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Pathogenic arterial remodeling: the good and bad of microRNAs

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[1]*Institute for Cardiovascular Prevention, Ludwig-Maximilians University Munich, Munich, Germany;* [2]*Cardiovascular Research Institute Maastricht, University Maastricht, Maastricht, The Netherlands; and* [3]*DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany*

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Wei Y, Schober A, Weber C. Pathogenic arterial remodeling: the good and bad of microRNAs. *Am J Physiol Heart Circ Physiol* 304: H1050–H1059, 2013. First published February 8, 2013; doi:10.1152/ajpheart.00267.2012.—A number of cardiovascular diseases, such as restenosis, aneurysm, and atherosclerosis, lead to vascular remodeling associated with complex adaptive reactions of different cell populations. These reactions include growth of smooth muscle cells, proliferation of endothelial cells, and the inflammatory response of macrophages. MicroRNAs (miRNAs), a class of short RNAs, play key roles in various biological processes and in the development of human disease by post-transcriptional regulation of gene expression. Here, we review the molecular mechanisms of a subset of miRNAs involved in vascular remodeling, including miR-143/145, miR-221/222, miR-126, miR-21, and miR-155. Some of these miRNAs, such as miR-143/145 and miR-126, have been shown to be protective during vascular remodeling, whereas others, such as miR-21, may promote the cellular response that leads to neointima formation. The increasing knowledge regarding the roles of miRNAs in vascular remodeling opens novel avenues for the treatment of various cardiovascular diseases. However, more in vivo studies on the functional roles of these miRNAs are required in the future.

atherosclerosis; microRNA; remodeling; vascular

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Introduction

The vascular system can be characterized into functional entities that are specialized either in converting the intermittent ejection of blood into continuous flow (aorta), conducting blood to organs (conduit vessels), regulating blood pressure and local blood flow (resistance vessels), controlling the exchange of volume and solutes (exchange vessels), or forming a reservoir of blood, which determines cardiac filling pressures (capacitance vessels) (65). The classical three-layer structure of the vessel wall including the intima, media, and adventitia bordered by the elastic laminae is adapted to the various functional requirements of the circulatory system. For instance, a highly muscularized media is characteristic of resistance vessels, whereas the required elasticity of the aorta is achieved by a much higher content of elastic fibers in the media (65). This highly specialized vascular network matures during development and structurally adapts to the concomitant increase in the perfusion pressure.

Vascular remodeling is a widely used term for the different adaptive processes of the vessel wall structure, e.g., embryonic angiogenesis or arteriogenesis (6). In addition to these physiological remodeling processes, different types of vascular injuries can cause maladaptive alterations primarily of the arterial vessel wall, which may result in disease processes (27). Following vascular injury, e.g., after coronary stent implantation, neointima formation occurs because of the accumulation of smooth muscle cells (SMCs), which may lead to restenosis (71). In pulmonary and arterial hypertension, the width of the tunica media increases, which raises the vascular resistance and therefore raises blood pressure (54, 55). Atherosclerosis is accompanied by profound remodeling of the vascular wall driven by the inflammatory response to the subendothelial accumulation of modified lipoproteins (88). These maladaptive processes are orchestrated by various vascular cell types, including endothelial cells (ECs) and SMCs. In addition, an inflammatory response, characterized primarily by the recruitment of monocytes and macrophages, promotes vascular remodeling through the activation of SMCs (70, 72, 73, 92).

The phenotype of SMCs is highly adaptable to environmental cues and ranges in a continuum between a contractile and a synthetic state (68). The transformation of the SMC phenotype from a contractile to a synthetic state is a hallmark in vascular remodeling and neointima formation (62). In contrast to contractile SMCs, the content of contractile proteins is reduced and the proliferative capacity is increased in synthetic SMCs (17). In addition, progenitor cells of SMCs from the circulation or the adventitia can be recruited to the neointima and differentiate into SMCs (74). Moreover, fibroblasts and vascular stem cells from the adventitia can migrate into the neointima and adopt a SMC phenotype (36, 44). ECs sense stimuli that induce vascular remodeling, such as hemodynamic stress or

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hyperlipidemia, and transmit these signals to the medial SMCs or promote the inflammatory response. For instance, low endothelial shear stress activates ECs toward a proatherogenic phenotype, whereas high shear stress preserves an anti-inflammatory phenotype (8).

The phenotypes of SMCs and ECs, as well as the inflammatory activation of macrophages, are regulated by noncoding, small RNAs (~22 nt) through post-transcriptional regulation of gene expression (22, 37, 75). Therefore, these microRNAs (miRNAs) are involved in the cellular response during vascular remodeling. Numerous miRNAs have been identified in SMCs, ECs, and macrophages that either promote or limit the structural changes that lead to vascular remodeling. In this review, we will summarize the effects of a subset of miRNAs in arterial remodeling and outline their therapeutic implications in cardiovascular diseases.

miR-143/145 and the Phenotypic Plasticity of SMCs

miR-143 and miR-145 are two distinct cardiovascular-specific miRNAs encoded by a bicistronic gene cluster, which play a crucial role together with other miRNAs, such as miR-1 or miR-24 in the differentiation and proliferation of SMCs (9, 11, 40, 90). The transcription factor serum response factor (SRF) and the coactivators myocardin and myocardin-related transcription factors are essential for the expression of SMC-specific proteins but also regulate the transcription of the miR-143/145 cluster via a CArG box in the miR-143/145 upstream enhancer (11, 90) (Fig. 1). Furthermore, the Jag-1/Notch pathway can transcriptionally induce miR-143/145 independently of SRF in SMCs, thus increasing differentiation of SMCs (5). Notably, both miR-143 and miR-145 are required to mediate the Jag-1/Notch effect on the SMC phenotype (5).

