSMCs, but also in monocytes/macrophages. In this review, we will focus on miRs that are expressed in monocytes/macrophages and have been shown to contribute to atherosclerosis. The sequence-specific effects of miRs allow the control of inflammatory mediators, monocyte recruitment, M1/M2 polarization, lipid uptake and efflux, foam cell formation, and plaque stability. These specific miR-effects on individual steps of the atherosclerotic process are discussed in detail in the following sections of this review (Figure 1).

In terms of therapeutic treatments, interference with miRs has great potential, as they can be very specifically suppressed by antagomiRs or enhanced by miRmimics. In addition, the development of carrier systems is the focus of current research with the aim to allow the preferred uptake of these oligonucleotides into certain cell types, such as macrophages. Cell-specific uptake, together with the sequence-specific regulation of signalling pathways may finally allow very targeted interventions in atherosclerotic processes. However, systems for cell-specific uptake of antagomiRs or miRmimics are still in their infancy and certainly require further research before they can be used in patients.

3.1 | Monocytic microRNAs controlling inflammatory mediators and recruitment of monocytes

There is plenty of evidence in the literature that monocytic miRs can influence inflammatory responses and atherosclerosis, which we will present in this section.

Cellular apolipoprotein E (ApoE) expression exerts anti-inflammatory properties protecting against atherosclerosis by enhancing miR-146a levels in monocytes and macrophages. Hyperlipidaemic ApoE/low-density lipoprotein receptor (LDLR) deficient (ApoE(−/−) LDLR(−/−)) mice display enhanced chronic inflammation. Cellular enrichment of miR-146a through the systemic delivery of miR-146a mimetics in ApoE(−/−)LDLR(−/−) mice attenuated monocyte/macrophage activation and atherosclerosis in the absence of plasma lipid reduction. This characterizes miR-146a as anti-inflammatory and antiatherosclerotic miR (Li et al., 2015).

Similarly to miR-146, miR-223 has anti-inflammatory and anti-atherosclerotic properties. In addition, it also impacts cholesterol metabolism. miR-223 overexpression antagonizes LPS-induced macrophage-derived foam cell formation and suppresses the TLR4/NFκB pathway leading to a reduction of proinflammatory cytokines in LPS-stimulated macrophages (Wang, Bai, et al., 2015). Furthermore, it suppresses NLRP3 inflammasome activation and IL-1β release (Haneklaus et al., 2012). Deficiency of miR-223 in bone marrow-derived cells results in increased numbers of circulating monocytes and enhanced cytokine release including **IL-6** (Nguyen et al., 2022). In humans with unstable carotid atherosclerotic plaques, miR-223 levels

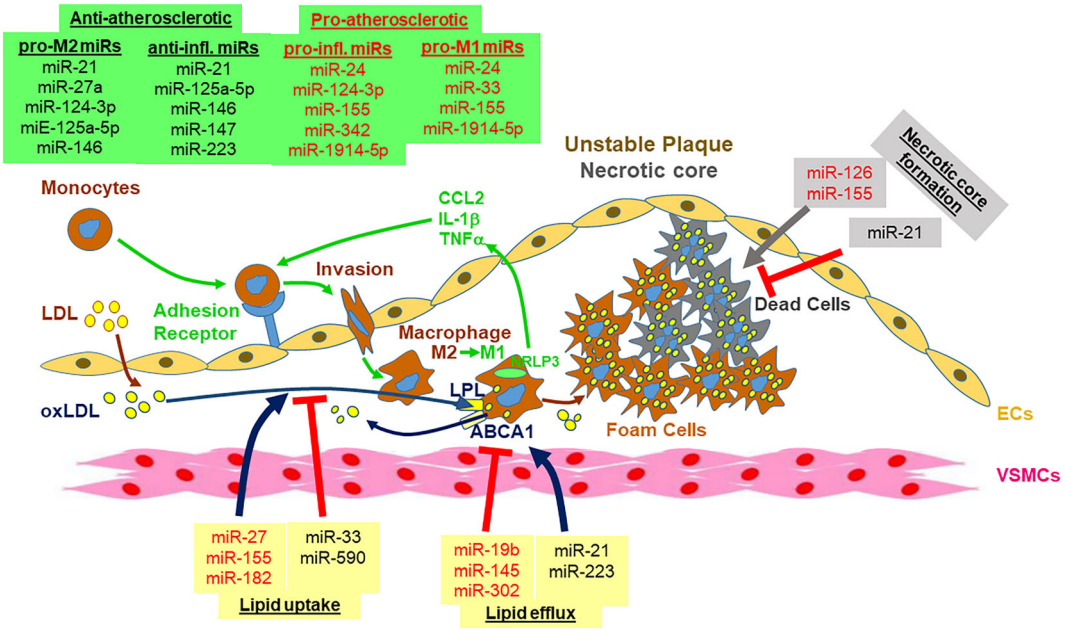

FIGURE 1 Interaction of monocytic microRNAs with atherosclerotic processes. The course of atherosclerosis is shown, starting with the entry of inflammatory monocytes into the tissue and their differentiation into macrophages (depicted in green), via the uptake of oxLDL and the formation of foam cells (depicted in yellow/blue), the death of the cells and the formation of an unstable plaque (depicted in grey). Monocytic microRNAs that stimulate atherosclerotic processes are written in red, and antiatherosclerotic microRNAs are written in black. MicroRNAs that modulate the inflammatory process of atherosclerosis are shown in green boxes, microRNAs that influence lipid uptake and foam cell formation are shown in yellow boxes, and those regulating cell death and necrotic core development are shown in grey boxes.

are increased. This up-regulation can be considered as a negative feedback loop to limit atherosclerosis progression (Nguyen et al., 2022).

