

MicroRNAs in Hypertension: Mechanisms and Therapeutic Targets

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Abstract Hypertension is a complex, multifactorial disease, and its development is determined by a combination of genetic susceptibility and environmental factors. Several mechanisms have been implicated in the pathogenesis of hypertension: increased activity of the sympathetic nervous system, overactivation of the renin-angiotensin aldosterone system (RAAS), dysfunction of vascular endothelium, impaired platelet function, thrombogenesis, vascular smooth muscle and cardiac hypertrophy, and altered angiogenesis. MicroRNAs are short, noncoding nucleotides regulating target messenger RNAs at the post-transcriptional level. MicroRNAs are involved in virtually all biologic processes, including cellular proliferation, apoptosis, and differentiation. Thus, microRNA deregulation often results in impaired cellular function and disease development, so microRNAs have potential therapeutic relevance. Many aspects of the development of essential hypertension at the molecular level are still unknown. The elucidation of these processes regulated by microRNAs and the identification of novel microRNA targets in the pathogenesis of hypertension is a highly valuable and exciting strategy that may eventually lead to the development of novel treatment approaches for hypertension. This article reviews the potential role of

microRNAs in the mechanisms associated with the development and consequences of hypertension and discusses advances in microRNA-based approaches that may be important in treating hypertension.

Keywords MicroRNA · Hypertension · Blood pressure · Vascular remodeling · Endothelial function · Renin-angiotensin-aldosterone system · RAAS · Heart · Kidney · Mechanisms · Treatment

Introduction

Hypertension, persistent elevation of systemic blood pressure, is one of the most common medical conditions involving the cardiovascular system. Despite continuous advancement in treatment options, it is an increasingly important health problem [1], affecting as many as 1 billion people worldwide with high associated morbidity and mortality. Chronic elevation of blood pressure is an undisputed risk factor for cardiovascular diseases such as myocardial infarction and stroke, and it is also a leading cause of chronic kidney failure [2].

Hypertension is classified as “essential,” with no obvious medical cause, in about 90% to 95% of cases. The remaining cases are caused by various identifiable medical conditions affecting the kidneys, the heart, or the endocrine system.

It is widely acknowledged that hypertension is a complex, multifactorial disease. Its development may involve many different genes, each with mild effects but reacting to environmental stimuli [3]. Blood pressure is tightly regulated in the cardiovascular system to ensure adequate perfusion to all tissues, and it is determined by the interaction of various neurohormonal, cardiac, renal, and vascular mechanisms. Hypertension develops on the basis

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of genetic susceptibility [3, 4•] and environmental factors through aberrant regulation of vascular tone and renal sodium excretion, leading to functional and structural changes in the cardiovascular system. These pathologic features may be present before the blood pressure elevation is sustained [5]. Several mechanisms have been implicated in the pathogenesis of hypertension: increased activity of the sympathetic nervous system, overactivation of the renin-angiotensin-aldosterone system (RAAS), dysfunction of the vascular endothelium, impaired platelet function, thrombogenesis, vascular smooth muscle and cardiac hypertrophy, and altered angiogenesis [6]. When hypertension persists, these underlying mechanisms are responsible for the gradual development of pathologic manifestations in the form of vascular and renal diseases such as arteriosclerotic vascular disease, retinopathy, vascular dementia, nephropathy, cardiac hypertrophy, stroke, and kidney and heart failure.

Since their discovery in 1993 as transcriptional regulators, microRNAs have been implicated in numerous biologic processes determining cell fate, apoptosis, proliferation, or stress response [7]. A multitude of studies have demonstrated associations between diseases and specific microRNAs, especially in the cardiovascular system [8•].

This review is aimed to provide an overview of the likely role of microRNAs in the development and consequences of hypertension, as well as advances in microRNA-based therapeutic approaches. Better understanding of the molecular processes in the pathogenesis of hypertension is valuable in guiding efforts to devise novel treatment strategies to prevent the consequences associated with hypertension.

MicroRNA Functions

MicroRNAs are evolutionarily conserved, short, noncoding RNAs. MicroRNAs regulate target messenger RNA translation by binding mainly to complementary sequences of the 3′-untranslated region (UTR) of target messenger RNA transcripts, thereby leading to RNA degradation and/or inhibition of protein synthesis [9]. MicroRNAs are abundant in all human cells, although at different levels; the estimated number of microRNA genes that the human genome encodes is well above 1,000, and they regulate the activity of about 50% of the genome [10, 11]. MicroRNA biogenesis has been extensively studied.