miR-145 expression is required for the myocardin-induced conversion of fibroblasts to SMCs. Introduction of miR-145 into neural crest cells and embryonic stem cells triggers their reprogramming to a SMC phenotype as determined by the expression of SMC markers such as smooth muscle α-actin, SM22 (also known as transgelin), and smooth muscle myosin heavy chain (11, 91). In a regulatory positive feedback loop, miR-145 can stimulate SMC differentiation in vitro by targeting Krüppel-like factor (KLF) 5, a transcriptional repressor of myocardin (10, 91) (Fig. 1). In addition to KFL5, several other targets of miR-145 have been identified by luciferase reporter assay, including KLF4, slit-robo GTPase-activating protein 1 and 2, adducin-3, slingshot 2 phosphatase, and calmodulin kinase IIδ, which are involved in the regulation of SRF activity and actin dynamics (11, 90). Interestingly, Cordes et al. (11) found that miR-145 can increase the expression of myocardin via interaction with a miR-145-specific binding site in the 3′-UTR of myocardin (Fig. 1). Although various targets of miR-145 are also suppressed by miR-143, despite the low sequence homology, the effects of miR-145 and miR-143 on SMCs seem to differ. miR-143 has been shown inhibit SMC proliferation by directly targeting ETS-like gene 1 (11).

Genetic deficiency in miR-143/145 in mice results in a shift of the SMC phenotype from a contractile state to a synthetic state (4, 19, 90). However, different phenotypic changes in the vessel wall of miR-143/145$^{-/-}$ mice have been described. Whereas Boettger et al. (4) and Elia et al. (19) found a reduction of SMC-specific contractile proteins, Xin et al. (90) reported a decrease in primarily actin stress fibers in medial SMCs. These controversial observations regarding miR-143/145 effects on the SMC phenotype may be related to differences in the genetic background of the transgenic mice. Moreover, the neomycin resistance cassette, which might affect the phenotype of transgenic mice, has only been removed in the mice generated by Xin et al. (4, 61, 64, 90). Regardless of the differences in the SMC phenotype, all three groups consistently described a thinning of the arterial media and a decrease in blood pressure in miR-145/143-deficient mice (19).

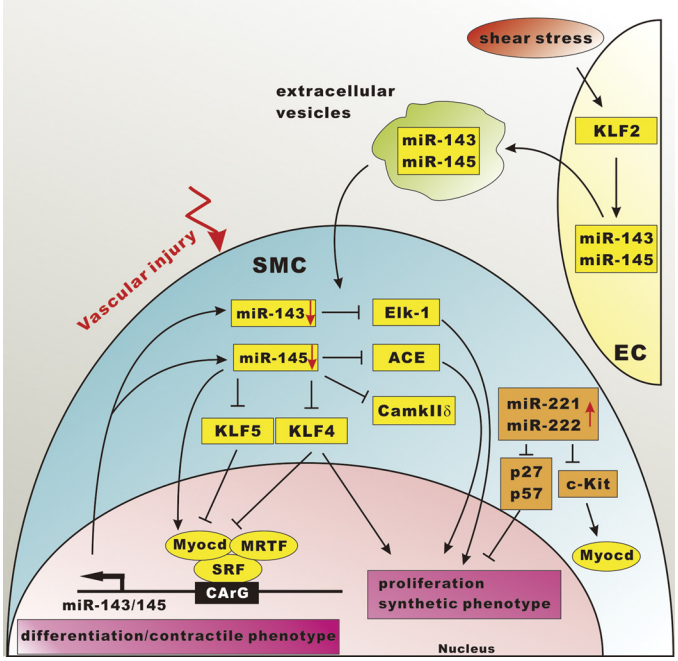

Fig. 1. The smooth muscle cell (SMC) phenotype is controlled by miR-143/145 and miR-221/222. The expression of miR-143/145 is positively regulated by SMC-specific transcription factors, and miR-145 represses the negative regulators of myocardin and Krüppel-like factor (KLF) 4 and 5. This constitutes a positive feedback loop in the differentiation of SMCs. In addition, KLF2 increases miR-143/145 in endothelial cells (ECs) and triggers their microvesicle-mediated transfer to SMCs, which confers atheroprotection. miR-221/222 promote a synthetic SMC phenotype with increased proliferative capacity because of suppression of p27 and p57 and a reduced expression of SMC markers due to suppression of c-Kit. The downregulation of miR-143/145 and the upregulation of miR-221/222 by vascular injury play a crucial role in neointima formation. Arrows indicate upregulation. Capped lines indicate suppression. Elk-1, ETS-like gene 1; ACE, angiotensin I-converting enzyme; CamkIIδ, calmodulin kinase II δ; CArG, DNA consensus sequence CC(A/T)$_6$GG; p27, cyclin-dependent kinase inhibitor 1B; p57, cyclin-dependent kinase inhibitor 1C; c-Kit, kit oncogene; Myocd, myocardin; MRTF, myocardin-related transcription factor; SRF, serum response factor.

In addition to the effects of miR-143/145 on normal SMC function, miR-143/145 are also important players in the responsiveness of SMCs to vascular injury. Several studies have shown that miR-143/145 are downregulated in injured vessels, e.g., following balloon injury or ligation of the carotid artery, atherosclerotic lesions, and experimentally induced aneurysms (10, 11, 19). The suppression of miR-143/145 in these vascular disease models might be due to increased KLF4 expression; inhibition of myocardin by NF-κB, which is constitutively activated in neointimal SMCs; or downregulation of the Notch signaling pathway (25, 28, 78, 97). Overexpression of miR-145 decreases neointimal formation in balloon-injured arteries by

regulating the expression of KLF5 (Table 1) (10). Accordingly, restoration of miR-143 and miR-145 inhibits neointimal growth in a rat model of acute vascular injury (19). Moreover, spontaneous formation of atherosclerotic lesions associated with the upregulation of angiotensin I-converting enzyme was observed in the femoral arteries of aged miR-143/145$^{-/-}$ mice (4). Accordingly, overexpression of miR-145 in SMCs reduces atherosclerosis in apolipoprotein E knockout (Apoe$^{-/-}$) mice and leads to plaque stabilization (49). In contrast, neointimal formation was reduced in miR-143$^{-/-}$ and miR-145$^{-/-}$ mice following carotid artery ligation, a well-established model of vascular injury, probably because the SMCs already had an altered phenotype before the injury (Table 1). This indicates that miR-143/145 can also promote vascular remodeling (90). Moreover, deficiency of miR-143/145 in other cell types than SMCs (e.g., in ECs) of miR-143/145$^{-/-}$ mice may also affect the vascular response to vascular injury.