Another antiatherogenic miR is miR-125a-5p. It was found to mediate lipid uptake and to decrease the secretion of some inflammatory cytokines (IL-2, IL-6, TNFα, transforming growth factor-beta [**TGFβ**]) in oxLDL-stimulated monocyte-derived macrophages (Chen et al., 2009). The reduced cytokine release may be due to the inhibition of M1 polarization by miR-125a.

MiR-147 is also one of the antiatherogenic miRs. It is induced in LPS-stimulated mouse macrophages via TLRs. Transfection of miR-147 mimics into macrophages significantly decreased LPS-induced TNFα and IL-6 production. These data demonstrate that miR-147 attenuates the inflammatory response of macrophages and thus serves as a negative feedback loop to prevent excessive inflammatory responses upon LPS stimulation (Liu et al., 2009).

A very prominent miR with antiatherosclerotic properties is miR-21. It is found to be increased in postefferocytotic peripheral blood monocyte-derived macrophages (Das et al., 2014). Following successful efferocytosis, miR-21 induction in macrophages results in elevated production of anti-inflammatory IL-10. Increasing miR-21 levels, using miRmimics, provoked a significant suppression of LPS-induced TNFα expression. Both processes account for a net anti-inflammatory phenotype (Das et al., 2014; Huang et al., 2018).

In addition to the anti-inflammatory miRs presented so far, a number of proinflammatory miRs exist. miR-124-3p is one that belongs to this group. Expression of miR-124-3p is increased in monocytes of smokers in association with a higher probability of atherosclerosis. It up-regulates the monocyte surface protein cluster of differentiation CD29, one component that binds to its ligand **VCAM-1** on the endothelium (de Ronde et al., 2017). This promotes monocyte recruitment to the vessel wall, an initial step towards atherosclerosis. However, opposite findings were presented by Manoharan et al. (2014), showing that anti-miR-124a increases the C-C-motiv ligand 2 (**CCL2**) mRNA levels in murine macrophages, thereby creating a proinflammatory and proatherosclerotic environment. Therefore, its mode of action may depend on the environmental conditions in vivo.

An increase in miR-155 levels has been shown in atherosclerosis-prone ApoE knock out (KO) mice under a high-fat diet. MiR-155 seems to have dual roles: either proinflammatory or anti-inflammatory. In the early stage of atherosclerosis, miR-155 suppressed lesion formation in ApoE KO mice via reducing the lesional macrophage content by inhibiting *Csf1r*-mediated macrophage proliferation (Wei et al., 2015). However, the proatherosclerotic action of miR-155 seems to prevail: Leukocyte-specific miR-155 deficiency reduced plaque size and the number of lesional macrophages in ApoE KO mice after partial carotid ligation via reduction of CCL2 expression (Nazari-Jahantigh et al., 2012). Furthermore, overexpression of miR-155 promoted the activation of the NLRP3 inflammasome and the release of IL-1β in oxLDL-induced macrophages. This proinflammatory action can explain the aggravation of atherosclerotic lesions by miR-155 in ApoE KO mice (Yin et al., 2019). Furthermore, miR-155 exerts proatherosclerotic functions not only in relation to inflammation but also in other areas such as lipid metabolism. In the context of miR-155, the function of miR-342 should be discussed, because macrophage-derived miR-342-5p promotes atherosclerosis and enhances the inflammatory stimulation of macrophages by suppressing the serine/threonine kinase Akt1-mediated inhibition of miR-155 expression, resulting in miR-155 up-regulation in ApoE KO mice and enhanced release of inflammatory cytokines like IL-6. Systemic treatment with an inhibitor of miR-342-5p reduced the progression of atherosclerosis in the aorta of ApoE KO mice (Wei et al., 2013).

MiR-21 is found to be highly expressed in monocytes. miR-21 negatively regulates the expression of various proinflammatory mediators including LPS and TNFα (Huang et al., 2018) and thus acts as anti-inflammatory and antiatherogenic.

Just recently, miR-1914-5p was identified to be down-regulated by IL-1β in a human monocytic cell line (THP-1). This enhanced adhesion to endothelial cells by up-regulation of macrophage 1-antigen (Mac1), a counter-ligand to ICAM-1. Furthermore, transmigration activity through the endothelial cell layer increased due to MCP-1. These effects were counteracted by transfection of monocytes with miR-1914-5p mimics (Toriuchi et al., 2023).

The transmigratory activity and thus the invasive capacity of macrophages can be influenced by MMPs, because they change the extracelluar matrix of the cells and thereby the motility and flexibility of the cells. MiR-24 has been shown to impact MMPs in macrophages. Unstable plaques contained lower miR-24 levels than stable plaques, and miR-24 colocalized with foam cell macrophages that exhibited low MMP-14 protein expression, which is a sign for an invasive subset of macrophages. Silencing miR-24 in macrophages significantly increased MMP-14 expression and enhanced their invasive capacity. In vivo, systemic miR-24 silencing in atherosclerotic ApoE KO mice accelerated atherosclerosis (Di Gregoli et al., 2014).

