Published in final edited form as:

Adv Exp Med Biol. 2015 ; 887: 53–77. doi:10.1007/978-3-319-22380-3_4.

microRNAs Distinctively Regulate Vascular Smooth Muscle and Endothelial Cells: Functional Implications in Angiogenesis, Atherosclerosis, and In-Stent Restenosis

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Abstract

Endothelial cells (EC) and vascular smooth muscle cells (VSMC) are the main cell types within the vasculature. We describe here how microRNAs (miRs)—noncoding RNAs that can regulate gene expression via translational repression and/or post-transcriptional degradation—distinctively modulate EC and VSMC function in physiology and disease. In particular, the specific roles of miR-126 and miR-143/145, master regulators of EC and VSMC function, respectively, are deeply explored. We also describe the mechanistic role of miRs in the regulation of the pathophysiology of key cardiovascular processes including angiogenesis, atherosclerosis, and in-stent restenosis post-angioplasty. Drawbacks of currently available therapeutic options are discussed, pointing at the challenges and potential clinical opportunities provided by miR-based treatments.

Keywords

Endothelium; Restenosis; VSMC; Neointima; Stent; DES; Circulation; Thrombosis; miR-126; miR-143/145; Diabetes; Myocardin; Myoregulin; Atherosclerosis; Angiogenesis; Inflammation; Angioplasty

Introduction

Endothelial cells (EC) and vascular smooth muscle cells (VSMC) constitute the main cell types within the vasculature [1, 2]. The endothelium forms the inner thin layer that represents an interface between circulating fluid in the lumen and the rest of the vessel wall. The endothelial monolayer lines the entire circulatory system, from the smallest capillaries to the heart [3]. The main functions of EC include regulation of vascular tone, fluid filtration, neutrophil recruitment, hormone trafficking, and hemostasis [4–6].

VSMC are able to contract or relax in response to various stimuli, and are thereby responsible for the redistribution of the blood within the body to areas where it is needed, including tissues with temporarily enhanced oxygen consumption [7–11]. Their main function is to regulate the caliber of the blood vessels: excessive vasoconstriction might lead to hypertension, whereas excessive vasodilation can induce hypotension (as in shock) [12, 13].

Coding or Noncoding: *Whether “Tis Nobler in the Mind to Suffer...”*

MicroRNAs (miRs) are small, generally noncoding RNAs, that regulate gene expression via post-transcriptional degradation or translational repression [14]. Unquestionably, miRs are fundamental regulators of numerous biological processes. See Chaps. 1 and 2 of this book for more detailed information on biological aspects of miRs.

More than 30,000 mature miR products have been identified (~200 in the human genome), and the number of published miR sequences continues to increase rapidly [15].

Importantly, some transcripts that originally appeared to be lncRNAs may actually encode micropeptides. For instance, a conserved micropeptide, named myoregulin, encoded by RNA that had been misannotated as noncoding, has been recently identified by Eric Olson [16]. The fact that putative noncoding RNA may actually harbor hidden micropeptides had been recently suggested by genome-wide analyses [17].

Consequently, scanning the microproteome embedded in “noncoding RNA,” whose coding capacity has been somehow ignored in gene annotations [18, 19], will certainly represent a main field of research in the next future, revisiting previously established annotations of noncoding RNAs and hopefully leading to the discovery of new mechanistically important micropeptides.

Fine Tuning of Vascular Biology via MicroRNA

Vascular endothelium plays a key role in regulating vessel biology and homeostasis. Alteration of its function can be the basis of cardiovascular disorders including hypertension, atherosclerosis, and impairment of the angiogenic processes [20–22]. Several events are involved in angiogenesis, including EC division, selective degradation of the basal membrane, and the surrounding extracellular matrix, with the subsequent EC migration with the formation of neovessels [7].