Although miR-143/145 is a SMC-specific miRNA cluster, a recent study indicates that miR-143/145 is transferred from ECs to SMCs by extracellular vesicles (10, 34). Interestingly, KLF2, a crucial regulator of flow-dependent gene expression in ECs, positively regulates expression of miR-143/145 by binding to its promoter in ECs and induces enrichment of miR-143/145 in extracellular vesicles that transfer miR-143/145 from ECs to SMCs (Fig. 1). Injection of vesicles from KLF2-overexpressing cells results in a reduction of atherosclerotic plaques in mice fed on high-fat diet, which is prevented by the inhibition of miR-143/145 (34).

Differential Effects of miR-221/222 in Vascular Cells

miR-221 and miR-222 are expressed by a common gene cluster at the X chromosome and share the same seed sequence. Both miRNAs are highly expressed in SMCs and ECs, and their expression is upregulated primarily in SMCs after balloon injury of carotid arteries in rats (43, 47, 102). This increased miR-221/-222 expression may be related to epidermal growth factor receptor-activated pathways, such as the RAS/RAF/MEK signaling cascade or NF-κB (26, 76, 99). Treatment of the injured carotid artery with a 2′-*O*-methyl-modified miR-222 or miR-221 by combined short-term infusion into the injured artery and perivascular application reduced the neointima formation by ~40% at 14 days (Table 1) (46, 47). Conversely, adenoviral overexpression of miR-221/222 significantly increased the neointimal growth (Table 1) (46). In vitro, the proliferation of SMCs is mediated by miR-221/222 through targeting of the cell-cycle inhibitors p27 (Kip1) and p57 (Kip2) (13, 47) (Fig. 1). Accordingly, both p27 (Kip1) and p57 (Kip2) are upregulated, and the number of proliferating cells is reduced in injured carotid arteries treated with miR-222 inhibitor, indicating that miR-222 promotes neointimal growth via enhanced proliferation of SMCs (47). Furthermore, miR-221 suppresses the expression of the receptor for stem cell factor (SCF) c-Kit in SMCs in vitro, which positively regulates the transcription of myocardin (13) (Fig. 1). Therefore, miR-221, which promotes a synthetic phenotype, plays a crucial role in the platelet-derived growth factor-induced dedifferentiation and proliferation of SMCs.

However, in contrast to the effect of miR-221/222 on SMCs, the proliferation of ECs is inhibited by miR-221/222, although the same targets such as p27 (Kip1), p57 (Kip2), and c-Kit are suppressed as in SMCs (46) (Fig. 2). Moreover, miR-221/222 decreases apoptosis and increases migration of SMCs, whereas the opposite effect on EC migration and apoptosis was observed (46). The cause for these differential effects of miR-221/222 on SMCs and ECs is unclear; however, differences in the expression of miR-221/222 target genes between SMCs and ECs may lead to opposite cellular effects of miR-221/222. For instance, c-Kit increases proliferation and is highly expressed in ECs, whereas the expression levels of the antiproliferative factors p27 (Kip1) and p57 (Kip2) are higher in SMCs than in ECs (46). Inhibition of miR-221/222 in injured carotid arteries enhances the endothelial recovery, which is generally assumed to limit neointimal growth (46, 83). Therefore, the effect of miR-221/222 on SMC proliferation appears to prevail over the enhanced endothelial repair during neointima formation. Additional functional targets of miR-221/222 in ECs have been described, such as signal transducer and activator of transcription 5A (STAT5A) (15). The downregulation of miR-222 in interleukin 3 (IL-3)- or basic fibroblastic growth factor-stimulated ECs is involved in the enhanced inflammation-mediated angiogenesis by derepression of STAT5A, probably by stimulating the proliferation of ECs (15) (Fig. 2). Furthermore, the expression of miR-222 in ECs from advanced atherosclerotic lesions is reduced, whereas STAT5A expression in ECs increases during the progression of atherosclerosis (15). Therefore, an atheroprotective effect of EC-derived miR-222 due to the limitation of intraplaque neovascularization has been postulated (15). Moreover, miR-221/222 inhibits the angiotensin II-induced inflammatory response in ECs in vitro by suppressing the expression of vascular cell adhesion mole-

Table 1. *Effect of microRNAs on arterial remodeling*

microRNA	Lesion Formation	Animal Model	Reference
miR-145 overexpression	Decreased	Balloon injury in rats	(10)
miR-143 and miR-145 overexpression	Decreased	Balloon injury in rats	(19)
SMC-specific miR-145 overexpression	Decreased	Diet-induced atherosclerosis in Apoe$^{-/-}$ mice	(49)
miR-143/145$^{-/-}$ mice	Decreased	Ligation of the carotid artery	(90)
miR-145-enriched endothelial microvesicles	Decreased	Diet-induced atherosclerosis in Apoe$^{-/-}$ mice	(34)
miR-221/222 inhibition	Decreased	Balloon injury of the carotid artery in rats	(46, 47)
miR-221/222 overexpression	Increased	Balloon injury of the carotid artery in rats	(46)
miR-126-enriched EC-derived apoptotic bodies	Decreased	Diet-induced atherosclerosis in Apoe$^{-/-}$ mice	(98)
miR-21 inhibition	Decreased	Balloon injury of the carotid artery in rats	(39)
miR-155$^{-/-}$ BM cells	Increased	Diet induced atherosclerosis in LDLR$^{-/-}$ mice	(16)
miR-155$^{-/-}$ BM cells	Decreased	Diet-induced atherosclerosis in Apoe$^{-/-}$ mice	(56)

SMC, smooth muscle cell; EC, endothelial cell; BM, bone marrow; Apoe$^{-/-}$, apolipoprotein E knockout; LDLR$^{-/-}$, LDL receptor knockout.