3.2 | Monocytic microRNAs controlling M1/M2 polarization

The polarization state of macrophages clearly regulates their influence on atherosclerosis because M1 macrophages have a proinflammatory character and M2 macrophages acts anti-inflammatory (Wu et al., 2023). Their polarization state can be influenced by monocytic miRs, as will be discussed in this section.

MiR-155 has been introduced above as acting proinflammatory via activation of the NLRP3 inflammasome and the release of IL-1β. Considering the influence of miR-155 on macrophage polarization, it also has a clear proinflammatory character, because it stimulates M1 polarization and simultaneously inhibits M2 polarization through the inhibition of *IL-13* and IL-4 pathway components (Pasca et al., 2020).

Another interesting miR in this context is miR-33. Via disruption of the balance of aerobic glycolysis and mitochondrial oxidative phosphorylation, miR-33 instructs macrophage polarization and shapes

innate and adaptive immune responses. It promotes an inflammatory M1-like macrophage phenotype that is associated with metabolic diseases such as atherosclerosis. Notably, inhibition of miR-33 metabolically reprograms macrophages to the M2 phenotype. This effect is independent of the role of miR-33 in regulating macrophage cholesterol efflux (Ouimet et al., 2015). Treatment of hypercholesterolemic mice with miR-33 inhibitors resulted in the accumulation of inflammation-suppressing M2 macrophages and regulatory T cells (Tregs) in plaques and reduced atherosclerosis progression (Ouimet et al., 2015). As regulatory T cells (Tregs) also reduce macrophage inflammation and promote differentiation to the M2 phenotype (Foks et al., 2015), the increased number of M2 macrophages in plaques of anti-miR-33-treated mice may initiate a positive feedback loop that further restores an anti-inflammatory low M1/M2 macrophage ratio.

The elevation of monocytic miR-124-3p in smokers in association with a higher probability of atherosclerosis, as described above, is also related to the up-regulation of cluster of differentiation CD206, a marker of M2 polarization (de Ronde et al., 2017). In murine monocytes miR-124-3p has been shown to mediate the elevation of the monocyte surface protein CD206, which is an indicator of M2 polarization (Veremeyko et al., 2013). Although this is a sign of antiatherosclerotic action (Wu et al., 2023), the increase in miR-124-3p in vivo is associated with atherosclerosis, indicating that the recruitment of monocytes to the vessel wall plays a leading role of miR-124-3p in atherosclerosis.

miR-27a can also change macrophage polarization, because exposure to alcohol provoked M2 polarization via miR-27a in human monocytes (Saha et al., 2015). However, whether this influence on M2 polarization is relevant for atherosclerosis has not yet been shown.

MiR-21 has already been introduced as an antiatherosclerotic miR. This holds true also in respect to macrophage polarization. Treatment of human peripheral blood mononuclear cells with LPS resulted in induction of miR-21. Transfection of cells with a miR-21 precursor blocked NFκB activity and promoted IL-10 production in response to LPS, thereby promoting M2 polarization and anti-inflammation (Sheedy et al., 2010). The same applies to miR-223. miR-223 represses cytokine release in macrophages, and this goes along with macrophage polarization towards the anti-inflammatory M2 phenotype (Nguyen et al., 2022).

As well, miR-146 acts antiatherogenic in LDLR KO mice and inhibits M1 polarization. However, mice deficient in miR-146 in the bone marrow have reduced atherosclerosis, indicated by suppression of endothelial cell activation and attenuated atherosclerotic plaque burden, presumably due to reduced haematopoiesis resulting in lower levels of circulating monocytes (Cheng, Besla, et al., 2017). These findings indicate that the influence of miRs on atherosclerosis is dependent of the localization of the expression. Whereas the antiatherogenic action was found under systemic application of miR-146-mimics, reduced atherosclerosis was evident when miR-146 was decreased only in bone marrow cells.

Overexpression of miR-125a-5p diminished M1 phenotype expression under LPS and promoted M2 marker expression induced by IL-4. miR-125a-5p targets KLF13 (Krüppel-like factor 13), a transcriptional factor that has an important role in T lymphocyte activation and inflammation (Banerjee et al., 2013). Through promotion of a low M1/M2 ratio, miR-125a-5p may have antiatherogenic potential.

3.3 | Monocytic microRNA controlling lipid metabolism, transport, and foam cell formation

Macrophage cholesterol homeostasis results from a balanced relationship between cholesterol synthesis, uptake, and efflux and is an essential component in the prevention of atherosclerosis. The influence of miRs on these processes becomes clear just when we consider their impact on ABCA1, a transporter responsible for cholesterol efflux from macrophages. ABCA1 is a target of about 10 different miRs, all of which provoke ABCA1 down-regulation and thus cholesterol accumulation in macrophages resulting in foam cell formation. As they have been reviewed by Tabaei and Tabaee (2021), we will not discuss all of them in detail.

