MicroRNAs and the response to injury in atherosclerosis

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Keywords
Endothelial proliferation, miRNA, macrophages, vascular wound healing

Summary
Endothelial cells (ECs) at arterial branching points are physiologically subjected to chronic damage by disturbed blood flow, which triggers a vascular wound healing response. Additional damage by hyperlipidemia perturbs this delicate balance of endothelial injury and regeneration, and the progressive accumulation of noxious modified lipoproteins leads to macrophage death. Several miRNAs such as miR-92a and miR-712, which modulate EC proliferation and inflammation, are up-regulated by disturbed flow in ECs, and contribute to atherosclerosis. In addition, reduced endothelial levels of miR-126–5p limit the regenerative capacity of ECs, which becomes apparent by insufficient endothelial repair under hyperlipidemic stress. In macrophages, miR-342–5p induces the expression of miR-155 during the progression of atherosclerosis, which promotes inflammatory gene expression and inhibits efferocytosis by targeting Bcl6, thus contributing to necrotic core formation. Deciphering the complex cell- and context-specific effects of miRNAs during vascular wound healing appears essential for the development of miRNA-based therapies of atherosclerosis.

Schlüsselwörter
Endotheliale Proliferation, miRNA, Makrophagen, vaskuläre Wundheilung

Zusammenfassung

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Pathological vascular wound healing and atherosclerosis

Arterial endothelial cells (ECs) are perfectly adapted to the hemodynamic stress in arteries characterized by high shear stress and pulsatile blood flow with a high-pressure amplitude. However, hemodynamics are grossly altered at branching points of arteries resulting in reduced shear stress and oscillatory flow patterns. Despite the enormous endothelial heterogeneity, ECs at arterial bifurcation sites cannot adjust to this physiologically disturbed flow. Instead, disturbed blood flow results in a constant low-grade injury of ECs and increased repair, contrasting the extremely low turnover rate of ECs throughout the other parts of the circulatory system (1, 2). Disturbed flow induces apoptosis and desquamation of ECs from the arterial wall by upregulating X-box binding protein 1 (XBP1), an important component of the endoplasmic reticulum (ER) stress response (3). XBP1 reduces the survival of ECs by suppressing Vascular Endothelial (VE)-cadherin, which plays an essential role in endothelial integrity and is one of the key elements of the mechanosensory complex that transduces high shear stress into a pro-survival signal by activating the v-akt murine thymoma viral oncogene homolog 1 (Akt1) (3–5). Accordingly, endothelial cell death through overexpression...
of XBP1 or genetic deletion of the Akt1 gene enhances atherosclerosis (3, 6). Endothelial desumation may abrogate the contact inhibition of EC proliferation and trigger endothelial repair by resident ECs (7, 8). In addition, dendritic cell-like macrophages, which are derived from recruited monocytes and express inflammatory genes, accumulate in the subintimal space at predilection sites of atherosclerosis in the absence of hyperlipidemia, indicating a role of these cells in the response to chronic EC damage (9). In fact, macrophages play crucial roles in tissue repair by the concerted switch from a pro-inflammatory M1 phenotype (induced by interferon-γ (IFN-γ) and Toll-like receptor (TLR) ligands) towards wound-healing M2 cells (induced by interleukin-4 (IL-4) and IL-13) (10).

Chronic endothelial damage at bifurcation sites is mostly clinically silent throughout the reproductive period in humans; however, it generates the Achilles heel of the arterial system, highly vulnerable to additional damage or impairment of endothelial growth (e.g., by hyperlipidemia). Oxidized low-density lipoproteins (oxLDL), reactive oxygen species, and chronic low-grade inflammation can induce endothelial senescence (i.e., a stable proliferation arrest) and thus compromise the regeneration of damaged ECs (11–13). Conversely, senescent cells express increased levels of inflammatory genes, such as chemokines, which promote the recruitment of inflammatory monocytes and may thus generate a vicious circle of inflammation and impaired EC regeneration (14). Accordingly, activation of the pro-inflammatory transcription module Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) in ECs, which is also activated in response to disturbed flow, increases endothelial senescence and enhances atherosclerosis (15, 16).

