Targeting smooth muscle microRNAs for therapeutic benefit in vascular disease.

Albinsson, Sebastian; Swärd, Karl

Published in:
Pharmacological Research

DOI:
10.1016/j.phrs.2013.04.003

2013

Draft 2012-12-20

Targeting smooth muscle microRNAs for therapeutic benefit in vascular disease

Sebastian Albinsson and Karl Swärd

Department of Experimental Medical Science, Lund University, Lund

Correspondence and proofs

Dr. Sebastian Albinsson

Department of Experimental Medical Science

Lund University

BMC D12

SE-221 84 Lund, Sweden

Tel: +46-46-2227765

Fax: +46-46-2113417

E-mail: sebastian.albinsson@med.lu.se

Abstract

In view of the bioinformatic projection that a third of all protein coding genes and essentially all biological pathways are under control of microRNAs (miRNAs), it is not surprising that this class of small RNAs plays roles in vascular disease progression. MiRNAs have been shown to be involved in cholesterol turnover, thrombosis, glucose homeostasis and vascular function. Some miRNAs appear to be specific for certain cells, and the role that such cell-specific miRNAs play in vascular disease is only beginning to be appreciated. A notable example is the miR-143/145 cluster which is enriched in mature and highly differentiated smooth muscle cells (SMCs). Here we outline and discuss the recent literature on SMC-expressed miRNAs in major vascular diseases, including atherosclerosis, neointima formation, aortic aneurysm formation, and pulmonary arterial hypertension. Forced expression of miR-145 emerges as a promising strategy for reduction and stabilization of atherosclerotic plaques as well as for reducing neointimal hyperplasia. It is concluded that if obstacles in the form of delivery and untoward effects of antimirs and mimics can be overcome, the outlook for targeting of SMC-specific miRNAs for therapeutic benefit in vascular disease is bright.

Smooth muscle specific miRNAs

Smooth muscle cells (SMCs) surround hollow organs such as blood vessels, the gastrointestinal tract, the airways and the urinary bladder. By contraction and relaxation the SMCs regulate the diameter of these organs, which in the vasculature is essential for blood pressure and blood flow. Normally, the vascular SMC is quiescent and specialized for contractile function. This is referred to as the contractile or differentiated phenotype. However, under certain circumstances, such as in vascular injury, the SMCs undergo phenotypic modulation and start to proliferate and migrate out of the vascular media. This is important for the vascular repair process but can also be detrimental such as in restenosis following angioplasty when the vessel is partially or completely occluded by proliferating SMCs.

In recent years, small non-coding RNAs called microRNAs (miRNAs) have been implicated in the regulation of SMC phenotype (Figure 1). Deletion of smooth muscle miRNAs by conditional (SM22-Cre mediated) knockout of Dicer results in embryonic lethality associated with loss of smooth muscle contractile differentiation and function, vascular remodeling and internal hemorrhage (1, 2). Deletion of Dicer in adult mice using Tamoxifen-inducible SMMHC-CreER^{T2}/Dicer$^{loxP/loxP}$ (Dicer KO) mice results in phenotypic modulation of SMCs and reduced contractility of arterial, venous and detrusor smooth muscle (3-5). Furthermore, we found that these effects were associated with a reduced vascular mechanotransduction, lower blood pressure and altered voiding pattern in Dicer KO mice. Eventually, inducible loss of smooth muscle miRNAs is lethal in adult mice. However, it is likely that a gastrointestinal and not a vascular phenotype is responsible for the lethality that follows 12-14 weeks after SMC-specific deletion of Dicer in adult mice (5), and for the lethality in three week old constitutive Dicer KO mice using Cre recombinase directed by the SMMHC promoter (6).

Several studies have shown that miR-143 and miR-145 are among the most highly expressed miRNAs in smooth muscle (7-10). These miRNAs are coordinately expressed and have been demonstrated to play an important role in regulation of calcium signaling, contractile differentiation, proliferation and migration of SMCs (1, 3, 7-11). The mir-143/145 cluster is reduced in atherosclerosis and neointima formation, consistent with a role in phenotype regulation (8).

Interestingly, overexpression of miR-145 alone is sufficient to rescue the loss of contractile differentiation in Dicer KO SMCs. Inhibition of miR-145 alone in wild type SMCs can similarly reduce the expression of e.g. the L-type calcium channel to a similar extent as Dicer KO (1, 3). These results suggest that some of the effects in Dicer KO mice are highly dependent on the loss of miR-145. However, although deletion of the miR-143/145 cluster results in effects similar to those seen after SMC-specific Dicer deletion, the phenotype is less severe, suggesting that other miRNAs are also involved in development and contractile differentiation of smooth muscle. Furthermore, the expression level of individual miRNAs and their targets may differ depending on the anatomical location of the SMCs. For this review we have compiled a list of smooth muscle miRNAs that are highly expressed in tissues from three different origins; detrusor, portal vein and aorta (Table 1). These miRNAs were more than 3-fold down-regulated SMC-specific Dicer KO detrusor suggesting high expression in SMCs (see also Table S1 in (4)). However, three highly expressed miRNAs were less affected by Dicer KO and most notable was miR-126, which was only reduced by approximately 20% in Dicer KO detrusor. This is in accordance with our earlier results, showing no reduction of miR-126 in Dicer KO aorta (5) and likely depends on the high and relatively specific expression of miR-126 in endothelial cells (12, 13). Thus it is important to consider the contribution of non-SMC types when evaluating expression levels of smooth muscle miRNAs in certain conditions, such as in disease models, which will be further discussed herein. Although conditional and constitutive deletion of Dicer and constitutive deletion of miR-143 and or miR-145 to our knowledge represent the only miRNA knockout mouse models where smooth muscle differentiation has been thoroughly investigated, a number of additional miRNAs are thought to play a role in smooth muscle phenotype switching and vascular disease.

SMC miRNAs and atherosclerosis

Atherosclerosis is a chronic disease of the vascular wall involving lipid retention and inflammation. It is an important, if not the major, cause of mortality in Western societies. A complex interplay between different cell types, the extracellular matrix, circulating lipoproteins and shear stress is required for the progression from the early so called fatty streaks to the complex “vulnerable” plaques that are responsible for the acute and often calamitous consequences of the disease. Nonmodifiable risk factors for atherosclerosis are

age, male gender, and a genetic predisposition, whereas risk factors that are amenable to intervention include hypertension, dyslipidemia, smoking, diabetes and inflammation. Recent studies have identified polymorphisms associated with atherosclerosis that impinge on the classical risk factors but that also suggest novel disease mechanisms (e.g. (14)). In view of the complex pathogenesis, it is not surprising that a plethora of miRNAs are implicated in various aspects of atheromatous plaque formation. These miRNAs affect cholesterol metabolism (15-17), glucose homeostasis (18, 19), macrophage regulation (20, 21), and thrombosis (22).

SMCs play roles during all phases of the atherogenic process (23, 24) and a role of SMC miRNAs is therefore to be expected. Indeed, recent studies lend substantial support to the notion that SMC miRNAs are critically involved in atherogenesis. The starting-point of one of these studies (25) is the well-established fact that endothelial shear stress is atheroprotective and that this may involve the transcription factor Krüppel-like factor 2 (*KLF2*). Viral *KLF2* transduction, shear stress, and statin treatment were all found to cause up-regulation of miR-143/145 in endothelial cells. This in turn led to release of miR-143/145 in ~100 nm exosome-like vesicles. The authors next demonstrated transfer of miR-143/145 from endothelial cells to SMCs in an *in vitro* co-culture system, and repression of miR-143/145 target genes in SMCs. The most robustly regulated genes in SMCs were phosphatase and actin regulator 4 (*PHACTR4*) and matrix metalloproteinase 3 (*MMP3*), but established miR-143/145-regulated targets such as Krüppel-like factor 4 (*KLF4*) and calcium/calmodulin-dependent protein kinase type II delta (*CAMK2d*) were reduced as well. It was finally demonstrated that vesicles derived from KLF2-transduced endothelial cells reduced aortic atherosclerosis in dyslipidemic mice. Thus, taken together, the study by Hergenreider et al. (25) suggests a novel route of atheroprotective communication between endothelium and SMCs, and supports the view that miR-143/145 may be targeted for therapeutic benefit in atherosclerosis.

A study focusing directly on miR-145 in SMCs provides additional support for the notion that the miR-143/145 cluster can be exploited for plaque prevention. Lovren et al. (26) used a lentiviral vector directing the expression of miR-145 to smooth muscle by use of the SM22α promoter to test if counteracting the reduction of miR-145 in atherosclerosis may be beneficial. Mir-145 or control vector was repeatedly injected into $ApoE^{-/-}$ and control mice

followed by feeding of a Western diet for 12 weeks after which atherosclerotic lesion size was analyzed. A sizeable reduction of plaque size was seen in the aortic root, in the aorta, and in brachiocephalic arteries without concomitant change in lipoprotein concentrations or blood pressure. Forced miR-145 expression moreover reduced the necrotic core of plaques, increased the fibrous cap, and increased the relative collagen-positive area; that is, plaques were not only reduced in size but also remodeled to become more stable. Mechanistically, these effects were linked to increased expression of myocardin and reduced expression of KLF4, both of which are transcription factors of central importance for contractile differentiation of SMCs (8, 27-29).

Several other studies suggest additional miRNAs that may play a role for plaque development. Reddy et al. (30) for example demonstrated upregulation of miR-200b in the aortic SMCs from db/db mice. This was found to be associated with repression of the transcription factor Zeb1 by miR-200b and with upregulation of cyclooxygenase 2 and monocyte chemoattractant protein-1 (MCP-1). Mir-200b mimic was moreover found to increase the interaction between macrophages and SMCs *in vitro*. This suggests that SMC-directed expression of an antimir targeting Mir-200b may be beneficial in diabetic vascular disease. An additional example provided by the same group is miR-125b. This microRNA was increased in db/db mice similar to miR-200b, but in that case an association with reduced expression of inflammatory mediators (IL-6, MCP-1) was demonstrated (31).