Similar events take place during embryogenesis, however in this context blood vessels are generated from the differentiation of EC precursors, the angioblasts, which associate to eventually form primitive vessels in a process known as vasculogenesis [23]. Endothelial maturation is finely guided by signals from other cell types [23]. Indeed, development of the vascular architecture involves the strict association between EC and mural cells, including VSMC and pericytes [24]. Under physiological conditions, the communication between these cell types leads to the maturation and stabilization of the vessel. These processes involve the action of different growth factors and heterotypic cellular interactions [25].

miR-143/145 vs. miR-126, Master Regulators of VSMC and EC Function

miR-143/145

A major advance in the smooth muscle biology field occurred with the report of the essential role of a bicistronic unit encoding for miR-143 and miR-145 in the regulation of smooth muscle physiology [26–41]. The miR-143/145 gene is located on chromosome 5 in humans and chromosome 18 in mice and rats. miR-143 and miR-145 are distinct in

sequence, yet transcribed together as one primary cluster. Being controlled by serum response factor (together with myocardin) and Nkx2.5, expression of miR-143/145 seems to occur primarily in VSMCs and is critical for maintaining their contractile phenotype [2, 26]. Its expression pattern correlates with a differentiated phenotype as defined by alpha-smooth muscle actin, smooth muscle myosin heavy chain, and calponin expression [39, 42, 43].

The joint suppression of specific target mRNAs by miR-143/145 contributes to a contractile phenotype, as demonstrated in mice with genetic deficiency of miR-143/145, which display reduced vascular tone and blood pressure control [44]. miR-143/145^{-/-} mice also have a tendency to develop neointimal lesions. Aortic VSMCs from the double knockout mouse lack myofilamentous cytoskeletal organization and have diffuse actin staining. A proteomic analysis of aortas from global miR-143/145^{-/-} mice revealed angiotensin-converting enzyme (ACE-1) as a target of the miR-143/145 cluster. Strikingly, pharmacological inhibition by ACE inhibitors or angiotensin 1 receptor blockers partially reverses vascular dysfunction normalizing gene expression in miR-143/145^{-/-} mice [45].

At the ultrastructural electron microscopic analysis, miR-143/145-deficient VSMC exhibit show greater presence of rough endoplasmic reticulum and less focal adhesions compared with wild-type cells. These differences have been associated with impaired contractility of femoral artery rings in response to angiotensin II and phenylephrine. Of note, the functional relevance of miR-143/145 in human vessel pathology had been suggested by the observed downregulation of the miR-143/145 cluster in human aortic aneurysms compared with normal aortic tissue [46].

As mentioned above, the cardiac muscle- and smooth muscle-specific transcriptional factor myocardin has been shown to up-regulate miR-143/145 expression [47, 48]. Similarly, the introduction of miR-143/145 potentiates myocardin-induced smooth muscle marker gene expression, calcium fluxes, and myofilament formation in response to endothelin-1. The repressor of myocardin-directed smooth muscle gene expression KLF4, which binds myocardin, has been identified as a potential target of miR-145 in murine VSMC [29]. However, human KLF4 mRNA contains no binding site for miR-143 and only a low-probability site for miR-145, whereas KLF5 (which has protein sequences highly conserved across human, mouse, and rat) contains a binding site for miR-145. In vivo experiments revealed that vascular injury causes downregulation of miR-145, which up-regulates KLF5 and inhibits myocardin, leading to VSMC dedifferentiation and increased neointimal lesion formation [26, 49]. Notably, PDGF receptor α , fascin, and protein kinase C ϵ , essential regulators of podosome formation, were revealed as direct targets of miR-143 and miR-145 [50].

