The role of 14q32 microRNAs in vascular remodelling

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Chapter 2

The multifactorial nature of microRNAs is vascular remodelling

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Abstract

Vascular remodelling is a multifactorial process which involves both adaptive and maladaptive changes of the vessel wall through amongst others, cell proliferation and migration, but also apoptosis and necrosis of the various cell types in the vessel wall. Vascular remodelling can be beneficial e.g. during neovascularization after ischemia, as well as pathological e.g. during atherosclerosis and aneurysm formation. In recent years, it has become clear that microRNAs are able to target many genes that are involved in vascular remodelling processes and either can promote or inhibit structural changes of the vessel wall. Since many different processes of vascular remodelling are regulated by similar mechanisms and factors, both positive and negative vascular remodelling can be affected by the same microRNAs. A large number of microRNAs has been linked to various aspects of vascular remodelling and indeed, several of these microRNAs regulate multiple vascular remodelling processes, including both the adaptive processes angiogenesis and arteriogenesis as well as maladaptive processes of atherosclerosis, restenosis and aneurysm formation. Here, we discuss the multifactorial role of microRNAs and microRNA clusters that were reported to play a role in multiple forms of vascular remodelling and are clearly linked to cardiovascular disease. The microRNAs reviewed are miR-126, miR-155 and the microRNA gene clusters 17-92, 23/24/27, 143/145 and 14q32. Understanding the contribution of these microRNAs to the entire spectrum of vascular remodelling processes is important, especially as these microRNAs may have great potential as therapeutic targets for treatment of various cardiovascular diseases.

Introduction

MicroRNAs

MicroRNAs are a class of endogenous noncoding RNA molecules of approximately 22 nucleotides in length. MicroRNAs inhibit translation of mRNAs into proteins by binding to specific sites in the 3'untranslated region (3'UTR) of their target mRNAs. Rather than completely silencing their target gene, binding of a microRNA leads to modest target downregulation. However, a single microRNA is able to downregulate the expression of numerous target genes, and by doing so, that single microRNA can regulate complex, multifactorial physiological processes[1]. MicroRNAs have been shown to play an important role in human diseases, including cardiovascular disease (CVD). In this review, we describe the multifactorial nature of microRNAs in the regulation of vascular remodelling, by discussing the different target genes and regulatory mechanisms that have been described for these microRNAs. Although many microRNAs play a role in some aspects of vascular remodelling, we focused on those microRNAs that to play a role in multiple forms of vascular remodelling ánd are clearly linked to CVD. The individual microRNAs miR-126 and miR-155, the microRNA gene clusters 17-92, 23/24/27, 143/145, and the largest known microRNA gene cluster 14q32, all met these criteria (Figure 1). An overview of confirmed target genes for these microRNAs is given in Table 1 and 2.

Vascular remodelling

Vascular remodelling comprises beneficial adaptive responses of the vessel wall to changes in hemodynamic forces, vasoactive stimuli or growth factors, but also maladaptive responses that can lead to CVD[2]. Thus, vascular remodelling can be divided into adaptive and maladaptive processes regarding vessel wall structure and blood supply towards downstream tissues[3]. For this review, we focused on neovascularization on the one hand and on atherosclerosis, post-interventional restenosis and aneurysm formation on the other. All of these processes are orchestrated by microRNAs[3].

When studying the role of microRNAs in these processes, there are several microRNAs that have been very well described. For example, one of the most promising microRNAs as therapeutic target for the treatment of atherosclerotic disease is miR-33a/b, (discussed below), as it controls cholesterol metabolism, a crucial mechanism in CVD. The phenotype of smooth muscle cells (SMCs), either contractile or proliferative, is also imperative for vascular remodelling and neointima formation. Several microRNAs, including miR-133, miR-125b, miR-26a, miR-663 and miR-1, have been shown to control SMC phenotype and function[4-8].

In aneurysm formation, the miR-29 family has been shown to play a major role by targeting genes that are involved in extracellular matrix homeostasis. Inhibition of miR-29b in two murine abdominal aortic aneurysm (AAA) models increased expression of genes encoding for collagen and elastin and reduced expression of matrix metalloproteinases, resulting in decreased aneurysm progression in these mice[9]. Similarly, miR-21 regulated AAA expansion through targeting of PTEN[10].

Therapeutic potential of microRNAs

Several microRNAs that gave promising results as therapeutic targets in murine models of CVD are now being studied in larger animal models. A relevant example is the miR-33 family, consisting of miR-33a and miR-33b. Both miR-33a and miR-33b regulate the expression of cholesterol transporter ABCA1, which mediates the efflux of cholesterol[11]. Inhibition or deficiency of miR-33a reduced progression of plaques and raised HDL levels in atherosclerotic mouse models[11-13]. Since rodents lack miR-33b, extrapolation of these results to a human situation was not straightforward. Systemic inhibition of miR-33a/b in African green monkeys, which do express miR-33b, led to increased expression of ABCA1 in the liver of treated animals and increased plasma HDL levels[14]. The authors also observed regulation of other genes involved in fatty acid oxidation and fatty acid synthesis, leading to a decrease in plasma VLDL levels, an effect that was not observed in mice[14]. Moreover, no overt toxicity was observed in animals treated with anti-miRs, supporting the development of anti-miR-33 therapeutics for treatment of atherosclerosis.

Janus phenomenon

However, caution is wanted when intervening in individual processes of vascular remodelling. This is best illustrated by the Janus phenomenon, named after the two-faced Roman deity Janus. The Janus phenomenon was first described by Epstein et al., who noticed that interventions used to stimulate arteriogenesis also increased atherosclerosis and vice versa[18]. The phenomenon is explained by the fact that there is a strong overlap in the mechanisms that underlie the various forms of vascular remodelling. One of the important mechanisms shared in vascular remodelling are the inflammatory responses. Since microRNAs can target numerous genes that may be involved in many processes, modulation of one microRNA could influence more than one form of vascular remodelling. This could be positive, for example when targeting a single microRNA inhibits various forms of maladaptive remodelling simultaneously. However, an unwanted effect could be that anti-atherogenic microRNAs also have anti-arteriogenic effects due to common pathways in atherosclerosis and arteriogenesis. The Janus phenomenon is a major drawback for many novel therapeutics designed to modulate vascular remodelling and must also be taken into account when exploring the therapeutic potential of microRNAs.

Therefore, we chose to discuss those microRNAs, miR-126, miR-155 and microRNA gene clusters 17-92, 23/24/27, 143/145 and 14q32, that play a confirmed role in multiple forms of vascular remodelling ánd are clearly linked to CVD.

Non-standard Abbreviations and Acronyms	
3'UTR	3'untranslated region
AAA	Abdominal aortic aneurysm
AAV	Adeno-associated virus
AngII	Angiotensin II
CAD	Coronary artery disease
CVD	Cardiovascular disease
EC	Endothelial cell
HDL	High density lipoprotein
IA	Intracranial aneurysm
LNA	Locked nucleic acid
miR	MicroRNA
MO	Morpholino
MP/MV	Microparticle/Microvesicle
MSC	Mesenchymal stem cell
oxLDL	Oxidized low density lipoprotein
PBMC	Peripheral blood mononuclear cell
siRNA	Small interfering RNA
TLR	Toll-like receptor
(V)LDL	(very) low density lipoprotein
(V)SMC	(vascular) smooth muscle cell
Abbreviations of NCBI-Annotated Target Genes	
ABCA1	ATP-binding cassette transporter A1
ACAT1	Acyl-CoA cholesterol acyltransferase-1
ACE	Angiotensin converting enzyme
ANGPTL3	Angiopoietin-like 3
ARF6	ADP ribosylation factor 6
AT1R	Angiotensin II type 1 receptor
BCL2 / 6	B-cell lymphoma 2 / 6
bFGF	Basic fibroblast growth factor
BIC	B cell integration cluster
BMP4	Bone morphogenetic protein 4
CCL2/MCP1	Monocyte chemoattractant protein 1
CD146	Cluster of differentiation 146 (melanoma cell adhesion molecule)
CDK4	Cyclin dependent kinase 4
CPT1α	Carnitine palmitoyl transferase 1α
CXCL12/SDF1	Stromal derived factor-1
CXCR4	Chemokine (C-X-C motif) receptor 4
DGAT2	Diacylglycerol O-acyltransferase 2
DLK1	NOTCH1 inhibitor delta-like 1 homolog
E2F1	E2F transcription factor 1
EFNB2	Ephrin B2
eNOS	Endothelial nitric oxide synthase
ERK1/2	Extracellular-signal-related kinase 1/2
ETS-1	V-ets avian erythroblastosis virus E26 oncogene homolog 1
FGFGR2	Fibroblast growth factor receptor 2
FLT1	VEGF receptor fms-related tyrosine kinase 1
FOXO3 / 4	Forkhead box O 3 / 4
FSR2	Fibroblast growth factor receptor substrate 2
FZD4	Frizzled class receptor 4
GATA2	GATA Binding Protein 2
GPAM	glycerol-3-phosphate acyltransferase 1 mitochondrial
HIF1α / HIF2α	Hypoxia inducible factor 1 / 2, alpha subunit
HKII	Hexokinase II
HMGB1	HMG box-transcription protein 1
HMOX1	Heme oxygenase 1
ICAM1	Intracellular cell adhesion molecule 1
IGF1	Insulin-like growth factor 1

IL33	Interleukin 33
INSIG1	Insulin induced gene 1
IRS1 / 2	Insulin receptor substrate 1 / 2
ITGβ8	Integrin β 8
JAK1	Janus Kinase 1
KLF2 / 4 / 5	Krüppel-like factor 2 / 4 / 5
LPL	Lipoprotein lipase
LRP6	LDL receptor related protein 6
MCP1/CCL2	Monocyte chemoattractant protein 1
MEF2a	Myocyte enhancer factor 2
MEG3	Maternally expressed gene 3
MIF	Macrophage migration inhibitory factor
MKK4	Mitogen-Activated Protein Kinase Kinase 4
MMP1 / 3	Matrix metalloproteinase 1 / 3
MRTFA	Myocardin related transcription factor A
Myd88	myeloid differentiation primary response gene
PAK4	p21-activated kinase 4
PIK3R2	Phosphoinositide-3-kinase, regulatory subunit 2
PPARγ	Proliferator-activator receptor gamma
PPP2R2A	Protein phosphatase 2 regulatory subunit B, alpha
RGS16	Regulator of G protein signalling 16
SDF1/CXCL12	Stromal derived factor-1
SEMA6A / 6D / 3B	Semaphorin 6A / 6D / 3B
SMAD3	SMAD family member 3
SOCS1 / 5	Suppressor of cytokine signalling 1 / 5
SPRED1	Sprouty-related, EVH1 domain containing 1
SREBPs	Sterol regulatory element-binding proteins
SRF	Serum response factor
TAB2	TGF-β activated kinase1/MAP3K7 binding protein 2
TGFβ(2)	Transforming growth factor beta (2)
TGFβR2	TGFβ receptor 2
TIMP3	Tissue inhibitor of metalloproteinase 3
TLR4	Toll like receptor 4
TNF-α	Tumor necrosis factor alpha
TRIF	TIR-domain-containing adapter-inducing interferon-β
uPA	urokinase-type plasminogen activator
VCAM1	Vascular cell adhesion molecule 1
VE-cadherin	Vascular endothelial cadherin
VEGF	Vascular endothelial growth factor

Table 1. Non standard abbreviations and acronyms.

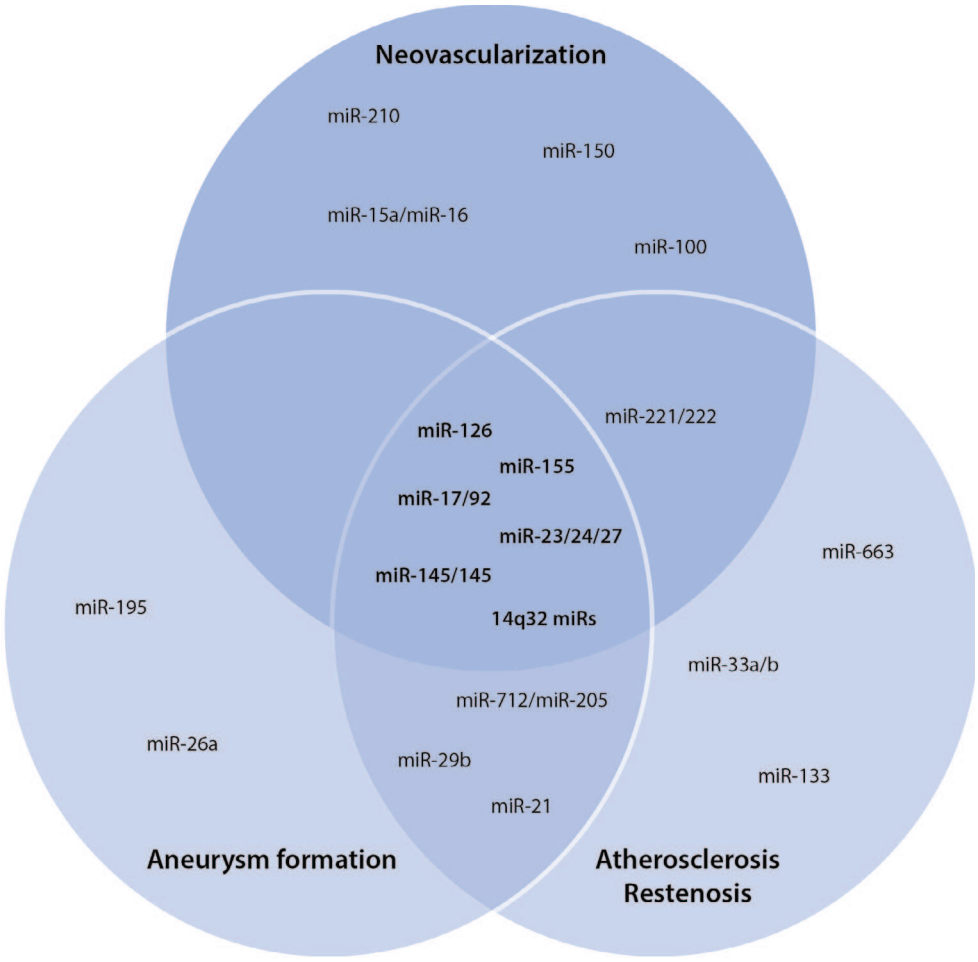

Figure 1. The top 5 microRNAs reported to play a role in each of the following vascular remodelling processes, atherosclerosis and restenosis formation, aneurysm formation and neovascularization, are shown. MicroRNAs that were reported to play a role in multiple forms of these processes were selected for this review and shown here in bold.