ABCA1 expression is regulated by miR-302a, amongst others (Meiler et al., 2015). It is regulated in primary macrophages by modified LDLs. Transfection of murine macrophages with miR-302a down-regulated ABCA1 expression and attenuated cholesterol efflux in response to apolipoprotein A-1 (ApoA-1). Long-term in vivo administration of anti-miR-302a to mice with LDLR deficiency, fed an atherogenic diet, led to an increase in ABCA1, reduced atherosclerotic plaque size, and a more stable plaque morphology with reduced signs of inflammation (Meiler et al., 2015). Also, miR-19b directly regulated the expression levels of endogenous ABCA1 in foam cells derived from human THP-1 macrophages and dramatically suppressed ApoA-1-mediated ABCA1-dependent cholesterol efflux. miR-19b precursor treatment increased aortic plaque size and lipid content in ApoE KO mice (Lv et al., 2014). Furthermore, the anti-inflammatory action of miR-223 is accompanied by promotion of cholesterol efflux from macrophages due to the up-regulation of ABCA1, thereby counteracting foam cell formation (Nguyen et al., 2022). miR-21 also influences ABCA1 expression. In the absence of miR-21 in macrophages, ABCA1 is down-regulated, reducing efflux of cholesterol within macrophages, and hence its dysfunction will promote the development of foam cells, thus building up the atherosclerotic plaque (Canfrán-Duque et al., 2017). Although miR-145 is mainly considered as VSMC specific, upon TNFα stimulation, it can be transferred from VSMCs to macrophages via transport in VSMC-derived EVs and thus then acts as monocytic miR. In EV-transfected macrophages, miR-145 decreases ABCA1 expression and cholesterol efflux and thereby contributes to foam cell formation (Sala et al., 2014).

miR-155 also affects macrophage lipid metabolism, but not via ABCA1. Its expression increased significantly in both plasma and macrophages from atherosclerotic (ApoE−/−) mice. oxLDL induced the expression and release of miR-155 in macrophages, which mediated oxLDL-induced lipid uptake and ROS production of macrophages. Inhibition of miR-155 by antagomiR-155 decreased lipid

loading in macrophages and thus foam cell formation, resulting in reduced atherosclerotic plaques in ApoE KO mice (Tian et al., 2014). Thus, in terms of lipid metabolism, miR-155 acts clearly proatherogenic.

Macrophage-derived lipoprotein lipase (LPL) may contribute to foam cell formation and the progression of atherosclerosis via modulation of the cell surface accumulation and subsequent cellular uptake of modified lipoproteins, especially oxLDL (Wang et al., 2007). MiR-27 has been shown to down-regulate LPL in macrophages (Zhang et al., 2014). However, in the proatherogenic environment, such as ApoE KO mice fed with high-fat diet, miR-27 is down-regulated. Application of miR-27 mimics in these mice down-regulated LPL and decreased aortic plaque size and lipid content (Xie et al., 2016). As well, miR-27 affected the ability of THP-1 macrophages to uptake DiI-oxLDL via LPL (Zhang et al., 2014). MiR-182 also affects LPL levels. It up-regulated LPL expression by directly targeting HDAC9 (histone deacetylase 9) in THP-1 macrophages. ApoE KO mice treated with miR-182 mimics presented increased plasma concentrations of proinflammatory cytokines and lipids and exhibited more severe atherosclerotic plaques (Cheng, Gong, et al., 2017). In contrast to miR-182 and miR-27 that augment LPL expression, miR-590 directly targets and represses macrophage LPL levels, thus retarding the formation of proinflammatory foam cell macrophages. Accordingly, in high fat-fed ApoE KO mice, systemic administration of miR-590 mimics prevented the progression of aortic atherosclerosis (He et al., 2015).

The selective loss of miR-33 in macrophages enhances the capacity of the cells to efflux cholesterol via enhanced ABCA1 expression and is sufficient to reduce monocyte recruitment, lipid accumulation, and atherosclerotic plaque burden under hyperlipidaemic conditions (Price et al., 2017). However, whole body depletion of miR-33 is proatherogenic, as it increases body weight and dyslipidaemia in LDLR−/− mice (Price et al., 2017). This indicates that systemic depletion of miR-33 affects other organs that counteract its antiatherogenic functions in macrophages.

3.4 | Monocytic microRNA controlling efferocytosis and thenecrotic core of the plaque

Impaired efferocytosis resulting in reduced elimination of dead cells in the plaque contributes to the growth of the necrotic core and plaque instability.

Impairment of efferocytosis is found under enhanced miR-155 levels. It diminishes B cell lymphoma 6 (*Bcl6*)-mediated phagocytosis of apoptotic cells during the advanced stage of atherosclerosis, leading to increased formation of a necrotic core and acceleration of atherosclerosis (Wei et al., 2015). As discussed above, miR-155 acts proatherosclerotic at multiple points (inflammation, lipid accumulation, and efferocytosis); so overall the main effect of miR-155 is proatherogenic. However, some anti-inflammatory effects in the early phase of atherosclerosis cannot be excluded. Therefore, treatment options with anti-miR-155 should preferably start in advanced stages of the disease.

miR-126 levels decrease in diabetic mice, which results in impaired efferocytosis in macrophages. Defective efferocytosis in diabetic conditions leads to increased inflammation and necrotic core formation that eventually contributes to atherosclerosis (Suresh Babu et al., 2016).