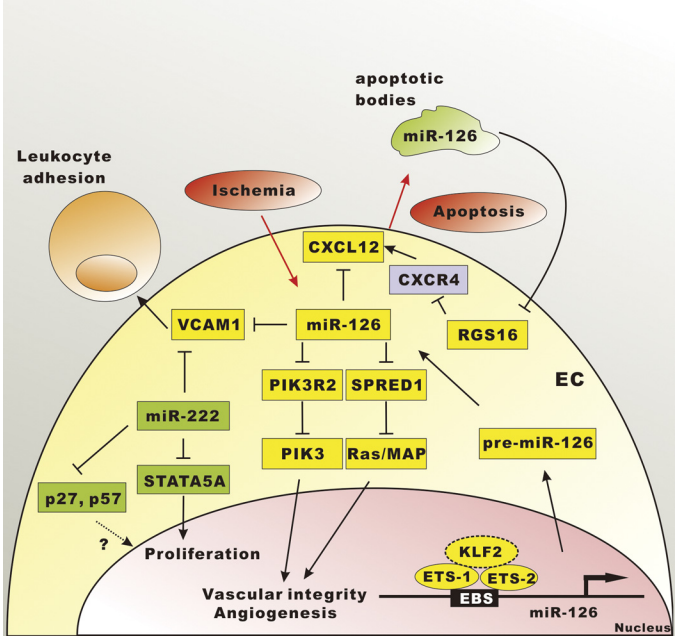

Fig. 2. The roles of miR-126 and miR-222 in ECs. miR-126 is transcriptionally regulated by E26 avian leukemia oncogene, 5′ domain (ETS-1) and E26 avian leukemia oncogene, 3′ domain (ETS-2) and KLF-2 in ECs. miR-126 promotes angiogenesis and vascular integrity during embryogenesis by targeting the inhibitors of VEGF signaling, phosphoinositol-3 kinase (PI3K) regulatory subunit 2 (PIK3R2), and sprouty-related protein EVH1 domain containing 1 (SPRED1). During ischemia-induced neoangiogenesis, miR-126-mediated suppression of chemokine (C-X-C motif) ligand 12 (CXCL12) reduces the recruitment of endothelial progenitor cells. However, apoptotic ECs release apoptotic bodies that are enriched in miR-126. Through microvesicle-mediated delivery, miR-126 suppresses regulator of G-protein signaling 16 (RGS16), an inhibitor of CXCR4, which unleashes CXCR4-dependent CXCL12 expression in ECs and thus enhances endothelial repair and limits atherosclerosis. Anti-inflammatory effects of miR-126 are due to the repression of vascular cell adhesion molecule 1 (VCAM-1), which reduces leukocyte adhesion. miR-222 inhibits EC proliferation during angiogenesis and vascular repair by targeting signal transducer and activator of transcription 5A (STATA5A) and p27/p57. However, the mechanism of reduced EC proliferation by miR-222-mediated suppression of p27/p57 remains unclear. Arrows indicate upregulation. Capped lines indicate suppression. In addition, miR-221/222 can reduce leukocyte adhesion and VCAM-1 expression. EBS, ETS binding site; MAP, mitogen-activated protein.

cule 1 (VCAM-1), monocyte chemotactic protein 1 (MCP1), and fms-related tyrosine kinase and reducing the adhesion of leukocytes (102). This anti-inflammatory effect of miR-221/222 may be due to the targeting of the angiotensin II-induced proinflammatory transcription factor E26 transformation-specific sequence (Ets) factor Ets-1 (102).

Taken together, miR-221/222 promotes neointima formation after vascular injury by enhancing the proliferation of SMCs, whereas EC-derived miR-221/222 may be atheroprotective by blocking intraplaque neovascularization and suppressing the inflammatory activation of ECs.

miR-126 Plays an Antiatherogenic Role by Enhanced Endothelial Repair

miR-126 is one of the most abundant miRNAs in ECs and plays a crucial role in regulating the function of ECs (31, 87). The transcription of miR-126 is regulated by several transcriptional factors, such as Ets-1 and Ets-2 and KLF2 (32, 57) (Fig. 2). Thus miRNAs that target Ets-1, such as miR-221/222 and miR-155, in ECs may affect the expression of miR-126. Increased expression of miR-126 via flow-dependent activation of KLF2 plays an important role in embryonic angiogenesis in zebrafish (57). It is noteworthy that KLF2-dependent regulation of miR-126 was absent in human ECs in vitro (34).

miR-126 deficiency causes leaky vessels and hemorrhage because of loss of vascular integrity in both zebrafish and mice during embryonic development, partly mediated by direct targeting of negative regulators of vascular endothelial growth factor (VEGF) signaling, such as sprouty-related protein and phosphoinositol-3 kinase regulatory subunit 2 (24, 87) (Fig. 2). Accordingly, defective development of aortic arch blood vessels was found in the zebrafish embryo by disturbed flow-mediated VEGF signaling when miR-126 was inhibited (57). These studies indicate that miR-126 plays a vital role during angiogenesis.