Yet another example of a miRNA that may be involved in atherogenesis was described by Chen et al. (32). These authors found that oxidized LDL up-regulates the oxidized LDL (lectin-like) receptor 1 (OLR1, LOX-1) via down-regulation of the microRNA Let-7g in human SMCs. Solid evidence implicating LOX-1 in atherosclerosis in both the aorta and coronary arteries has been presented previously (33, 34). Overexpression of Let-7g or a mimic in vivo may thus repress LOX-1 expression and reduce atherosclerosis. A highly relevant aspect of this story is that LOX-1 appears to be rather dramatically regulated by blood pressure (35) and by SMC stretch in vitro (36), findings that in part may explain the synergy between hypertension and other classical risk factors in atherosclerosis. This is, in our view, of considerable interest because hypertension remains the most prevalent risk factor for atherosclerosis.

SMC miRNAs and neointima formation

Neointimal expansion can occur in response to altered mechanical load or following vascular injury such as in atherosclerosis. It is also a common secondary consequence of balloon dilatation or stenting of arteries that have become obstructed due to atherosclerotic plaques (37). Smooth muscle proliferation and migration play major roles in neointimal expansion and therapeutic strategies have therefore been focused on local delivery of antiproliferative drugs such as Sirolimus and Paclitaxel using drug-eluting stents. 5-year follow-ups of drug eluting stents show that the need for target vessel revascularization is reduced by only about 50% compared to bare metal stents (38). Primary and secondary neointimal disease thus remains an important problem and novel therapeutic targets are needed.

The importance of miRNAs in neointimal hyperplasia was initially studied by the group of Dr. Chunxiang Zhang in a series of pioneering studies (39-41). In the first report by Ji et al., miRNA expression in the vascular wall after balloon angioplasty of the rat carotid artery was examined using microarray analysis (40). MiR-21 and miR-221/222 were found to be both highly expressed in normal arteries and significantly up-regulated after balloon angioplasty (40, 41). Furthermore, partial inhibition of miR-21 and miR-221/222 *in vivo* significantly reduced neointimal formation, which for the first time demonstrated the potential of smooth muscle miRNAs as therapeutic targets for treatment of vascular disease. It was shown that these miRNAs down-regulated the tumor suppressor proteins PTEN (miR-21) and p27(Kip1)/p57(Kip2)(miR-221/222) in proliferating SMCs (40-42).

Cheng et al. identified miR-145 as the most abundant miRNA in normal smooth muscle and moreover found this miRNA to be significantly down-regulated in neointimal lesions (39). Overexpression of miR-145 in balloon-injured rat carotid arteries reduced neointimal hyperplasia, likely via down-regulation of KLF5 and up-regulation of myocardin. However, in a separate study by Xin et al. (10), neointimal hyperplasia after vascular injury was shown to be completely abolished in miR-145 mutant mice, which demonstrates that a certain level of miR-145 expression is required for the vascular repair process after injury. Reduced actin polymerization and migratory ability of the SMCs was forwarded as a molecular explanation for these findings (10).

In the future, we can expect a growing list of smooth muscle miRNAs that regulate proliferation and neointimal hyperplasia. The most recent additions to this list are miR-146a

and miR-195 (43, 44). MiR-146a is up-regulated in proliferating SMCs and inhibition of miR-146a reduced neointimal formation following balloon injury of rat carotid arteries *in vivo*. The effect of the miR-146a inhibitor was suggested to depend on up-regulation of its target KLF4, which has been shown to inhibit smooth muscle proliferation (45, 46).

Additional miRNAs such as miR-1 and miR-133 have also been shown to reduce smooth muscle proliferation *in vitro* and loss of these miRNAs may thus be involved in the development of neointimal hyperplasia (47, 48). The miRNAs discussed in this section are summarized in Figure 1.

SMC miRNAs and aortic aneurysm formation

Aortic aneurysm formation is a process driven by hemodynamic forces that act in concert with risk factors and genetic predisposition to cause progressive localized dilatation or ballooning of the aorta (49). The risk factors for aortic aneurysms include age, male gender, blood pressure, and a family history (50). Smoking accounts for nearly 80% of excess prevalence (50). Whether atherosclerosis is an independent risk factor for aortic aneurysms is less clear (50, 51), and diabetes is, somewhat surprisingly, protective (50, 52). The latter association has been attributed to glycation of extracellular matrix proteins (53). Similar to the situation in atherosclerosis, large genome wide association scans have uncovered unexpected genetic associations that point to novel disease mechanisms in aneurysm formation (e.g. (54)).

The site of origin of aortic aneurysms can be anywhere along the length of the vessel. A local weakness in the wall, such as an atherosclerotic plaque with increased levels of macrophage-derived proteases or a defect in the composition of the extracellular matrix (such as in Marfan's syndrome), are plausible triggering factors. Once the aneurysm has formed it grows almost linearly in size. The law of Laplace states that the wall tension in a tube equals the product of the pressure and the tube radius; it follows that the local dilatation will increase the tension in the wall simply as a consequence of the increase in radius. This is likely to be an important driving force for aneurysm progression. Added to this is the change of flow from laminar to turbulent at the dilated site. This affects the shear stress on the endothelium in a pro-atherogenic fashion (see section on atherosclerosis for miRNAs of relevance in this context), which will further promote macrophage accumulation, protease

activity and dedifferentiation of SMCs. When left untreated, aneurysms may eventually rupture with catastrophic consequences. Initiatives to screen for aortic aneurysms using ultrasound in elderly men have therefore been initiated.

Much of the current thinking regarding aneurysm formation is centered on the role of the extracellular matrix and its turnover. Because SMCs play an important role in matrix deposition SMC miRNAs are likely to play a role in aneurysm formation. Several studies have centered on miR-29 (a, b, c). This miRNA cluster is likely expressed in fibroblasts, but our array data from SMC Dicer KO organs suggest an even higher expression in SMCs (cf. Table 1). MiR-29a and miR-29c for example are knocked down by ~80% following smooth muscle-specific deletion of Dicer (unpublished and (4)) arguing that they are more abundant in smooth muscle than in other cell types. MiR-29a-c are derived from two independent bicistronic transcripts (55). In pioneering work, Boon et al. (55) demonstrated that miR-29 levels were increased in aged aortas. Interestingly, these miRNAs were the only ones out of 18 identified to be regulated by aging that had a significant impact on mRNA levels. Many of the targets of miR-29 can be found among extracellular matrix proteins (*FBN1*, *COL4A1*, *LAMC1*, *COL15A1*, *COL3A1* (56, 57)). Thus, up-regulation of miR-29 in aging is expected to be associated with weakening of the extracellular matrix in the in the aorta. In keeping with this hypothesis, the authors found reduced expression of collagen and elastin in aged aortas, and *in vivo* silencing of miR-29 using a locked nucleic acid construct (LNA-29) had the opposite effect (55). LNA-29 additionally inhibited dilatation of the aorta induced by *in vivo* treatment with angiotensin II.

In another study on human aneurysm tissue, miR-29a was found to be reduced (58). This is in contrast to the study by Boon et al. ((55), discussed by (59)) but in keeping with later studies on miR-29b (60). Thus, the majority of data seems to suggest that once the aneurysmal process has started, miR-29 is down-regulated in the vascular wall. This may represent feedback inhibition of further aneurysmal dilatation via an increase in matrix deposition (Figure 2). In keeping with this hypothesis, blockade of miR-29b in three different in vivo models of aortic aneurysm formation was beneficial and associated with increased matrix deposition (55, 60, 61). All in all, these studies identify blockade of miR-29 as a novel therapy to combat aortic aneurysm progression and suggest a mechanism by which aging promotes formation of aortic aneurysms via up-regulation of miR-29.

Recent work identified miR-21 as an additional modifier of smooth muscle proliferation and apoptosis in abdominal aortic aneurysm formation (62). In murine aneurysm models miR-21 was found to increase in parallel with aneurysm growth. Overexpression of miR-21 using a lentiviral vector led to increased SMC proliferation and reduced apoptosis which curtailed further aneurysm expansion. One of the targets of miR-21 was PTEN; its down-regulation promoted Akt-dependent pro-proliferative and anti-apoptotic pathways which presumably imparted on the aortic wall a greater tensile strength. Conversely, a locked nucleic acid-modified antagomir against miR-21 increased aneurysm size. Altogether this is in keeping with the recently demonstrated protective role of Akt2 in aneurysm formation and dissection via a shift in the balance between MMP-9 and TIMP-1 in favor of the latter (63). These findings support strategies to increase miR-21 in the aortic wall for aneurysm prevention.

SMC miRNAs and pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is characterized by an increase in pulmonary arterial pressure (systolic pressure >25 mmHg), which can be caused both by excessive constriction and hypertrophic remodeling of the pulmonary vascular smooth muscle. PAH ultimately leads to right heart failure and death (64). The primary cause of this disease is not completely understood but in some cases PAH has been associated with a mutation in the bone morphogenic protein receptor 2 (BMPR2) (65). Treatment strategies against pulmonary hypertension are focused on vasodilator substances such as endothelin receptor antagonists, phosphodiesterase inhibitors and prostacyclin derivatives but since there is currently no cure for PAH, novel targets for therapeutic intervention are needed. A number of promising compounds are currently under investigation including guanylate cyclase activators, tyrosine kinase inhibitors, and serotonin antagonists. In addition, recent studies have shown that miRNA-related therapies may be effective against PAH, offering an alternative therapeutic principle.