Transcription of the primary microRNAs by RNA polymerase II forms a hairpin structure called precursor microRNA. The precursor microRNA associates with exportin 5 and Ran-GTP and is transported into the cytoplasm, where it is cleaved by Dicer, and processed into a double-stranded product consisting of 22 nucleotides.

The guide strand of the mature microRNA is incorporated into the RNA-induced silencing complex (RISC). The RISC-microRNA complex specifically targets mRNAs and leads to suppression of protein synthesis or mRNA degradation [11, 12].

One important aspect of the transcriptional regulation by microRNAs is the fact that each individual microRNA regulates a comprehensive set of genes, thus affecting cellular pathways and governing biologic function.

Endothelial MicroRNAs in Hypertension

The vascular endothelium contributes to the pathogenesis of hypertension in several ways. In the hypertensive state, the steady exposure to high intraluminal pressure leads to the activation of endothelial cells, the release of inflammatory and procoagulant mediators, and the adherence of neutrophils and platelets. As a result, the endothelium becomes dysfunctional, leading to impaired vasodilatation and a proinflammatory and prothrombic phenotype of the vessel wall [13]. Endothelial dysfunction is closely associated with organ damage and clinical prognosis in patients with hypertension [14]. Endothelial cells are also crucial players in the development, maintenance, and remodeling of vascular networks. A typical endothelium-mediated pathogenetic feature of hypertension is the loss of functional microvessels (rarefaction) [15], and defective angiogenesis at the target organ level. One of the fundamental pathways that is altered in hypertension is vascular endothelial growth factor (VEGF) signaling. VEGF, the main angiogenic factor, is also involved in blood pressure regulation. Importantly, the main adverse effect of anti-VEGF therapies is the development of hypertension.

Growing evidence suggests that microRNA biogenesis, as well as specific microRNAs, may be important in the pathogenesis of endothelial dysfunction and reduced angiogenic capacity in hypertension. In endothelial cells, Dicer is constitutively expressed and has a prominent role in angiogenesis. The lack of Dicer both in vitro and in vivo results in profoundly dysregulated angiogenesis and redox signaling [16–18].

Many specific microRNAs and their targets that are involved in angiogenesis have been recently identified. The endothelial-specific microRNA, miR-126, has been found to be necessary for vascular integrity and angiogenesis. Targeted deletion of miR-126 leads to leaky vessels, hemorrhage, and partial embryonic lethality [19]. Also, miR-126 controls endothelial response to VEGF through inhibition of angiogenic kinases such as SPRED-1 and/or the PIK3 regulatory subunit-2 [19, 20], and it also regulates the expression of vascular cell adhesion molecule VCAM-1, a mediator in inflammatory adhesion [21]. In addition, miR-126, transferred

by endothelial apoptotic bodies, induces the production of the chemokine CXCL12, conferring protection against apoptosis and mobilization of endothelial progenitor cells [22]. A recent study demonstrated that miR-21 plays a role in the integration of haemodynamics and VEGF signaling during angiogenesis [23•].

In another study, miR-21 has been found to inhibit angiogenesis by targeting RhoB expression in endothelial cells [24]. Upregulation of miR-21 is also found in circulating angiogenic cells in patients with coronary artery disease, leading to cellular dysfunction [25]. MicroRNA profiling from plasma samples of stroke patients showed differential regulation of many microRNAs involved in angiogenesis, indicating that endothelial function is indeed an essential determinant of stroke recovery [26, 27].

Senescence of endothelial cells may contribute to cardiovascular diseases, including hypertension. MiR-217 was found to be directly involved in endothelial senescence by affecting the expression of silent information regulator 1 (SirT1), leading to the loss of its function on its major endothelial targets, FoxO1 and endothelial nitric oxide synthase (eNOS) [28].