Although miR-126 does not affect the differentiation of ECs, inhibition of miR-126 increases the rate of EC proliferation and inhibits VEGF-induced reduction in apoptosis and VEGF-induced migration of ECs in zebrafish (24). In contrast, the proliferation of ECs in miR-126$^{-/-}$ mouse embryos is significantly reduced (87). Moreover, silencing of miR-126 increases the expression of stromal-derived factor-1 (CXCL12) in ECs, which enhances the migration of CD34^{+} progenitor cells in vitro and increases the number of circulating Sca-1^{+}/Lin^{-} progenitor cells in a murine model of hindlimb ischemia (82) (Fig. 2). Furthermore, miR-126 targets VCAM-1 in ECs and thereby negatively regulates leukocyte adherence to ECs in vitro (31) (Fig. 2). miR-126-mediated repression of VCAM-1 upon inflammatory stimulation occurs in glomerular ECs and sinusoidal ECs of the liver, but not in the heart or lung, indicating that the anti-inflammatory effect of miR-126 is restricted to a subset of ECs (3).

Apoptosis of ECs results in the release of apoptotic microvesicles, which are enriched in miR-126 (98). These EC-derived apoptotic microvesicles transfer miR-126 to cells in atherosclerotic lesions and thereby inhibit the progression of atherosclerosis (Table 1) (98). Mechanistically, this microvesicle-mediated transfer of miR-126 suppresses RGS16, a negative regulator of CXCR4 signaling (98) (Fig. 2). Thus miR-126 triggers enhanced CXCR4 signaling and initiates an autoregulatory feedback loop, which finally leads to upregulation of CXCL12 (98) (Fig. 2). This miR-126-induced CXCL12 expression by endothelial apoptotic bodies reduces atherosclerotic lesion formation and enhances lesional EC repair through the recruitment of Sca-1^{+} EC progenitor cells in a mouse model of atherosclerosis (98). Reduced expression of miR-126 has been demonstrated in the circulation of patients with coronary artery disease and type 2 diabetes (23, 96). Aberrant plasma levels of circulating miR-126 in patients are probably due to the defective enrichment of miR-126 in apoptotic bodies released from ECs (98). Therefore, reduced transfer of miR-126 by endothelial-derived apoptotic bodies may be a pathogenic factor for the accelerated atherosclerosis in patients with diabetes. Furthermore, during transcoronary passage through the culprit vessel in patients with myocardial injury, miR-126 levels decrease (14), indicating that circulating miR-126 is transferred to cells in the ruptured lesion. In contrast, the levels of miR-126 in the systemic circulation increase in patients with acute coronary syndromes, probably because of an elevated

generation of apoptotic microvesicles via the systemic activation of ECs (14).

In summary, apoptotic or activated ECs release miR-126 in microvesicles and trigger an atheroprotective intercellular communication by the transfer of miR-126. Defective miR-126 packaging into microvesicles may play a role in the accelerated atherogenesis in patients with diabetes. Therefore, this microvesicle-dependent miRNA carrier system may be exploited for novel treatment strategies in patients with increased risk for atherosclerosis.

miR-21 Controls the SMC Phenotype and Affects the EC Response to Shear Stress

miR-21 is another important miRNA involved in vascular remodeling that affects both SMCs and ECs. Following vascular injury, such as ligation of the carotid artery or balloon angioplasty, and in human atherosclerotic lesions, the expression of miR-21 is increased (39, 66, 93). Differentiation of human pulmonary SMCs induced by bone morphogenic proteins (BMPs) in vitro is mediated by miR-21, partly through the suppression of the miR-21 target, programmed cell death 4 (12) (Fig. 3). Interestingly, transforming growth factor-β (TGF-β) and BMPs increase the expression of miR-21 in SMCs at a post-transcriptional level by the recruitment of SMAD signal transducers to the microprocessor complex that processes the pri-miR-21 (12). In contrast, differentiation of human or rat aortic SMCs under serum-deprived conditions in vitro reduced the expression of miR-21 (39, 93). Conversely, the overexpression of miR-21 in SMCs induced a synthetic SMC phenotype by repression of specificity protein-1, which in turn leads to the downregulation of cystathionine γ-lyase, an enzyme known to be involved in SMC differentiation by serum deprivation (93) (Fig. 3). Similarly, silencing of miR-21 inhibits cell proliferation and increases cell apoptosis in rat aortic SMCs in vitro and in injured rat carotid arteries (39). This effect of miR-21 on the growth of SMCs may be the cause for the diminished neointima formation observed after inhibition of miR-21 in injured rat carotid arteries (Table 1) (39). Although the direct targets of miR-21 that mediate its effect on neointima formation have not been determined, it has been demonstrated that the suppression of phosphatase and tensin homolog and the upregulation of B-cell leukemia/lymphoma-2 expression by miR-21 are involved in neointimal growth (39) (Fig. 3). Alternatively, inhibition of miR-21 also enhances apoptosis and reduces proliferation of adventitial fibroblasts and myofibroblasts by derepressing programmed cell death 4 expression and thus activates the JNK/c-Jun pathway (86). More recently, miR-21 has been found to promote the growth of human pulmonary SMCs in vitro (95). In SMCs, in which SRF was inactivated, miR-21 is upregulated by the reduced expression of miR-143, which in turn leads to derepression of the Fos-related antigen 1 (35), indicating that the effects of miR-143 and miR-21 are functionally connected. Taken together, the roles of miR-21 on the SMC phenotype appear to be diverse and may depend on the respective stimulus (e.g., BMP vs. serum deprivation).