Various reports revealed that cells can release miRs which can then exert their specific effects by modulating processes in recipient cells. Thus, miRs have intercellular signal transduction capabilities. Most recently, Climent and colleagues elegantly demonstrated that VSMC communicate with EC via miR-143 and miR-145: cell-to-cell VSMC/EC contacts induce the activation of miR-143/145 transcription in VSMC, promoting the transfer of these miRNAs to the endothelium [51]. In particular, VSMC can deliver miR-143/145 to EC via fine intercellular tubes, named membrane nanotubes or tunneling nanotubes [51]. Indeed, the

level of miR-143/145, but not that of its precursor molecule (pri-miR-143/145), rose substantially in EC when these cells were cultured together with VSMC. A specific molecular pathway has been elucidated, in which secretion of transforming growth factor- β by EC stimulates the transfer of miR-143/145 from VSMC to EC, where VSMC-derived miR-143/145 represses hexokinase II and integrin β 8 and thereby the angiogenic potential of the recipient cell [51]. Notably, the expression of miR-143/145 in EC could not be achieved by the simple transfer of conditioned medium or VSMC-derived exosomes and was not sensitive to gap junction uncoupling agents (both exosomes and gap junctions had been reported as potential routes for intercellular transfer of miRs). Instead, the transfer of miR-143/145 was sensitive to latrunculin A, an inhibitor of the formation of tunneling nanotubes, tiny membrane connections that cultured cells form among each other. The intercellular transfer of miRs through tunneling nanotubes had been previously reported in ovarian cancer [52]. High-resolution imaging allowed the direct visualization of tunneling nanotubes between EC and VSMC and the transport of miRs within them [51].

miR-126

miR-126 is encoded by intron 7 of the EGF-like domain 7 (EGFL7) gene, a.k.a. vascular endothelial-statin (VE-statin), which is under the transcriptional control of the E-26 family of transcription factors ETS1/2 [53–65]. In resting conditions, ETS1 is expressed at a very low level whereas during angiogenesis or re-endothelialization, it is transiently expressed at high levels. During replicative senescence an increased expression of ETS1 could induce the increasing of miR-126 expression. Interestingly, one of the main targets of miR-126 is the host gene EGFL7, which regulates the proper spatial organization of ECs within each sprout and influences their collective movement. The cardiovascular phenotype of EGFL7-deficient mice is recapitulated by the ablation of miR-126, causing ruptured blood vessels, multifocal hemorrhages, and systemic edema (~40 % of miR-126 KO mice die embryonically) [57].

miR-126 has been extensively studied in plasma and circulating cells because its expression is very high in EC [2], endothelial progenitor cells (EPCs), and platelets [59, 66–80]. Most recently, miR-126 has been identified as an efficient marker in the detection and purification of EC [81]. This miR plays a critical role in modulating vascular development and homeostasis, targeting specific mRNAs including the Sprouty-related protein 1 (SPRED-1), CXCL12, SDF-1, and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2) [82–87]. Confirming its key role in maintaining vascular integrity, amidst the other targets of miR-126 there is a key mediator of leukocyte adhesion and inflammation: vascular cell adhesion molecule 1 (VCAM-1). miR-126 has also been related with the endothelial dysfunction associated with the development of diabetes and its complications [88].

Circulating miR-126 acts as an intercellular messenger mainly released by EC and internalized primarily by monocytes and VSMC [57]. Its transfer modulation may be an important strategy to prevent or delay endothelial dysfunction [88–92]. A significant increase in circulating miR-126 has been detected in patients with acute myocardial infarction and angina whereas miR-126 downregulation has been reported in plasma from patients with diabetes, heart failure, or cancer [14]. Hence, circulating miR-126 can be modulated by diverse stimuli inducing dissimilar cellular fates in different cell types [60, 85,

93–97]. Most recently, Italian researchers demonstrated that circulating miR-126-3p (see Chap. 1 for miR nomenclature) can be considered a biomarker of physiological endothelial senescence in normoglycemic elderly subjects and appears to underlie a mechanism that may be disrupted in aged diabetic patients [98]. Intriguingly, diabetes mellitus leads to dysregulated activation of ETS, which blocks the functional activity of progenitor cells and their commitment toward the endothelial cell lineage [99].