miR-126

MiR-126 is one of the most abundantly expressed microRNAs in endothelial cells (ECs)[15]. The miR-126 gene is located on human chromosome 9 and gives rise to two mature microRNAs, miR-126-3p and miR-126-5p. Generally, the role of miR-126 in vascular remodelling as described in the literature corresponds to miR-126-3p (Figure 2). MiR-126 is also abundantly expressed in platelets, suggesting a role for miR-126 in vascular homeostasis and inflammation[16]. Platelets are a major source of circulating miR-126[17]. Consequently, levels of circulating miR-126 are influenced by the use of platelet inhibitors, such as aspirin[17]. The delivery of miR-126 by platelet microparticles (MPs) to primary human macrophages was reported recently and miR-126 derived from these platelet MPs influences macrophage gene expression and function[18]. Levels of miR-126 are differentially expressed in plasma samples of patients with coronary artery disease (CAD)[19].

Neovascularization

The first studies from 2008 that investigated the role of miR-126 in EC function demonstrated that miR-126 targets VCAM1[20]. Increased expression of adhesion molecules such as VCAM1 and increased leukocyte adherence to ECs are necessary for the initiation of angiogenesis. Both mechanisms are stimulated by inhibition of miR-126[20]. In HUVECs, inhibition of miR-126 led to increased proliferation and migration[21]. Furthermore, injection of miR-126 inhibitors into zebrafish embryos affected blood vessel integrity, as was demonstrated by collapsed lumens and compromised endothelial tube organization[21].Studies in mice showed that inhibition of miR-126 decreased recovery after myocardial infarction and impaired angiogenic capacity in a hind limb ischemia model[22-24]. These effects were partially mediated via inhibitors of VEGF-signalling, namely SPRED1 and PIK3R2[21-23]. Both Spred1 and Pik3r2 are upregulated in absence of miR-126, causing an increase in vascular permeability and leakage[21, 23]. MiR-126 was also shown to target CXCL12[25]. Silencing miR-126 induced CXCL12 expression which enhanced migration of $CD34^+$ progenitor cells *in vitro* and increased the number of circulating bone marrow-derived progenitor cells after hind limb ischemia *in vivo*[25, 26].

In addition, exosomes from human $CD34^+$ cells, which are rich in miR-126, have great angiogenic capacity both *in vitro* and *in vivo*[27]. Mocharla *et al.* showed that $CD34^+$ peripheral blood mononuclear cells (PBMCs) secrete microvesicles and exosomes that are enriched with miR-126[28]. These microvesicles and exosomes are taken up by ECs and facilitate the proangiogenic effects of miR-126[28].

Atherosclerosis

Atherosclerotic plaque progression is often accompanied by apoptosis of (vascular) cells in the plaque[29]. During apoptosis, ECs release microvesicles that are enriched with miR-126[25]. Delivery of miR-126 to recipient vascular cells inhibits the progression of atherosclerosis, presumably via suppression of RGS16, which is a negative regulator of CXCR4. Subsequent upregulation of CXCR4 led to the production of CXCL12. This reduced lesion formation by decreasing the number of macrophages and apoptotic cells in the plaque and increasing the recruitment of endothelial progenitor cells for repair in a mouse model for atherosclerosis[25]. Vesicle-independent transfer of miR-126 from ECs

to SMCs was also reported, increasing miR-126 levels in SMCs (Figure 2). Decreased expression of miR-126 target genes FOXO3, BCL2 and IRS1 led to increased proliferation of SMCs[30]. Subjecting ECs to laminar shear stress or miR-126 inhibition abolished these effects. In miR-126$^{-/-}$ mice, neointima formation was attenuated compared to wildtype mice after ligation of the left common carotid artery[30]. Recently, the contribution of miR-126-5p to atherosclerosis formation was demonstrated by Schober *et al.*[31]. Hypercholesterolemic miR-126$^{-/-}$ApoE$^{-/-}$ and miR-126$^{+/+}$ApoE$^{-/-}$ mice were subjected to endothelial denudation. After 14 and 28 days, lesion area was increased in miR-126$^{-/-}$ApoE$^{-/-}$ mice compared to control animals[31]. Moreover, endothelial recovery of the carotid lumen was impaired in miR-126$^{-/-}$ApoE$^{-/-}$ animals due to reduced EC proliferation[31]. In these animals, expression of multiple miR-126-5p predicted target genes was increased, whereas expression of known miR-126-3p targets was not[31]. The authors confirmed targeting of DLK1 by miR-126-5p and demonstrated that inhibition of miR-126-5p increased Dlk1 expression and reduced EC proliferation[31]. To identify the specific role of miR-126-3p and miR-126-5p in endothelial repair, denuded arteries of ApoE$^{-/-}$ mice were treated with either miR-126-3p-, miR-126-5p- or control-miR inhibitors. Treatment with anti-miR-126-5p, but not anti-miR-126-3p, significantly increased the lesion area and impaired endothelial recovery and EC proliferation[31]. In untreated ApoE$^{-/-}$ mice, disturbed flow led to decreased miR-126-5p levels and increased Dlk1 mRNA and protein levels in the carotid artery, whereas miR-126-3p levels were unaltered[31]. The authors proposed that miR-126-5p plays a role in regulating EC proliferation at non-predilection sites, whereas miR-126-3p presumably regulates the replicative capacity of ECs at predilection sites[31]. Finally, in human atherosclerotic lesions miR-126-5p levels were found to inversely correlate with DLK1 expression and the number of lesional macrophages and positively correlated with EC proliferation, suggesting an atheroprotective effect of increased miR-126-5p levels in humans[31].

Aneurysm

Although miR-126 is upregulated in AAA and upregulation correlated with decreased TNF-α expression, the exact function of miR-126 in AAA pathogenesis is still unknown[32]. In plasma of patients with AAA, miR-126 was significantly downregulated compared to plasma of healthy volunteers, but not compared to patients with CAD[32].

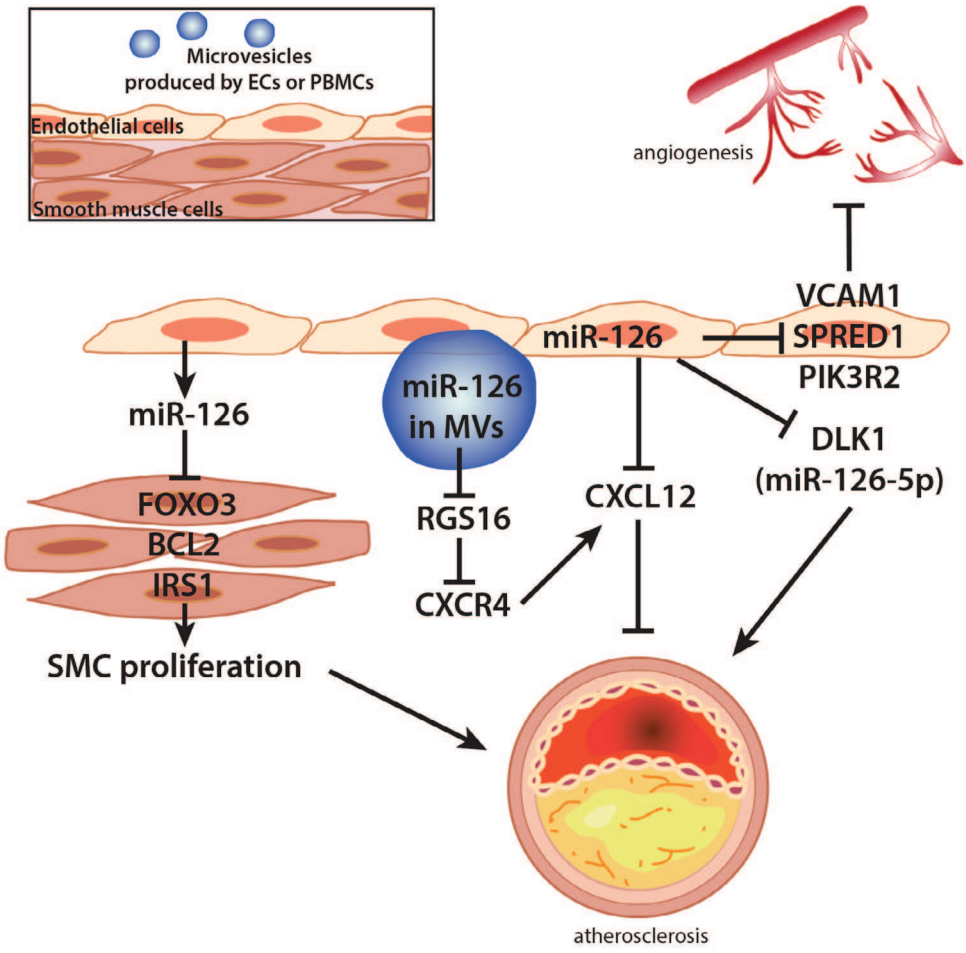

Figure 2. The role of endothelial miR-126 in vascular remodelling. MiR-126 regulates angiogenesis and vascular integrity via targeting of VCAM1 and targeting the inhibitors of VEGF signalling;SPRED1and PIK3R2. Via microvesicle-mediated delivery from ECs to neighbouring vascular cells, miR-126 inhibits RGS16, an inhibitor of CXCR4, resulting in the expression of CXCL12 and reducing atherosclerosis. Paracrine secretion of miR-126 from ECs to SMCs leads to inhibition of FOXO3, BCL2 and IRS1 target genes and increases proliferation of SMCs, which contributes to the atherogenic actions of miR-126. In addition, miR-126-5p suppresses the NOTCH1 inhibitor DLK1, thereby limiting atherosclerosis. Arrows indicate upregulation. Capped lines indicate inhibition. MV; microvesicle, EC; endothelial cell, SMC; smooth muscle cell. PBMCs; peripheral blood mononuclear cells. For full target gene names, see Table 1.

miR-155

The miR-155 gene is located within an exon of the non-coding RNA BIC on human chromosome 21. MiR-155 is highly expressed by activated B and T cells, but also by monocytes and macrophages[33, 34]. In addition, miR-155 is expressed in ECs and SMCs[35].

MiR-155 is upregulated in macrophages via TLR ligands, such as LPS[34]. MiR-155 exerts pro-inflammatory effects via targeting of the anti-inflammatory SOCS1[36]. In contrast, anti-inflammatory effects of miR-

155 signalling have also been described via targeting of TAB2[37] (Figure 3).

In 2012, Corsten *et al.* described a role for miR-155 in CVD, demonstrating upregulation of miR-155 during the acute inflammatory phase of viral myocarditis[38]. Systemic inhibition of miR-155 reduced cardiac monocyte/macrophage infiltration, decreased T lymphocyte activation and reduced myocardial damage in a mouse model of acute viral myocarditis[38].

Neovascularization

MiR-155 is co-expressed with AT1R in HUVECs and SMCs, where it represses AT1R expression[39]. Interestingly, a single nucleotide polymorphism (+1166 A/C), which is associated with CVD, was found to disrupt a miR-155 target site in the 3'UTR of AT1R[39]. Overexpression of miR-155 reduced migration of HUVECs in response to Angiotensin II (AngII) via targeting of the AT1R.

ETS-1 has two potential binding sites for miR-155 in its 3'UTR and is another target of miR-155 in HUVECs[35]. ETS-1 and its downstream target genes VCAM1, MCP1 and FLT1 were induced in HUVECs upon stimulation with AngII. Overexpression of miR-155 abrogated this effect[35].

Recent work by Pankratz *et al.* demonstrated that miR-155 exerts both antiangiogenic and proarteriogenic functions after induction of hind limb ischemia in mice[40, 41]. MiR-155 was upregulated seven days after femoral artery ligation in mice. Inhibition of miR-155 in HUVECs resulted in increased EC proliferation and tube formation[40]. These results were confirmed in aortic ring assays, as well as in *in vivo* matrigel plug assays using miR-155$^{-/-}$ mice. In miR-155$^{-/-}$ ECs, expression of AT1R was increased. AT1R expression could be manipulated by overexpression or inhibition of miR-155 in both human and murine ECs. The authors concluded that the antiangiogenic properties of miR-155 are mediated via AT1R[40] (Figure 2). Despite the antiangiogenic properties of miR-155, blood flow recovery after hind limb ischemia was impaired in miR-155$^{-/-}$ mice. MiR-155 deficiency decreased migration of bone-marrow derived macrophages. MiR-155$^{-/-}$ macrophages showed significantly reduced expression levels of proarteriogenic cytokines and chemokines upon LPS stimulation, compared to wildtype cells. SOCS1 was identified as a potential mediator, as this was the most upregulated target gene in miR-155$^{-/-}$ BMDMs. Knockdown of SOCS1 indeed reversed the effects of miR-155 deficiency on proarteriogenic cytokine production[40].