A number of miRNAs have been demonstrated to be differentially expressed in animal models of pulmonary hypertension and in human patients (66, 67), and a major challenge is now to identify the functional importance of these miRNAs in the development of PAH. The most well studied miRNA in the context of PAH is miR-21. However, conflicting results have

been reported and at this point it is difficult to conclude if this miRNA is protective or detrimental for the pathogenesis of PAH. MiR-21 was initially shown to be down-regulated in both human and rat lungs during PAH (66). However, several groups recently reported increased miR-21 expression in mouse lung and distal pulmonary arteries during PAH (68-70) as well as in human plexiform and concentric lesions from PAH patients (71). The discrepancies between the studies may originate from the method used to induce pulmonary hypertension (hypoxia± SU5416 vs. monocrotaline), the species used (rat vs. mouse or human) or the origin of the sample (lung homogenate vs. pulmonary arteries). In the study by Yang et al., *in vivo* treatment with miR-21 inhibitor was shown to reduce pulmonary hypertension (using Fulton Index: [RV/(LV + S)] as a substitute for pulmonary pressure) and to attenuate hypoxia-induced pulmonary vascular remodeling (68). Furthermore, a miR-21 mimic enhanced proliferation of PASMCs in vitro, in accordance with earlier observations in aortic SMCs (40). In accordance with these results, Pullamsetti et al. demonstrated a significant reduction in right ventricular pressure after treating PAH in mice with miR-21 antagomir (69). However, Parikh et al. who also report increased levels of miR-21 in pulmonary vessels during PAH, argue that this up-regulation serves a protective role in the disease (70). In support of this hypothesis they demonstrate that knockout of miR-21 in mice results in increased right ventricular pressure and media thickness of pulmonary arteries compared to wild type mice after exposure to the vascular endothelial growth factor receptor-2 antagonist SU5416 in combination with chronic hypoxia. Furthermore, miR-21 is suggested to exert some of its protective effects against PAH by down-regulating RhoB, which has been recently implicated in the development of PAH (72). Consistent with this view, mutations in the BMP pathway results in decreased miR-21 expression in PASMC (73) and dysregulation of the BMP-miR-21-RhoB axis may thus predispose for PAH. To summarize, most studies show an increased expression of miR-21 in pulmonary arteries from humans and rodents with PAH while miR-21 levels in the whole lung differ depending on experimental model. Possibly, reduced levels of miR-21 in healthy rodents can increase susceptibility to PAH while inhibition of miR-21 during in PAH may alleviate symptoms.

In addition to miR-21 some other miRNAs have been predicted or demonstrated to play a role in in the pathogenesis of PAH including the miR-17/92 cluster and mir-204 (67, 69, 70, 74, 75). Recently, miR-204 was shown to be reduced in human and rodent PAH (67).

Interestingly, the down-regulation correlated with the severity of the disease in both human patients and murine models. Furthermore, restoration of miR-204 levels in mouse PAH by intratracheal nebulization of miR-204 mimic, reduced pulmonary artery wall thickness and normalized pulmonary arterial pressure. The miR-204 mimic was shown to directly target the Src activator SHP2 and down-regulate the pro-proliferative and anti-apoptotic Src–STAT3–NFAT pathway, which is promoted during PAH.

The miR-17-92 cluster of miRNAs is known to play an important role for endothelial cell proliferation and angiogenesis (12, 76, 77). Certain members of the miR-17-92 cluster including miR-17 and miR-20 are promoted by STAT3 transcription factor and down-regulate BMPR2, suggesting a role in PAH (69, 74, 75). The expression of miR-17 is modestly and transiently up-regulated in the lungs from mice with hypoxia-induced PAH (69) while miR-20 expression is unchanged (75). However, as indicated previously, the effect on expression level in the vasculature can be masked by other cell types in the lung homogenate. Treatment of pulmonary hypertensive mice or rats with a miR-17 antagomir resulted in a reduction of right ventricular pressure and right heart hypertrophy, and an improvement in right ventricular function (69). Similar results were obtained in mice treated with miR-20 antimir (75). The mechanism behind the effects of the miR-17 and miR-20 antimirs involves up-regulation of the cyclin-dependent kinase inhibitor p21 and attenuated smooth muscle proliferation as well as reduced muscularization of pulmonary arteries. Interestingly, delivery of mesenchymal stromal cells (MSC), derived from the bone marrow, has been reported to attenuate experimentally induced PAH in rat and mouse (78, 79). It was recently reported that the beneficial effect of MSCs may depend on the release of exosomes, which are microvesicles that are released into the environment by multiple cell types following fusion of multivesicular bodies with the plasma membrane (80). Furthermore, it was demonstrated that the effect of MSC exosomes was mediated at least in part through inhibition of STAT3 and subsequent down-regulation of miR-17 and miR-20, and up-regulation of miR-204. However, identifying the mechanism for STAT3 inhibition by exosomes will require further investigation.

Despite its crucial role in smooth muscle development and function, only one study has so far focused on the importance of miR-145 in PAH (81). The expression of miR-143/145 was shown to be increased in the lungs and pulmonary arteries of mice with hypoxia-induced

PAH by as well as in human patients with heritable or idiopathic PAH. Interestingly, increased expression of miR-143/145 was also found in the lungs of mice with BMPR2-mutation, which exhibit spontaneous PAH, as well as in cultured PASMC from patients with heritable PAH caused by BMPR2-mutation (81). Since the BMP signaling pathway is known to induce miR-143/145 expression via the myocardin-related transcription factor (MRTF), it seems contradictory that loss of BMP receptors also induces the expression of these miRNAs (82). It is possible that the increased muscularization of pulmonary arteries in BMPR2 mutant mice results in a relative increase of the smooth muscle specific miR-143/145. An alternative explanation may be a compensatory increase in mediators of TGF-β-induced signaling, which also promotes miR-143/145 expression (82, 83).

Knockout of miR-143/145 or subcutaneous injection of LNA anti-miR-145 resulted in significant protection from the development of PAH while anti-miR-143 had no effect (81). The effect of miR-145 deletion was associated with a reduced hypoxia-induced vascular remodeling of the pulmonary arteries and may involve inhibition of Wnt/β-catenin signaling pathway. Several inhibitors of this pathway were up-regulated in miR-143/145 KO mice after hypoxia (81) and Wnt signaling has recently been suggested to play an important role in smooth muscle proliferation and pulmonary disease (84). It remains to be addressed if forced expression of miR-145 promotes PAH. This is an important experiment given the promise of this strategy for treatment of atherosclerosis.

Besides the miRNAs mentioned previously, some additional miRNAs have recently been suggested to be involved in the pathogenesis of PAH including miR-206 (85), miR-210 (86) and miR-328 (87). The role of miR-328 was tested *in vivo* using transgenic over expression and miR-328 was shown to have a modest protective effect against hypoxia-induced PAH by targeting the L-type calcium channel on PASMCs. The effects of miR-206 and miR-210 were not tested *in vivo* but they were shown to be down- and up-regulated, respectively, in hypoxia-induced PAH. Transfection with miR-206 mimic decreased proliferation and increased apoptosis of PASMCs in vitro (85) while miR-210 inhibitor reduced cell number mainly by an effect on apoptosis (86). Further studies evaluating the effects of these miRNAs *in vivo* are warranted in order to assess their therapeutic potential against PAH.

In summary, there is a growing list of miRNAs that are involved in the pathogenesis of PAH. The therapeutic effect of inhibitors or mimics of these miRNAs in animal models is quite astonishing and holds promise for future pharmacological intervention against this disease.

SMC miRNAs and systemic arterial hypertension

As noted above, systemic arterial hypertension is the most prevalent risk factor for cardiovascular disease with close to half of all cases of stroke and ischemic heart disease being directly attributable to high blood pressure (88). It is evident from our previous discussion that SMC miRNAs play essential roles in blood pressure control. It is however less clear that they are also good targets for therapeutic intervention to combat chronic systemic hypertension as such. A good example is the miR-143/145 cluster. Deletion of this cluster leads to reduction of blood pressure (7, 10), and to a reduction of the hypertensive response to angiotensin II (7, 9). Targeted reduction of these miRNAs *in vivo* by antimirs is therefore expected to reduce arterial blood pressure or, at least, curtail the blood pressure elevating effect of angiotensin II. However, because the mechanism of action in part involves phenotypic modulation of SMCs, this would likely promote atherosclerosis (c.f. (7, 26)) and therefore ischemic heart disease. While this could be an acceptable tradeoff in PAH, which is associated with considerable morbidity, it is unacceptable for treatment of primary arterial hypertension alone. SMC-specific overexpression of miR-145 does not raise systemic blood pressure (26), possibly because the effect on SMC differentiation is saturated by endogenous miR-145 levels in the healthy systemic arteries, but inhibits SMC dedifferentiation in the plaques as discussed above.

As in the case of atherosclerosis there are several SMC miRNAs that, based on indirect evidence, qualify as candidate targets for therapeutic intervention in hypertension. MiR-130a mimic, for example, was shown to affect proliferation of vascular SMCs *in vitro* and mir-130a levels were found to correlate with vascular remodeling *in vivo* (89). We have chosen not to discuss such examples because decisive *in vivo* evidence is still lacking. It should also be kept in mind that systemic arterial blood pressure is the consequence of heart, kidney, endocrine and endothelial activity. Hence, miRNAs in these organs and tissues also need to be considered for therapeutic intervention (reviewed by (90)).

Conclusions

Obstacles in the form of stability, cellular specificity and uptake have to be addressed before miRNA-based therapies will become established as a strategy to treat vascular disease. For antimirs, valuable progress has been made, but the technical development of miRNA mimicry has taken less decisive strides forward (91). Unwanted side effects of antimir designs on blood clotting, complement activation, innate immunity and liver toxicity need to be addressed. Another issue of relevance for patient compliance is that the size and charge of current candidate antimirs preclude oral delivery (91). When these obstacles are overcome, however, a novel treatment modality can likely be added to the therapeutic weaponry for combatting atherosclerosis, pulmonary arterial hypertension and aortic aneurysm formation.

Acknowledgements

The authors sincerely thank Karolina Turczynska and for assistance with the miRNA arrays. The authors also thank Dr. Per Hellstrand, Dr. Bengt-Olof Nilsson, Dr. Catarina Rippe and Karolina Turczynska for critical reading of the manuscript.

Work in the authors' laboratories is supported by The Swedish Research Council, The Heart and Lung Foundation, The Greta and Johan Kock's Foundation, The Crafoord Foundation; The Royal Physiographic Society; The Åke Wiberg Foundation; The Tore Nilson Foundation; The Magnus Bergvall Foundation, The Lars Hierta Memorial Foundation and The Jeansson Foundation.

Figure legends

Figure 1. MicroRNAs are involved in the regulation of smooth muscle phenotype

Summary of highly expressed miRNAs in contractile smooth muscle cells and miRNAs that are up-regulated during phenotypic modulation of smooth muscle cells into synthetic and proliferative cells in neointimal formation following vascular injury.

Figure 2. Role of miR-29 in mechanical distension and vascular stiffness

Hypothetical model showing distension-induced repression of miR-29 (1) and de-repression of extracellular matrix proteins (2,3) resulting in increased tissue stiffness and inhibiting further distension (4). The depicted feedback regulation could explain down-regulation of miR-29 in aortic aneurysm expansion and in infarcted heart, both of which are conditions associated with distension.

Table I. MiRNAs that are highly expressed in portal vein, aorta and detrusor.