The Phenotypic Switch of Vascular Smooth Muscle Cells: A Key Event in Atherosclerosis and Restenosis

VSMC can modulate their phenotype) in response to the environmental stimuli via a process characterized by decreased gene expression of VSMC contractile markers and increased proliferation, migration, and matrix synthesis [100]. Such a phenotypic switch represents one of the main cellular events underlying various VSMC-related pathological conditions, including hypertension, atherosclerosis, post-angioplasty restenosis, and angiogenesis. Unraveling the mechanisms involved in VSMC phenotypic switch is an important step toward better understanding the pathophysiology of these disorders and ultimately designing therapeutic agents for their treatment and prevention.

The switch between the contractile (differentiated, quiescent) and synthetic (dedifferentiated, proliferative) VSMC phenotypes are orchestrated via a synergistic molecular regulatory network. Overall, contractile smooth muscle proteins contain an evolutionarily conserved *cis*-elements, the CArG box (CC(A/T)₆GG) in the promoter–enhancer region of the gene. This has been demonstrated for α -smooth muscle actin, desmin, smooth muscle myosin heavy chain, SM22- α , and calponin.

The serum response factor (SRF), a ubiquitously expressed transcription factor, binds the CArG box driving transcription. The interaction between SRF and CArG boxes in response to environmental changes is generally coordinated by the interaction of SRF with additional transcription factors, divided in co-activators (myocardin, PRX1, and GATA factors) and co-repressors (ELK-1, KLF4, YY1, and Foxo factors).

Virtually all of the VSMC-specific contractile protein genes alongside with many other genes involved in migration, proliferation, and extracellular matrix production during the process of VSMC phenotypic switch, containing CArG boxes within their promoters. The balance between those positive and negative SRF cofactors finely regulates the dynamic VSMC gene expression in response to environmental signals. Amidst these regulatory components, myocardin, which binds SRF in a 1:2 stoichiometric ratio inducing CArG-dependent VSMC gene transcription, is widely considered to be the master gene regulating VSMC differentiation.

The TGF- β and bone morphogenetic proteins (BMPs) also contribute to VSMC phenotypic switch by promoting VSMC contractility enhancing the expression of the contractile apparatus [101]. In particular, TGF- β superfamily of growth factors triggers VSMC differentiation by post-transcriptionally increasing the expression of a subset of miRs

including miR-21. Treatment of VSMC with TGF- β and BMP4 results in change in the expression of miRs, by finely regulating DROSHA complex [102].

Intriguingly, the TGF- β signaling pathway is also under the control of miRs. Other studies demonstrated that the inhibition of miR-26a promote VSMC apoptosis and phenotypic switch to a contractile status while inhibiting proliferation and migration [103]. Intriguingly, miR-24 is involved into both TGF- β and PDGF-BB signaling pathways, which respectively represent the distinguishing trigger of VSMC differentiation and proliferation [104]: PDGF-BB induces miR-24 expression, which subsequently leads to Tribbles-like protein-3 (TRB3) downregulation by a post-transcriptional effect, with a decrease in BMP and TGF β signaling, promoting VSMC proliferation. On the contrary, inhibition of miR-24 enhances TRB3 expression and impairs VSMC proliferative activity [104]. Of note, TGF- β Signaling has been also implied in the mineralization of VSMC induced by oxidized LDL [105].

The specific roles of miRs in the regulation of VSMC phenotypic switch are summarized in Table 4.1 .

Several miRs have been shown to modulate VSMC proliferation and migration, key aspects in the pathogenesis of atherosclerosis. For instance, miR-133 [136] and miR-136 [137] promote whereas miR-365 [117] and miR-1298 (which has been shown to be regulated by DNA methylation [138]) inhibit VSMC proliferation and migration.

Importantly, the role of VSMC varies depending on the stage of atherosclerotic disease, playing a maladaptive role in early lesion development and progression and a beneficial adaptive role within the fibrous cap by stabilizing advanced plaques in the face of end-stage disease events such as plaque rupture [139]. Indeed, VSMC can synthesize components of the fibrous cap, which separates circulating blood from the thrombogenic plaque interior [140]. In humans, ruptured atherosclerotic plaques show less VSMC compared with stable lesions, indicating an active contribution of VSMC to plaque stability [139]. Promoting a quiescent VSMC phenotype might lead to increased fibrous cap integrity, and in this respect, miRs represent an opportunity for positive VSMC regulation (see Chap. 5 of this book for a systematic overview of miRs involved in the atherosclerotic process).