Atherosclerosis

Expression of miR-155 was upregulated in human atherosclerotic plaques, predominantly in pro-inflammatory macrophages[42, 43]. However, circulating levels of miR-155 were significantly lower in patients with CAD compared to healthy volunteers[19]. In several studies, treatment of macrophages with oxidized LDL and IFN-γ led to upregulation of miR-155, whereas suppression of miR-155 by oxLDL treatment has also been reported[42, 44-46]. Nazari-Jahantigh *et al.* demonstrated that miR-155 targets BCL6, a transcription factor that attenuates pro-inflammatory NF-kB signalling and directly represses CCL2. Leukocyte-specific deletion of miR-155 decreased Ccl2 signalling and reduced atherosclerotic plaque formation in ApoE$^{-/-}$ mice[42]. Recently, Tian *et al.* showed that miR-155 targets HMGB1, which supresses MIF and increases uptake of oxLDL by macrophages[47]. Elevated miR-155 levels enhanced oxLDL induced foam cell formation by targeting HMGB1. Systemic inhibition of miR-155 in ApoE$^{-/-}$ mice resulted in smaller atherosclerotic plaques that contained less lipid-laden macrophages[47]. However,

opposite findings on the role of miR-155 in atherosclerosis have also been reported. LDL-R$^{-/-}$ mice transplanted with miR-155$^{-/-}$ bone marrow developed larger lesions compared to mice transplanted with wildtype bone marrow[48]. Increased numbers of macrophages and neutrophils were present in these lesions as well as increased numbers of granulocytes and inflammatory monocytes in the circulation[48]. Apparently, miR-155 can have opposite effects in macrophages, being either pro- or anti-inflammatory (Figure 3).

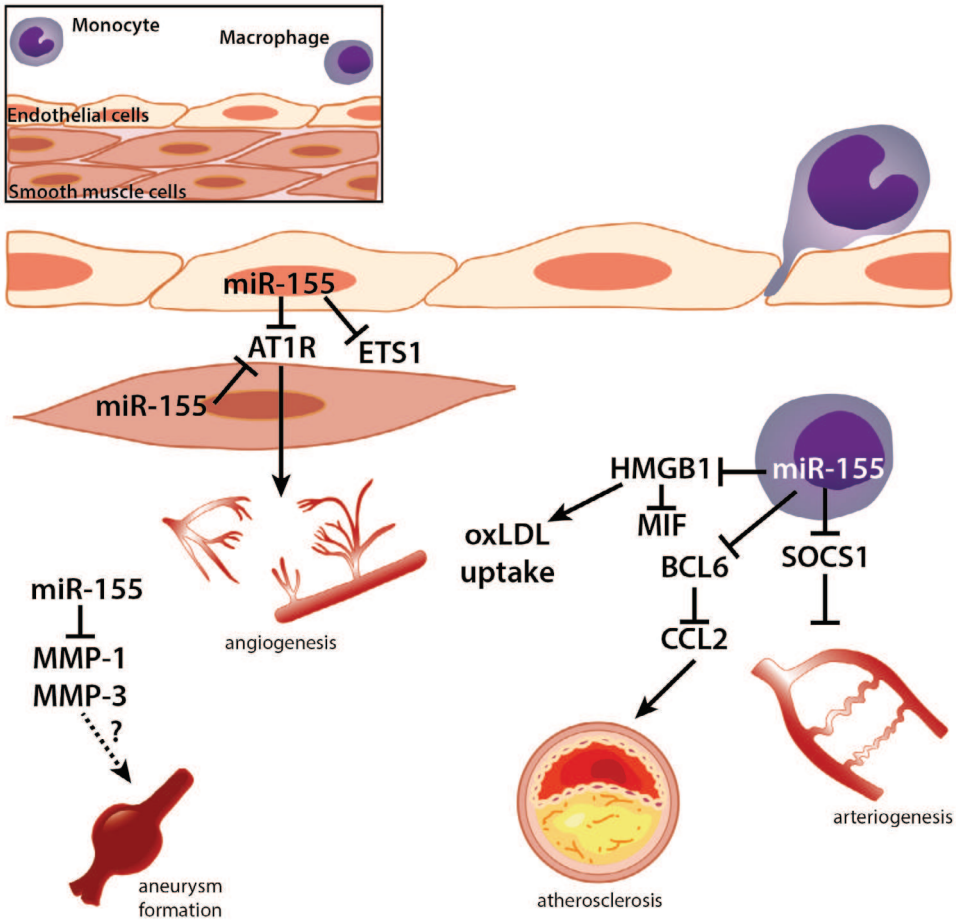

Figure 3. The inflammatory miR-155 in vascular remodelling. MiR-155 is co-expressed with AT1R in HUVECs and SMCs and inhibits expression of AT1R in these cells. ETS1 transcription factor is also targeted by miR-155 in HUVECs. Via these targets, miR-155 affects angiogenesis. In addition, miR-155 has been demonstrated to affect arteriogenesis. This effect is mediated by inhibition of SOCS1 in macrophages, resulting in upregulation of proarteriogenic cytokines. MiR-155 in atherosclerotic plaques is predominantly expressed in (pro-inflammatory) macrophages, where it suppresses the transcription factor BCL6. In addition, miR-155 targets HMGB1, increasing oxLDL uptake by macrophages. MiR-155 reduces matrix metalloproteinases MMP-1 and MMP-3, which could reduce matrix degradation and progression of aneurysm formation. Arrows indicate upregulation. Capped lines indicate inhibition. The dashed line indicates possible interactions that have not been confirmed yet. (HUV)EC; (human umbilical venous) endothelial cell, SMC; smooth muscle cell. For full target gene names, see Table 1.

Aneurysm

MiR-155 is significantly upregulated in AAA tissue[32]. However, expression of miR-155 was lower in plasma of patients with AAA compared to plasma levels of healthy controls ánd of patients with CAD[32]. In models for rheumatoid arthritis, overexpression of miR-155 led to downregulation of MMP1 and MMP3[49]. This suggests that overexpression of miR-155 in AAA may function as an endogenous rescue mechanism that inhibits matrix degradation and progression of aneurysm formation[49].

miR-17-92 cluster

The miR-17-92 gene cluster is located within intron 3 of the C13orf25 gene on human chromosome 13 and encodes six individual microRNAs, namely miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a[50] (Figure 4). Recently, it was shown that expression of the miR-17-92 cluster in ECs is stimulated by VEGF, via activation of the Erk/Elk1 pathway[51]. Upon stimulation, expression of miR-17-92 contributed to endothelial proliferation and angiogenic sprouting *in vitro* and physiological angiogenesis *in vivo*[51].

Neovascularization

In 2009, Bonauer *et al.* showed that miR-92a is highly expressed in human ECs and overexpression of miR-92a in ECs blocked sprouting in a three-dimensional angiogenesis model. *In vivo* inhibition of miR-92a increased the number of perfused vessels in matrigel plugs and improved blood flow recovery after hind limb ischemia[52]. ITGA5 was identified as a direct target of miR-92a[52]. To elucidate the specific function of the other members of the miR-17-92 cluster in angiogenesis, Doebele *et al.* overexpressed or blocked individual members of the cluster both *in vitro* and *in vivo*[53]. *In vitro* inhibition of all miR-17-92 members, except miR-19, resulted in increased sprouting of EC spheroids[53]. Combined inhibition of miR-17 and miR-20a was shown to promote angiogenesis in matrigel plugs *in vivo,* whereas inhibition of other members showed trends but no significant effects on angiogenesis[53]. Expression of JAK1 was reduced at mRNA and protein level upon miR-17 overexpression and inhibition of JAK1 using siRNAs was shown to reduce *in vitro* angiogenesis. Using luciferase assays, JAK1 was confirmed as a direct target of miR-17[53].

The contribution of the miR-17-92 cluster to physiological and pathological arteriogenesis was studied by Landskroner-Eiger *et al.*[54]. Endothelial specific knockout of miR-17-92 in mice showed that these animals had more pre-existent collateral arterioles. Consequently, these animals showed improved blood flow recovery after ischemia. MiR-19a targets components of WNT signalling, namely FZD4 and LRP6. Inhibition of miR-19a improved post-ischemic blood flow recovery[54].

Expression of the miR-17-92 cluster is repressed by HDAC9 in ECs[55]. Inhibition of HDAC9 reduced neovascularization *in vitro* and *in vivo*. Inhibition of HDAC9, using either a broad spectrum HDAC inhibitor or siRNAs against HDAC9, increased expression of the miR-17-92 cluster, suggesting that the antiangiogenic effects of HDAC9 inhibition are mediated through the miR-17-92 cluster. Indeed, inhibition of miR-17-20a combined, but not of miR-17 alone, completely rescued the reduced sprouting and network formation in HDAC9 deficient ECs[55].

Atherosclerosis

Several studies showed that miR-17-92 cluster members are regulated by changes in shear stress[56, 57]. Upregulation of miR-19a by laminar shear stress has an antiproliferative effect on ECs via targeting of Cyclin D1[57]. MiR-92a expression was reduced in HUVECs that were subjected to atheroprotective laminar shear stress, leading to upregulation of KLF2[58]. Expression of KLF2 targets eNOS and thrombomodulin were decreased upon miR-92a overexpression[58].

MicroRNA expression profiling in HUVECs revealed upregulation of miR-92a upon low shear stress conditions and the presence of oxLDL[59]. Accordingly, miR-92a expression was higher in the vasculature of both mice and humans in atheroprone regions with low shear stress[59]. MiR-92a inhibition reduced atherosclerosis formation in hypercholesterolemic $LDLR^{-/-}$ mice. Expression of target genes Klf2 and Klf4 was increased upon anti-miR-92a treatment. The authors identified SOCS5 as a novel target of miR-92a, which is involved in the regulation of endothelial inflammation[58]. Furthermore, circulating ICAM-1 levels were reduced in anti-miR-92a treated animals. These results suggest that upregulation of miR-92a by oxLDL in atheroprone regions promotes endothelial dysfunction and atherosclerosis formation[59].

Interestingly, inhibition of miR-92a in rats reduced neointima formation in carotid arteries after vascular injury[60]. MiR-92a inhibition increased EC proliferation and migration, improving reendothelialization after balloon injury or arterial stenting. Expression of KLF4 and MKK was upregulated by miR-92a inhibition[60]. MiR-92a is a promising therapeutic target to reduce atherosclerosis development and post-interventional restenosis.

Aneurysm

Two members of the 17-92 cluster, miR-20a and miR-92a, were significantly upregulated in ECs of AAA tissue, but a causative role has yet to be confirmed[32].

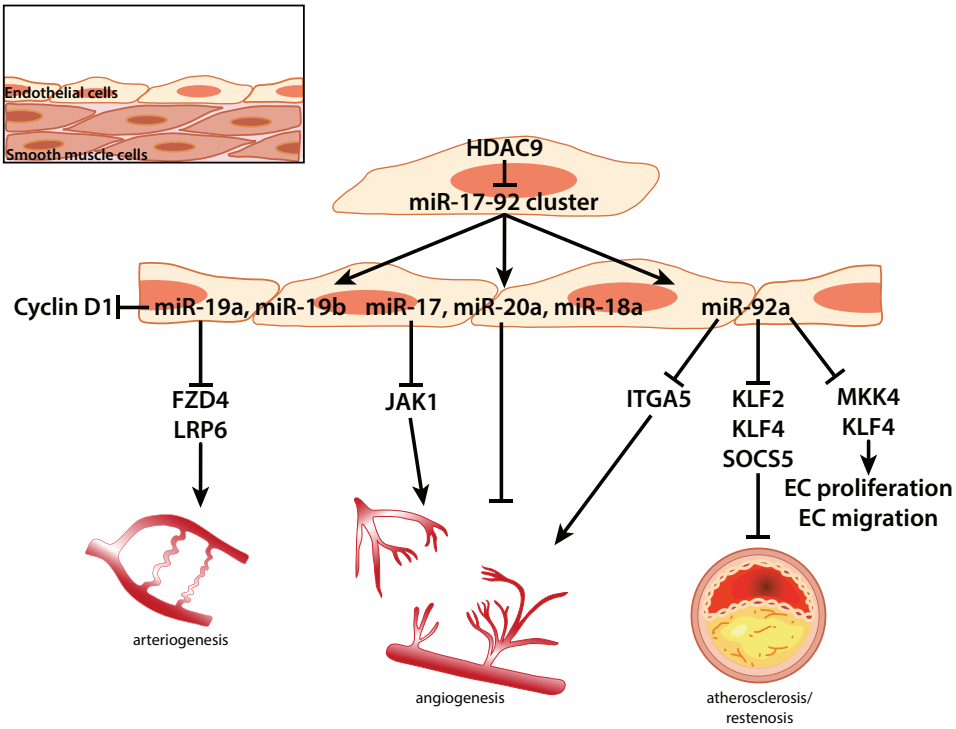

Figure 4. The role of the miR-17-92 cluster in vascular remodelling. MiR-17-92 cluster members regulate angiogenesis via suppression of several target genes. MiR-17 reduces expression of JAK1. ITGA5 is targeted by miR-92a. In addition, miR-92a targets KLF2, KLF4 and SOCS5, promoting atherosclerosis formation. Proliferation and migration of endothelial cells is regulated by targeting of MKK4 and KLF4 by miR-92a. MiR-19a has an antiproliferative effect in ECs via suppression of Cyclin D1. Other target genes of miR-19a include FZD4 and LRP6, regulators of WNT signalling. Targeting of these genes by miR-19a affects collateral artery formation and bloodflow recovery after ischemia. Arrows indicate upregulation. Capped lines indicate inhibition. EC; endothelial cell. For full target gene names, see Table 1.

miR-23/24/27 family

The miR-23/24/27 family consists of two separate microRNA gene clusters. The mouse intergenic miR-23a-27a-24-2 cluster lies on chromosome 8; in humans this cluster is located on chromosome 19. The miR-23b-27b-24-1 cluster has an intronic location on mouse chromosome 13, chromosome 9 in humans[61, 61, 62]. Members of the miR-23/24/27 family are highly expressed in vascularized tissues and ECs[63] (Figure 5). Laminar flow and unidirectional shear stress increase the expression of miR-23b, miR-27a/b and miR-24 in ECs[56, 64, 65]. Increased expression of miR-23b and miR-27b by pulsatile shear flow was found to correlate with EC growth arrest. The expression of cell cycle gene E2F1 was downregulated by miR-23b and miR-27b[56]. Moreover, phosphorylation of the Rb protein was blocked by miR-23b. Decreased Rb phosphorylation reduces EC proliferation and inhibits cell cycle progression[56]. Anti-miR-23b, but not anti-miR-27, treatment of HUVECs resulted in partial reversal of shear stress induced growth arrest[55].