A defective or insufficient endothelial repair process increases the accumulation of cytotoxic modified lipoproteins, thus extending the damage to the whole vessel wall. Subintimal macrophages engulf these modified lipoproteins, which may ameliorate lipid-induced damage of vascular cells. Notably, lipid uptake and degradation by macrophages can promote a phenotypic switch into anti-inflammatory M2 cells, which expand through proliferation in a colony stimulating factor- (CSF) and scavenger receptor class A- (SRA) dependent manner and can contribute to lesion formation (17–21).

The ongoing influx of lipoproteins, however, overwhelms the lipid-handling capacity of macrophages and results in the failure of the macrophage-based lipid removal system. The increasing intracellular accumulation of cholesterol induces inflammatory activation and apoptosis in macrophages due to accumulation of cholesterol in the ER membrane and lipid-induced ER stress in concert with activation of toll-like receptor 2 (TLR2) and 4 (TLR4), respectively (22, 23). Disposal of apoptotic macrophages by efferocytosis becomes progressively impaired during the progression of atherosclerosis and leads to secondary necrosis of the apoptotic cells and increased inflammation (24). Moreover, lipoproteins and cholesterol accumulate in the extracellular space, which form together with the apoptotic cell debris the necrotic core of atherosclerotic lesions. In this stage of atherosclerosis, the formation of a fibrous cap by smooth muscle cells (SMCs) isolates the cytotoxic and highly thrombogenic necrotic core content from the vascular cells and the blood. Thus, atherogenesis can be described as a chronic wound healing process of the vasculature based on the physiological damage to ECs at bifurcation sites of arteries by disturbed flow and additional damage by hyperlipidemia. In this Review, we will describe the crucial role of miRNAs in the vascular wound healing response during atherosclerosis.

MicroRNA biogenesis and function

MicroRNAs (miRNAs) are small non-coding RNAs (21–25 nucleotides) and play crucial roles during vascular development and in the inflammatory response through regulation of gene expression primarily as post-transcriptional repressors (25). Following transcription of miRNA genes, the primary pri-miRNA transcripts are cleaved by Drosha, an RNaseIII enzyme, into ~70-nucleotide precursors called pre-miRNAs. The precursors are transported into the cytoplasm where they are further processed by Dicer into mature miRNA duplexes that bind to the argonaute protein 2 (AGO2), a part of the RNA-induced silencing complex (RISC). Whereas one strand of the miRNA duplex is usually released and degraded (i.e., the passenger strand), the guide strand interacts with the 3′-untranslated region of target mRNAs in a sequence-specific manner in the RISC resulting in mRNA degradation or translational inhibition (26). Due to the short sequence by which the miRNA binds to its mRNA target (nucleotides 2–7 at the 5′ end of the miRNA), one miRNA can theoretically target a large number of mRNAs (that can be part of a shared pathway), which confers great versatility to miRNA-mediated regulation of gene expression (27).

In addition, miRNAs sharing the same target site for a specific miRNA can compete for binding to this miRNA and thereby mutually influence the expression of each other (28). Therefore, one miRNA can mediate different effects in distinct cell types due to the expression of a diverse set of mRNA targets. Important contributions of miRNAs in endothelial cells, macrophages, and smooth muscle cells to atherogenesis by regulating proliferative and inflammatory processes have been described (27, 29, 30).

miR-126: a miRNA pair controls the regeneration of arterial endothelial cells

miR-126 is the most abundant endothelial miRNA and deletion of the Mir126 gene in mice results in severe defects in blood vessel development, including delayed angiogenic sprouting, and partial embryonic lethality (31, 32). The passenger strand miR-126–5p, but not the guide strand miR-126–3p, is selectively downregulated in ECs from atherosclerosis-prone regions of the arterial tree compared to regions protected from atherosclerosis, probably due to the suppression of the krüppel-like factor 2 (KLF2) by disturbed flow (33). The reduced expression of miR-126–5p limits the capability of ECs at predilection sites to respond to hyperlipidemic stress by increased proliferation by upregulation of its
target delta-like 1 homolog (Dlk1), a NOTCH1 inhibitor and negative regulator of EC proliferation (34). Accordingly, the inhibition of EC proliferation by Dlk1 correlates with reduced NOTCH1 activation in atherosclerotic ECs. These data suggest that increased endothelial repair at branching points is counterbalanced by the activation of an anti-proliferative mechanism in which disturbed flow-induced suppression of miR-126–5p inhibits NOTCH1-mediated regenerative growth of ECs through Dlk1. However, this control of regenerative growth impedes endothelial repair upon additional damage, such as hyperlipidemia. Conversely, high shear stress increases the regenerative capacity of ECs by miR-126–5p-mediated suppression of Dlk1 at non-predilection sites of atherosclerosis. Pharmacological treatment with miR-126–5p mimics can rescue EC proliferation at predilection sites and reduce atherogenesis, thus demonstrating that insufficient EC proliferation promotes atherosclerosis (33).