Following LPS treatment, the expression of miR-21 was increased in monocyte-derived macrophages that engulfed apoptotic cells. miR-21 is directly implicated in switching wound-associated macrophages to an anti-inflammatory mode following successful engulfment of apoptotic cells at the site of injury (Das et al., 2014). Vice versa macrophage miR-21 deficiency provokes vascular inflammation (Canfrán-Duque et al., 2017).

4 | CROSS TALK OF MONOCYTES/MACROPHAGES WITH OTHER CELLS VIA EXTRACELLULAR VESICLES (EVs)

EVs are microscopic phospholipid bilayer-encircled particles ranging in size from ∼30 nm to 5 μm and include exosomes, microvesicles/microparticles, and apoptotic bodies. They contain proteins, lipids, small molecules, and nucleic acids, including miRs (Li et al., 2018). They are secreted by cells and serve as intercellular communicators. Many EVs, derived from endothelial, vascular smooth muscle, and mesenchymal stem cell, that target monocytes/macrophages and thereby influence atherosclerotic processes, have been described. The majority of EVs detected in peripheral blood are derived from platelets, followed by mononuclear phagocytes, including macrophages (Hunter et al., 2008). RNA molecules, contained in macrophage-derived EVs, can target monocytes, endothelial cells, and fibroblasts and thus enhance intercellular communication between these cells. For example, M2-derived EVs transfer miR-148a to inhibit ischaemia–reperfusion injury in the heart, probably via the reduction of the NLPR-inflammasome (Dai et al., 2020). Also in atherosclerosis, macrophage-derived EVs play a role: EVs released from macrophages drove the differentiation of human THP-1 monocytes and naive monocytes to the macrophage state and increased their adhesion properties to endothelial cells. Encapsulated miR-223 was the prevailing miR in these EVs and may thus play a major role in this process that serves as a positive feedback loop to locally activate and intensify the innate immune response (Ismail et al., 2013). EVs from bone marrow-derived macrophages, containing miR-99a/146b/378a, suppressed NFκB and TNFα signalling, enhanced M2 polarization, and attenuated the inflammatory response in atherosclerosis (Bouchareychas et al., 2020).

Often, M2-derived EVs, encapsulating miR221-3p or miR199a-5p or others, affect endothelial or VSMCs and act antiatherosclerotic via modulation of cell adhesion, cytokine release, apoptosis, migration, or proliferation (reviewed by Wang et al., 2023). However, EVs derived from mouse and human M1 macrophages treated with an atherogenic stimulus (oxLDL) were enriched in miR-146a, miR-128, miR-185, miR-365, and miR-503. Delivery of such EVs to naive macrophages decreased cell migration and

promoted macrophage entrapment in the vessel wall, thereby accelerating the risk for development of atherosclerosis. Inhibition of miR-146a, the most enriched miR in atherogenic EVs, reduced the inhibitory effect of EVs on macrophage migratory capacity (Nguyen et al., 2018).

5 | CURRENT TREATMENT OPTIONS FOR ATHEROSCLEROSIS

Chronic inflammation, provoked by enhanced cholesterol levels, modification of lipoproteins by oxidation or glycation, and activation of endothelial cells, is the main driver of atherosclerosis. Cells of the adaptive and innate immune systems are involved in atherosclerosis. Helper T cells of the Th1 subtype, as well as B-lymphocytes and monocytes/macrophages in particular, play a prominent role in this process. Current therapies are predominantly directed against elevated LDL-C levels as a primary and secondary prevention for atherosclerotic disease. LDL-C is one driver of immune cell activation (Virani et al., 2020). Therefore, blocking of LDL-C or cholesterol synthesis is a useful therapeutic target for the treatment of atherosclerosis. Indeed, statins that interfere with cholesterol biosynthesis via inhibition of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA-reductase) are standard drugs in the treatment of atherosclerosis (Hermida & Balligand, 2014) (Figure 2). Statin treatment of patients results in lowering of the LDL-C level, reduced inflammation, and slowing of the atherosclerotic process (Kinlay et al., 2003).

An important addition is the identification of very high-risk patients who may benefit with risk reduction from the addition of nonstatins (as **Ezetimibe** ± **proprotein convertase subtilisin/kexin 9** [PCSK9] inhibitor) to maximally tolerated statin therapy, because, in clinical practice, >70% of patients with established atherosclerotic cardiovascular disease and statin therapy do not reach an LDL-C < 70 mg·dl^{-1} (Mach et al., 2020). In addition, nonstatins are an alternative therapy for patients with partial or complete intolerance to statins, because up to 25% of currents statin users report adverse musculoskeletal effects that prevent them from using statins or limit their ability to receive guideline-recommended doses (Jacobson et al., 2018).

Ezetimibe is an elective cholesterol absorption inhibitor, which reduces the delivery of cholesterol to the liver and blood. Its primary target of action is the cholesterol transport protein Nieman Pick C1 like 1 protein (**NPC1L1**) in the intestine (Figure 2) (Jia et al., 2011). As a result, Ezetemibe promotes the synthesis of LDL receptors in the intestine with a subsequent incremental reduction of serum LDL-C by 13% to 20% (Grundy et al., 2019).