Neovascularization

Knockdown of miR-23a/b and miR-27a/b decreased *in vitro* EC sprouting and *ex vivo* aortic ring sprouting[62]. Anti-angiogenic genes SEMA6A, SEMA6D and SPROUTY2 are targeted by miR-23a/b and miR-27a/b , as shown by luciferase gene reporter assays[62]. Urbich *et al.* showed that i*n vivo* angiogenesis was also affected upon inhibition of miR-27a/b. Anti-miR-27a/b treatment decreased the number of perfused vessels in matrigel plugs[66]. Moreover, inhibition of miR-27a/b impaired vasculogenesis in zebrafish embryos. *In vitro* experiments showed additional targeting of SEMA3B but *in vivo,* only SEMA6A was a target of miR-27a/b[66]. Young *et al.* showed that miR-27a also targets VE-cadherin, both *in vitro* and *in vivo*[67].

MiR-24 is expressed in cardiac ECs[68]. The expression of miR-24 is induced upon hypoxia and miR-24 is enriched in cardiac ECs compared to other cardiac cells after cardiac ischemia[68, 69]. Overexpression of miR-24 in HUVECs increased apoptosis and impaired tube formation, sprouting, migration and proliferation[68]. The endothelium-enriched transcription factors GATA2 and PAK4 were validated as targets of miR-24. Inhibition of miR-24 increased vascularity and decreased myocardial infarct size in mice[68]. Another confirmed target gene for miR-24 in ECs is NDST1. Inhibition of NDST1 by miR-24 decreased sulfation of HSPGs and subsequently the binding affinity of HSPGs for VEGFA. MiR-24 mediated suppression of NDST1 lowered VEGFR2 levels and reduced EC responsiveness to VEGFA[70]. Via these mechanisms, miR-24 affects EC responsiveness to VEGFA. MiR-24 also affected apoptosis, proliferation and function of SMCs, partially through HMOX1[69].

Atherosclerosis, restenosis and lipid metabolism

The effects of miR-24 and 27b as described here are predominantly on lipid metabolism, which will ultimately also influence atherosclerosis[71-73]. MiR-24 and miR-27b are upregulated in livers of high-fat diet (HFD) fed mice[71, 72]. Inhibition of miR-24 in HFD fed mice reduced plasma triglyceride levels and lipid accumulation in the liver, but did not affect plasma cholesterol levels. This effect was mediated via increased expression of INSIG1 in the liver and subsequent decreased expression of SREBPs and other lipogenic genes[72].

MiR-27b targets several additional lipogenic genes, including PPARγ, ANGPTL3 and GPAM. However, direct binding of miR-27b to the 3'UTR of the mRNAs of these genes was not demonstrated[71]. Upregulation of hepatic miR-27b was observed in HFD $ApoE^{-/-}$ mice and expression of miR-27b target genes Angptl3 and Gpam was reduced[71]. Experiments performed in the THP-1 human monocyte cell line showed that miR-27a/b regulates cholesterol homeostasis[73]. MiR-27a/b targeting of ABCA1 affected apoA1-mediated cholesterol efflux in macrophages[73]. Lipid uptake was also affected by miR-27a/b, as was shown by reduced oxLDL binding to macrophages after miR-27a/b overexpression. This was mediated by miR-27a/b target gene LPL. Finally, cholesteryl-ester formation was reduced by miR-27a/b via targeting of ACAT1[73].

The contribution of miR-23b to SMC phenotypic switching upon vascular injury was recently reported by Iaconetti *et al.*[74]. Expression of miR-23b was reduced after carotid injury in rats. Increased proliferation and migration of SMCs was observed upon miR-23b inhibition, whereas overexpression of miR-23b led to reduced proliferation and migration[74]. Overexpression of miR-23b resulted in decreased neointima formation in rat carotid arteries after balloon angioplasty and target genes uPA, SMAD3 and FOXO4

were downregulated in these animals[74].

Aneurysm

MicroRNA expression profiling revealed decreased expression of the miR-23b/miR-24-1 cluster, in human intracranial aneurysmal (IA) samples[75, 76]. In murine AAA models, the miR-23b-27b-24 cluster is also downregulated. MiR-24 was most significantly downregulated, leading to upregulation of the inflammatory target gene CHI3L1[77]. *In situ* hybridization showed localization of miR-24 in adventitial macrophages of aneurysmal aortic mouse tissue. MiR-24 co-localized with CHI3L1 in activated macrophages, where CHI3L1 drives inflammatory gene expression[78]. Modulation of miR-24 levels in murine AAA models using either pre-miR-24 or anti-miR-24 led to reduced and increased AAA formation respectively[77]. In summary, miR-23b-24-27b family members are downregulated in human IA samples and murine AAA models and modulation of miR-24 influences aortic inflammation, thereby contributing to AAA development[75-77]. This renders the miR-23-24-27 family a potentially interesting therapeutic target for AAA treatment.

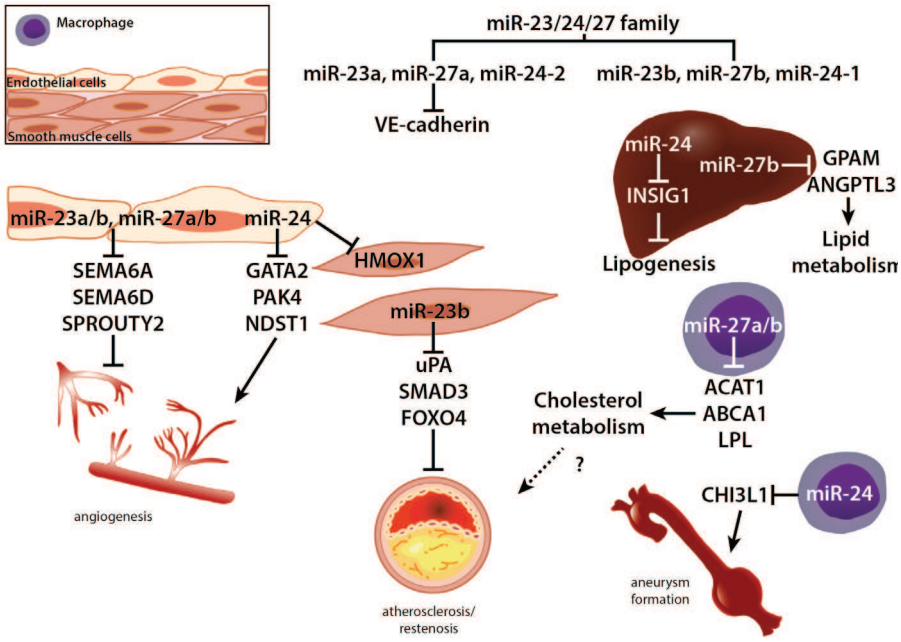

Figure 5. Different roles in vascular remodelling for microRNAs of the miR-23/24/27 family. The miR-23/24/27 family consists of two miR clusters, namely the miR-23a-27a-24-2 cluster and the miR-23b-27b-24-1 cluster. MiR-23a/b and miR-27a/b target the anti-angiogenic genes SEMA6A, SEMA6D and SPROUTY2. In addition, miR-27a inhibits expression of VE-cadherin. MiR-24 inhibits proliferation, migration and sprouting of HUVECs via the endothelium-enriched transcription factor GATA2, PAK4 and NDST1. Moreover, miR-24 affects SMC apoptosis, proliferation and function via HMOX1. Expression of miR-23b in SMCs was found to target uPA, SMAD3 and FOXO4, which results in decreased proliferation and migration of SMCs. Overexpression of miR-23b decreases neointima formation upon balloon injury in rats. Members of the miR-23/24/27 family also play an important role in lipid metabolism. Cholesterol metabolism is affected by miR-27a/b through suppression of ACAT1, ABCA1 and LPL in macrophages. In the liver, miR-27b targets the lipogenic genes GPAM and ANGPTL3, whereas miR-24 suppresses expression of INSIG1. Of the miR-23/24/27 family members, only miR-24 has been described to affect AAA formation. MiR-24 is expressed in macrophages in the adventitia of murine aneurysmal tissue, where it is co-localized with and inhibits expression of the CHI3L1 gene. Arrows indicate upregulation. Capped lines indicate inhibition. The dashed lines indicates an interaction that has not been confirmed yet. (HUV)EC; (human umbilical venous) endothelial cell, SMC; smooth muscle cell. For full target gene names, see Table 1.

miR-143/145 cluster

The miR-143/145 gene cluster contains two highly conserved microRNAs, which are located on human chromosome 5. In 2007, these microRNAs were first described as downregulated in rat carotid arteries after induction of balloon injury[78]. Restoration of miR-143 and miR-145 expression levels using an adenoviral vector reduced neointima formation upon balloon injury in rat carotids[79, 80]. MiR-145 is the most abundantly expressed microRNA in healthy rat carotid arteries, where it is predominantly localized in SMCs[81]. During SMC differentiation from multipotent stem cells, high transcript levels of miR-143 and miR-145 are observed[82]. Upregulation of these microRNAs allows for SMC differentiation, whereas their expression is downregulated upon proliferation[82]. Together, these microRNAs play an important role in the differentiation and proliferation of SMCs. Differentiation of SMCs is induced via SRF, Myocardin and myocardin-related transcription factors, but can also be induced via the Jag-1/ Notch signalling pathway[83, 84]. These factors also regulate the transcription of the miR-143/145 cluster, further promoting differentiation of SMCs[83, 84] (Figure 6, upper panel). Expression of contractile genes is mediated via (amongst other factors) KLF4, which is directly targeted by miR-145[82]. Inhibition of KLF4 by miR-145 increases expression of SMC markers [82]. MiR-143 can directly inhibit proliferation of SMCs via targeting of ELK1[84].

In accordance with these findings, Boettger *et al.* described that miR-143/145 deficient mice have a thinner arterial medial layer and a decreased blood pressure[85]. In general, the miR-143/145 cluster has proven essential for SMC function and controls the phenotypic switch of contractile SMCs towards synthetic VSMCs[79, 81, 82, 85] (Figure 6, upper panel).

Neovascularization

MiR-145 inhibits tumour angiogenesis via targeting of IGF1, the IRS1 pathway and its downstream genes N-RAS and VEGF-A[86, 87]. MiR-143 was found to inactivate AKT, which is a downstream signalling molecule in the IGF1 receptor pathway and thereby regulates angiogenesis and tumorigenesis[88]. Inactivation of AKT by miR-143 resulted in decreased protein levels of HIF-1α and reduced VEGFA expression[88]. In neuroblastoma samples, miR-145 expression was also downregulated, which was inversely correlated with HIF-2α expression[89]. The authors showed that miR-145 can directly target HIF-2α and suppress angiogenesis, which was demonstrated by tube formation of neuroblastoma cells[89].

Although these findings relate mainly to pathological angiogenesis, many fundamental mechanisms are shared with physiological angiogenesis, such as receptor signalling cascades (e.g. HIF-1α), proliferation and migration of vascular cells and tube formation[90]. Indeed, Wang *et al.* found that miR-145 was transiently downregulated *in vivo* following coronary artery occlusion in mice and *in vitro* upon hypoxia treatment of cardiac fibroblasts[91]. Inhibition of miR-145 increased infarct scar size at 7 and 28 days after myocardial infarction in mice. However, reduced differentiation of cardiac fibroblasts towards myofibroblasts, and not decreased angiogenesis, most likely mediated these effects[91]. The authors demonstrated that transfection with miR-145 increased the number of α-SMA positive cells in fibroblast cultures, thus inducing transdifferentiation of fibroblasts into myofibroblasts. KLF5 is

a direct target of miR-145. Transfection with miR-145 decreased expression of KLF5 and increased Myocardin expression. These data suggest that miR-145 mediates differentiation of cardiac fibroblasts to myofibroblasts through the KLF5-Myocardin pathway[91].