Genetic predisposition to essential hypertension has been linked to altered endothelial function and decreased L-arginine and nitric oxide (NO) metabolism due to a polymorphism in the 3'-UTR of human L-arginine transporter SLC7A1 [29]. The minor allele contains one more potential miR-122 binding site and significantly attenuates reporter gene expression, lowering SLC7A1 levels. Thus, miR-122 likely contributes to the endothelial dysfunction seen in hypertensive individuals, but clinical relevance still needs to be confirmed [30]. The eNOS inhibitor asymmetric dimethylarginine (ADMA) also leads to microRNA deregulation in circulating angiogenic cells and impairs their function by a miR-21-dependent mechanism [25]. Hypoxia-mediated upregulation of miR-24 was shown to result in impaired endothelial function and reduced capillary density, which could be improved by treatment with miR-24 antagonists, chemically modified oligonucleotides targeting the microRNA [31].

Many other microRNAs have been found to be pro-angiogenic (miR-27b, -130a, -210, -378, -17–92 cluster and let-7f) or to have anti-angiogenic effects (miR-15, -16, -20a, -20b, -24, -221, -222), as reviewed elsewhere [32, 33].

Renal MicroRNAs in Hypertension

Kidneys have a complex role in the maintenance of blood pressure: hypertension can be the cause or the result of renal disease. The vascular peripheral resistance and cardiac output, which determine blood pressure, are regulated by renal mechanisms involving sodium homeostasis, blood

volume, and arteriolar resistance mainly through the RAAS. Persistent hypertension induces localized damage to the glomeruli, and the development of necrotic glomerulosclerosis is the hallmark of hypertensive kidney damage. On the other hand, hypertension is often the result of kidney disease or genetic or developmental kidney malformation. Indeed, decreased glomerular number at birth is often the cause of hypertension [34].

The striking phenotype of Dicer and specific-microRNA knockout animals has implicated the fundamental role of microRNAs in kidney development and function. The role of microRNAs in kidney physiology and disease has been recently reviewed [35, 36, 37••].

In the well-established model of the Dahl salt-sensitive form of hypertension, no difference has been found between normotensive and hypertensive animals in the expression level of a studied pool of 118 microRNAs [38]. Another study, however, identified five microRNAs that were differentially expressed between Dahl salt-sensitive hypertensive animals and consomic SS-13^{BN} controls; the downregulation of miR-29b was confirmed in the hypertensive animals. MiR-29b has been identified as a master regulator of several collagen genes and genes related to the extracellular matrix, such as matrix metalloproteinase 2 (*Mmp2*) and integrin β 1 (*Itgb1*), which are functionally important in renal pathology [36, 39]. The authors concluded that miR-29b has a broad effect on a large number of collagens and genes related to the extracellular matrix, and miR-29b is presumably involved in protection from renal medullary injury in normotensive SS-13^{BN} rats.

Differential expression of microRNAs may become a useful biomarker for disease diagnosis. Indeed, in patients with hypertensive nephrosclerosis, intrarenal expression of several microRNAs (miR-200a, miR-200b, miR-141, miR-429, miR-205, and miR-192) has been found to be increased, and the degree of upregulation correlated with disease severity [40]. However, the functional significance of these microRNAs in disease pathology remains to be determined.

MicroRNAs Targeting the RAAS in Hypertension

The RAAS has an essential role in blood pressure regulation by affecting cardiac contractility, vascular resistance, and blood volume. RAAS overactivation is a major pathogenetic factor in hypertension. As an endocrine system, RAAS encompasses several enzymes, peptides, and receptors that are important therapeutic targets in hypertension. Angiotensin II, a vasoconstrictor peptide, induces cell growth through protein kinase C in smooth muscle cells and in cardiomyocytes in conditions of endothelial damage such as hypertension [41]. It also

facilitates the release of other hormones such as aldosterone and vasopressin. The gene encoding the angiotensin II receptor, type 1 (*AGTR1*) is the most important receptor for angiotensin II. Aldosterone stimulates tubular sodium reuptake, leading to increased salt and water content in the circulation. Vasopressin, acting through vasopressin-1A receptor (*AV1*), confers vasoconstriction, and activation of vasopressin-2 receptor induces water resorption in the collecting ducts. The bradykinin receptor 2 (*B2R*) counteracts RAAS by inducing vasodilatation and by inhibiting water reabsorption by aquaporin-2. Thromboxane A₂, acting via the thromboxane A₂ receptor (which is encoded by the *TBXA2R* gene), is a potent vasoconstrictor.