MiRNA analyses were performed in each tissue separately using qPCR based arrays as described (4). Left column shows microRNAs that are highly expressed in mouse aortic, portal vein and detrusor smooth muscle cells (SMCs), and that are more than 3-fold down-regulated in SMC-specific Dicer knockout detrusor. Middle column shows a selection of predicted targets in man (from a TargetScan 5.2 survey of top 100 predicted targets, irrespective of site conservation, ranked left to right by total context score) that may influence differentiation or function of SMCs. Bolded targets have been more extensively examined than others in the context of smooth muscle function. Italicized genes are targeted by multiple SMC miRNAs. Confirmed targets of relevance for SMC differentiation and function are given in the right column and discussed in the text. The confirmed targets were selected by a Pubmed search using the individual miRNA and smooth muscle as keywords.

References

1. S. Albinsson, Y. Suarez, A. Skoura, S. Offermanns, J.M. Miano, and W.C. Sessa. MicroRNAs are necessary for vascular smooth muscle growth, differentiation, and function. Arterioscler Thromb Vasc Biol. 30:1118-1126 (2010).
2. Y. Pan, L. Balazs, G. Tigyi, and J. Yue. Conditional deletion of Dicer in vascular smooth muscle cells leads to the developmental delay and embryonic mortality. Biochem Biophys Res Commun. 408:369-374 (2011).
3. K.M. Turczynska, M.K. Sadegh, P. Hellstrand, K. Sward, and S. Albinsson. MicroRNAs are essential for stretch-induced vascular smooth muscle contractile differentiation via microRNA (miR)-145-dependent expression of L-type calcium channels. J Biol Chem. 287:19199-19206 (2012).
4. M.K. Sadegh, M. Ekman, C. Rippe, B. Uvelius, K. Sward, and S. Albinsson. Deletion of dicer in smooth muscle affects voiding pattern and reduces detrusor contractility and neuroeffector transmission. PLoS One. 7:e35882 (2012).
5. S. Albinsson, A. Skoura, J. Yu, A. Dilorenzo, C. Fernandez-Hernando, S. Offermanns, J.M. Miano, and W.C. Sessa. Smooth Muscle miRNAs Are Critical for Post-Natal Regulation of Blood Pressure and Vascular Function. PLoS One. 6:e18869 (2011).
6. C. Park, W. Yan, S.M. Ward, S.J. Hwang, Q. Wu, W.J. Hatton, J.K. Park, K.M. Sanders, and S. Ro. MicroRNAs dynamically remodel gastrointestinal smooth muscle cells. PLoS One. 6:e18628 (2011).
7. T. Boettger, N. Beetz, S. Kostin, J. Schneider, M. Kruger, L. Hein, and T. Braun. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. J Clin Invest. 119:2634-2647 (2009).
8. K.R. Cordes, N.T. Sheehy, M.P. White, E.C. Berry, S.U. Morton, A.N. Muth, T.H. Lee, J.M. Miano, K.N. Ivey, and D. Srivastava. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature. 460:705-710 (2009).
9. L. Elia, M. Quintavalle, J. Zhang, R. Contu, L. Cossu, M.V. Latronico, K.L. Peterson, C. Indolfi, D. Catalucci, J. Chen, S.A. Courtneidge, and G. Condorelli. 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).
10. M. Xin, E.M. Small, L.B. Sutherland, X. Qi, J. McAnally, C.F. Plato, J.A. Richardson, R. Bassel-Duby, and E.N. Olson. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev. 23:2166-2178 (2009).
11. M. Quintavalle, L. Elia, G. Condorelli, and S.A. Courtneidge. MicroRNA control of podosome formation in vascular smooth muscle cells in vivo and in vitro. J Cell Biol. 189:13-22 (2010).
12. Y. Suarez, C. Fernandez-Hernando, J. Yu, S.A. Gerber, K.D. Harrison, J.S. Pober, M.L. Iruela-Arispe, M. Merkenschlager, and W.C. Sessa. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc Natl Acad Sci U S A. 105:14082-14087 (2008).
13. S. Wang, A.B. Aurora, B.A. Johnson, X. Qi, J. McAnally, J.A. Hill, J.A. Richardson, R. Bassel-Duby, and E.N. Olson. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell. 15:261-271 (2008).
14. H. Schunkert, I.R. Konig, S. Kathiresan, M.P. Reilly, T.L. Assimes, H. Holm, M. Preuss, A.F. Stewart, M. Barbalic, C. Gieger, D. Absher, Z. Aherrahrou, H. Allayee, D. Altshuler, S.S. Anand, K. Andersen, J.L. Anderson, D. Ardissino, S.G. Ball, A.J. Balmforth, T.A. Barnes, D.M. Becker, L.C. Becker, K. Berger, J.C. Bis, S.M. Boekholdt, E. Boerwinkle, P.S. Braund, M.J. Brown, M.S. Burnett, I. Buysschaert, J.F. Carlquist, L. Chen, S. Cichon, V. Codd, R.W. Davies, G. Dedoussis, A. Dehghan, S. Demissie, J.M. Devaney, P. Diemert, R. Do, A. Doering, S. Eifert, N.E. Mokhtari, S.G. Ellis, R. Elosua, J.C. Engert, S.E. Epstein, U. de Faire, M. Fischer, A.R. Folsom, J. Freyer, B. Gigante, D. Girelli, S. Gretarsdottir, V. Gudnason, J.R. Gulcher, E. Halperin, N. Hammond, S.L. Hazen, A. Hofman, B.D. Horne, T. Illig, C. Iribarren, G.T. Jones, J.W. Jukema, M.A. Kaiser, L.M.

Kaplan, J.J. Kastelein, K.T. Khaw, J.W. Knowles, G. Kolovou, A. Kong, R. Laaksonen, D. Lambrechts, K. Leander, G. Lettre, M. Li, W. Lieb, C. Loley, A.J. Lotery, P.M. Mannucci, S. Maouche, N. Martinelli, P.P. McKeown, C. Meisinger, T. Meitinger, O. Melander, P.A. Merlini, V. Mooser, T. Morgan, T.W. Muhleisen, J.B. Muhlestein, T. Munzel, K. Musunuru, J. Nahrstaedt, C.P. Nelson, M.M. Nothen, O. Olivieri, R.S. Patel, C.C. Patterson, A. Peters, F. Peyvandi, L. Qu, A.A. Quyyumi, D.J. Rader, L.S. Rallidis, C. Rice, F.R. Rosendaal, D. Rubin, V. Salomaa, M.L. Sampietro, M.S. Sandhu, E. Schadt, A. Schafer, A. Schillert, S. Schreiber, J. Schrezenmeir, S.M. Schwartz, D.S. Siscovick, M. Sivananthan, S. Sivapalaratnam, A. Smith, T.B. Smith, J.D. Snoep, N. Soranzo, J.A. Spertus, K. Stark, K. Stirrups, M. Stoll, W.H. Tang, S. Tennstedt, G. Thorgeirsson, G. Thorleifsson, M. Tomaszewski, A.G. Uitterlinden, A.M. van Rij, B.F. Voight, N.J. Wareham, G.A. Wells, H.E. Wichmann, P.S. Wild, C. Willenborg, J.C. Witteman, B.J. Wright, S. Ye, T. Zeller, A. Ziegler, F. Cambien, A.H. Goodall, L.A. Cupples, T. Quertermous, W. Marz, C. Hengstenberg, S. Blankenberg, W.H. Ouwehand, A.S. Hall, P. Deloukas, J.R. Thompson, K. Stefansson, R. Roberts, U. Thorsteinsdottir, C.J. O'Donnell, R. McPherson, J. Erdmannand N.J. Samani. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 43:333-338 (2011).

15. C. Esau, S. Davis, S.F. Murray, X.X. Yu, S.K. Pandey, M. Pear, L. Watts, S.L. Booten, M. Graham, R. McKay, A. Subramaniam, S. Propp, B.A. Lollo, S. Freier, C.F. Bennett, S. Bhanot, and B.P. Monia. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab. 3:87-98 (2006).
16. K.J. Rayner, Y. Suarez, A. Davalos, S. Parathath, M.L. Fitzgerald, N. Tamehiro, E.A. Fisher, K.J. Moore, and C. Fernandez-Hernando. MiR-33 contributes to the regulation of cholesterol homeostasis. Science. 328:1570-1573 (2010).
17. K.J. Rayner, F.J. Sheedy, C.C. Esau, F.N. Hussain, R.E. Temel, S. Parathath, J.M. van Gils, A.J. Rayner, A.N. Chang, Y. Suarez, C. Fernandez-Hernando, E.A. Fisher, and K.J. Moore. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. J Clin Invest. 121:2921-2931 (2011).
18. M.N. Poy, L. Eliasson, J. Krutzfeldt, S. Kuwajima, X. Ma, P.E. Macdonald, S. Pfeffer, T. Tuschl, N. Rajewsky, P. Rorsman, and M. Stoffel. A pancreatic islet-specific microRNA regulates insulin secretion. Nature. 432:226-230 (2004).
19. M. Trajkovski, J. Hausser, J. Soutschek, B. Bhat, A. Akin, M. Zavolan, M.H. Heim, and M. Stoffel. MicroRNAs 103 and 107 regulate insulin sensitivity. Nature. 474:649-653 (2011).
20. T. Chen, Z. Huang, L. Wang, Y. Wang, F. Wu, S. Meng, and C. Wang. MicroRNA-125a-5p partly regulates the inflammatory response, lipid uptake, and ORP9 expression in oxLDL-stimulated monocyte/macrophages. Cardiovasc Res. 83:131-139 (2009).
21. M. Nazari-Jahantigh, Y. Wei, H. Noels, S. Akhtar, Z. Zhou, R.R. Koenen, K. Heyll, F. Gremse, F. Kiessling, J. Grommes, C. Weber, and A. Schober. MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. J Clin Invest. 122:4190-4202 (2012).
22. P. Landry, I. Plante, D.L. Ouellet, M.P. Perron, G. Rousseau, and P. Provost. Existence of a microRNA pathway in anucleate platelets. Nat Struct Mol Biol. 16:961-966 (2009).
23. A.C. Doran, N. Meller, and C.A. McNamara. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. Arterioscler Thromb Vasc Biol. 28:812-819 (2008).
24. M.C. Clarke, N. Figg, J.J. Maguire, A.P. Davenport, M. Goddard, T.D. Littlewood, and M.R. Bennett. Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. Nat Med. 12:1075-1080 (2006).
25. E. Hergenreider, S. Heydt, K. Treguer, T. Boettger, A.J. Horrevoets, A.M. Zeiher, M.P. Scheffer, A.S. Frangakis, X. Yin, M. Mayr, T. Braun, C. Urbich, R.A. Boon, and S. Dimmeler. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol. 14:249-256 (2012).
26. F. Lovren, Y. Pan, A. Quan, K.K. Singh, P.C. Shukla, N. Gupta, B.M. Steer, A.J. Ingram, M. Gupta, M. Al-Omran, H. Teoh, P.A. Marsden, and S. Verma. MicroRNA-145 targeted therapy reduces atherosclerosis. Circulation. 126:S81-90 (2012).