Work by Climent *et al.* suggests that miR-143 and miR-145 are transferred from SMCs to ECs via membrane protrusions[92]. TGFβ induces the transfer of miR-143/145, as inhibition of either the TGFβ pathway or TGFβR2 reduced miR-143/145 transfer towards ECs[92]. Overexpression of miR-143 and miR-145 in ECs reduced proliferation and the ability to form capillary-like structures on matrigel[92]. The authors identified HKII and ITGβ8 as direct targets of miR-143/145 that modulate the angiogenic potential of ECs[92] (Figure 6).

Atherosclerosis and restenosis

MiR-143/145$^{-/-}$ mice develop spontaneous neointimal lesions in the femoral arteries at older age[85]. Angiotensin converting enzyme (ACE) was identified as a target for miR-143/145. Increased expression of ACE in miR-143/145$^{-/-}$ mice resulted in increased AngII levels, which subsequently contributed to the synthetic phenotype of miR-143/145$^{-/-}$ SMCs[85]. ApoE$^{-/-}$ mice treated with SMC-specific lentiviral miR-145 showed a reduction in plaque size and an increase in atherosclerotic plaque stability[93]. This is in line with the finding that overexpression of miR-145 decreased neointima formation in balloon injured arteries by modulation of KLF5 expression[80]. However, reduced neointima formation after carotid artery ligation in miR-143/145$^{-/-}$ mice has also been reported[94]. The authors explained this by the fact that the SMCs in their knockout model were already deficient in miR-145 at the onset of injury, whereas in the overexpression model, miR-145 expression was normal at the onset of the experiment[94].

In humans, miR-145 levels were significantly lower in plaques than in atherosclerosis-free regions[93]. Cholesterol loading of mouse aortic SMCs resulted in downregulation of SMC markers, whereas expression of macrophage markers was increased[95]. Expression of miR-143 and miR-145, as well as the expression of SRF and Myocardin, were downregulated[95]. Cholesterol loading, via downregulation of the miR-143/145/SRF/Myocardin axis, causes reprogramming of SMCs towards a macrophage like phenotype[95]. Moverover, statin treatment, which is the most common form of anti-atherosclerotic therapy today, increases expression of miR-143/145 in ECs[96].

In addition to intercellular transfer of miR-143/145 from SMCS to ECs, transport of miR-143/145 in the opposite direction has also been described[92]. Increases in laminar shear stress lead to upregulation of KLF2, which subsequently induces transcription of the miR-143/145 cluster in ECs[96]. Upregulation of miR-145 in ECs was shown to repress JAM-A, which reduces leukocyte recruitment and infiltration and thus atherosclerosis formation[97]. KLF2 also triggered release of EC derived MVs, which transfer miR-143/145 from ECs to SMCs. Injection of MVs that are rich in miR-143/145 in HFD fed mice, led to a reduction of plaque formation[96]. In contrast, miR-143/145$^{-/-}$ mice developed smaller atherosclerotic lesions compared to LDLR$^{-/-}$ controls[98]. Plaques of miR-143/145$^{-/-}$ mice contained less macrophages and analysis of plasma cholesterol levels revealed decreased VLDL and LDL fractions. ABCA1 was confirmed as miR-145 target in this study, but this was not reflected by increased HDL levels in miR-143/145$^{-/-}$ mice[98].

Aneurysm

MiR-145 was downregulated in IA tissues[75]. Elia *et al.* demonstrated that both miR-143 and miR-145 were reduced in human thoracic aorta aneurysms, which correlated with SMC function[79, 99]. In AAA however, expression levels of miR-145 and miR-143 were similar to those in normal abdominal aortic tissues[32].

Homozygous miR-143/145$^{-/-}$ mice showed structural defects in the SMC layer of the aorta[74]. Additionally, the SMCs in the media of aortas from miR-143/145$^{-/-}$ mice had a dedifferentiated phenotype, demonstrated by increased migration and proliferation and an increased protein synthesis[74].

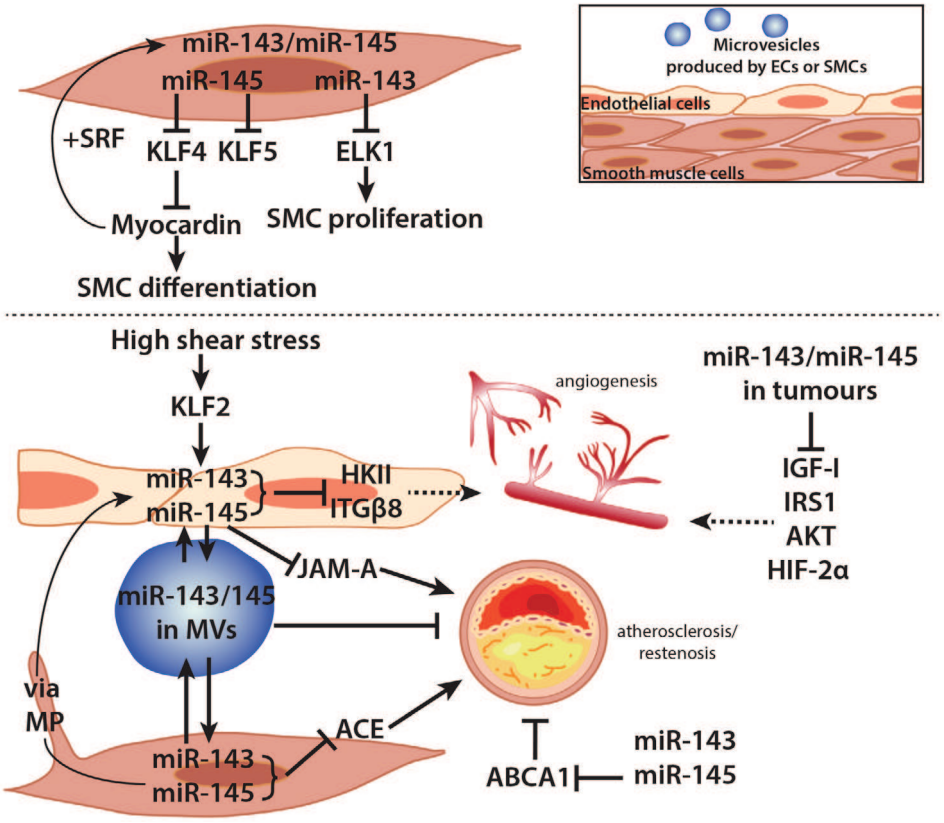

Figure 6. The miR-143/145 cluster in vascular remodelling. MiR-143 and miR-145 control SMC phenotype. Differentiation of SMCs is regulated by SRF, Myocardin and myocardin-related transcription factors (MRTFs). Via a feedback loop, these factors also regulate expression of the miR-143/miR-145 cluster itself (upper panel). Inhibition of KLF4 and KLF5 by miR-145 results, via Myocardin, in SMC differentiation and myofibroblast transdifferentiation, whereas targeting of ELK1 by miR-143 inhibits proliferation of SMCs. TGFβ and BMP4 are also able to activate expression of the miR-143/miR-145 cluster (not shown here). In addition, high shear stress, via KLF2, increases expression of miR-143/miR-145 in ECs (figure 4, lower panel).
In tumours, miR-145 suppresses IGF1 and the IRS1 pathway, affecting angiogenesis. In addition, miR-145 targets HIF2α and suppresses angiogenesis. MiR-143 targets AKT, thereby decreasing HIF1α and VEGFA expression. Transfer of miR-143/145 from VSMCs to ECs via membrane protrusions (MP) decreases the expression of HKII and ITGβ8 and via this mechanism presumably modulates angiogenesis. Intercellular transfer of miR-143/145 via MVs has also been described. MVs rich in miR-143/145 inhibit atherosclerotic plaque formation. ACE is another target of miR-143/145 which affects atherosclerosis. Arrows indicate upregulation. Capped lines indicate inhibition. The dashed lines indicate interactions that have not been confirmed yet. MP; membrane protrusion, MV; microvesicle, EC; endothelial cell , SMC; smooth muscle cell. For full target gene names, see Table 1.

14q32 microRNA gene cluster

The 14q32 microRNA cluster is the largest known mammalian microRNA gene cluster, located on human chromosome 14 and mouse chromosome 12. The cluster consists of 54 microRNAs in humans and 61 in mice[100, 101] (Figure 7). It is assumed that transcription of the 14q32 microRNA gene cluster is controlled by the long noncoding RNA MEG3, also located on human chromosome 14, as deletion of MEG3 leads to downregulation of 14q32 microRNAs, as was shown in MEG3$^{-/-}$ mice[102]. MEG3$^{-/-}$ embryos have increased expression of VEGF pathway genes and increased cortical microvessel density[102].

Neovascularization

Using a reverse target prediction analysis, where we looked for putative microRNA binding sites in the 3'UTRs of a set of nearly 200 neovascularization genes, our research group observed enrichment of binding sites for 14q32 microRNAs in the 3' UTRs of these genes. Microarray analyses performed on adductor muscle tissue of mice that underwent single ligation of the femoral artery as a model for effective neovascularization showed upregulation of 14q32 microRNAs following three different expression patterns[101]. We observed so called early responders, microRNAs whose expression was upregulated 24 hours after induction of ischemia, late responders whose expression was upregulated from 72 hours after ischemia induction and non-responders. Inhibition of early responders miR-487b, miR-494, late responder miR-329 and non-responder miR-495 led to increased neovascularization and an improved blood flow recovery after hind limb ischemia in mice. Inhibition of miR-329, miR-487b and miR-495 increased proliferation of human umbilical arterial ECs[101]. *In vivo,* inhibition of miR-329 led to upregulation of target genes TLR4, VEGFA, FGFR2 and MEF2A, whereas TLR4, VEGFA, ARF6, EFNB2 and FGFR2 were upregulated upon inhibition of miR-494. Using dual luciferase reporter gene assays, direct binding of miR-494 to the 3'UTRs of VEGFA, EFNB2 and FGFR2 was demonstrated. MiR-329 directly targets MEF2a and although VEGFA was regulated by miR-329, this was an indirect effect[101]. MiR-495 directly targets the 3'UTR of CCL2 and via this mechanism, proliferation and apoptosis of HUVECs is affected[103]. Inhibition of miR-329, miR-487b, miR-494 and miR-495 also increased sprouting in aortic ring assays[101]. Wang *et al.* showed that miR-329 is a negative regulator of angiogenesis by targeting CD146, which functions as co-receptor for VEGFR2. Inhibition of miR-329 increased angiogenesis in this study, both *in vitro* and *in vivo*[104].

In a model for cerebral ischemia, 14q32 miR-376b-5p also regulates angiogenesis. Expression of miR-376b-5p was decreased following middle cerebral artery occlusion (MCAO) in rats and miR-376b-5p inhibited angiogenesis *in vivo*, as well as in HUVEC cultures, via targeting of the HIF-1α mediated VEGFA/Notch-1 signalling pathway [105]. In another study, 14q32 microRNA miR-377 was identified as the most significantly downregulated microRNA in hypoxia-treated mesenchymal stem cells (MSCs) in rats[106]. Knockdown of miR-377 in HUVECs promoted angiogenesis *in vitro*, via direct targeting of VEGFA. To elucidate whether hypoxia-associated miR-377 regulated MSC induced myocardial angiogenesis in ischemic hearts, the authors transduced rat MSCs with lentiviral vectors in order to overexpress or suppress miR-377 expression. MSCs with lentiviral miR-377, anti-miR-377 or empty vector were then injected into ischemic rat hearts after ligation of the left anterior descending coronary artery.

Inhibition of miR-377 in MSCs enhanced angiogenesis, decreased the area of fibrosis and improved cardiac function of these animals[106].

Atherosclerosis

MicroRNA expression profiling in symptomatic versus asymptomatic human atherosclerotic plaques, showed upregulated expression of 14q32 microRNA miR-127[107]. Our group also investigated expression of 14q32 microRNAs in stable versus unstable plaques of patients who underwent carotid endarterectomy surgery. We observed upregulation of 14q32 miR-494 in unstable atherosclerotic plaques. Inhibition of miR-494 led to reduced plaque formation in mice, while plaque stability was increased[108]. Moreover, total plasma cholesterol and VLDL fractions were decreased in these animals. Inhibition of miR-494 led to upregulation of target genes TGFB2, TIMP3 and IL33.

In addition,14q32 microRNA miR-136 was upregulated in human atherosclerotic plaques . This microRNA is also highly expressed in synthetic SMCs *in vitro*[109]. MiR-136 targets PPP2R2A, resulting in increased ERK1/2 phosphorylation and increased proliferation of SMCs. The authors proposed that via this mechanism, miR-136 contributes to abnormal proliferation of SMCs, which is often observed in atherosclerosis[109].

In a study performed by Ramirez *et al.*, the 14q32 microRNA miR-758 regulated ABCA1 in macrophages. Transfection of J774-macrophages with miR-758 reduced cholesterol efflux[110]. MiR-758 levels were furthermore regulated by dietary cholesterol *in vivo*. High dietary fat repressed miR-758 expression in the liver as well as in peritoneal macrophages, whereas ABCA1 levels were increased[110]. In another study, miR-758 levels were upregulated in hypercholesterolemic human plaques compared to normocholesterolemic plaques[111]. ABCA1 mRNA levels were also increased in hypercholesterolemic patients, whereas protein levels were similar to normocholesterolemic patients, suggesting strong posttranscriptional regulation of ABCA1 by miR-758. These human data suggest a role for miR-758 as ABCA1 modulator in human atherosclerotis[111].