Complementary to miR-126–5p, the sister strand miR-126–3p is athero-protective through upregulation of the chemokine (C-X-C motif) ligand 12 (CXCL12) in ECs. Apoptotic bodies secreted from ECs are enriched with miR-126–3p and can transfer this miRNA to neighboring cells where it promotes the autoinduction of CXCL12 by targeting the regulator of G-protein signaling 16 (RGS16) (35). Although the mechanism by which CXCL12 reduces atherosclerosis is currently incompletely understood, it may enhance endothelial regeneration through direct effects on the proliferation of ECs or the recruitment of angiogenic myeloid cells (35–37). Of note, endogenous miR-126–3p can also directly target CXCL12, suggesting that transfer by microparticles may change the target preference of miR-126–3p and results in opposing effects (35). Although endogenous miR-126–3p plays a minor role in endothelial repair following endothelial denudation, delivery of miR-126–3p through systemic treatment with endothelial microparticles enhances re-endothelialization, presumably by targeting the Sprouty-related, EVH1 domain-containing protein 1 (Spred1) (33, 38). The abundance of miR-126–3p in circulating endothelial microparticles is reduced in patients with diabetes, suggesting that defec-

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**Fig. 1** Biogenesis and mechanisms of miRNAs: RNAPolII-mediated transcription of the miRNA genes or splicing of introns results in the primary pri-miRNAs, which are further processed into precursor-miRNAs (pre-miRNAs) by the microprocessor complex composed of the RNase Drosha and the double-stranded RNA binding protein DGCR8. Exportin 5, a RanGTP-dependent dsRNA-binding protein, mediates nuclear export of pre-miRNAs, which are further processed into mature miRNA duplex (miRNA/miRNA*) by a complex composed of TRBP and Dicer. During the binding of miRNA duplex to the Ago2 protein in the RISC complex, miRNA passenger strands (miRNA*) are usually degraded and the guide strands interact with the 3'-untranslated regions of their target transcripts resulting in inhibition of translation or degradation of the target mRNA. RNAPOLII: RNA polymerase type II; DGCR8: DiGeorge Syndrome Critical Region 8; TRBP: human immunodeficiency virus transactivating response RNA-binding protein; RISC: RNA-inducing silencing complex; Ago2: Argonaute RISC catalytic component 2; miRNA/miRNA*: miRNA guide strand/miRNA passenger strand.
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The packaging of miR-126–3p into microparticles may accelerate atherosclerosis progression in diabetes (38, 39). Similarly, circulating levels of miR-126–3p are decreased in patients with coronary artery disease (CAD) (40), most likely due to the reduced loading of microparticles with miR-126–3p or to an increased uptake into atherosclerotic lesions (35, 41, 42). Accordingly, increased expression levels of miR-126 in circulating microparticles are associated with a reduced occurrence of a first major adverse cardiac event (43). By contrast, increased circulating levels of miR-126–3p have been found in patients with unstable angina or angiographically documented CAD (44, 45); however, it is unclear whether the increased abundance of circulating miR-126 in these studies is attributable to changes of its expression level in microparticles. Notably, the abundance of miR-126–3p is not increased in high shear stress-induced endothelial microvesicles, which limit atherosclerosis by the transfer of miR-143 and –145 to SMCs (46). Moreover, miR-126–3p/Ago2 complexes can be transmitted from ECs to SMCs independent of vesicles and increase the proliferation of SMCs, thus contributing to neointima formation following complete blood cessation (47). However, this mechanism may be specific for this model of SMC hyperplasia, because silencing miR-126–3p expression did not affect wire-induced neointima formation (33).