27. Z. Wang, D.Z. Wang, G.C. Pipes, and E.N. Olson. Myocardin is a master regulator of smooth muscle gene expression. Proc Natl Acad Sci U S A. 100:7129-7134 (2003).
28. T. Yoshida, S. Sinha, F. Dandre, B.R. Wamhoff, M.H. Hoofnagle, B.E. Kremer, D.Z. Wang, E.N. Olson, and G.K. Owens. Myocardin is a key regulator of CArG-dependent transcription of multiple smooth muscle marker genes. Circ Res. 92:856-864 (2003).
29. Y. Liu, S. Sinha, O.G. McDonald, Y. Shang, M.H. Hoofnagle, and G.K. Owens. Kruppel-like factor 4 abrogates myocardin-induced activation of smooth muscle gene expression. J Biol Chem. 280:9719-9727 (2005).
30. M.A. Reddy, W. Jin, L. Villeneuve, M. Wang, L. Lanting, I. Todorov, M. Kato, and R. Natarajan. Pro-inflammatory role of microrna-200 in vascular smooth muscle cells from diabetic mice. Arterioscler Thromb Vasc Biol. 32:721-729 (2012).
31. L.M. Villeneuve, M. Kato, M.A. Reddy, M. Wang, L. Lanting, and R. Natarajan. 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. 59:2904-2915 (2010).
32. K.C. Chen, I.C. Hsieh, E. Hsi, Y.S. Wang, C.Y. Dai, W.W. Chou, and S.H. Juo. Negative feedback regulation between microRNA let-7g and the oxLDL receptor LOX-1. J Cell Sci. 124:4115-4124 (2011).
33. K. Inoue, Y. Arai, H. Kurihara, T. Kita, and T. Sawamura. Overexpression of lectin-like oxidized low-density lipoprotein receptor-1 induces intramyocardial vasculopathy in apolipoprotein E-null mice. Circ Res. 97:176-184 (2005).
34. J.L. Mehta, N. Sanada, C.P. Hu, J. Chen, A. Dandapat, F. Sugawara, H. Satoh, K. Inoue, Y. Kawase, K. Jishage, H. Suzuki, M. Takeya, L. Schnackenberg, R. Beger, P.L. Hermonat, M. Thomas, and T. Sawamura. Deletion of LOX-1 reduces atherogenesis in LDLR knockout mice fed high cholesterol diet. Circ Res. 100:1634-1642 (2007).
35. A. Nakano, N. Inoue, Y. Sato, N. Nishimichi, K. Takikawa, Y. Fujita, A. Kakino, K. Otsui, S. Yamaguchi, H. Matsuda, and T. Sawamura. LOX-1 mediates vascular lipid retention under hypertensive state. J Hypertens. 28:1273-1280 (2010).
36. Z. Zhang, M. Zhang, Y. Li, S. Liu, S. Ping, J. Wang, F. Ning, F. Xie, and C. Li. Simvastatin inhibits the additive activation of ERK1/2 and proliferation of rat vascular smooth muscle cells induced by combined mechanical stress and oxLDL through LOX-1 pathway. Cell Signal. 25:332-340 (2013).
37. R. Komatsu, M. Ueda, T. Naruko, A. Kojima, and A.E. Becker. Neointimal tissue response at sites of coronary stenting in humans: macroscopic, histological, and immunohistochemical analyses. Circulation. 98:224-233 (1998).
38. S. Silber, A. Colombo, A.P. Banning, K. Hauptmann, J. Drzewiecki, E. Grube, D. Dudek, and D.S. Baim. Final 5-year results of the TAXUS II trial: a randomized study to assess the effectiveness of slow- and moderate-release polymer-based paclitaxel-eluting stents for de novo coronary artery lesions. Circulation. 120:1498-1504 (2009).
39. Y. Cheng, X. Liu, J. Yang, Y. Lin, D.Z. Xu, Q. Lu, E.A. Deitch, Y. Huo, E.S. Delphin, and C. Zhang. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. Circ Res. 105:158-166 (2009).
40. R. Ji, Y. Cheng, J. Yue, J. Yang, X. Liu, H. Chen, D.B. Dean, and C. Zhang. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. Circ Res. 100:1579-1588 (2007).
41. X. Liu, Y. Cheng, S. Zhang, Y. Lin, J. Yang, and C. Zhang. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. Circ Res. 104:476-487 (2009).
42. B.N. Davis, A.C. Hilyard, P.H. Nguyen, G. Lagna, and A. Hata. Induction of microRNA-221 by platelet-derived growth factor signaling is critical for modulation of vascular smooth muscle phenotype. J Biol Chem. 284:3728-3738 (2009).

43. S.G. Sun, B. Zheng, M. Han, X.M. Fang, H.X. Li, S.B. Miao, M. Su, Y. Han, H.J. Shi, and J.K. Wen. miR-146a and Kruppel-like factor 4 form a feedback loop to participate in vascular smooth muscle cell proliferation. EMBO Rep. 12:56-62 (2011).
44. Y.S. Wang, H.Y. Wang, Y.C. Liao, P.C. Tsai, K.C. Chen, H.Y. Cheng, R.T. Lin, and S.H. Juo. MicroRNA-195 regulates vascular smooth muscle cell phenotype and prevents neointimal formation. Cardiovasc Res. 95:517-526 (2012).
45. S. Wassmann, K. Wassmann, A. Jung, M. Velten, P. Knuefermann, V. Petoumenos, U. Becher, C. Werner, C. Mueller, and G. Nickenig. Induction of p53 by GKLF is essential for inhibition of proliferation of vascular smooth muscle cells. J Mol Cell Cardiol. 43:301-307 (2007).
46. T. Yoshida, K.H. Kaestner, and G.K. Owens. Conditional deletion of Kruppel-like factor 4 delays downregulation of smooth muscle cell differentiation markers but accelerates neointimal formation following vascular injury. Circ Res. 102:1548-1557 (2008).
47. J. Chen, H. Yin, Y. Jiang, S.K. Radhakrishnan, Z.P. Huang, J. Li, Z. Shi, E.P. Kilsdonk, Y. Gui, D.Z. Wang, and X.L. Zheng. Induction of microRNA-1 by myocardin in smooth muscle cells inhibits cell proliferation. Arterioscler Thromb Vasc Biol. 31:368-375 (2011).
48. D. Torella, C. Iaconetti, D. Catalucci, G.M. Ellison, A. Leone, C.D. Waring, A. Bochicchio, C. Vicinanza, I. Aquila, A. Curcio, G. Condorelli, and C. Indolfi. MicroRNA-133 controls vascular smooth muscle cell phenotypic switch in vitro and vascular remodeling in vivo. Circ Res. 109:880-893 (2011).
49. N. Sakalihasan, R. Limet, and O.D. Defawe. Abdominal aortic aneurysm. Lancet. 365:1577-1589 (2005).
50. F.A. Lederle, G.R. Johnson, S.E. Wilson, E.P. Chute, F.N. Littooy, D. Bandyk, W.C. Krupski, G.W. Barone, C.W. Acher, and D.J. Ballard. Prevalence and associations of abdominal aortic aneurysm detected through screening. Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Group. Ann Intern Med. 126:441-449 (1997).
51. J.F. Blanchard, H.K. Armenian, and P.P. Friesen. Risk factors for abdominal aortic aneurysm: results of a case-control study. Am J Epidemiol. 151:575-583 (2000).
52. A.R. Brady, S.G. Thompson, F.G. Fowkes, R.M. Greenhalgh, and J.T. Powell. Abdominal aortic aneurysm expansion: risk factors and time intervals for surveillance. Circulation. 110:16-21 (2004).
53. J. Golledge, M. Karan, C.S. Moran, J. Muller, P. Clancy, A.E. Dear, and P.E. Norman. Reduced expansion rate of abdominal aortic aneurysms in patients with diabetes may be related to aberrant monocyte-matrix interactions. Eur Heart J. 29:665-672 (2008).
54. A. Helgadottir, G. Thorleifsson, K.P. Magnusson, S. Gretarsdottir, V. Steinthorsdottir, A. Manolescu, G.T. Jones, G.J. Rinkel, J.D. Blankensteijn, A. Ronkainen, J.E. Jaaskelainen, Y. Kyo, G.M. Lenk, N. Sakalihasan, K. Kostulas, A. Gottsater, A. Flex, H. Stefansson, T. Hansen, G. Andersen, S. Weinsheimer, K. Borch-Johnsen, T. Jorgensen, S.H. Shah, A.A. Quyyumi, C.B. Granger, M.P. Reilly, H. Austin, A.I. Levey, V. Vaccarino, E. Palsdottir, G.B. Walters, T. Jonsdottir, S. Snorradottir, D. Magnusdottir, G. Gudmundsson, R.E. Ferrell, S. Sveinbjornsdottir, J. Hernesniemi, M. Niemela, R. Limet, K. Andersen, G. Sigurdsson, R. Benediktsson, E.L. Verhoeven, J.A. Teijink, D.E. Grobbee, D.J. Rader, D.A. Collier, O. Pedersen, R. Pola, J. Hillert, B. Lindblad, E.M. Valdimarsson, H.B. Magnadottir, C. Wijmenga, G. Tromp, A.F. Baas, Y.M. Ruigrok, A.M. van Rij, H. Kuivaniemi, J.T. Powell, S.E. Matthiasson, J.R. Gulcher, G. Thorgeirsson, A. Kong, U. Thorsteinsdottir, and K. Stefansson. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet. 40:217-224 (2008).
55. R.A. Boon, T. Seeger, S. Heydt, A. Fischer, E. Hergenreider, A.J. Horrevoets, M. Vinciguerra, N. Rosenthal, S. Sciacca, M. Pilato, P. van Heijningen, J. Essers, R.P. Brandes, A.M. Zeiher, and S. Dimmeler. MicroRNA-29 in aortic dilation: implications for aneurysm formation. Circ Res. 109:1115-1119 (2011).