Several microRNAs have been shown to interact with the RAAS. MiR-155 has been found to regulate *AGTR1* expression [42]. MiR-155 is located on chromosome 21. Interestingly, trisomy 21 is commonly associated with decreased blood pressure. Analysis of monozygotic twins has found that *AGTR1* protein expression is downregulated and miR-155 is upregulated in trisomy 21 patients [43]. The miR-155 target site in the 3'-UTR of human *AGTR1* contains a single nucleotide polymorphism (SNP). It has been suggested that the rs5186(C) allele is associated with increased risk for essential hypertension in female Caucasian populations [44]. Interestingly, miR-155 was found to downregulate only the normal allele of *AGTR1* [43]. In a very recent study, the associations between SNPs located in microRNA binding sites in genes of RAAS and arterial blood pressure and the risk of myocardial infarction has been investigated in a large study population from the Study of Myocardial Infarctions Leiden (SMILE) [45]. Ten SNPs in eight RAAS genes have been identified. Four SNPs, located in the arginine vasopressin 1A receptor (*AVPR1A*), *B2R* (*BDKRB2*), and *TBXA2R* genes, were associated with changes in blood pressure. In addition, a rare allele of *AVPR1A* was associated with increased blood pressure, whereas the rare alleles of two linked *BDKRB2* SNPs and of the *TBXA2R* SNP were associated with decreased blood pressure. Although not associated with blood pressure, a rare allele of the mineralocorticoid receptor (*NR3C2*) SNP was associated with increased risk of myocardial infarction in men younger than 50 years. Luciferase assays showed that the allele containing a SNP interferes with the regulation of *AVPR1A* gene expression by hsa-miR-526b and hsa-miR-578. Hsa-miR-34a and hsa-miR-34c were able to repress luciferase activity for the common allele of *BDKRB2*. MiR-765-induced gene repression was reduced for an allele of *TBXA2R* containing a SNP. Gene repression by hsa-miR-383 was reduced for the allele of *NR3C2* containing the SNP rs5534. This SNP was not associated with an increase in blood pressure, but elevated plasma levels of von Willebrand factor and factor VIII and increased risk of myocardial infarction were observed in the affected patients. Of note, the

results regarding the *AGTR1* SNP were inconclusive in this study.

The hypertrophy-inducing effect of the mineralocorticoid aldosterone in cardiac and vascular smooth muscle cells is well known. In a recent study, it was shown that part of this hypertrophic pathway is the nuclear factor of activated T cells c3 (NFATc3) and myocardin, and both can be targeted by miR-9 [46]. Myocardin expression is elevated in response to aldosterone stimulation, and NFATc3 can bind to the promoter region of myocardin and transcriptionally activate its expression. miR-9, which is able to suppress myocardin expression, is downregulated as a result of hypertrophic stimulus. The authors suggest that miR-9 could be targeted to reverse the hypertrophic transformation in cells of the cardiovascular system.

The mineralocorticoid receptor gene *NR3C2* is a ligand-dependent transcription factor that regulates water and ion transporter expression. It regulates blood pressure by promoting renal salt retention, centrally modulating sympathetic tone, blood volume, and salt appetite [47]. A recent article highlighted the role of microRNAs in the translational regulation of *NR3C2*. MiR-124 and miR-135a independently repressed the translation of *NR3C2* without affecting mRNA levels. The authors proposed that miR-124 and miR-135a may contribute to the modulation of the RAAS and thereby to blood pressure regulation [48].

MicroRNAs Targeting Vascular Smooth Muscle Cells in Hypertension

Vascular smooth muscle cells (VSMCs) have a fundamental role in the development of hypertension. Peripheral vascular resistance is determined by neuronal and hormonal factors in the wall of small arterioles [41]. As contractile elements of the vascular wall, VSMCs determine vascular tone and thus regulate vascular resistance, tissue perfusion, and blood pressure.

VSMCs maintain remarkable plasticity even in the mature cardiovascular system, able to react to various forms of vascular stress or injury by switching from the differentiated contractile phenotype to a proliferating, synthetic, dedifferentiated phenotype [49]. Proliferation of VSMCs in response to stress results in vascular remodeling, a key structural feature in sustained hypertension [50].