A role for the 14q32 microRNA miR-370 in lipid metabolism and atherosclerosis was first described by Iloupoulos *et al.,* mainly via direct targeting of CPT1α, an important enzyme in fatty acid β-oxidation[112]. Other lipogenic genes such as SREBP-1c and DGAT2 were also regulated by miR-370. Using transfection experiments with sense and antisense miR-370, this regulation was mediated indirectly via miR-122[112]. Finally, extensive hypomethylation of the 14q32 locus was observed in human atherosclerotic plaques, which resulted in upregulation of several 14q32 microRNAs[113]. These findings suggest a role for epigenetic modulation of the 14q32 microRNA cluster in atherosclerosis[113].

Aneurysm

MiR-487b is involved in hypertension-induced outward remodelling of the aorta. Chronic hypertension induced via AngII infusion led to significant upregulation of miR-487b in the aortae of rats[114]. MiR-487b was predominantly expressed in the adventitia and co-localized with the vasoactive IRS1. Using luciferase reporter gene assays, miR-487b was shown to directly target the IRS1 3'UTR, both in rats and humans. MiR-487b downregulated expression of IRS1 in aortae of hypertensive rats, both at mRNA and protein level[114].

Although further research into this extraordinarily large microRNA cluster is necessary, it is clear that the 14q32 microRNAs play important but diverse roles in the multiple processes of vascular remodelling, opening up new possibilities for prevention, detection and treatment of CVD[115] (Figure 7).

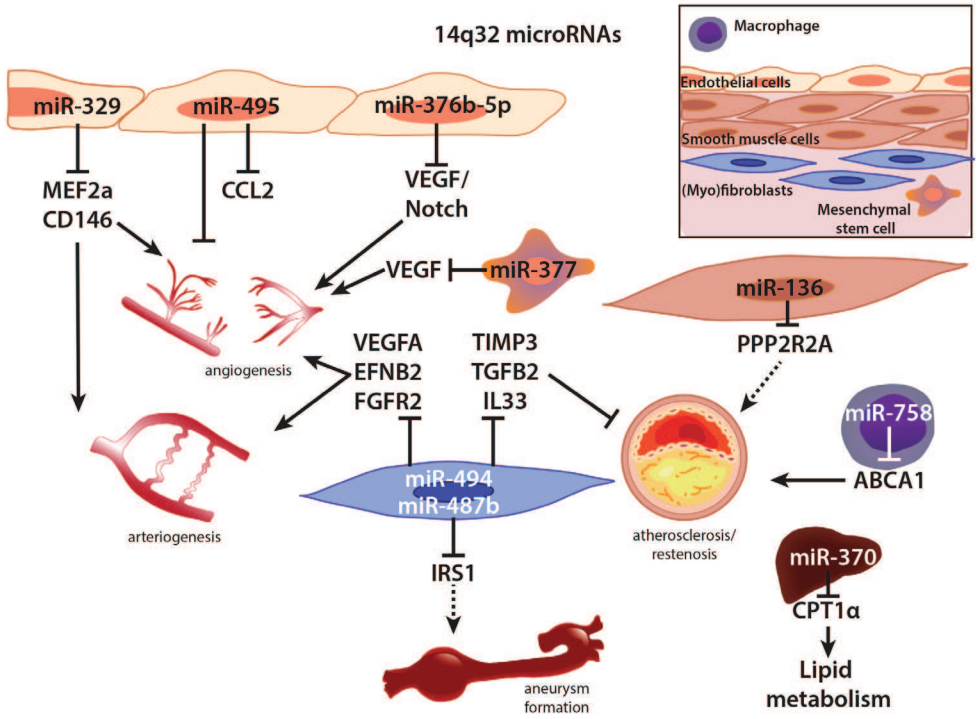

Figure 7. Roles for 14q32 miRs in vascular remodelling. MiR-329 inhibits angiogenesis and arteriogenesis via targeting of the co-receptor for VEGFR2, CD136 and MEF2a. Arteriogenesis and angiogenesis are also inhibited by miR-494, which suppresses VEGFA, EFNB2 and FGFR2. MiR-495 inhibits both arteriogenesis and angiogenesis. Proliferation and migration of HUVECs is affected by miR-495, via targeting of CCL2. VEGF signalling is further influenced through targeting of the HIF1α mediated VEGF/Notch signalling pathway by miR-376-5p and via direct targeting of VEGF by miR-377. MiR-494 also influences atherosclerosis, through the inhibition of several target genes namely TIMP3, TGFβ2 and IL33. MiR-136 is upregulated in human atherosclerotic plaques, where it targets PPP2R2A. Furthermore, cholesterol metabolism is affected by miR-758 through suppression of ABCA1 and by miR-370 via direct targeting of CPT1α. MiR-487b in highly expressed in the adventitia of rat aortae during chronic hypertension. Here, miR-487b targets IRS1, where it is thought to contribute to outward remodelling of the aorta. Arrows indicate upregulation. Capped lines indicate inhibition. MV; microvesicle, (HUV)EC; (human umbilical venous) endothelial cell , SMC; smooth muscle cell layer. For full target names, see Table 1.

Circulating microRNAs

Several of the microRNAs discussed in this review are also expressed in the circulation and could be used as biomarkers for vascular remodelling and cardiovascular disease. For example, circulating miR-155 was expressed at significantly lower levels in CAD patients compared to healthy volunteers[19]. Likewise, circulating levels of miR-126, miR-17, miR-92a were decreased in these patients[19, 115]. Several miRs, including miR-126, were significantly increased in plasma of patients with insufficient collateral artery function[116]. Furthermore, circulating levels of several 14q32 microRNAs, including miR-134,

miR-328, miR-370, miR-487a and miR-480 may have a diagnostic value for acute myocardial infarction, coronary artery disease and cardiac death[117-121]. 14q32 miR-487b was increased in circulating leukocytes of patients with acute ischemic stroke[122]. Other 14q32 microRNAs, including miR-665 and miR-541 have been reported to play a role in heart failure and cardiac hypertrophy, respectively[123, 124] Nevertheless, these findings need to be confirmed in large prospective cohort studies to determine the potential use of these microRNAs as biomarkers for CVD.

Future perspectives

In this review, we have described the multifactorial nature of microRNAs in vascular remodelling, as demonstrated by their role in multiple remodelling processes. Adaptive remodelling, such as arteriogenesis and angiogenesis, is stimulated by miR-126 and by the miR-23/24/27 family. Correspondingly, these microRNAs inhibit pathological remodelling, including atherosclerosis and restenosis. MiR-17/92, the 14q32 miRs and miR-143/145 induce pathological remodelling, while they inhibit adaptive remodelling (except miR-143/145, for which different effects on pathological remodelling have been described). MiR-155 was found to inhibit angiogenesis but stimulated arteriogenesis and was also reported to play contradicting roles in atherosclerosis formation.
The role of these microRNAs in the molecular mechanisms leading to aneurysm formation however, are still poorly described; except for miR-24 and miR-487b, the microRNAs discussed here were only described to be differentially regulated in aneurysms, and a causative role has yet to be elucidated (Figure 8). As mentioned in the introduction, a single microRNA is able to target numerous genes and can regulate complex (patho)physiological processes. Often in literature, a single target gene is validated to explain the observed *in vivo* and *in vitro* effects upon microRNA modulation. However, it is far more plausible that the observed effects are caused by modulation of many genes involved in these (patho)physiological processes rather than one gene. Since microRNAs only modestly downregulate the expression of their target genes, it can be difficult to confirm significant changes in target gene expression. Nevertheless, the multifactorial nature of microRNAs in both adaptive and maladaptive vascular remodelling offers great opportunities for development of future therapeutics for treatment and prevention of CVD. In conclusion, the multifactorial effects on vascular remodelling observed for the microRNAs discussed here, will hopefully stimulate the continued efforts to explore the potential of microRNAs as therapeutic target.

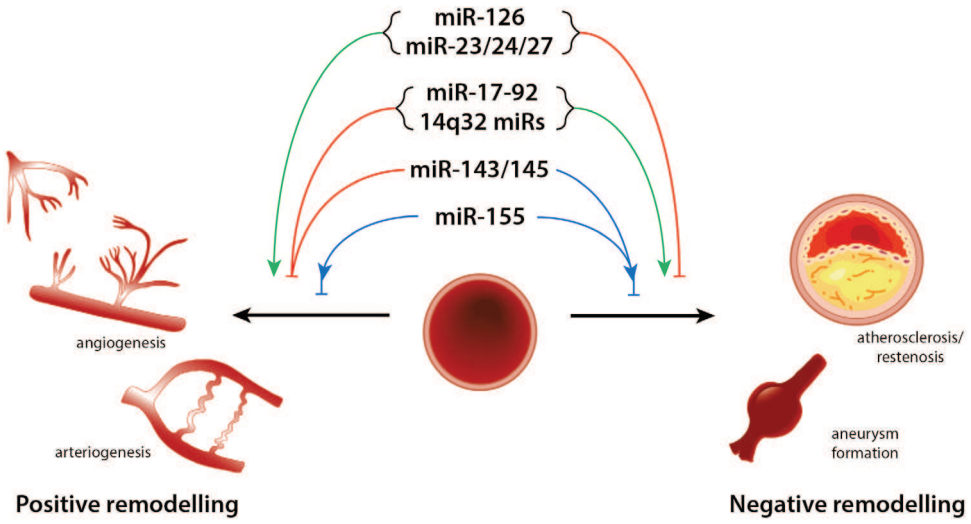

Figure 8. Graphical abstract. Several vascular microRNAs can influence multiple processes of vascular remodelling. MiR-126 is both pro-angiogenic as well as anti-atherosclerotic, like the miR-23-24-27 family. On the other hand, the 17-92 microRNA cluster and 14q32 miRs are anti-angiogenic but pro-atherosclerotic. For miR-155 and miR-143/145, both pro- and anti-angiogenic functions have been reported, as well as pro- and anti-atherosclerotic roles. Green arrows indicate a stimulatory function, whereas red arrows represent an inhibitory function. Blue arrows are used when both stimulatory as well as inhibitory roles have been reported.

MicroRNA		Confirmed Targets	Biological process affected
miR-126		VCAM1[20], SPRED1 and PIK3R2[21-23]	Angiogenesis, vascular integrity
		SDF1/CXCL12[25, 26]	Migration of $CD34^+$ progenitor cells
		RGS16[25]	Recruitment of Sca-1+ endothelial progenitor cells, atherosclerosis
		FOXO3, BCL2 and IRS1[30]	VSMC turnover
		DLK1 (miR-126-5p)[31]	Endothelial repair, atherosclerosis
miR-155		SOCS1[36]	Proinflammatory signalling
		TAB2[37]	Anti-inflammatory signalling
		PU.1[38]	Monocyte/macrophage infiltration, T lymphocyte activation
		AT1R[39], ETS-1[35]	HUVEC activation and migration
		AT1R[40], SOCS1[40]	Angiogenesis, Arteriogenesis
		BCL6, CCL2[42]	Atherosclerosis
		HMGB1[47]	Foam cell formation
		MMP1 and MMP3[49]	Matrix degradation
miR-23-24-27	miR-23 and miR-27	SEMA6A, SEMA6D, SPROUTY2[62]	EC sprouting, angiogenesis
	miR-23b	E2F1[56]	Rb phosphorylation, EC growth arrest
		uPA, SMAD3, FOXO4[74]	VSMC phenotypic switching
	miR-24	GATA2, PAK4[68]	Vascularity, cardiac function and infarct size after myocardial infarction
		NDST1[70]	HSPG sulfation and affinity of HSPGs for VEGF, endothelial cell responsiveness to VEGFA
		HMOX1[69]	SMC apoptosis and proliferation
		INSIG1[72]	Lipid accumulation and plasma triglyceride levels
		CHI3L1[77]	Inflammation, AAA formation
	miR-27a	VE-cadherin[67]	Vascular leakage
	miR-27b	ABCA1, LPL, ACAT1[73]	Cholesterol efflux, lipid uptake and cholesteryl ester formation
miR-17-92	miR-17-92	TSP1, CTGF[125]	Tumour angiogenesis
	miR-17/20	JAK1[53]	Angiogenesis
	miR-19a	CyclinD1[57]	EC proliferation
		FZD4 and LRP6[54]	WNT signalling, arteriogenesis
	miR-92a	KLF2, KLF4, SOCS5[59]	Endothelial homeostasis, atherosclerosis
		ITGA5[52]	Blood flow recovery after ischemia and LV function after myocardial infarction
		KLF4 and MKK[60]	EC proliferation and migration
miR-143/145	miR-143/145	HKII, ITGβ8[92]	Angiogenesis, vessel stability
		ACE[85]	Atherosclerosis
	miR-143	ELK1[84]	VSMC proliferation
		AKT[88]	Angiogenesis, tumorigenesis
	miR-145	IGF-I, IRS1[86, 87]	Tumour angiogenesis
		HIF2α[89]	Angiogenesis
		KLF5[91]	Transdifferentiation of fibroblasts to myofibroblasts, neointima formation
		KLF4[82]	VSMC differentiation
		JAMA1[97]	Leukocyte recruitment
		ABCA1[98]	Cholesterol efflux
14q32 miRs	miR-329	MEF2a[101], CD146[104]	Angiogenesis, Arteriogenesis, EC proliferation
	miR-494	VEGFA, EFNB2, FGFR2[101]	Angiogenesis, Arteriogenesis, myofibroblast proliferation
		TIMP3, TGFB2, IL33[108]	Atherosclerosis
	miR-376b-5p	HIF1a/VEGF signalling pathway[105]	Angiogenesis
	miR-377	VEGFA[106]	Angiogenesis
	miR-136	PPP2R2A[109]	VSMC proliferation
	miR-758	ABCA1[110]	Cholesterol efflux
	miR-370	CPT1α[112]	Fatty acid β oxidation
	miR-487b	IRS1[114]	Outward remodelling of the aorta

Table 2. Overview of confirmed target genes for microRNAs discussed.