Thus, the pair of miRNAs generated from the pre-miR-126 has synergistic effects on endothelial regeneration and protect from atherosclerosis.

miR-126–3p can also affect endothelial inflammation by suppressing the vascular cell adhesion molecule-1 (VCAM-1) and thereby reduces leukocyte adhesion to activated ECs (48). In mice, this effect of miR-126–3p on VCAM-1 expression appears to be tissue-dependent and was not found in aortas of Mir126 knockout mice fed a high fat diet (HFD). Moreover, miR-126–5p has been implicated in increased adhesion and reduced transmigration of leukocytes in vitro by targeting the set domain containing 5 (SetD5) and the activated leukocyte cell adhesion molecule (ALCAM), respectively (49). Suppression of ALCAM by miR-126–5p was found in the lung but not in the retina, and decreases leukocyte accumulation in the lung following LPS treatment (49). By contrast, inhibition of miR-126–5p increased the expression of SetD5 in the retina but not in the lung or the heart of newborn mice, and resulted in vascular defects in the retina, suggesting that miR-126–5p plays a role in angiogenesis (49). Moreover, the expression of both miR-126–3p and –5p in cancer cells reduces the recruitment of inflammatory monocytes into the tumour-associated stroma by suppressing chemokine (C-C motif) ligand 2 (CCL2) in a CXCL12-dependent manner (50). Taken together, these data suggest that the roles of miR-126–3p and –5p on endothelial inflammation are highly context- and tissue-specific, and may not substantially contribute to atherosclerosis.

miR-92a: increased inflammation and reduced endothelial repair promote vascular disease

In contrast to miR-126–5p, miR-92a is upregulated at predilection sites of atherosclerosis and suppressed by pulsatile laminar flow in ECs (51–53). Moreover, oxLDL further enhances the upregulation of miR-92 in ECs exposed to low shear stress, probably via activation of the signal transducer and activator of transcription 3 (STAT3), and thereby promotes inflammatory activation of ECs and the adhesion of monocytes to the endothelium (53). Current evidence indicates that the effect of miR-92 on endothelial activation results from the targeting of multiple transcription factors. Endothelial miR-92a can target both KLF2 and KLF4, and activate NF-κ B (56). In addition, the suppressor of cytokine signalling 5 (SOCS5) has been identified as a novel miR-92a target involved in the regulation of endothelial inflammation (53); however, the role of SOCS5 in ECs is incompletely understood.

In line with its pro-inflammatory properties, the abundance of miR-92a is increased in endothelial-derived microparticles from patients with acute coronary syndrome (44) and with unstable angina (42, 55). In accordance, pharmacological inhibition of miR-92a reduces atherosclerosis and promotes a stable plaque phenotype in mice (53). Moreover, miR-92a impairs endothelial regeneration following vascular injury by suppressing endothelial proliferation, indicating a role of this miRNA in endothelial repair (56). Since KLF2 inhibits endothelial proliferation, alternative miR-92 targets, such as integrin-α5 and sirtuin-1, may mediate the effects of miR-92a on endothelial repair (57). Taken together, miR-92a plays a crucial role in the maladaptation of ECs to disturbed flow and oxLDL-induced endothelial damage by suppressing several targets, such as KLF2, KLF4, and SOCS5.

Opposite effects of miR-181b and miR-712 on endothelial inflammation and atherosclerosis

In contrast to miR-92a, miR-181b is not regulated by shear stress (46, 51, 52). However, inflammatory stimulation by tumor necrosis factor-α (TNF-α) or hyperlipidemia decreases miR-181b expression in ECs, which results in derepression of importin-α3 and thus increases NF-κB activation through enhanced translocation of p65 from the cytoplasm into the nucleus (58, 59). Notably, systemic delivery of miR-181b reduces atherosclerosis and endothelial inflammation, but did not affect NF-κB activation in leukocytes, which requires importin-α5 rather than importin-α3 for nuclear translocation (59).