Bempedoic acid is another nonstatin, an ATP citrate lyase inhibitor that targets cholesterol synthesis upstream of HMG-CoA-reductase, the enzyme inhibited by statins, which reduce LDL-C levels in the circulation (Pinkosky et al., 2016). Furthermore, as a prodrug that is activated in the liver and not in most peripheral tissues, including skeletal muscle, the bempedoic acid therapy is associated with a low incidence of muscle-related adverse events (Lim, 2023). Therapy with bempedoic acid is not only a factor that may reduce the potential for adverse effects on muscles by statin-intolerant patients but is also associated with a lower risk of major adverse cardiovascular events (Nissen et al., 2023).

PCSK9 inhibitors have recently become a new therapeutic option as nonstatins and on top therapy for very high-risk patients (Patriki et al., 2022). PCSK9 binds to the LDL receptor, induces its degradation, and thereby increases circulating blood LDL-C. The PCSK9 inhibitors (Evolocumab and Alirocumab) reduce LDL-C by ~60% and are administered either once or twice monthly by subcutaneous injection. Through the reduction of LDL-C levels by PCSK9 inhibitors, inflammation and atherosclerotic processes are reduced (Ragusa et al., 2021).

Inclisiran is a novel small interfering RNA (siRNA) therapy that inhibits the translation and production of the PCSK9 protein in hepatocytes, leading to decreased concentrations of PCSK9 and plasma concentrations of LDL-C (Ray et al., 2017). This process is facilitated via entry of the siRNA molecules into a cell (hepatocyte). There they

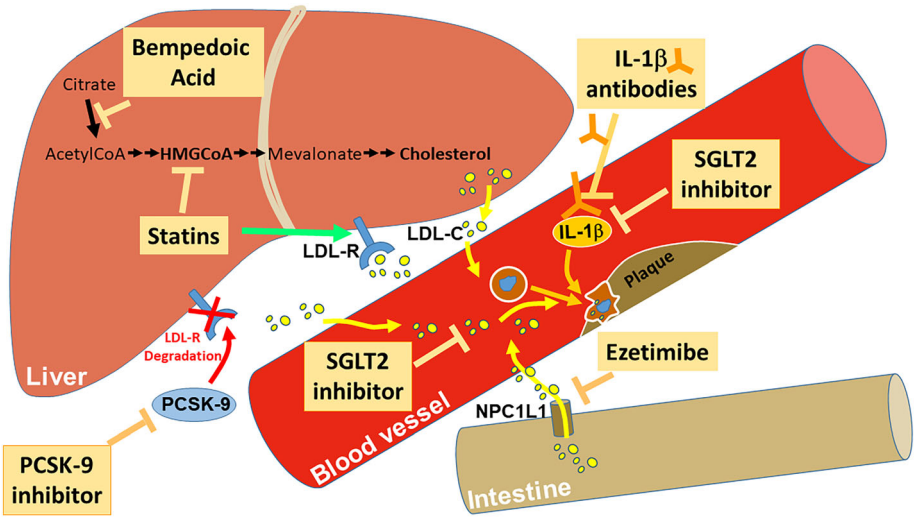

FIGURE 2 Current treatment options of atherosclerosis. Statins and bempedoic acid inhibit steps of cholesterol synthesis in the liver. At the same time LDL-R expression is enhanced. Both processes contribute to lowering of LDL levels. Ezetimibe inhibits cholesterol uptake from the intestine by blocking the cholesterol transporter NPC1L1. PCSK-9 inhibitors protect from LDL-R degradation. IL-1β antibodies act anti-inflammatory and SGLT-2 inhibitors have anti-inflammatory and cholesterol lowering effects.

are loaded into the RISC complex and undergo separation of the guide and passenger strands (Alshaer et al., 2021). After separation, the passenger strand is discarded and the guide strand/RISC complex travels to the target mRNA where it cleaves the PCSK9 specific mRNA, inhibiting the translation of target PCSK9 protein. This is a unique mechanism, because the guide strand remains in the RISC and it is able to continue to bind and degrade mRNA for an extended period of time. This allows lower and less frequent dosing than previous PCSK9 inhibitors. Thus, inhibition of translation and production of the PCSK9 protein prevents the destruction of LDL receptors present on hepatocytes. This ensures the presence of the LDL receptor on the surface of hepatocytes, through which the uptake of LDL-C from circulation is increasing and serum LDL-C levels are consequently decreasing. As a new nonstatin strategy for intensive LDL-C lowering, Inclisiran is administered subcutaneously as a 284-mg injection on day 1, followed by a second injection on day 90, and further injections every 6 months. This infrequent and thus more convenient dosing increases patient compliance. Inclisiran significantly lowers total cholesterol, LDL-C by up to 50%, and triglycerides and is associated with an 18%–25% reduction in lipoprotein(a) (LP(a)) levels (Soffer et al., 2022). The safety and tolerability profile of the drug is similar to placebo, although mild to moderate, transient injection-site adverse reactions were more frequent with Inclisiran (Frampton, 2023). However, there are no completed cardiovascular outcome studies for Inclisiran to date. The ORION-4 and VICTORIAN-2P are such ongoing trials for Inclisiran with completion dates anticipated in 2026 and 2027, respectively.