Mechanistic Importance of Endothelium in Restenosis and in Vascular Remodeling

Stent implantation and/or balloon angioplasty, interventions that are used routinely to treat coronary artery disease, lead to mechanical EC damage. A normal operative endothelium is crucial due to its participation in the regulation of vascular tone, alongside with its role in suppressing intimal hyperplasia by inhibiting inflammation, thrombus formation, and VSMC proliferation and migration [2, 141, 142]. Thus, the endothelium provides a selectively permeable barrier that protects against potentially detrimental circulating factors [143–145]. Endothelial denudation and medial wall injury are generally considered the initial effects of angioplasty-induced injury [146–149].

Given the essential role of EC in suppressing inflammation and thrombosis [150, 151] and overall in controlling vascular tone and function [4], the restoration of a healthy endothelial layer is an imperative therapeutic goal in order to prevent restenosis and to avoid the detrimental consequences of in-stent thrombosis [2, 152].

Re-endothelialization of injured arteries occurs naturally via outgrowth of local EC [153]. The recruitment of circulating bone marrow-derived endothelial progenitor cells in this process is controversial [154–156], and the actual contribution of this cell population continues to be uncertain [157].

The effect of stent deployment on EC behavior remains poorly understood. Unquestionably, re-endothelialization of injured coronary arteries is affected by the presence of a stent since such a structure provides a nonphysiological surface for adhesion and generates perturbations in blood flow [158, 159].

The issue of EC repair has been somehow brought into sharp relief in the era of drug-eluting stents (DES), which can release cytostatic compounds that inhibit cell cycle progression [160, 161]. Albeit DES are associated with reduced restenosis rates via inhibition of VSMC proliferation [162–164], they have also been linked to lethal late-stage thrombotic events, which are associated with EC injury [165–167]. Ergo, there is an urgent need to develop new therapeutic interventions to promote EC repair in stented arteries and thereby reduce the incidence of late thrombosis and avoid serious risks associated with prolonged administration of systemic antiplatelet treatments [168, 169], as discussed in detail in the section “ Angioplasty, Stents, and miRs ” of this chapter.

The precise mechanisms of endothelial repair following angioplasty-related injury have been the focus of a plethora of studies. As mentioned above, the potential regenerative capacity of endothelial progenitor cells remains controversial [170–172], and current research focuses on the complex interaction of circulating cells and mature vessel-wall residual EC. In this context, the emerging functional role of microparticles, tiny membrane fragments of activated and apoptotic cells, has been recently investigated: in brief, EC injury triggers the release of EC-derived microparticles, which act as important carriers of bioactive molecules playing crucial roles in cell–cell cross talk. Indeed, microparticles can trigger antiapoptotic effects on EC, and are able to transfer microRNAs, such as miR-126, to target EC, ultimately enhancing endothelial repair mechanisms [170].

Under hyperglycemic conditions, EC microparticles exhibit reduced regenerative capacity, suggesting that hyperglycemia not only directly harms the endothelium, but also indirectly promotes vascular damage by altering endogenous vascular regeneration mechanisms [170]. Analysis of microRNA-126 level in patients with stable coronary artery disease confirmed that diabetes mellitus reduces microRNA-126 expression in circulating microparticles. Moreover, genetic downregulation of microRNA-126 reduces endothelial microparticle-mediated EC repair both in vivo and in vitro [170, 173].

The endothelium plays a fundamental role in angiogenesis [20–22, 25, 174] and numerous studies investigated the role of miRs in regulating this process. For simplicity, the most

important miRs heretofore implicated in EC-mediated angiogenetic process are reported in Table 4.2, alongside with their target gene(s) and function(s).