Dicer is constitutively expressed in VSMCs and has a crucial role in maintaining vascular function. VSMC-specific deletion of Dicer in mice results in late embryonic lethality associated with extensive internal hemorrhage. The vascular structure and function in these mice is severely impaired; the vessels are dilated and are thin-walled owing to reduced cellular proliferation [51]. A recent follow-up study using a mouse model of VSMC-specific tamoxifen-inducible deletion

of Dicer resulted in profound hypotension *in vivo*, with abolished contractile responses in the arteries [51, 52]. As the authors suggested, continual microRNA turnover regulates the levels of key genes in VSMCs that control contraction, remodeling, and phenotypic modulation [52].

Mice lacking both miR-143 and miR-145 have a phenotype that is very similar to that of the VSMC-specific Dicer knockout mice, but nonlethal and less severe. Both the VSMC-specific Dicer knockout mice and the miR-143/145 double-knockout mice have decreased blood pressure, reduced contractile response to vasoconstrictors, decreased medial thickness, and reduced contractile differentiation. MiR-143/145 double-knockout mice have been developed and studied concurrently by several groups. The vascular wall in these mice shows severe reduction in the number of contractile VSMCs and an increase in synthetic VSMCs in the aorta and the femoral artery, with profoundly impeded neointima formation in response to vascular injury [53–55]. The VSMCs showed disarray of actin stress fibers and ultrastructure typical of synthetically active VSMCs [54, 55]. Fascinatingly, the miR-143/145 double-knockout mice had upregulation of angiotensin-converting enzyme (ACE) in VSMCs but no systemic elevation of angiotensin II levels. The loss of miR-145 in this model presumably leads to upregulation of membrane-bound ACE in VSMCs. The chronic stimulation of VSMCs by angiotensin II contributes to the transformation of the muscular layer, but on the other hand, results in desensitization and “angiotensin resistance” of VSMCs, which would explain the absence of elevated blood pressure in these mice.

MiR-143 and miR-145 are important determinants of VSMC differentiation and function, but they are not essential for VSMC development. Indeed, in isolated VSMCs lacking Dicer, overexpression of miR-145 almost completely rescued differentiation into the contractile phenotype [51].

As opposed to other microRNAs, the miR-143/145 cluster has been found to promote differentiation and can direct smooth muscle fate. The miR-143/145 cluster is highly expressed in VSMCs under normal conditions, but vascular injury leads to marked downregulation with concomitant change of miR-21 expression in the opposite direction (see below) [56]. MiR-145 targets the Krüppel-like factor 5 (KLF5), an inhibitor of myocardin, which has a role in smooth muscle differentiation [54, 57]. Also, very interestingly, miR-145 binds to a seed sequence in the myocardin 3'-UTR to activate translation [56], as opposed to the more common function of microRNAs as translational repressors. Other identified targets of the miR-143/145 cluster are myocardin-related transcription factor-B (MRTF-B) and calmodulin kinase II- δ .

MicroRNA-21, implicated in cardiac remodeling [58], has been found to be a critical regulator of VSMC

proliferation and an inhibitor of apoptosis by targeting phosphate and tensin homolog (PTEN) and Bcl-2 expression [59]. A detailed review of the targets of miR-21 can be found in [60]. In a recent study, several microRNAs (miR-21, miR-26b, miR-98, and miR-1826) were implicated in NO and atrial natriuretic peptide signaling in VSMCs. In particular, miR-21 promotes VSMC proliferation and relaxation via cGMP signaling [61].

VSMC differentiation can also be regulated by the miR-221/222 cluster, likely by modulating the expression of p27 (Kip1), p57(Kip2), and/or c-kit. MiR-221/222 expression induced by platelet-derived growth factor or by overexpression results in a phenotypic switch to a proliferative/dedifferentiated phenotype, whereas downregulation of these microRNAs had the opposite effect in VSMCs [52, 62, 63].