Disturbed flow also up-regulates the non-canonical processing of the murine specific miR-712 from the spacer region of rRNAs due to decreased degradation by XRN1, which is downregulated by low shear stress (60). miR-712 and its human homolog miR-205 target the tissue inhibitor of metalloproteinases inhibitor 3 (TIMP3) in ECs and thereby trigger the release of TNF-α, promote leukocyte adhesion, and increase endothelial permeability, probably due to the increased activity of a disintegrin and metalloproteinase domain-containing protein (ADAMs) and ADAM17 (60). Moreover, pharmacological inhibition of miR-712 reduces atherosclerosis in mice and increases the expression of TIMP3 (60). However, TIMP3 expression was increased also in medial SMCs, indicating that the effect of miR-712 may not be EC specific. In addition, angio-
tensin II up-regulates miR-712 and miR-205 in ECs, which mediates the formation of aortic aneurysms and increased vascular inflammation by targeting TIMP3 and the reversion-inducing-cysteine-rich protein with kazal motifs (RECK) (61).

**Additional miRNAs that mediate disturbed flow-induced endothelial damage**

Although miR-23b is, like miR-126–5p, up-regulated by KLF2 in ECs under flow conditions, it suppresses EC proliferation in contrast to miR-126–5p (46, 62). In vitro, miR-23b targets the short 3´-untranslated region of cyclin H, a member of the Cyclin-Dependent Kinase (CDK)-activating kinase complex (CAK complex), and thus limits the activation of CDK2 and 4, and RNA polymerase II in ECs (63). The inactivation of CDK2/4 by miR-23b increases the hypophosphorylated form of the Retinoblastoma protein (Rb), inhibits E2F1 activation (that remains linked to Rb), and promotes cell cycle arrest (63). In addition to miR-23b, miR-19a and miR-10a are up-regulated by high shear stress in a KLF2-independent manner (46, 64, 65). Whereas miR-19a inhibits endothelial cell proliferation under high shear stress conditions by targeting cyclin D1, reduced expression levels of miR-10a increase the activation of NF-κB through suppression of the transforming growth factor-beta (TGF-β)–activated kinase 1 (TAK1) and the beta-transducin repeat containing E3 ubiquitin protein ligase (BTRC), which both promote proteosomal degradation of the NF-κB inhibitor-α (IκBα) and p65 nuclear translocation (51, 64). However, the effect of reduced expression of miR-23b, miR-19a, and miR-10a in ECs from predilection sites on atherosclerosis is unclear.

**miR-155 and the lesional accumulation of macrophages: a matter of time**

A broad range of inflammatory mediators, including mildly oxidized LDL (moxLDL), can induce the expression of miR-155 in macrophages, which in turn promotes the expression of inflammatory genes. miR-155 belongs to a small group of miRNAs that is upregulated in M1 macrophages and is highly expressed in atherosclerotic lesions in mice and humans mainly in macrophages (66, 67). Several studies demonstrated that genetic deficiency of miR-155 in macrophages or pharmacological treatment with miR-155 inhibitors reduces atherosclerosis (67–69). Accordingly, circulating levels of miR-155 are elevated in patients with acute coronary syndrome (55). However, studies from Fichtlscherer and colleagues showed a significant down-regulation of circulating miR-155 in patients with CAD, most likely due to increased uptake from circulation into atherosclerotic that might promote progression of atherosclerosis (40, 42).

Macrophage-derived miR-342–5p positively regulates miR-155 expression in atherosclerotic lesions by suppressing Akt1, an inhibitor of miR-155 expression, thus promoting atherosclerosis and enhancing the inflammatory stimulation of macrophages (70). Targeting of Akt1 by miR-342–5p is activated in M1 macrophages due to the transcriptional down-regulation of the competing miR-342–5p target Bmp2 (Bone morphogenetic protein receptor type II) after treatment with LPS and IFNγ. Although the expression of miR-342–5p does not change during M1 polarization, the increased availability of miR-342–5p in the absence of Bmp2 allows targeting of Akt1 and thus upregulates miR-155 (70).