Angioplasty, Stents, and miRs

Coronary artery disease represents a leading cause of mortality worldwide [200–203]. Percutaneous coronary intervention (PCI) is one of the most commonly performed interventions [204], representing the main option for revascularization in a series of cardiovascular disorders, from unstable angina and myocardial infarction, to multivascular disease [204].

Milestones in Interventional Cardiology

Recurrent lumen narrowing has been a substantial limitation of open and percutaneous methods of arterial reconstruction from their inception.

The term restenosis, commonly used since 1950 in reference to recurrent cardiac valvular stenosis [205], was later adopted to define lumen re-narrowing after open arterial reconstruction such as carotid endarterectomy.

With the development of peripheral angioplasty by Dotter in the 1960s [206] and then the application of percutaneous approach to angioplasty of renal, coronary, and iliac arteries by Gruentzig and colleagues [207, 208] the problem of restenosis grew exponentially: as angioplasty became widely adopted in the 1980s, restenosis after the intervention was reported in up to 60 % of patients [209, 210].

A major breakthrough in the field was the introduction of bare metal stents (BMS), which revolutionized interventional cardiology preventing the elastic recoil of the treated vessels [211] and, more importantly, significantly reducing the phenomenon of restenosis, as demonstrated by two milestone prospective randomized clinical trials [212, 213]: compared with angioplasty, stenting massively reduced restenosis, reaching percentages of 22 %.

However, the major drawback of this procedure is the induction of proliferation/migration and subsequent accumulation of VSMC [214], macrophages [215], and lymphocytes [216] in the arterial wall, eventually leading to restenosis [217].

Once the molecular mechanisms underlying the restenosis process were better comprehended (and mainly attributable to the proliferation of VSMC), DES were introduced in the clinical scenario, in order to deliver in situ a drug that can inhibit cell proliferation [218, 219].

Nowadays, millions of procedures to intervene on occlusive vascular lesions are performed worldwide each year (~700,000 angioplasties are performed annually in the USA) and 70–90 % of all angioplasty patients receive a stent, inserted permanently at the site of the vascular blockage to form an internal scaffolding that keeps the angioplastied vessel from closing.

The introduction of DES significantly reduced rates of restenosis [218, 220, 221]. Though, concerns have been raised over the long-term safety of the DES, with particular reference to stent thrombosis, essentially due to impaired re-endothelization caused by the nonselective antiproliferative properties of DES [222–224]. As such, when the obstacle of restenosis seemed finally overcome, enthusiasm and euphoria were tempered by epidemiologic data reporting that DES did not ameliorate mortality rates when compared to BMS [225]. Basic research revealed that the essential cause of the increased mortality observed in patients receiving DES, despite the achieved prevention of restenosis, was mainly attributable to the fact that the antiproliferative drugs eluted by the stents were nonselective, inhibiting not only the proliferation and migration of the “bad” cells responsible for restenosis (VSMC), but also the growth and mobility of the “good” EC, utterly indispensable for the healing of the vessel following the stent implantation [226]. The lack of endothelial coverage eventually leads to an increased risk of thrombosis, with catastrophic clinical consequences for the patients.

Drugs eluted by the stents currently available in clinical practice (Fig. 4.1) are not able to differentiate EC from VSMC, T-cells or macrophages [227–229], and the inhibition of proliferation and migration affects all these cellular types [228, 230, 231], leading to an increased risk for late thrombosis, due to delayed/incomplete re-endothelization [204, 214, 232]. Thus, impaired endothelial coverage after angioplasty prolongs the window of vulnerability to thrombosis, requiring thereby a prolonged dual antiplatelet therapy.

Several vasculoprotective approaches have been proposed to overcome the restenosis problem after angioplasty, preserving endothelial function [233–235]. However, vascular restenosis and thrombosis continue to be a major problem of interventional cardiology, limiting the effectiveness of revascularization procedures. The ideal eluting stent should display a selective antiproliferative effect on VSMC, macrophages, and T-lymphocytes, without affecting EC [204].