Vaccine-based approaches to reduce LDL-C is one of multiple strategies, which have been most recently described to inhibit PCSK9, such as monoclonal antibodies, inhibitors, or siRNA (Fowler et al., 2023). This bivalent virus-like particle (VLP)-based vaccine is a form of active immunotherapy with two different peptides of PCSK9 that elicits anti-PCSK9 antibodies, reduces serum levels of PCKS9, increases liver LDL receptor expression, and reduces circulating LDL-C in multiple animal models without requiring coadministration of statins (Fowler et al., 2023). The vaccination is the most novel trend in therapy against atherosclerosis and needs to be further developed.

Furthermore, during treatment of diabetic patients with sodium glucose cotransporter 2 (**SGLT2**)-inhibitors, besides their blood glucose reducing effects, antiatherosclerotic properties via favourable influences on lipid metabolism, reduction of systemic inflammation, and improvement of endothelial function were observed (Pahud de Mortanges et al., 2021; Xu et al., 2023).

In patients with high levels of C-reactive protein (CrP) (2 mg or more per litre), lowering circulating cytokines is an additional option to LDL-C-lowering therapies. An efficient reduction of atherothrombotic events by such a treatment, in addition to the standard LDL-C lowering therapy, was proved in the CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) trial: An anti-inflammatory therapy targeting the innate immunity pathway by monoclonal antibodies against IL-1β led to a significantly lower rate of recurrent cardiovascular events in post-myocardial infarction patients with high C-reactive protein (CrP) levels (Figure 2). However, patients experienced a higher incidence of fatal infections and sepsis due to the overall reduction in inflammatory responses (Ridker et al., 2017), indicating that a systemic intervention in the inflammatory systems causes unwanted side effects, and a more specific approach to target proinflammatory macrophages in the plaque would be a better solution to treat atherosclerotic patients. In this context, it must be considered that during atherosclerosis not only the number of infiltrating monocytes/macrophages is crucial for plaque formation but also their polarization state. Basically, two main polarization states can be distinguished, the proinflammatory M1 and the anti-inflammatory M2 macrophages. A more sophisticated fine tuning of M1/M2 ratios in atherosclerosis could, therefore, be a better treatment than a general immunosuppression. Furthermore, the possibility of specific interventions in foam cell formation or necrotic core building, in which macrophages are involved, could provide new treatment options.

6 | TREATMENT OPTIONS USING microRNAs

6.1 | Treatment options in the cardiovascular system

Anti-RNA-based drugs, like antisense oligonucleotides, which specifically target mRNA sequences in order to repress specific disease promoting protein expression, are already established and food and drug administration (FDA) approved. With regard to the cardiovascular system, Mipomersen, that targets the mRNA coding for Apolipoprotein B-100, or Patisiran, directed against a mRNA causing transthyretin-mediated amyloidosis, can be mentioned (Huang et al., 2020), as well as Inclisiran, that targets PCSK9 mRNA, thereby interfering with atherosclerotic processes (Ray et al., 2017).

In contrast to the great progress made with antisense oligonucleotides, the use of miRs as therapeutics are not yet that far developed. But studies in animal models and first clinical trials already exist. One example is the use of antagomiR-92a to reduce infarct size in an animal model of ischaemia/reperfusion in pigs (Hinkel et al., 2013). Clinical trials for safety measurements in healthy volunteers have been done. miR-21 is a prominent example that entered clinical trials. It was discovered as a profibrotic miR in myocardial disease (Thum et al., 2008). AntagomiR-21 (Lademirsen) is in clinical trial against Alport syndrome, which causes fibrotic kidney disease (NCT02855268). Further antagomiRs exist that have entered the clinical trial stage (for a detailed overview, see Huang et al., 2020). One major problem in the transfer of antagomiR drugs into therapeutic use is the administration system. While local administration of antagomiRs, like intramyocardial injections, is quite promising in animal studies, intravenous or subcutaneous injection is preferred for most clinical applications. However, when applied systemically, antagomiRs enter various organ systems and cause off-target effects. Thus, tissue-specific drug delivery systems have to be developed.

6.2 | Treatment options using monocytic microRNAs

The wide range of targets in the atherosclerotic signalling cascade that is regulated by one miR can be an advantage in the treatment of atherosclerosis. As described above, some miRs, like miR-21 or miR-223, target different steps in atherosclerosis and might therefore be particularly efficient when used in therapeutic treatments. AntagomiRs or miRmimics efficiently and specifically inhibit or activate these miRs and can thereby interfere with the atherosclerotic process (Islas & Moreno-Cuevas, 2018). They can be applied systemically via intravenous injections, as it is often done in animal experiments. The advantage of the easy systemic application, however, also brings with it a disadvantage: the antagomiRs/miRmimics are delivered into various organs, where they can cause unwanted side effects (Krützfeldt et al., 2005). Therefore, cell-specific delivery systems to introduce the oligonucleotides specially in monocytes/macrophages, either circulating or in the plaque, have to be developed to optimize their treatment potential in atherosclerosis.