56. H.C. Dietz, G.R. Cutting, R.E. Pyeritz, C.L. Maslen, L.Y. Sakai, G.M. Corson, E.G. Puffenberger, A. Hamosh, E.J. Nanthakumar, S.M. Curristin, and et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 352:337-339 (1991).
57. S. Sengupta, J.A. den Boon, I.H. Chen, M.A. Newton, S.A. Stanhope, Y.J. Cheng, C.J. Chen, A. Hildesheim, B. Sugden, and P. Ahlquist. MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. Proc Natl Acad Sci U S A. 105:5874-5878 (2008).
58. J.A. Jones, R.E. Stroud, E.C. O'Quinn, L.E. Black, J.L. Barth, J.A. Elefteriades, J.E. Bavaria, J.H. Gorman, 3rd, R.C. Gorman, F.G. Spinale, and J.S. Ikonomidis. Selective microRNA suppression in human thoracic aneurysms: relationship of miR-29a to aortic size and proteolytic induction. Circ Cardiovasc Genet. 4:605-613 (2011).
59. R.A. Boonand S. Dimmeler. MicroRNAs and aneurysm formation. Trends Cardiovasc Med. 21:172-177 (2011).
60. L. Maegdefessel, J. Azuma, R. Toh, D.R. Merk, A. Deng, J.T. Chin, U. Raaz, A.M. Schoelmerich, A. Raiesdana, N.J. Leeper, M.V. McConnell, R.L. Dalman, J.M. Spin, and P.S. Tsao. Inhibition of microRNA-29b reduces murine abdominal aortic aneurysm development. J Clin Invest. 122:497-506 (2012).
61. D.R. Merk, J.T. Chin, B.A. Dake, L. Maegdefessel, M.O. Miller, N. Kimura, P.S. Tsao, C. Iosef, G.J. Berry, F.W. Mohr, J.M. Spin, C.M. Alvira, R.C. Robbins, and M.P. Fischbein. miR-29b participates in early aneurysm development in Marfan syndrome. Circ Res. 110:312-324 (2012).
62. L. Maegdefessel, J. Azuma, R. Toh, A. Deng, D.R. Merk, A. Raiesdana, N.J. Leeper, U. Raaz, A.M. Schoelmerich, M.V. McConnell, R.L. Dalman, J.M. Spin, and P.S. Tsao. MicroRNA-21 blocks abdominal aortic aneurysm development and nicotine-augmented expansion. Sci Transl Med. 4:122ra122 (2012).
63. Y.H. Shen, L. Zhang, P. Ren, M.T. Nguyen, S. Zou, D. Wu, X.L. Wang, J. Coselli, and S.A. Lemaire. AKT2 Confers Protection Against Aortic Aneurysms and Dissections. Circ Res (2012).
64. M.R. Macleanand Y. Dempsie. The serotonin hypothesis of pulmonary hypertension revisited. Adv Exp Med Biol. 661:309-322 (2010).
65. K.B. Lane, R.D. Machado, M.W. Pauciulo, J.R. Thomson, J.A. Phillips, 3rd, J.E. Loyd, W.C. Nichols, and R.C. Trembath. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. Nat Genet. 26:81-84 (2000).
66. P. Caruso, M.R. MacLean, R. Khanin, J. McClure, E. Soon, M. Southgate, R.A. MacDonald, J.A. Greig, K.E. Robertson, R. Masson, L. Denby, Y. Dempsie, L. Long, N.W. Morrell, and A.H. Baker. Dynamic changes in lung microRNA profiles during the development of pulmonary hypertension due to chronic hypoxia and monocrotaline. Arterioscler Thromb Vasc Biol. 30:716-723 (2010).
67. A. Courboulin, R. Paulin, N.J. Giguere, N. Saksouk, T. Perreault, J. Meloche, E.R. Paquet, S. Biardel, S. Provencher, J. Cote, M.J. Simard, and S. Bonnet. Role for miR-204 in human pulmonary arterial hypertension. J Exp Med. 208:535-548 (2011).
68. S. Yang, S. Banerjee, A. Freitas, H. Cui, N. Xie, E. Abraham, and G. Liu. miR-21 regulates chronic hypoxia-induced pulmonary vascular remodeling. Am J Physiol Lung Cell Mol Physiol. 302:L521-529 (2012).
69. S.S. Pullamsetti, C. Doebele, A. Fischer, R. Savai, B. Kojonazarov, B.K. Dahal, H.A. Ghofrani, N. Weissmann, F. Grimminger, A. Bonauer, W. Seeger, A.M. Zeiher, S. Dimmeler, and R.T. Schermuly. Inhibition of microRNA-17 improves lung and heart function in experimental pulmonary hypertension. Am J Respir Crit Care Med. 185:409-419 (2012).
70. V.N. Parikh, R.C. Jin, S. Rabello, N. Gulbahce, K. White, A. Hale, K.A. Cottrill, R.S. Shaik, A.B. Waxman, Y.Y. Zhang, B.A. Maron, J.C. Hartner, Y. Fujiwara, S.H. Orkin, K.J. Haley, A.L. Barabasi, J. Loscalzo, and S.Y. Chan. MicroRNA-21 integrates pathogenic signaling to control pulmonary hypertension: results of a network bioinformatics approach. Circulation. 125:1520-1532 (2012).

71. C.L. Bockmeyer, L. Maegel, S. Janciauskiene, J. Rische, U. Lehmann, U.A. Maus, N. Nickel, A. Haverich, M.M. Hoeper, H.A. Golpon, H. Kreipe, F. Laenger, and D. Jonigk. Plexiform vasculopathy of severe pulmonary arterial hypertension and microRNA expression. J Heart Lung Transplant. 31:764-772 (2012).
72. B. Wojciak-Stothard, L. Zhao, E. Oliver, O. Dubois, Y. Wu, D. Kardassis, E. Vasilaki, M. Huang, J.A. Mitchell, L.S. Harrington, G.C. Prendergast, and M.R. Wilkins. Role of RhoB in the regulation of pulmonary endothelial and smooth muscle cell responses to hypoxia. Circ Res. 110:1423-1434 (2012).
73. K.M. Drake, D. Zygmunt, L. Mavrakis, P. Harbor, L. Wang, S.A. Comhair, S.C. Erzurum, and M.A. Aldred. Altered MicroRNA processing in heritable pulmonary arterial hypertension: an important role for Smad-8. Am J Respir Crit Care Med. 184:1400-1408 (2011).
74. M. Brock, M. Trenkmann, R.E. Gay, B.A. Michel, S. Gay, M. Fischler, S. Ulrich, R. Speich, and L.C. Huber. Interleukin-6 modulates the expression of the bone morphogenic protein receptor type II through a novel STAT3-microRNA cluster 17/92 pathway. Circ Res. 104:1184-1191 (2009).
75. M. Brock, V.J. Samillan, M. Trenkmann, C. Schwarzwald, S. Ulrich, R.E. Gay, M. Gassmann, L. Ostergaard, S. Gay, R. Speich, and L.C. Huber. AntagomiR directed against miR-20a restores functional BMPR2 signalling and prevents vascular remodelling in hypoxia-induced pulmonary hypertension. Eur Heart J (2012).
76. A. Bonauer, G. Carmona, M. Iwasaki, M. Mione, M. Koyanagi, A. Fischer, J. Burchfield, H. Fox, C. Doebele, K. Ohtani, E. Chavakis, M. Potente, M. Tjwa, C. Urbich, A.M. Zeiher, and S. Dimmeler. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science. 324:1710-1713 (2009).
77. C. Doebele, A. Bonauer, A. Fischer, A. Scholz, Y. Reiss, C. Urbich, W.K. Hofmann, A.M. Zeiher, and S. Dimmeler. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. Blood. 115:4944-4950 (2010).
78. O.D. Liang, S.A. Mitsialis, M.S. Chang, E. Vergadi, C. Lee, M. Aslam, A. Fernandez-Gonzalez, X. Liu, R. Baveja, and S. Kourembanas. Mesenchymal stromal cells expressing heme oxygenase-1 reverse pulmonary hypertension. Stem Cells. 29:99-107 (2011).
79. S.R. Baber, W. Deng, R.G. Master, B.A. Bunnell, B.K. Taylor, S.N. Murthy, A.L. Hyman, and P.J. Kadowitz. Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction. Am J Physiol Heart Circ Physiol. 292:H1120-1128 (2007).
80. C. Lee, S.A. Mitsialis, M. Aslam, S.H. Vitali, E. Vergadi, G. Konstantinou, K. Sdrimas, A. Fernandez-Gonzalez, and S. Kourembanas. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. Circulation. 126:2601-2611 (2012).
81. P. Caruso, Y. Dempsie, H.C. Stevens, R.A. McDonald, L. Long, R. Lu, K. White, K.M. Mair, J.D. McClure, M. Southwood, P. Upton, M. Xin, E. van Rooij, E.N. Olson, N.W. Morrell, M.R. MacLean, and A.H. Baker. A role for miR-145 in pulmonary arterial hypertension: evidence from mouse models and patient samples. Circ Res. 111:290-300 (2012).
82. B.N. Davis-Dusenbery, M.C. Chan, K.E. Reno, A.S. Weisman, M.D. Layne, G. Lagna, and A. Hata. 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. 286:28097-28110 (2011).
83. X. Longand J.M. Miano. Transforming growth factor-beta1 (TGF-beta1) utilizes distinct pathways for the transcriptional activation of microRNA 143/145 in human coronary artery smooth muscle cells. J Biol Chem. 286:30119-30129 (2011).
84. E.D. Cohen, K. Ihida-Stansbury, M.M. Lu, R.A. Panettieri, P.L. Jones, and E.E. Morrisey. Wnt signaling regulates smooth muscle precursor development in the mouse lung via a tenascin C/PDGFR pathway. J Clin Invest. 119:2538-2549 (2009).