MicroRNAs in Other Etiological Factors of Hypertension

Association of between human cytomegalovirus (HCMV) infections and cardiovascular disorders through impaired endothelial dysfunction has been proposed, although a direct link was established only very recently. By comparing healthy controls and patients with essential hypertension, Li et al. [64•] found several differentially expressed microRNAs, out of which miR-296-5p, let-7e, and an HCMV-encoded microRNA, hcmv-miR-UL112, were validated. Also, HCMV seropositivity and quantitative titers, which were elevated in the hypertension group along with hcmv-miR-UL112, were independently associated with increased risk of hypertension [64•]. The authors also demonstrated that hcmv-miR-UL112 directly targets the transcription factor interferon regulatory factor 1 (IRF-1) [64•], which is involved in apoptosis, angiogenesis, neointimal formation, and vascular disease mechanisms [65].

Chromogranin A (CHGA), stored and released from secretory granules of chromaffin cells and neurons, is a precursor of catestatin, a catecholamine release-inhibitory neuroendocrine protein. Catestatin release is reduced and the plasma ratio of CHGA to catestatin is increased in the hypertensive population, suggesting an impairment of CHGA processing in this disorder [66]. A common polymorphism, T+3246C (rs938671), in the 3'-UTR region of the *ATP6V0A1* gene, is associated with CHGA/catestatin secretion and systemic blood pressure in the population. *ATP6V0A1*, a component of the vacuolar ATPase, has an important role in controlling the vacuolar pH, thereby affecting sympathochromaffin exocytosis. In a recent study, it has been found that T+3246C is located in a binding motif for microRNA hsa-miR-637, at which the C allele may impair translation of *ATP6V0A1* mRNA. Consequently, the secretion of CHGA/catestatin may be impaired. As these

authors point out, the results shed new light on the role of chromaffin granule function in autonomic control of the circulation and, more broadly, in the pathogenesis of cardiovascular disease conditions such as hypertension [66].

Therapeutic Implications

The altered expression of tissue-specific or cell-specific microRNA in various disease conditions provides the rationale to use microRNA technologies in disease states to reinstate the balance of gene regulation at the cellular

level. In hypertension, microRNAs may affect disease development and prognosis in many ways (Table 1). In the past several years, chemically modified oligonucleotides known as antagomirs or anti-miRs have been developed to silence specific endogenous microRNAs. Upon intravenous systemic administration, antagomirs have been shown to effectively inhibit the activity of target microRNAs in a sequence-specific manner in many cell types [67, 68]. In the cardiovascular field, our group has demonstrated that systemic administration of antagomirs against miR-21 successfully blocked the development of cardiac fibrosis. Later, inhibitors against miR-21 [58] were

Table 1 MicroRNAs in hypertension

MicroRNAs	Target Genes/Pathway/Signaling mechanism	References
Endothelial microRNAs		
miR-126	SPRED-1; PIK3 regulatory subunit-2; VCAM-1; <i>CXCL12</i> ; RhoB	[19–22, 23•, 24]
miR-217	Sirt1	[28]
miR-122	<i>SLC7A1</i>	[29, 30]
miR-21	Nitric oxide pathway	[25]
miR-24	Hypoxia-induced mechanism	[31]
miR-27b, -130a, -210, -378, -17–92, let-7f	Pro-angiogenic	Reviewed in [32]
miR-15, -16, -20a, -20b, -24, -221, -222	Anti-angiogenic	Reviewed in [32]
Renal microRNAs		
miR-29b	Fibrotic pathway; collagen genes; <i>Mmp2</i> ; <i>Itgb1</i>	[36, 39]
miR-200a, miR-200b, miR-141, miR-429, miR-205, miR-192	Biomarkers of nephrosclerosis	[40]
MicroRNAs targeting the renin-angiotensin-aldosterone system (RAAS)		
miR-155	<i>AGTR1</i>	[42]
miR-526b and -578	<i>AVPR1A</i>	[45]
miR-34a, and -34c	<i>BDKRB2</i>	[45]
miR-765	<i>TBXA2R</i>	[45]
miR-383	<i>NR3C2</i>	[45]
miR-9	NFATc3	[46]
miR-124 and miR-135a	<i>NR3C2</i>	[48]
MicroRNAs targeting vascular smooth muscle cells		
miR-143 and miR-145	Actin stress fibers; ACE; KLF5; myocardin; MRTF-B; calmodulin kinase II- δ	[53–57, 61]
miR-21	PTEN; Bcl-2; cGMP signaling	[59, 60]
miR-21, -26b, -98, and -1826	Nitric oxide and ANP pathway	
miR-221 and -222	p27(Kip1), p57(Kip2) and/or c-kit	[52, 62, 63]
MicroRNAs in other etiologic factors		
miR-296-5p, let-7e, hcmv-miR-UL112	Association with hypertension	[64•]
hcmv-miR-UL1	IRF-1	[64•]
miR-637	<i>ATP6V0A1</i> , chromaffin granule function	[66]