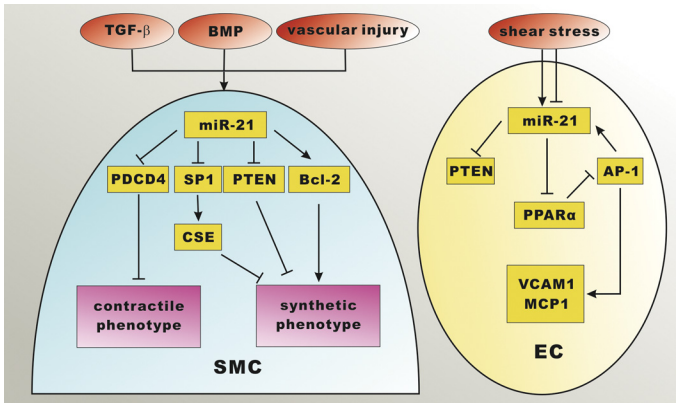

Fig. 3. The functional roles of miR-21 in SMCs and ECs. Transforming growth factor-β (TGF-β) and bone morphogenetic protein (BMP)-induced differentiation of SMCs is mediated by the upregulation of miR-21. In contrast, serum deprivation downregulates miR-21, which leads to activation of specificity protein-1 (SP1)/cystathionine γ-lyase (CSE)-mediated SMC differentiation in vitro. The increased expression of miR-21 following vascular injury promotes neointimal growth probably by suppressing phosphatase and tensin homolog (PTEN) and upregulating B cell leukemia/lymphoma 2 (Bcl-2). In ECs, miR-21 induced by high shear stress (HSS) suppresses PTEN. In contrast, low shear stress can also upregulate miR-21, which targets peroxisome proliferator-activated receptor-α (PPARα) and triggers a positive feedback loop by derepressing Jun oncogene (AP-1) that induces endothelial inflammation. Arrows indicate upregulation. Capped lines indicate suppression. PDCD4, programmed cell death 4; MCP1, monocyte chemotactic protein 1.

miR-21 is highly expressed in ECs and regulation of miR-21 expression by shear stress has been demonstrated (43, 85, 89, 101). However, the results regarding the expression of miR-21 in ECs exposed to different types of shear stress are conflicting. Whereas miR-21 is significantly upregulated in human umbilical vein ECs exposed to prolonged unidirectional shear stress (USS) at 15 dyn/cm^2, pulsatile shear stress at 12 dyn/cm^2 was found to transiently suppress the expression of miR-21 (89, 101). Moreover, oscillatory shear stress (OSS) at 0.5 dyn/cm^2 increases the expression of miR-21 via activation of c-Jun/AP-1 and promotes the association of miR-21 with Argonaut proteins in human umbilical vein ECs compared with static incubation (101). The effects of miR-21 were also different in ECs exposed to USS and OSS. Inhibition of miR-21 increased the diminished phosphatase and tensin homolog expression in ECs exposed to USS, which may suggest that miR-21 plays a role in reducing apoptosis in ECs exposed to USS (89) (Fig. 3). These conflicting observations on the regulation of miR-21 in ECs may be related to additional effects of pulsatile shear stress, which also creates cyclic strain in ECs, compared with laminar flow (1). Furthermore, increased nitric oxide production and endothelial nitric oxide synthase phosphorylation was found in ECs that overexpress miR-21, indicating that miR-21 is involved in the formation of an atheroprotective phenotype in ECs exposed to USS (89). In contrast, OSS-induced miR-21 suppresses expression of peroxisome proliferator-activated receptor-α, an inhibitor of AP-1, and thereby constitutes a positive feedback loop that increases miR-21 expression and the expression of VCAM-1 and MCP-1 in an AP-1-dependent manner (101) (Fig. 3). Thus miR-21 exacerbates the inflammatory response and increases the adhesion of monocytes to OSS-exposed ECs (101).

Recently, a role for miR-21 in pulmonary vascular remodeling that leads to pulmonary hypertension (PH) was described (63, 95). In pulmonary ECs, miR-21 is upregulated by hypoxia and signaling via BMP receptor 2, two crucial pathogenic factors in PH (63). Moreover, the expression of miR-21 is increased in the media and adventitia of distal pulmonary

arteries in mice exposed to chronic hypoxia (95). miR-21 deficiency in mice exacerbated hypoxia-induced PH and enhanced the remodeling of the pulmonary vessels by increasing the medial thickness, indicating a protective role for miR-21 in PH (63). In contrast, intratracheal administration of locked nucleic acid (LNA)-modified miR-21 inhibitor ameliorates hypoxia-induced PH and reduces the muscularization of distal arteries of the lung (95). Whether the differences in the effect of miR-21 on PH are due to a compensatory adaptation of genetically deficient miR-21 mice or to an insufficient specificity of the LNA-miR-21 inhibitor remain to be determined.