85. S. Jalali, G.K. Ramanathan, P.T. Parthasarathy, S. Aljubran, L. Galam, A. Yunus, S. Garcia, R.R. Cox, Jr., R.F. Lockey, and N. Kolliputi. Mir-206 regulates pulmonary artery smooth muscle cell proliferation and differentiation. PLoS One. 7:e46808 (2012).
86. D. Gou, R. Ramchandran, X. Peng, L. Yao, K. Kang, J. Sarkar, Z. Wang, G. Zhou, and J.U. Raj. miR-210 has an antiapoptotic effect in pulmonary artery smooth muscle cells during hypoxia. Am J Physiol Lung Cell Mol Physiol. 303:L682-691 (2012).
87. L. Guo, Z. Qiu, L. Wei, X. Yu, X. Gao, S. Jiang, H. Tian, C. Jiang, and D. Zhu. The microRNA-328 regulates hypoxic pulmonary hypertension by targeting at insulin growth factor 1 receptor and L-type calcium channel-alpha1C. Hypertension. 59:1006-1013 (2012).
88. C.M. Lawes, S. Vander Hoorn, and A. Rodgers. Global burden of blood-pressure-related disease, 2001. Lancet. 371:1513-1518 (2008).
89. W.H. Wu, C.P. Hu, X.P. Chen, W.F. Zhang, X.W. Li, X.M. Xiong, and Y.J. Li. MicroRNA-130a mediates proliferation of vascular smooth muscle cells in hypertension. Am J Hypertens. 24:1087-1093 (2011).
90. S. Batkaiand T. Thum. MicroRNAs in hypertension: mechanisms and therapeutic targets. Curr Hypertens Rep. 14:79-87 (2012).
91. E. van Rooijand E.N. Olson. MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. Nat Rev Drug Discov. 11:860-872 (2012).
92. M.S. Karbalaei, C. Rippe, S. Albinsson, M. Ekman, A. Mansten, B. Uvelius, and K. Sward. Impaired contractility and detrusor hypertrophy in cavin-1-deficient mice. Eur J Pharmacol. 689:179-185 (2012).
93. M.L. Yu, J.F. Wang, G.K. Wang, X.H. You, X.X. Zhao, Q. Jing, and Y.W. Qin. Vascular smooth muscle cell proliferation is influenced by let-7d microRNA and its interaction with KRAS. Circ J. 75:703-709 (2011).
94. M. Imamura, Y. Sugino, X. Long, O.J. Slivano, N. Nishikawa, N. Yoshimura, and J.M. Miano. Myocardin and MicroRNA-1 modulate bladder activity through connexin 43 expression during post-natal development. J Cell Physiol (2013).
95. C. Xie, H. Huang, X. Sun, Y. Guo, M. Hamblin, R.P. Ritchie, M.T. Garcia-Barrio, J. Zhang, and Y.E. Chen. MicroRNA-1 regulates smooth muscle cell differentiation by repressing Kruppel-like factor 4. Stem Cells Dev. 20:205-210 (2011).
96. T. Minami, K. Kuwahara, Y. Nakagawa, M. Takaoka, H. Kinoshita, K. Nakao, Y. Kuwabara, Y. Yamada, C. Yamada, J. Shibata, S. Usami, S. Yasuno, T. Nishikimi, K. Ueshima, M. Sata, H. Nakano, T. Seno, Y. Kawahito, K. Sobue, A. Kimura, and R. Nagai. Reciprocal expression of MRTF-A and myocardin is crucial for pathological vascular remodelling in mice. EMBO J. 31:4428-4440 (2012).
97. B.N. Davis, A.C. Hilyard, G. Lagna, and A. Hata. SMAD proteins control DROSHA-mediated microRNA maturation. Nature. 454:56-61 (2008).
98. J. Sarkar, D. Gou, P. Turaka, E. Viktorova, R. Ramchandran, and J.U. Raj. MicroRNA-21 plays a role in hypoxia-mediated pulmonary artery smooth muscle cell proliferation and migration. Am J Physiol Lung Cell Mol Physiol. 299:L861-871 (2010).
99. K.U. Kotlo, B. Hesabi, and R.S. Danziger. Implication of microRNAs in atrial natriuretic peptide and nitric oxide signaling in vascular smooth muscle cells. Am J Physiol Cell Physiol. 301:C929-937 (2011).
100. M. Wang, W. Li, G.Q. Chang, C.S. Ye, J.S. Ou, X.X. Li, Y. Liu, T.Y. Cheang, X.L. Huang, and S.M. Wang. MicroRNA-21 regulates vascular smooth muscle cell function via targeting tropomyosin 1 in arteriosclerosis obliterans of lower extremities. Arterioscler Thromb Vasc Biol. 31:2044-2053 (2011).
101. G. Yang, Y. Pei, Q. Cao, and R. Wang. MicroRNA-21 represses human cystathionine gamma-lyase expression by targeting at specificity protein-1 in smooth muscle cells. J Cell Physiol. 227:3192-3200 (2012).
102. H. Kang, B.N. Davis-Dusenbery, P.H. Nguyen, A. Lal, J. Lieberman, L. Van Aelst, G. Lagna, and A. Hata. Bone morphogenetic protein 4 promotes vascular smooth muscle contractility by

activating microRNA-21 (miR-21), which down-regulates expression of family of dedicator of cytokinesis (DOCK) proteins. J Biol Chem. 287:3976-3986 (2012).
103. M.C. Chan, A.C. Hilyard, C. Wu, B.N. Davis, N.S. Hill, A. Lal, J. Lieberman, G. Lagna, and A. Hata. Molecular basis for antagonism between PDGF and the TGFbeta family of signalling pathways by control of miR-24 expression. EMBO J. 29:559-573 (2010).
104. J.S. Mohamed, M.A. Lopez, and A.M. Boriek. Mechanical stretch up-regulates microRNA-26a and induces human airway smooth muscle hypertrophy by suppressing glycogen synthase kinase-3beta. J Biol Chem. 285:29336-29347 (2010).
105. N.J. Leeper, A. Raiesdana, Y. Kojima, H.J. Chun, J. Azuma, L. Maegdefessel, R.K. Kundu, T. Quertermous, P.S. Tsao, and J.M. Spin. MicroRNA-26a is a novel regulator of vascular smooth muscle cell function. J Cell Physiol. 226:1035-1043 (2011).
106. P. Zhang, A. Huang, J. Ferruzzi, R.P. Mecham, B.C. Starcher, G. Tellides, J.D. Humphrey, F.J. Giordano, L.E. Niklason, and W.C. Sessa. Inhibition of microRNA-29 enhances elastin levels in cells haploinsufficient for elastin and in bioengineered vessels--brief report. Arterioscler Thromb Vasc Biol. 32:756-759 (2012).
107. Y. Du, C. Gao, Z. Liu, L. Wang, B. Liu, F. He, T. Zhang, Y. Wang, X. Wang, M. Xu, G.Z. Luo, Y. Zhu, Q. Xu, and W. Kong. Upregulation of a disintegrin and metalloproteinase with thrombospondin motifs-7 by miR-29 repression mediates vascular smooth muscle calcification. Arterioscler Thromb Vasc Biol. 32:2580-2588 (2012).
108. K.C. Chen, Y.S. Wang, C.Y. Hu, W.C. Chang, Y.C. Liao, C.Y. Dai, and S.H. Juo. OxLDL up-regulates microRNA-29b, leading to epigenetic modifications of MMP-2/MMP-9 genes: a novel mechanism for cardiovascular diseases. FASEB J. 25:1718-1728 (2011).
109. J.A. Balderman, H.Y. Lee, C.E. Mahoney, D.E. Handy, K. White, S. Annis, D. Lebeche, R.J. Hajjar, J. Loscalzo, and J.A. Leopold. Bone morphogenetic protein-2 decreases microRNA-30b and microRNA-30c to promote vascular smooth muscle cell calcification. J Am Heart Assoc. 1:e003905 (2012).
110. C. Goettsch, M. Rauner, N. Pacyna, U. Hempel, S.R. Bornstein, and L.C. Hofbauer. miR-125b regulates calcification of vascular smooth muscle cells. Am J Pathol. 179:1594-1600 (2011).
111. X. Wang, G. Hu, and J. Zhou. Repression of versican expression by microRNA-143. J Biol Chem. 285:23241-23250 (2010).
112. H.N. Horita, P.A. Simpson, A. Ostriker, S. Furgeson, V. Van Putten, M.C. Weiser-Evans, and R.A. Nemenoff. Serum response factor regulates expression of phosphatase and tensin homolog through a microRNA network in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 31:2909-2919 (2011).
113. L. Zeng, A.D. Carter, and S.J. Childs. miR-145 directs intestinal maturation in zebrafish. Proc Natl Acad Sci U S A. 106:17793-17798 (2009).