miR-155 enhances the expression of inflammatory mediators, such as CCL2, in macrophages by targeting the B-cell lymphoma 6 protein (Bcl6), a negative regulator of NF-κB activation (67). Although miR-155 suppresses multiple miRNAs in macrophages, mainly the targeting of Bcl6 plays a role in the progression of atherosclerosis (67). Moreover, miR-155-mediated repression of Bcl6 inhibits the phagocytosis of apoptotic cells by macrophages, thus enhancing necrotic core formation in advanced lesions of Apoe−/− mice after feeding a HFD for 24 weeks (Wei Y. et al. unpublished data). Therefore, increased levels of miR-155 link increased inflammatory gene expression and impaired efferocytosis capacity in M1 macrophages. In addition, miR-155 enhances the production of reactive oxygen species and the intracellular accumulation of lipids by targeting the HMG-box transcription factor 1 (HBPI), which downregulates p47phox and the macrophage migration inhibitory factor (MIF) (69).

However, in Ldlr−/− mice, which develop less advanced lesions than Apoe−/− mice anti-atherogenic effects of miR-155 have been described, after 10 weeks of a HFD (71). We could recently confirm that genetic deficiency of miR-155 in macrophages increases atherosclerosis in Apo eo−/− mice after 12 weeks of a HFD; however, lesion progression was prevented in miR-155 knockout mice between 12 and 24 weeks of the HFD (Wei Y. et al. unpublished data), indicating a stage specific role of miR-155 in atherosclerosis. The anti-atherogenic effect of miR-155 is linked to reduced lesional macrophages proliferation by targeting the macrophage-CSF receptor (Wei Y. et al. unpublished data). Therefore, therapeutic strategies that target miR-155 in atherosclerosis should be tailored to inhibit specific interactions of miR-155 in macrophages, like that with Bcl6.

**miRNAs and the pathological vascular repair during atherosclerosis**

Atherosclerosis can be considered as a chronic wound healing process of the vasculature in response to disturbed flow and a second hit by hyperlipidaemia or other factors that damage vascular cells, such as hyperglycaemia, smoking or hypertension. Accordingly, we suggest to divide the vascular wound healing process into three phases. In the first, which we call the priming phase, a continuous cycle of endothelial injury and regeneration by disturbed flow confers increased susceptibility to additional damage. In the second, the impact phase, hyperlipidaemia further injures the endothelium, exhausts the regenerative capacity of ECs, and results in the accumulation of modified LDL in macrophages. In the third, the appeasement phase, vascular wound healing fails and excessive lipid accumulation in macrophages induces chronic inflammatory activation, apopto-
miR-92a and miR-712 modulate endothelial proliferation and inflammation and contribute to atherosclerosis. The miRNAs described in this article contribute to these different phases and may be promising targets for miRNA-based therapies of this devastating disease (Tab. 1).

In the priming phase, the effects of disturbed flow on the balance between the anti-inflammatory transcription factors KLF2 and KLF4 and the pro-inflammatory NF-κB module are mediated by the up-regulation of miR-92a and miR-712. Suppression of KLF2 by miR-92a not only increases the expression of inflammatory genes in ECs, but may also mediate the downregulation of the pro-proliferative miR-126–5p and the anti-proliferative miR-23b, and thus balances regenerative endothelial growth. Moreover, activation of ADAMs by miR-712-mediated suppression of TIMPs may promote endothelial damage and apoptosis through cleavage of VE-cadherin and increasing soluble TNF-α levels. The inflammatory activation of ECs induced by miR-92a and miR-712 increases the recruitment of monocytes, which may accumulate in the intima as dendritic cell-like macrophages. Whether these macrophages increase endothelial injury by inducing apoptotic cell death or promote en-

### Topics discussed in this review
- vascular wound healing response induced by disturbed flow
- pathological vascular wound healing induced by hyperlipidemia
- miRNAs-dependent modulation of vascular wound healing during atherosclerosis

### What is known about this topic?
- miR-92a and miR-712 modulate endothelial proliferation and inflammation and contribute to atherosclerosis.

### What does this paper add?
- Consideration of atherosclerosis as a pathological vascular wound healing in which proliferation and inflammation are strictly interconnected.
- miRNAs are here described as conductors of chronic vascular wound healing during atherosclerosis, suggesting important implications and providing new perspectives in terms of therapeutic interventions.