miR-155 Regulates Inflammatory Signaling in Macrophages

The expression of miR-155 is upregulated in human atherosclerotic lesions, but circulating levels of miR-155 are reduced in patients with coronary artery disease (23, 66). miR-155 is expressed in SMCs, ECs, and activated macrophages and thus may affect most of the cell types involved in vascular remodeling (51, 59, 102). The recruitment of monocytes to the arterial wall via ECs activated by modified LDL and/or low shear stress, followed by their differentiation into macrophages and dendritic cells, is a crucial feature of the inflammatory response in the vessel wall during remodeling (53, 72, 88). During the proinflammatory activation of macrophages, a small group of miRNAs is specifically upregulated including miR-155 (29, 56). miR-155 is upregulated by several Toll-like receptor ligands through myeloid differentiation primary response gene (MyD88)- or TIR domain-containing adapter-inducing IFN-β-dependent signaling and is one of the most important miRNAs modulating the inflammatory response in macrophages (Fig. 4) (21, 58, 59). Moreover, upregulation of miR-155 was described in monocytes and dendritic cells in response to lipopolysaccharide (LPS) stimulation (7, 77). In bone marrow (BM)-derived macrophages, LPS promotes the maturation of miR-155 by the single-strand RNA-binding protein KH-type splicing regulatory protein (69). Furthermore, LPS-induced miR-155 expression in dendritic cells inhibits the Toll-like receptor/IL-1 signaling pathway by suppressing TGF-β-activated kinase 1/MAP3K7-binding protein-2 expression, which transduces inflammatory signals (7). Furthermore, the anti-inflammatory cytokine IL-10 inhibits miR-155 expression in a STAT3-dependent manner, which in turn increases the expression of the miR-155 target inositol polyphosphate-5-phosphatase and downregulates the expression of proinflammatory mediators, indicating that the anti-inflammatory effects of IL-10 are mediated by the suppression of miR-155 (52). The serine/threonine kinase Akt (also known as protein kinase B)/phosphoinositide-3 kinase (PI3K) signaling pathway plays a key role in the inflammatory function of macrophages (30, 50). In response to LPS stimulation, Akt inhibits the expression of miR-155, which in turn induces expression of proinflammatory mediators via suppression of suppressor of cytokine signaling (2). miR-155 also modulates TGF-β signaling in macrophages via targeting SMAD2 (48). Overexpression of miR-155 reduces TGF-β-induced SMAD2 phosphorylation and the expression of some its downstream transcriptional targets, such as IL-1β (48). The silencing of oxidized LDL-induced expression of miR-155 in THP-1 macrophages enhances the uptake of lipids and increases the expression of scavenger receptors and cytokines, including IL-6 and TNF-α (59). Furthermore, treatment with mildly oxidized LDL and IFN-γ promotes a proinflammatory M1-type macrophage polarization mainly through upregulation of miR-155 (56). However, miR-155 was also found to be downregulated in oxidized LDL-treated macrophages (56, 94). Therefore, various pro- and anti-inflammatory effects of miR-155 in macrophages have been described, and the net effect of miR-155 on macrophage function during vascular remodeling remains unclear.

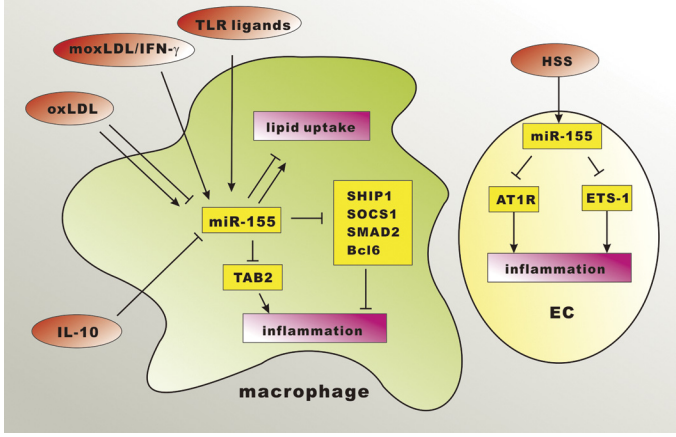

Fig. 4. miR-155 regulates the inflammatory response and lipid uptake of macrophages. In macrophages, the expression of miR-155 is induced by Toll-like receptor (TLR) ligands, such as lipopolysaccharide (LPS), or by mildly oxidized LDL (moxLDL)/interferon-γ (IFN-γ) and inhibited by interleukin-10 (IL-10). Upregulation and downregulation of miR-155 in response to oxidized LDL (oxLDL) in macrophages has been described presumably because of differences of degree of LDL oxidation. miR-155 can promote and inhibit the inflammatory macrophage activation in vitro by targeting several mediators of inflammatory signaling, such as inositol polyphosphate-5-phosphatase (SHIP1), suppressor of cytokine signaling 1 (SOCS1), MAD homolog 2 (SMAD2), and TGF-β activated kinase 1/MAP3K7 binding protein 2 (TAB2). In vivo, suppression of B-cell leukemia/lymphoma 6 (Bcl6) by miR-155 increases the inflammatory response in lesional macrophages and thereby promotes lesion formation. The uptake of lipids by macrophages in vitro is inhibited by miR-155, whereas the lipid accumulation in lesional miR-155$^{-/-}$ macrophages is reduced. Unidirectional HSS upregulates miR-155 in ECs in which miR-155 limits the inflammatory response to angiotensin II by suppressing angiotensin II type-1 receptor (AT_1R) and ETS-1. Arrows indicate upregulation. Capped lines indicate suppression.

Accordingly, opposing results of genetic deficiency of miR-155 in BM cells on atherosclerosis were described. Whereas LDL receptor knockout (LDL-R$^{-/-}$) mice harboring miR-155$^{-/-}$ BM cells developed increased atherosclerosis after 10 wk of a high-fat diet, miR-155 deficiency in BM cells of Apoe$^{-/-}$ mice resulted in reduced lesion formation induced by acutely disturbed flow in the carotid artery (16, 56). Although the molecular mechanism of increased atherosclerosis in LDL-R$^{-/-}$ mice is unclear, increased lesional recruitment of neutrophil and immature macrophages were found in this model. Because neutrophils play a crucial role only during early lesion formation, these findings may indicate that miR-155 in neutrophils inhibits the initiation of atherosclerosis (18). In contrast, impaired inflammatory stimulation of macrophages in Apoe$^{-/-}$ mice harboring miR-155$^{-/-}$ BM cells reduces advanced and stenotic lesions, which develop rapidly in partially ligated carotid arteries, because of derepression of the NF-κB counterregulator B-cell leukemia/lymphoma 6 (56). Moreover, the lipid accumulation in lesional macrophages was reduced in the absence of miR-155 expression, indicating that miR-155 also promotes foam cell formation during atherosclerosis (56).

In ECs, miR-155 is upregulated by USS and targets the angiotensin-II type-1 receptor and Ets-1, which reduces the proinflammatory activity of angiotensin II on ECs (Fig. 4) (51, 89, 102). Moreover, the suppression of angiotensin-II type-1 receptor by miR-155 in SMCs may also ameliorate the effects of angiotensin II on vascular remodeling (33). Although miR-155 is expressed in lesional SMCs, the development of atherosclerosis was not affected in miR-155$^{-/-}$/Apoe$^{-/-}$ mice harboring miR-155$^{+/+}$ BM cells, suggesting that miR-155 expressed in vascular cells does not play a crucial role in atherogenesis (56). However, further in vivo studies are needed to dissect the roles of EC- and SMC-derived miR-155 in cardiovascular disease.