Since EC injury is a fundamental element in the pathophysiology of atherogenesis, understanding EC repair is of critical importance in order to develop novel therapeutic strategies to preserve endothelial integrity and vascular health. In this sense, miRs and their intrinsic properties represent a wonderful opportunity to obtain a specific attenuation of neointimal formation.

Several miRs have been implicated in modification of vessel restenosis after interventional endothelial injury: Antisense knockdown of miR-21, which is moderately increased after vessel injury [123], can blunt the formation of neointimal lesions in response to balloon injury of the carotid artery. Inducing miR-221 in VSMCs on PDGF- β stimulation causes p27^{Kip1} inhibition [236], thereby increasing VSMC proliferation contributing to the formation of a neointimal lesion after arterial injury. Overexpression of miR-145 promotes neointima formation in response to balloon injury [237].

The fundamental role of miRs in the restenosis process has been also confirmed by Baker and colleagues, who identified multiple miRs, including miR-21, miR-146, and miR142-3p, aberrantly expressed in stented pig arteries. Using a mouse vascular stent model, they

demonstrate that the knockout of miR-21 can attenuate neointimal formation post-stenting modulating inflammation and VSMC response [238].

Harnessing the EC-specific expression of miR-126, we were able to obtain in one fell swoop both the inhibition of restenosis, attacking VSMC, and the prevention of restenosis and thrombosis, preserving the endothelial function [2].

A major challenge in the field is the delivery of miR-based therapies. In preclinical studies, antagomiRs and miR mimetics have been successfully [239–243] delivered systemically (intravenously injected) showing beneficial effects on cardiac remodeling; however, they are preferentially targeted to the liver, spleen, and kidney. The specific application of miR-based agents to the vasculature, for instance during cardiac catheterization for angioplasty, can be considered as an effective therapeutic strategy.

Potential alternatives [93, 244–250] to the direct intravascular delivery (which ideally could be combined to the new generation bioresorbable stents with biodegradable scaffolds [251–255]) include the stabilization of the miR-based agent (various chemical modifications of the nucleotides can enhance stability in vivo, for instance by using cholesterol-conjugated, 2'-O-methyl-modified antagomiRs) or the conjugation to targeting molecules including antibodies, peptides, or other bioactive molecules, which may promote the specific homing of the miR-based drug to the site of the injury.

Conclusion

Accumulating evidence establishes that miRs are becoming one of the most fascinating areas of biology, given their fundamental roles in several pathophysiological processes. The relative role of different miRs in vascular biology as direct or indirect post-transcriptional regulators of genes implied in structural remodeling, inflammation, angiogenesis, atherosclerosis, in-stent restenosis, and thrombosis indicate that miRs may serve as promising drug targets or potential biomarkers in prevention and management of vascular disorders.

Acknowledgements

Dr. Gaetano Santulli is supported by the National Institutes of Health (K99DK107895) and by the American Heart Association (AHA 15SDG25300007).

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Fig. 4.1.

Chemical modifications of the macrocyclic ring in order to obtain drugs with potent antiproliferative and immunosuppressive effects, including rapamycin (sirolimus), everolimus, zotarolimus, umirolimus (biolimus A9), novolimus, and myolimus. *Purple*: FKBP12 (a.k.a. Calstabin1) binding site; *Green*: mTOR binding site