References

1. Rajewsky N. microRNA target predictions in animals. Nat Genet 2006;38 Suppl:S8-13.
2. Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. N Engl J Med 1994;330:1431-1438.
3. Wei Y, Schober A, Weber C. Pathogenic arterial remodeling: the good and bad of microRNAs. Am J Physiol Heart Circ Physiol 2013;304:H1050-H1059.
4. Torella D, Iaconetti C, Catalucci D, Ellison GM, Leone A, Waring CD, et al. MicroRNA-133 controls vascular smooth muscle cell phenotypic switch in vitro and vascular remodeling in vivo. Circ Res 2011;109:880-893.
5. Villeneuve LM, Kato M, Reddy MA, Wang M, Lanting L, Natarajan R. Enhanced levels of microRNA-125b in vascular smooth muscle cells of diabetic db/db mice lead to increased inflammatory gene expression by targeting the histone methyltransferase Suv39h1. Diabetes 2010;59:2904-2915.
6. Leeper NJ, Raiesdana A, Kojima Y, Chun HJ, Azuma J, Maegdefessel L, et al. MicroRNA-26a is a novel regulator of vascular smooth muscle cell function. J Cell Physiol 2011;226:1035-1043.
7. Liu K, Ying Z, Qi X, Shi Y, Tang Q. MicroRNA-1 regulates the proliferation of vascular smooth muscle cells by targeting insulin-like growth factor 1. Int J Mol Med 2015;36:817-824.
8. Li P, Zhu N, Yi B, Wang N, Chen M, You X, et al. MicroRNA-663 regulates human vascular smooth muscle cell phenotypic switch and vascular neointimal formation. Circ Res 2013;113:1117-1127.
9. Maegdefessel L, Azuma J, Toh R, Merk DR, Deng A, Chin JT, et al. Inhibition of microRNA-29b reduces murine abdominal aortic aneurysm development. J Clin Invest 2012;122:497-506.
10. Maegdefessel L, Azuma J, Toh R, Deng A, Merk DR, Raiesdana A, et al. MicroRNA-21 blocks abdominal aortic aneurysm development and nicotine-augmented expansion. Sci Transl Med 2012;4:122ra22.
11. Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, et al. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. Science 2010;328:1566-1569.
12. Rayner KJ, Sheedy FJ, Esau CC, Hussain FN, Temel RE, Parathath S, et al. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. J Clin Invest 2011;121:2921-2931.
13. Rayner KJ, Suarez Y, Davalos A, Parathath S, Fitzgerald ML, Tamehiro N, et al. MiR-33 contributes to the regulation of cholesterol homeostasis. Science 2010;328:1570-1573.
14. Rayner KJ, Esau CC, Hussain FN, McDaniel AL, Marshall SM, van Gils JM, et al. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. Nature 2011;478:404-407.
15. Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ Res 2007;101:59-68.
16. Gatsiou A, Boeckel JN, Randriamboavonjy V, Stellos K. MicroRNAs in platelet biogenesis and function: implications in vascular homeostasis and inflammation. Curr Vasc Pharmacol 2012;10:524-531.
17. de Boer HC, van Solingen C, Prins J, Duijs JM, Huisman MV, Rabelink TJ, et al. Aspirin treatment hampers the use of plasma microRNA-126 as a biomarker for the progression of vascular disease. Eur Heart J 2013;34:3451-3457.
18. Laffont B, Corduan A, Rousseau M, Duchez AC, Lee CH, Boilard E, et al. Platelet microparticles reprogram macrophage gene expression and function. Thromb Haemost 2015;115.
19. Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C, et al. Circulating microRNAs in patients with coronary artery disease. Circ Res 2010;107:677-684.
20. Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci U S A 2008;105:1516-1521.
21. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 2008;15:272-284.
22. van Solingen C., Seghers L, Bijkerk R, Duijs JM, Roeten MK, van Oeveren-Rietdijk AM, et al. Antagomir-mediated silencing of endothelial cell specific microRNA-126 impairs ischemia-induced angiogenesis. J Cell Mol Med 2009;13:1577-1585.
23. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 2008;15:261-271.
24. Katare R, Rawal S, Munasinghe PE, Tsuchimochi H, Inagaki T, Fujii Y, et al. Ghrelin Promotes Functional Angiogenesis in a Mouse Model of Critical Limb Ischemia Through Activation of Proangiogenic MicroRNAs. Endocrinology 2015:en20151799.
25. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci Signal 2009;2:ra81.
26. van Solingen C., de Boer HC, Bijkerk R, Monge M, van Oeveren-Rietdijk AM, Seghers L, et al. MicroRNA-126 modulates endothelial SDF-1 expression and mobilization of Sca-1(+)/Lin(-) progenitor cells in ischaemia. Cardiovasc Res 2011;92:449-455.
27. Sahoo S, Klychko E, Thorne T, Misener S, Schultz KM, Millay M, et al. Exosomes from human CD34(+) stem cells mediate their proangiogenic paracrine activity. Circ Res 2011;109:724-728.
28. Mocharla P, Briand S, Giannotti G, Dorries C, Jakob P, Paneni F, et al. AngiomiR-126 expression and secretion from circulating CD34(+) and CD14(+) PBMCs: role for proangiogenic effects and alterations in type 2 diabetics. Blood 2013;121:226-236.

29. Stoneman VE, Bennett MR. Role of apoptosis in atherosclerosis and its therapeutic implications. Clin Sci (Lond) 2004;107:343-354.
30. Zhou J, Li YS, Nguyen P, Wang KC, Weiss A, Kuo YC, et al. Regulation of vascular smooth muscle cell turnover by endothelial cell-secreted microRNA-126: role of shear stress. Circ Res 2013;113:40-51.
31. Schober A, Nazari-Jahantigh M, Wei Y, Bidzhekov K, Gremse F, Grommes J, et al. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat Med 2014;20:368-376.
32. Kin K, Miyagawa S, Fukushima S, Shirakawa Y, Torikai K, Shimamura K, et al. Tissue- and plasma-specific MicroRNA signatures for atherosclerotic abdominal aortic aneurysm. J Am Heart Assoc 2012;1:e000745.
33. Turner M, Vigorito E. Regulation of B- and T-cell differentiation by a single microRNA. Biochem Soc Trans 2008;36:531-533.
34. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci U S A 2007;104:1604-1609.
35. Zhu N, Zhang D, Chen S, Liu X, Lin L, Huang X, et al. Endothelial enriched microRNAs regulate angiotensin II-induced endothelial inflammation and migration. Atherosclerosis 2011;215:286-293.
36. Lu LF, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, et al. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity 2009;30:80-91.
37. Ceppi M, Pereira PM, Dunand-Sauthier I, Barras E, Reith W, Santos MA, et al. MicroRNA-155 modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells. Proc Natl Acad Sci U S A 2009;106:2735-2740.
38. Corsten MF, Papageorgiou A, Verhesen W, Carai P, Lindow M, Obad S, et al. MicroRNA profiling identifies microRNA-155 as an adverse mediator of cardiac injury and dysfunction during acute viral myocarditis. Circ Res 2012;111:415-425.
39. Sethupathy P, Borel C, Gagnebin M, Grant GR, Deutsch S, Elton TS, et al. Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3' untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. Am J Hum Genet 2007;81:405-413.
40. Pankratz F, Bemtgen X, Zeiser R, Leonhardt F, Kreuzaler S, Hilgendorf I, et al. MicroRNA-155 Exerts Cell-Specific Antiangiogenic but Proarteriogenic Effects During Adaptive Neovascularization. Circulation 2015;131:1575-1589.
41. Welten SM, Quax PH, Nossent AY. Letter Regarding Article, "MicroRNA-155 Exerts Cell-Specific Antiangiogenic but Proarteriogenic Effects During Adaptive Neovascularization". Circulation 2015;132:e375.
42. Nazari-Jahantigh M, Wei Y, Noels H, Akhtar S, Zhou Z, Koenen RR, et al. MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. J Clin Invest 2012;122:4190-4202.
43. Raitoharju E, Lyytikainen LP, Levula M, Oksala N, Mennander A, Tarkka M, et al. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. Atherosclerosis 2011;219:211-217.
44. Huang RS, Hu GQ, Lin B, Lin ZY, Sun CC. MicroRNA-155 silencing enhances inflammatory response and lipid uptake in oxidized low-density lipoprotein-stimulated human THP-1 macrophages. J Investig Med 2010;58:961-967.
45. Zhu GF, Yang LX, Guo RW, Liu H, Shi YK, Wang H, et al. miR-155 inhibits oxidized low-density lipoprotein-induced apoptosis of RAW264.7 cells. Mol Cell Biochem 2013;382:253-261.
46. Yang K, He YS, Wang XQ, Lu L, Chen QJ, Liu J, et al. MiR-146a inhibits oxidized low-density lipoprotein-induced lipid accumulation and inflammatory response via targeting toll-like receptor 4. FEBS Lett 2011;585:854-860.
47. Tian FJ, An LN, Wang GK, Zhu JQ, Li Q, Zhang YY, et al. Elevated microRNA-155 promotes foam cell formation by targeting HBP1 in atherogenesis. Cardiovasc Res 2014;103:100-110.
48. Donners MM, Wolfs IM, Stoger LJ, van der Vorst EP, Pottgens CC, Heymans S, et al. Hematopoietic miR155 deficiency enhances atherosclerosis and decreases plaque stability in hyperlipidemic mice. PLoS One 2012;7:e35877.
49. Stanczyk J, Pedrioli DM, Brentano F, Sanchez-Pernaute O, Kolling C, Gay RE, et al. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis Rheum 2008;58:1001-1009.
50. Concepcion CP, Bonetti C, Ventura A. The microRNA-17-92 family of microRNA clusters in development and disease. Cancer J 2012;18:262-267.
51. Chamorro-Jorganes A, Lee MY, Araldi E, Landskroner-Eiger S, Fernandez-Fuertes M, Sahraei M, et al. VEGF-Induced Expression of miR-17~92 Cluster in Endothelial Cells is Mediated by ERK/ELK1 Activation and Regulates Angiogenesis. Circ Res 2015.
52. Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 2009;324:1710-1713.
53. Doebele C, Bonauer A, Fischer A, Scholz A, Reiss Y, Urbich C, et al. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. Blood 2010;115:4944-4950.
54. Landskroner-Eiger S, Qiu C, Perrotta P, Siragusa M, Lee MY, Ulrich V, et al. Endothelial miR-17 approximately 92 cluster negatively regulates arteriogenesis via miRNA-19 repression of WNT signaling. Proc Natl Acad Sci 2015;112:12812-12817.
55. Kaluza D, Kroll J, Gesierich S, Manavski Y, Boeckel JN, Doebele C, et al. Histone deacetylase 9 promotes angiogenesis by targeting the antiangiogenic microRNA-17-92 cluster in endothelial cells. Arterioscler Thromb Vasc Biol 2013;33:533-543.
56. Wang KC, Garmire LX, Young A, Nguyen P, Trinh A, Subramaniam S, et al. Role of microRNA-23b in flow-regulation of Rb phosphorylation and endothelial cell growth. Proc Natl Acad Sci U S A 2010;107:3234-3239.