One possibility to increase miR delivery at specific points in the body is the local administration of miRs. This has the advantage that the antagomiRs/miRmimics will be present in high concentrations at the site of vessel damage. Such an approach has been used to deliver anti-miR21 via coated stents in a humanized animal model with myointimal hyperplasia that is characterized by augmented proliferation and migration of VSMCs, narrowing the vessel volume. Anti-miR-21-coated-stents reduced luminal obliteration without systemic effects in other organs (Wang, Deuse, et al., 2015). Although this is an interesting approach for local delivery of antagomiRs/miRmimics for the prevention of in-stent stenosis, it might not be the optimal method for the treatment of atherosclerosis, because atherosclerosis is a systemic disease and plaques will emerge at several locations in different vessels (Gusev & Sarapultsev, 2023).

An interesting approach for therapeutic use is the encapsulation of miRs in liposomes. Ho et al. (2023) treated macrophages with miR-146 using this method. This treatment decreased the production of proinflammatory cytokines, TNFα and IL-1β, reduced oxLDL uptake and foam cell formation. However, this approach is not limited to macrophages, endothelial and smooth muscle cells were also transfected by this liposome construct, resulting in reduced ICAM expression and decreased monocyte adhesion. In this case, as miR-146 acts antiatherosclerotic in all these cell types, use of pleiotropic transfection is useful. However, this is not always the case. Furthermore, the study of Ho and coworkers was done in isolated cell culture systems, and it remains to be investigated whether negative side effects on other cell types/organs can be excluded when applied in animals (Ho et al., 2023).

Interestingly, some miRs are transported in the bloodstream by HDL. At present, over 20 endogenous miRs have been identified (Ben-Aicha et al., 2020). Export and uptake of miRs by HDL seems to be cell type and sequence specific. Treatment with HDL can therefore alter the cellular miR landscape via the delivery, export, or modulation of the endogenous expression of miRs sequence and may also be a useful therapeutic tool. Reconstituted HDL (rHDL) or synthetic HDL (sHDL) nanoparticles have been developed. They are highly effective at supporting cholesterol efflux from lipid-loaded cells and at targeting SR-BI-expressing cells (Ben-Aicha et al., 2020). Such particles were loaded with specific miRs in order to deliver their cargo to epithelial or tumour cells for support of wound healing or tumour regression. However, these approaches are still in their infancies but may become an interesting new route for disease treatments, also in atherosclerosis (Graham, 2023).

Engineered EVs are another possibility for efficient miR transfer. Packaging of miRs in EVs increases miR stability, because they are protected against degradation by circulating nucleases (Nguyen et al., 2019). Macrophages exposed to anti-miR33-5p containing EVs were shown to reduce atherosclerosis progression (Stamatikos et al., 2020). If EVs or liposomes can be established in such a way that they would only transfer their content to certain cell types or organs, this would surely enhance the applicability of miR treatments in atherosclerosis in future.

An interesting new approach in this direction for the delivery of antisense oligos into the atherosclerotic plaque was recently presented by Zhang et al. (2022): They used pH low-insertion peptides (pHLIP) as vehicle to deliver miR-33 antisense oligos to the plaque. pHLIPs target areas of high acidity at the cell surface. Due to the hypoxic conditions in macrophage foam cells, an acidic environment is created in the lipid core. This is why pHLIP are preferably taken up by macrophages residing in the plaque. Thus, miR-33 antisense, attached to pHLIP, were preferentially delivered to macrophages located in the plaque, and improved atherosclerosis regression by increasing collagen and decreasing lipid accumulation in advanced plaques without deleterious effects on other tissues (Zhang et al., 2022). In contrast, systemic silencing of miR-33, besides its positive effects on atherosclerosis regression, provoked deleterious lipid accumulation in the liver and moderate hepatic steatosis. Therefore, the targeted delivery systems by pHLIPs could prove incredibly valuable for the development of safe and reliable miR-based therapies for the treatment of atherosclerosis.

7 | CONCLUSIONS

As newly emerging gene regulators, monocytic miRs could be involved in the specific regulation of genes contributing to the development and progression of human atherosclerosis, including inflammation, cholesterol levels, foam cell formation, and cell death. The use of specific antagomiRs or miRmimics to antagonize or amplify their effects in atherosclerosis bear great potential for the development of specific antiatherosclerotic drugs. We found that the expression of miR-21 was significantly up-regulated in both coronary and carotid atherosclerotic plaques when compared with A. mammaria interna (Markus et al., 2016; Parahuleva et al., 2018). These data from our recent gene expression array studies have been used now in order to identify miR-21 as potential target to become a novel class of antiatherosclerosis drug for primary prophylaxis of coronary artery diseases (CAD) or

miRs-eluting stents for direct local treatment in different stages of coronary atherosclerotic plaque development (Circulating Monocytic Cell study in progress). In this context, aspirin, which proved to be a remarkable anti-inflammatory and antithrombotic agent and one of the most widely used drugs in pharmaceutical history, is highly effective in the secondary prevention of cardiovascular events, but not in the primary prevention (Ridker, 2018). Therefore, miR-based medical treatment could provide a new era in pharmaceutical development that could be used for patients at different stages of CAD, in particular as a primary prevention in early subclinical stable CAD with anatomically and haemodynamically less severe coronary stenosis (<50%).

7.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Fabbro, et al., 2023a, b, c; Alexander, Kelly, et al., 2023).

AUTHOR CONTRIBUTIONS

M. Parahuleva and G. Euler wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data availability is not applicable to this article, because it is a review article. No new data were created or analysed.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis and as recommended by funding agencies, publisher, and other organizations engaged with supporting research.

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