Therapeutic Role of miRNA Mimics and Inhibitors in Vascular Remodeling

Considering the crucial role of miRNAs in vascular remodeling, therapeutic strategies to either increase or inhibit miRNAs might be a promising approach. When compared with other molecular targets, the targeting of miRNAs has several theoretical advantages: *1*) the antisense oligonucleotides to inhibit endogenous miRNAs are short, which makes packaging and delivery easier; *2*) cell-specific expression of miRNAs and suppression of disease-specific targets may attenuate off-target effects of the treatment; and *3*) because of regulatory effects of miRNAs, therapeutic modulation of miRNA levels may allow more rapid and subtle interference with molecular disease mechanisms.

Several methods for miRNA loss-of-function in vivo studies using modified antisense oligonucleotides have been developed. Intravenous administration of "antagomirs," a class of nucleotides chemically modified with 2′-*O*-methyl phosphoramidites, and a hydroxyprolinol-linked cholesterol solid support, suppresses the expression of endogenous miRNA in mice in a specific, effective, and long-lasting way (up to 4 wk) (42). Locked nucleic acid-modified oligonucleotide (LNA-anti-miR) is another miRNA inhibitor effectively used for modulating endogenous miRNA expression in vivo (20). "Tiny" LNA oligonucleotides, which are complementary to the miRNA seed region, have been delivered to breast tumor cells to silence the expression of tumor-associated miR-21 (60). However, the specificity of the tiny LNA is limited because all the members of a miRNA family will be inhibited, likely causing off-target effects. The systematic delivery of 2′-fluoro/methoxyethyl-modified antisense oligonucleotides with a phosphorothioate backbone against miR-33 reduces atherosclerotic plaque size and lipid content associated with increased markers of plaque stability and decreased inflammatory gene expression (67). This study demonstrates the potential application of miRNA inhibitors in the treatment of vascular diseases, including atherosclerosis. Due to the excellent in vivo stability and uptake of the different types of miRNA inhibitors, packaging to support the delivery appears not necessary. Treatment with miRNAs mimics that enhance the disease suppressing effect of endogenous miRNAs is also a promising therapeutic approach. When compared with the number of miRNA inhibitors that have been developed, studies that used therapeutically administered miRNA mimic in vivo are still scarce. Notably, synthetic miR-34a and let-7 mimics packaged in a novel neutral lipid nanoparticle were successfully delivered into mice by intravenous tail-vein injection to treat lung cancer metastasis (79). In addition, packaging in cell-derived microparticles is an effective way to deliver miRNAs via a systemic route (34, 80, 98, 100). Mechanisms involved in the enrichment of certain miRNAs in microvesicles and in the efficient delivery to target cells are currently under investigation. The identification of these processes may allow the generation of synthetic microvesicles for the treatment with miRNA mimics. Moreover, endogenous lipoproteins contain and transfer specific miRNAs, which may also play a role in vascular diseases and may serve as another blueprint for the development of miRNA carriers (84). Restoring miRNA function by viral delivery of miRNAs ameliorates vascular disease in animal models (49); however, this therapeutic strategy faces the same challenges as the traditional gene therapy by the delivery of protein-coding genes (41).

Although the achievements in the administration of miRNA via synthetic antisense oligonucleotides in vivo are remarkable, the shortcomings of current approaches are obvious. First, side effects of the application of miRNA mimics or inhibitors have not been thoroughly investigated. The most thoroughly studied anti-miRNA treatment strategy is the systemic application of LNA-anti-miR-122 to treat hypercholesterolemia and hepatitis C virus infection (45). In nonhuman primates, three intravenous injections of 3 or 10 mg/kg LNA-anti-miR-122 (also called mirsavirsen) decrease plasma cholesterol levels up to 2 mo (20). This dosing regimen is not associated with liver or kidney toxicities as assessed by clinical chemistry and histology (20). Moreover, no severe adverse effects were reported in two *phase 1* studies (single doses up to 12 mg/kg and 5 doses of 5 mg/kg of mirsavirsen) and one *phase 2a* (5 weekly doses of up to 7 mg/kg of mirsavirsen) study (38, 45). However, these results were only published as abstracts so far, and long-term effects are still unknown. In addition, the dosages of the miRNA inhibitors that were applied in most studies are rather high, probably because of a high renal excretion rate, which makes unwanted effects more likely considering the fact that one miRNA can target multiple mRNAs (81). In this regard, local application to the vasculature might be promising because of reduced off-target effects. Furthermore, tissue-specific delivery approaches should be considered in the development of new miRNA-based therapeutic strategies.

Conclusions

Although several studies have demonstrated the functional roles of miRNAs in vascular remodeling, the often contradictory findings on the effect of miRNAs requires more detailed investigations to solve these discrepancies, probably in a more defined context of vascular disease. We also believe that it is important to verify and evaluate the current in vivo delivery technologies for synthetic miRNA inhibitors or mimics and to develop novel, more efficient, probably cell- or tissue- specific treatment strategies interfering with disease-related miRNAs. In addition to identifying new methods to modify the oligonucleotides themselves in order to keep them stable and easier to be delivered into cells, developing package technology is another relevant option for study. In this regard, microvesicles, which are secreted by cell populations in atherosclerotic lesions and could enrich and deliver miRNAs, may serve as an ideal template for novel package strategies to deliver miRNA

mimics or inhibitors in vivo. However, microvesicle-based mechanisms of miRNA packaging and delivery need to be identified in more detail.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Y.W., A.S., and C.W. conception and design of research; Y.W., A.S., and C.W. drafted manuscript; Y.W., A.S., and C.W. approved final version of manuscript; A.S. and C.W. edited and revised manuscript.

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