Figure 1.tiff
Click here to download high resolution image

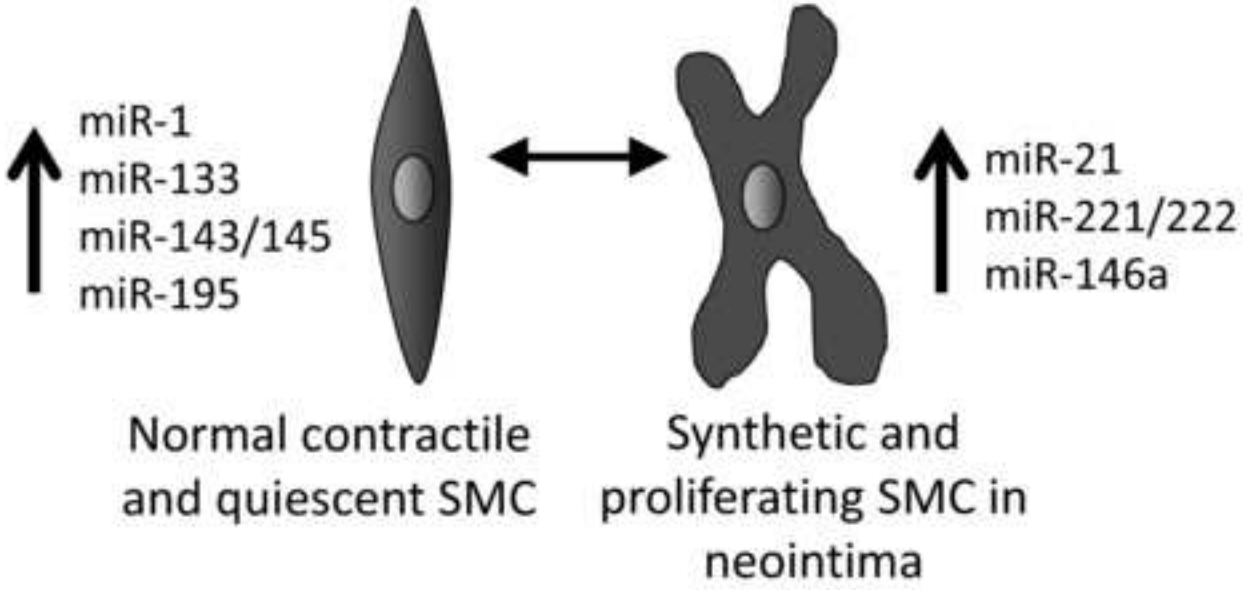

Figure 2.tiff
Click here to download high resolution image

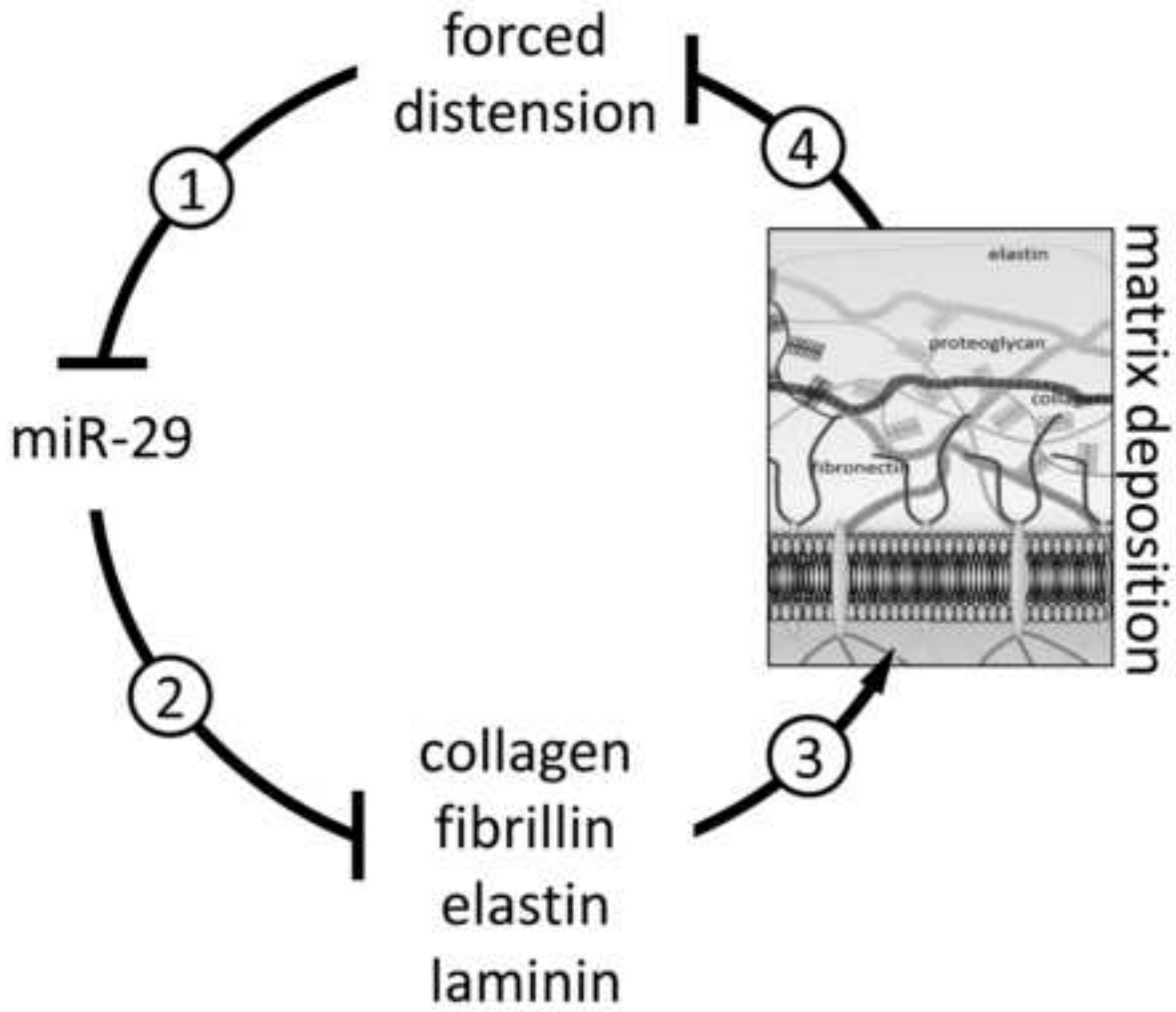

Table1 R1.docx

SMC miR(s)	Selected predicted targets (official human gene symbols are given)	Selected experimentally validated targets in smooth muscle
let-7acdef-5p	HMGA2, *YOD1*, **ADRB3**, DIAPH2, *ACVR1C*, **IGF1R**, **TGFBR1**, LIN28, **ADRB2**, *MAPK6*, **MAP3K1**, ***COL3A1***, **COL1A2**	KRAS (93)
miR-1a-3p	***COL4A3***, **GJA1**, DIAPH2, CORO1C , ***CAV2***, IGF1, FN1, LPPR4, ***FRS2***	GJA1 (94), PIM1 (47), KLF4 (95), MKL1 (96)
miR-16/195a-5p	AFAP1L1, **ATP7A**, ACTR2, PAPPA, PRKAR2A, ASH1L, **KL**, LRP6, **HTR4**, INSR, USP15, **PURA**, *SEMA3A*, SLC7A2, CCNE1, WEE1, *SSTR1*	CDC42, CCND1 (44),
miR-21a-5p	*YOD1*, WISP1, PBRM1, SKI, CCL20, SPRY1, KLF3, RECK, **VCL**, NEDD4, ITGA4, PGR, ***FRS2***, **SMAD7**	PTEN (40), PDCD4 (97), PPARA, SPRY2 (98), MPRIP, PRKG1, CFL2 (99), TPM1 (100), SP1 (101), DOCK4,5,7 (102)
miR-22-3p	CLIC4, **PTGS1**, HRH1, VEZF1, TRPC1, **ROCK2**, RCOR1, CSPG4, **AKT3**, KCND1, **VCAM1**, **SIRT1**, ARPC5, PGR, *OGN*	
miR-23ab-3p	PDE4B, CUGBP2, ROBO2, CTCF, PPP1R3C, ABI2, **HAS2**, **PPARGC1A**, **CAB39**, *TNRC6A*, **MAP3K1**, **ATP7A**, PPM1D, CD55, *ACVR1C*, ***CAV2***, **PTGER4**, ITGB8	
miR-24-3p	IL15RA, PTHLH, NTSR1, HSPB7, NDST1, **CDKN1B**, FASLG, HOXC11, *ACVR1C*, AHSG, **PDE1A**, KCNE1, GSTT1, **ADIPOR2**, TMEM173, **MKL2**, DIAPH1	TRIB3 (103)
miR-26ab-5p	DOCK4, PLOD2, NAB1, ULK1, PTGS2, SLC7A11, ***PTEN***, CHFR, SGCB, ULK2, **PRKCQ**, LHFPL3, **STK39**, ADAM9, **TLR3**, VANGL2, SLC38A2, LRRC16A, *ACVR1C*, *KCNJ2*, **ATF2**, *MAPK6*, **JAG1**	GSK3B (104), SMAD1,4 (105)
miR-27ab-3p	*ACVR1C*, **ADORA2B**, FGF5, *OGN*, RUNX1, ***KRAS***, HOXB8, AHR, CXCL11, *TNFSF4*, MKLN1, **FGF1**, **PPARG**, EYA1	
miR-29abc-3p	**COL4A5**, **ELN**, **FBN1**, ***COL4A3***, COL11A1, ***COL3A1***, COL5A1, HBP1, TDG, **COL1A1**, HAS3, **VEGFA**, **IGF1**, COL5A2, ***PTEN***, ADAMTS9, PXDN, CD109, **RND3**, CLDN1, SESTD1, **HDAC4**	COL1A1, COL3A1 (55) ELN (106), ADAMTS7 (107), DNMT3B (108)
miR-30abcd -5p	CYP24A1, *TNRC6A*, **RUNX2**, LEPR, SGCB, SCN9A, *RAPGEF4*, NFAT5, CCNE2, *TNFSF4*, **NT5E**, **CTH**, RUNX1, RARG, PRKAA2, GLI2, DDAH1, **CALCR**, **ABCC9**, STIM2	RUNX2 (109)
miR-125ab-5p	**ACHE**, *TNFSF4*, LFNG, ENPEP, **VDR**, STARD13, TRIM71, VPS4B, LMOD1, SPEG	SUV39H1 (31), SP7 (110),
miR-143-3p	MAP9, ABL2, ***KRAS***, PGR, **MMP16**, ARHGEF15, ERBB4, *SSTR1*, **IGFBP5**, **ADAMTS1**, *SSH2*, ASB5, FADS1, CLCN3	ELK1 (8), ADD3, SSH2, MKL2 (10), PDGFRA, PRKCE (11), VCAN (111), KLF4 (82), FOSL1 (112)
miR-145a-5p	**FSCN1**, ADCYAP1, DAB2, *SEMA3A*, YES1, ARF6, INSIG1, **MKL2**, *SSH2*, SLC24A4, ITGB8, **PPP3CA**, XRN1, PHACTR2, QKI, **SMAD3**, **NTRK2**, ZFYVE9	KLF5 (39), KLF4, CAMK2D, MYOCD (8), SRGAP1,2 (10), ACE (7), GATA6 (113), FSCN1 (11), PHACTR4 (25)
miR-365-3p	E2F2, RICTOR, *KCNJ2*, **CNTF**, *RAPGEF4*, **SGK1**, ARRDC3, **MYLK**, **ARRB2**, **NR3C2**, ***IGF1***, ANKRD17, OAZ2, SDHC, **ADM**, CYP19A1	

Graphical abstract.tif
Click here to download high resolution image