### Table 1 Functional overview of miRNAs involved in pathological vascular wound healing

<table>
<thead>
<tr>
<th>miRNA</th>
<th>target</th>
<th>effector cell</th>
<th>function</th>
<th>phase of vascular wound healing</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-126–5p</td>
<td>Dlk1</td>
<td>endothelial cells</td>
<td>↑ proliferation</td>
<td>↓ priming and impact phases</td>
<td>(33, 34)</td>
</tr>
<tr>
<td>miR-126–3p</td>
<td>RGS16</td>
<td>endothelial cell-derived apoptotic bodies</td>
<td>↑ proliferation, ↑ recruitment of progenitor cells</td>
<td>↑ priming and impact phases</td>
<td>(34, 35, 40, 42)</td>
</tr>
<tr>
<td>miR-92a</td>
<td>KLF2</td>
<td>endothelial cells</td>
<td>↑ inflammation</td>
<td>↑ priming and impact phases</td>
<td>(53, 54)</td>
</tr>
<tr>
<td>miR-181b</td>
<td>importin-a3</td>
<td>endothelial cells</td>
<td>↓ inflammation, ↓ replicative senescence</td>
<td>↓ impact phase</td>
<td>(58, 59)</td>
</tr>
<tr>
<td>miR-712</td>
<td>TIMP3</td>
<td>endothelial cells</td>
<td>↑ inflammation, ↑ apoptosis, ↑ leukocyte adhesion</td>
<td>↑ priming and impact phases</td>
<td>(60, 61)</td>
</tr>
<tr>
<td>miR-155</td>
<td>CSFR</td>
<td>macrophages (M2)</td>
<td>↓ proliferation</td>
<td>= impact phase</td>
<td>(71)</td>
</tr>
<tr>
<td></td>
<td>Bcl6</td>
<td>macrophages (M1)</td>
<td>↑ inflammation (M2-to-M1 switch)</td>
<td>↑ appeasement phase</td>
<td>(67)</td>
</tr>
<tr>
<td>miR-342–5p</td>
<td>Akt1</td>
<td>macrophages (M1)</td>
<td>↑ inflammation (miR-155-dependent M2-to-M1 switch)</td>
<td>↑ appeasement phase</td>
<td>(70)</td>
</tr>
<tr>
<td>miR-23b</td>
<td>Cyclin H</td>
<td>endothelial cells</td>
<td>↓ proliferation</td>
<td>↓ priming phase</td>
<td>(63)</td>
</tr>
<tr>
<td>miR-10a</td>
<td>TAK1 and BTRC</td>
<td>endothelial cells</td>
<td>↓ inflammation</td>
<td>unknown</td>
<td>(51, 64)</td>
</tr>
<tr>
<td>miR-19a</td>
<td>Cyclin D1</td>
<td>endothelial cells</td>
<td>↓ proliferation</td>
<td>unknown</td>
<td>(51, 64)</td>
</tr>
</tbody>
</table>

DLK1: delta-like 1 homolog; RGS16: regulator of G-protein signaling 16; KLF2: Krüppel-like factor 2; TIMP3: tissue inhibitor of metalloproteinases inhibitor 3; CSFR: colony stimulating factor receptor; Bcl6: B-cell lymphoma 6 protein; Akt1: v-akt murine thymoma viral oncogene homolog 1; TAK1: transforming growth factor-beta (TGF-β) activated kinase 1; BTRC: beta-transducin repeat containing E3 ubiquitin protein ligase.
Disturbed flow at arterial branching points in-

doletial repair through the release of an-
giogenic factors is currently unclear (72, 73) (▶ Fig. 2, panel A).