Table 4.1

miRNAs involved in the VSMC phenotypic switch

	Effect of injury on miRNA level	Main targets	References
<i>Pro-contractile miRNAs (quiescent, differentiated state)</i>			
miR-1	Reduction	PIM1, KLF4	[106, 107]
miR-10	Unknown	HDAC4, MAP3K7, β -TRC	[108, 109]
miR-15b	Increase	MAP2K4	[110]
miR-18a-5p	Increase	Syndecan4	[111]
miR-21	Increase	PDCD4	[112, 113]
miR-132	Increase	LRRFIP1	[114]
miR-133a	Reduction	MSN, SP-1	[115, 116]
miR-143/145	Reduction	FRA1, Elk1, CALMK, KLF4, ACE, KLF5, MRTFB, myocardin	[26, 42, 49]
miR-365	Reduction	Cyclin D1	[117]
miR-424/322	Increase	STIM1, calumenin	[118]
miR-490-3p	Reduction	PAPP-A	[119]
miR-638	Unknown	NOR1	[120]
miR-663	Unknown	JunB	[121, 122]
<i>Pro-synthetic miRNAs (proliferative, dedifferentiated state)</i>			
miR-21	Increase	PTEN, Bcl-2	[102, 123, 124]
miR-24	Reduction	TRB3	[104, 125, 126]
miR-26a	Increase	SMAD1/4	[103, 127, 128]
miR-31	Increase	LATS2, CREG	[129–131]
miR-146	Increase	HuR, NF- κ B	[132]
miR-221/222	Increase	p27, p57, c-kit	[102, 133–135]

β TRC β transducin repeat-containing gene, CREG cellular repressor of E1A-stimulated genes, Elk1 ETS domain-containing protein-1, KLF4 Kruppel-like factor 4, Trib3 Tribbles-like protein-3, LATS2 large tumor suppressor homolog 2, LRRFIP1 leucine-rich repeat in Flightless-1 interacting protein-1, MAP2K4 mitogen-activated protein kinase kinase 4, MAP3K7 mitogen-activated kinase kinase kinase 7, MSN moesin, NOR1 orphan nuclear receptor, PAPP-A pregnancy-associated plasma protein A, PDCD4 Programmed Cell Death 4, SMAD small mother against decapentaplegic, STIM1 stromal-interacting molecule 1, TRB3 Tribbles-like protein-3

Table 4.2

Proangiogenic and antiangiogenic miRNAs

	Targets	Main effect	References
<i>Pro-angiogenic miRNAs</i>			
miR-9	SOCS5	Enhanced EC migration	[175]
miR-10a	MAP3K7; HOXA1; bTRC	Anti-inflammatory	[109, 176, 177]
miR-23/27	SEMA6A; SPROUTY2	Pro-angiogenic	[178]
miR-126	PI3KR2; SPRED1	Pro-angiogenic	[57, 89, 98, 179–182]
	VCAM1; SDF1	Anti-inflammatory	
miR-130	HOXA5, GAX	Migration and proliferation	
miR-210	EFNA3	Pro-angiogenic	[183]
miR-217	SIRT1-FOXO/eNOS	Pro-angiogenic	[184]
miR-424	HIF-1 α	Chemotaxis, proliferation	[185]
<i>Anti-angiogenic miRNAs</i>			
miR-17	JAK-1	Anti-angiogenic	[186]
miR-21	RhoB	Antiproliferative	[187]
	PPAR γ	Pro-inflammatory	[188]
miR-24	GATA-2;	Anti-angiogenic	[189]
	PAK4	Pro-apoptotic	[190]
miR-92a	SIRT1; ITGA5	Anti-angiogenic	[191]
	KLF4 and MKK4	Inhibited re-endothelialization	[192]
miR-200	Ets-1; IL-8; CXCL1	Anti-angiogenic	[193, 194]
miR-221/222	STA5a; c-KIT; eNOS	Anti-angiogenic	[195–197]
miR-492	eNOS	Anti-angiogenic	[198]
14q32 miR cluster (329, 487b, 494, 495)	Multiple neovascularization genes	Anti-angiogenic	[199]

EFNA3 Ephrin-A3, *eNOS* Endothelial Nitric Oxide Synthase, *JAK1* Janus kinase 1, *KLF4* Kruppel-like factor 4, *PPAR- γ* Peroxisome proliferator-activated receptor gamma, *RhoB* Ras homolog gene family, member B, *SIRT1* sirtuin (silent mating type information regulation 2 homolog) 1, *SOCS5* suppressor of cytokine signaling 5, *PI3KR2* phosphoinositol-3 kinase regulatory subunit 2, *SEMA6A* Semaphorin-6A, *VCAM-1* vascular cell adhesion molecule 1