57. Qin X, Wang X, Wang Y, Tang Z, Cui Q, Xi J, et al. MicroRNA-19a mediates the suppressive effect of laminar flow on cyclin D1 expression in human umbilical vein endothelial cells. Proc Natl Acad Sci U S A 2010;107:3240-3244.
58. Wu W, Xiao H, Laguna-Fernandez A, Villarreal G, Jr., Wang KC, Geary GG, et al. Flow-Dependent Regulation of Kruppel-Like Factor 2 Is Mediated by MicroRNA-92a. Circulation 2011;124:633-641.
59. Loyer X, Potteaux S, Vion AC, Guerin CL, Boulkroun S, Rautou PE, et al. Inhibition of microRNA-92a prevents endothelial dysfunction and atherosclerosis in mice. Circ Res 2014;114:434-443.
60. Iaconetti C, Polimeni A, Sorrentino S, Sabatino J, Pironti G, Esposito G, et al. Inhibition of miR-92a increases endothelial proliferation and migration in vitro as well as reduces neointimal proliferation in vivo after vascular injury. Basic Res Cardiol 2012;107:296.
61. Bang C, Fiedler J, Thum T. Cardiovascular importance of the microRNA-23/27/24 family. Microcirculation 2012;19:208-214.
62. Zhou Q, Gallagher R, Ufret-Vincenty R, Li X, Olson EN, Wang S. Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23~27~24 clusters. Proc Natl Acad Sci U S A 2011;108:8287-8292.
63. Neth P, Nazari-Jahantigh M, Schober A, Weber C. MicroRNAs in flow-dependent vascular remodelling. Cardiovasc Res 2013;99:294-303.
64. Weber M, Baker MB, Moore JP, Searles CD. MiR-21 is induced in endothelial cells by shear stress and modulates apoptosis and eNOS activity. Biochem Biophys Res Commun 2010;393:643-648.
65. Ni CW, Qiu H, Jo H. MicroRNA-663 upregulated by oscillatory shear stress plays a role in inflammatory response of endothelial cells. Am J Physiol Heart Circ Physiol 2011;300:H1762-H1769.
66. Urbich C, Kaluza D, Fromel T, Knau A, Bennewitz K, Boon RA, et al. MicroRNA-27a/b controls endothelial cell repulsion and angiogenesis by targeting semaphorin 6A. Blood 2012;119:1607-1616.
67. Young JA, Ting KK, Li J, Moller T, Dunn L, Lu Y, et al. Regulation of vascular leak and recovery from ischemic injury by general and VE-cadherin-restricted miRNA antagonists of miR-27. Blood 2013;122:2911-2919.
68. Fiedler J, Jazbutyte V, Kirchmaier BC, Gupta SK, Lorenzen J, Hartmann D, et al. MicroRNA-24 regulates vascularity after myocardial infarction. Circulation 2011;124:720-730.
69. Fiedler J, Stohr A, Gupta SK, Hartmann D, Holzmann A, Just A, et al. Functional MicroRNA Library Screening Identifies the HypoxaMiR MiR-24 as a Potent Regulator of Smooth Muscle Cell Proliferation and Vascularization. Antioxid Redox Signal 2013.
70. Kasza Z, Fredlund FP, Tamm C, Eriksson AS, O'Callaghan P, Heindryckx F, et al. MicroRNA-24 suppression of N-deacetylase/N-sulfotransferase-1 (NDST1) reduces endothelial cell responsiveness to vascular endothelial growth factor A (VEGFA). J Biol Chem 2013;288:25956-25963.
71. Vickers KC, Shoucri BM, Levin MG, Wu H, Pearson DS, Osei-Hwedieh D, et al. MicroRNA-27b is a regulatory hub in lipid metabolism and is altered in dyslipidemia. Hepatology 2013;57:533-542.
72. Ng R, Wu H, Xiao H, Chen X, Willenbring H, Steer CJ, et al. Inhibition of microRNA-24 expression in liver prevents hepatic lipid accumulation and hyperlipidemia. Hepatology 2014;60:554-564.
73. Zhang M, Wu JF, Chen WJ, Tang SL, Mo ZC, Tang YY, et al. MicroRNA-27a/b regulates cellular cholesterol efflux, influx and esterification/hydrolysis in THP-1 macrophages. Atherosclerosis 2014;234:54-64.
74. Iaconetti C, De Rosa S, Polimeni A, Sorrentino S, Gareri C, Carino A, et al. Down-regulation of miR-23b induces phenotypic switching of vascular smooth muscle cells in vitro and in vivo. Cardiovasc Res 2015;107:522-533.
75. Jiang Y, Zhang M, He H, Chen J, Zeng H, Li J, et al. MicroRNA/mRNA profiling and regulatory network of intracranial aneurysm. BMC Med Genomics 2013;6:36.
76. Liu D, Han L, Wu X, Yang X, Zhang Q, Jiang F. Genome-wide microRNA changes in human intracranial aneurysms. BMC Neurol 2014;14:188.
77. Maegdefessel L, Spin JM, Raaz U, Eken SM, Toh R, Azuma J, et al. miR-24 limits aortic vascular inflammation and murine abdominal aneurysm development. Nat Commun 2014;5:5214.
78. Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, et al. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. Circ Res 2007;100:1579-1588.
79. Elia L, Quintavalle M, Zhang J, Contu R, Cossu L, Latronico MV, et al. The knockout of miR-143 and -145 alters smooth muscle cell maintenance and vascular homeostasis in mice: correlates with human disease. Cell Death Differ 2009;16:1590-1598.
80. Cheng Y, Liu X, Yang J, Lin Y, Xu DZ, Lu Q, et al. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. Circ Res 2009;105:158-166.
81. Zhang C. MicroRNA-145 in vascular smooth muscle cell biology: a new therapeutic target for vascular disease. Cell Cycle 2009;8:3469-3473.
82. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature 2009;460:705-710.
83. Boucher JM, Peterson SM, Urs S, Zhang C, Liaw L. The miR-143/145 cluster is a novel transcriptional target of Jagged-1/Notch signaling in vascular smooth muscle cells. J Biol Chem 2011;286:28312-28321.
84. Davis-Dusenbery BN, Chan MC, Reno KE, Weisman AS, Layne MD, Lagna G, et al. down-regulation of Kruppel-like factor-4 (KLF4) by microRNA-143/145 is critical for modulation of vascular smooth muscle cell phenotype by transforming growth factor-beta and bone morphogenetic protein 4. J Biol Chem 2011;286:28097-28110.
85. Boettger T, Beetz N, Kostin S, Schneider J, Kruger M, Hein L, et al. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. J Clin Invest 2009;119:2634-2647.

86. Zou C, Xu Q, Mao F, Li D, Bian C, Liu LZ, et al. MiR-145 inhibits tumor angiogenesis and growth by N-RAS and VEGF. Cell Cycle 2012;11:2137-2145.
87. La Rocca G., Shi B, Badin M, De Angelis T., Sepp-Lorenzino L, Baserga R. Growth inhibition by microRNAs that target the insulin receptor substrate-1. Cell Cycle 2009;8:2255-2259.
88. Qian X, Yu J, Yin Y, He J, Wang L, Li Q, et al. MicroRNA-143 inhibits tumor growth and angiogenesis and sensitizes chemosensitivity to oxaliplatin in colorectal cancers. Cell Cycle 2013;12:1385-1394.
89. Zhang H, Pu J, Qi T, Qi M, Yang C, Li S, et al. MicroRNA-145 inhibits the growth, invasion, metastasis and angiogenesis of neuroblastoma cells through targeting hypoxia-inducible factor 2 alpha. Oncogene 2014;33:387-397.
90. Anand S, Cheresh DA. Emerging Role of Micro-RNAs in the Regulation of Angiogenesis. Genes Cancer 2011;2:1134-1138.
91. Wang YS, Li SH, Guo J, Mihic A, Wu J, Sun L, et al. Role of miR-145 in cardiac myofibroblast differentiation. J Mol Cell Cardiol 2014;66:94-105.
92. Climent M, Quintavalle M, Miragoli M, Chen J, Condorelli G, Elia L. TGFbeta Triggers miR-143/145 Transfer From Smooth Muscle Cells to Endothelial Cells, Thereby Modulating Vessel Stabilization. Circ Res 2015;116:1753-1764.
93. Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta N, et al. MicroRNA-145 targeted therapy reduces atherosclerosis. Circulation 2012;126:S81-S90.
94. Xin M, Small EM, Sutherland LB, Qi X, McAnally J, Plato CF, et al. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev 2009;23:2166-2178.
95. Vengrenyuk Y, Nishi H, Long X, Ouimet M, Savji N, Martinez FO, et al. Cholesterol loading reprograms the microRNA-143/145-myocardin axis to convert aortic smooth muscle cells to a dysfunctional macrophage-like phenotype. Arterioscler Thromb Vasc Biol 2015;35:535-546.
96. Hergenreider E, Heydt S, Treguer K, Boettger T, Horrevoets AJ, Zeiher AM, et al. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol 2012;14:249-256.
97. Schmitt MM, Megens RT, Zernecke A, Bidzhekov K, van den Akker NM, Rademakers T, et al. Endothelial junctional adhesion molecule-a guides monocytes into flow-dependent predilection sites of atherosclerosis. Circulation 2014;129:66-76.
98. Sala F, Aranda JF, Rotllan N, Ramirez CM, Aryal B, Elia L, et al. MiR-143/145 deficiency attenuates the progression of atherosclerosis in Ldlr-/-mice. Thromb Haemost 2014;112:796-802.
99. Boon RA, Dimmeler S. MicroRNAs and aneurysm formation. Trends Cardiovasc Med 2011;21:172-177.
100. Benetatos L, Hatzimichael E, Londin E, Vartholomatos G, Loher P, Rigoutsos I, et al. The microRNAs within the DLK1-DIO3 genomic region: involvement in disease pathogenesis. Cell Mol Life Sci 2013;70:795-814.
101. Welten SM, Bastiaansen AJ, de Jong RC, de Vries MR, Peters EA, Boonstra MC, et al. Inhibition of 14q32 MicroRNAs miR-329, miR-487b, miR-494, and miR-495 increases neovascularization and blood flow recovery after ischemia. Circ Res 2014;115:696-708.
102. Gordon FE, Nutt CL, Cheunsuchon P, Nakayama Y, Provencher KA, Rice KA, et al. Increased expression of angiogenic genes in the brains of mouse meg3-null embryos. Endocrinology 2010;151:2443-2452.
103. Liu D, Zhang XL, Yan CH, Li Y, Tian XX, Zhu N, et al. MicroRNA-495 regulates the proliferation and apoptosis of human umbilical vein endothelial cells by targeting chemokine CCL2. Thromb Res 2015;135:146-154.
104. Wang P, Luo Y, Duan H, Xing S, Zhang J, Lu D, et al. MicroRNA 329 suppresses angiogenesis by targeting CD146. Mol Cell Biol 2013;33:3689-3699.
105. Li LJ, Huang Q, Zhang N, Wang GB, Liu YH. miR-376b-5p regulates angiogenesis in cerebral ischemia. Mol Med Rep 2014;10:527-535.
106. Wen Z, Huang W, Feng Y, Cai W, Wang Y, Wang X, et al. MicroRNA-377 regulates mesenchymal stem cell-induced angiogenesis in ischemic hearts by targeting VEGF. PLoS One 2014;9:e104666.
107. Cipollone F, Felicioni L, Sarzani R, Ucchino S, Spigonardo F, Mandolini C, et al. A unique microRNA signature associated with plaque instability in humans. Stroke 2011;42:2556-2563.
108. Wezel A, Welten SM, Razaway W, Lagraauw, de Vries MR, Goossens E.A.C., et al. Inhibition of microRNA-494 reduces atherosclerotic lesion development and increases plaque stability. Ann Surg 2015.
109. Zhang CF, Kang K, Li XM, Xie BD. MicroRNA-136 Promotes Vascular Muscle Cell Proliferation Through the ERK1/2 Pathway by Targeting PPP2R2A in Atherosclerosis. Curr Vasc Pharmacol 2014.
110. Ramirez CM, Davalos A, Goedeke L, Salerno AG, Warrier N, Cirera-Salinas D, et al. MicroRNA-758 regulates cholesterol efflux through posttranscriptional repression of ATP-binding cassette transporter A1. Arterioscler Thromb Vasc Biol 2011;31:2707-2714.
111. Mandolini C, Santovito D, Marcantonio P, Buttitta F, Bucci M, Ucchino S, et al. Identification of microRNAs 758 and 33b as potential modulators of ABCA1 expression in human atherosclerotic plaques. Nutr Metab Cardiovasc Dis 2014.
112. Iliopoulos D, Drosatos K, Hiyama Y, Goldberg IJ, Zannis VI. MicroRNA-370 controls the expression of microRNA-122 and Cpt1alpha and affects lipid metabolism. J Lipid Res 2010;51:1513-1523.
113. Aavik E, Lumivuori H, Leppanen O, Wirth T, Hakkinen SK, Brasen JH, et al. Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster. Eur Heart J 2014.
114. Nossent AY, Eskildsen TV, Andersen LB, Bie P, Bronnum H, Schneider M, et al. The 14q32 MicroRNA-487b Targets the Antiapoptotic Insulin Receptor Substrate 1 in Hypertension-Induced Remodeling of the Aorta. Ann Surg 2013.
115. Dimmeler S, Yla-Herttuala S. 14q32 miRNA cluster takes center stage in neovascularization. Circ Res 2014;115:680-682.

116. Hakimzadeh N, Nossent AY, van der Laan AM, Schirmer SH, de Ronde MW, Pinto-Sietsma SJ, et al. Circulating MicroRNAs Characterizing Patients with Insufficient Coronary Collateral Artery Function. PLoS One 2015;10:e0137035.
117. He F, Lv P, Zhao X, Wang X, Ma X, Meng W, et al. Predictive value of circulating miR-328 and miR-134 for acute myocardial infarction. Mol Cell Biochem 2014;394:137-144.
118. Hoekstra M, van der Lans CA, Halvorsen B, Gullestad L, Kuiper J, Aukrust P, et al. The peripheral blood mononuclear cell microRNA signature of coronary artery disease. Biochem Biophys Res Commun 2010;394:792-797.
119. Li C, Fang Z, Jiang T, Zhang Q, Liu C, Zhang C, et al. Serum microRNAs profile from genome-wide serves as a fingerprint for diagnosis of acute myocardial infarction and angina pectoris. BMC Med Genomics 2013;6:16.
120. Matsumoto S, Sakata Y, Nakatani D, Suna S, Mizuno H, Shimizu M, et al. A subset of circulating microRNAs are predictive for cardiac death after discharge for acute myocardial infarction. Biochem Biophys Res Commun 2012;427:280-284.
121. Wang J, Pei Y, Zhong Y, Jiang S, Shao J, Gong J. Altered serum microRNAs as novel diagnostic biomarkers for atypical coronary artery disease. PLoS One 2014;9:e107012.
122. Jickling GC, Ander BP, Zhan X, Noblett D, Stamova B, Liu D. microRNA expression in peripheral blood cells following acute ischemic stroke and their predicted gene targets. PLoS One 2014;9:e99283.
123. Mohnle P, Schutz SV, Schmidt M, Hinske C, Hubner M, Heyn J, et al. MicroRNA-665 is involved in the regulation of the expression of the cardioprotective cannabinoid receptor CB2 in patients with severe heart failure. Biochem Biophys Res Commun 2014;451:516-521.
124. Liu F, Li N, Long B, Fan YY, Liu CY, Zhou QY, et al. Cardiac hypertrophy is negatively regulated by miR-541. Cell Death Dis 2014;5:e1171.
125. Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat Genet 2006;38:1060-1065.