In the impact phase, hyperlipidaemia results in the deposition of modified LDL and Very Low Density Lipoproteins (VLDL) in the subendothelial space, which increases endothelial damage and inhibits the proliferation of ECs. Notably, hyperlipidaemia further upregulates the expression of miR-92a in ECs at predilection sites and thus aggravates the disturbed flow-induced endothelial dysfunction. However, the limited regenerative reserve of ECs due to the downregulation of miR-126–5p prevents endothelial regeneration in response to hyperlipidaemic stress. This insufficient endothelial regeneration may be partially compensated by the up-regulation of CXCL12 by miR-126–3p transferred from apoptotic cells via microvesicles. CXCL12 can enhance endothelial regeneration by the recruitment of APCs or by enhancing the proliferation of neighboring ECs (Figure 2, panel B). In addition, hyperlipidaemia activates endothelial NF-κB by downregulating miR-181b expression, which may also impair endothelial repair by inducing replicative senescence (▶ Fig. 2, panel B, left). Concomitantly, the accumulation of modified lipoproteins by subintimal macrophages may trigger an anti-inflammatory and highly proliferative M2 phenotype (M2φ). miR-155 can limit the proliferation of macrophages in early lesions by targeting Csf1r and thus reduces lesion formation (▶ Fig. 2, panel B, left).

The appeasement phase is characterized by a switch of M2 into M1 macrophages due to the miR-342–5p mediated upregulation of miR-155. In contrast to the impact phase, Csf1r is not involved in macrophage proliferation in the later phase and the increased levels of miR-155 can also target Bcl6, which results in increased inflammatory activation and impaired efferocytosis. Thus, the role of miR-155 also switches from being anti-atherogenic to pro-atherogen-
cic during the progression of atherosclerosis. In this way, engulfment of apoptotic macrophages by efferocytosis is progressively compromised, leading to secondary necrosis of apoptotic cells, enhanced inflammation and an increased necrotic core (▶ Fig. 2, panel B, right).

Fig. 2 Hypothetical model of the role of miRNAs in vascular wound healing during atherosclerosis: Disturbed flow at arterial branching points induces EC damage and apoptosis, and is accompanied by the recruitment of monocyte-derived inflammatory macrophages (M1φ), presumably mediated by the flow-dependent up-regulation of the pro-inflammatory miR-92a and miR-712. Moreover, suppression of KLF2 promotes endothelial proliferation by downregulating miR-23b (A); however, the role of miR-23b is unclear. Concurrently, reduced expression of miR-126–5p at predilection sites of atherosclerosis controls the regenerative growth of ECs, which leads to increased atherosclerosis upon hyperlipidemic stress (B) due to impaired EC proliferation. miR-126–3p released from apoptotic ECs in apoptotic bodies (a.b.) may partially compensate the reduced level of miR-126–5p, presumably by promoting EC regeneration through up-regulation of CXCL12, which triggers the recruitment of angiogenic progenitor cells (APC). In addition, hyperlipidaemia downregulates miR-181b and thereby increases the inflammatory activation of ECs through activation of NF-κB. Uptake of modified lipoproteins induces an anti-inflammatory macrophage phenotype, which may enhance lesional macrophage accumulation by increased proliferation. miR-155 reduces macrophage proliferation during early atherosclerosis by targeting Csf1r and thus limits lesion formation. However, progressive lipid accumulation and inflammatory activation by IFN-γ and TLR4 ligands, such as moxLDL, induce phenotypic switch to M1 cells in advanced lesions. This switch is orchestrated by the miR-342–5p-mediated upregulation of miR-155, which enhances inflammatory gene expression and decreases phagocytic capability by targeting Bc16. (C) Graphical representation of time-dependent progression of EC damage (red) and proliferation (green). The black lines indicate the phase-dependent prevalence of macrophage subtypes (M1φ: solid line; M2φ: dashed line). Arrows indicate up- or down-regulation; miRNA colors indicate the effect on athero-progression (green: promoters; red: inhibitors; black: unknown). LDL: low density lipoproteins. moxLDL: mildly oxidized-LDL. EC: endothelial cell. M1/M2 macrophages.
Conclusion

Taken together, miRNAs orchestrate chronic vascular wound healing during atherosclerosis by regulating increasing endothelial regeneration and macrophage polarization. Although the whole network of miRNAs involved in this process is still far from being discovered, current evidence suggests that therapeutic replacement of miR-126–5p or miR-181b may restore endothelial health and reduce atherosclerosis. Moreover, selective targeting of the interaction between miR-155 and Bcl6 in macrophages may be a promising approach to prevent the progression of atherosclerotic lesions.

Conflict of interest

The authors declare that they have no conflict of interest.

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