ACE angiotensin-converting enzyme; *AGTR1* angiotensin II receptor, type 1; *AVPR1A* arginine vasopressin 1A receptor; *IRF-1* interferon regulatory factor 1; *KLF5* Krüppel-like factor 5; *MRTF-B* myocardin-related transcription factor-B; *NFATc3* nuclear factor of activated T cells c3; *PTEN* phosphate and tensin; *Sirt1* sirtuin 1; *TBXA2R* thromboxane A2 receptor; *VCAM* vascular cell adhesion molecule

also used to block renal and pulmonary fibrosis [69, 70], suggesting that blocking miR-21 has general antifibrotic effects.

Although there has been great success in designing antagomirs, drug delivery issues remain an obstacle to their therapeutic use, especially for approaches to target microRNAs in the cardiovascular disease states. In the recent years, the stability, target specificity, and pharmacokinetic properties of the oligonucleotides have been greatly improved. By using intravenously administered cholesterol-conjugated antagomirs in mice, our group has shown that uptake and subsequent microRNA inhibition is highly efficient [58], and recently, the efficacy of various microRNA inhibitor chemistries was compared in a cardiac disease model [71]. To circumvent the complex steps of cholesterol conjugation, a delivery method by gold nanoparticles was recently developed [72].

A different class of miRNA inhibitors is presented by the locked-nucleic-acid (LNA)—modified oligonucleotides. These anti-miRs have proven safety and efficacy, and studies using LNA-based oligonucleotides targeting miR-122, for instance, are in phase II clinical testing in patients with hepatitis C virus infection [73].

The improved stability of antagomirs allows prolonged intracellular storage; combined with the relative long half-life of microRNAs, the result is efficient silencing of target microRNAs in various organ systems. Differences in oligonucleotide chemistries may lead to considerable differences in the biologic activity of antagomirs, however. In a head-to-head comparison, various miR-21 inhibitors had different effects on cardiac fibrosis [71].

As an alternative to chemically modified oligonucleotides, microRNA inhibitors termed *microRNA sponges* have been developed. Sponge microRNAs, which are produced from transgenes within cells, are competitive inhibitor transcripts designed by inserting multiple, tandem binding sites into the 3'-UTR of the microRNA of interest [74]. The binding sites of a sponge are specific to the seed region of the microRNA, allowing them to block the whole family of related microRNAs. Recently, noncoding RNAs as endogenous sponge RNAs have also been discovered in plants and prokaryotes as well as in animal systems [75].

MicroRNAs often are repressed in diseases, such as in tissues affected by hypertension. A possible strategy is to increase the level of these microRNAs by introducing so-called microRNA mimics, synthetic double-stranded precursor-microRNA molecules in which one strand is identical to the native microRNA and functions as a guide strand [76]. However, this technology is in its infancy.

The development of hypertension offers several targets for microRNA-based therapies (Table 1). One of the most important roles for microRNAs is their contribution to cell differentiation and organ development. Endothelial micro-

RNAs are potential targets to tackle capillary rarefaction and defective angiogenesis. Cell differentiation and dedifferentiation of smooth muscle cells in the vascular wall are crucial mechanisms associated with vascular alterations such as intima thickening in hypertension. MiR-21 could be a potential target in hypertension [61]. Mimics of miR-9 potentially could reverse hypertrophy of the heart or vascular wall owing to the proposed role of miR-9 in hypertrophy [46]. At the renal level, miR-29b, a key regulator of collagen expression, and microRNA mimics may be used to block the development of fibrosis. A novel aspect of microRNAs in hypertension that has not only epidemiologic but also pharmacologic implications are the various polymorphisms located in microRNA binding sites of genes involved in blood pressure regulation and the development of hypertension, such as the RAAS and mineralocorticoid receptors.

Conclusions

In spite of the continuous efforts of the scientific community to understand the pathogenesis of essential hypertension, many aspects at the molecular level remain elusive. The elucidation of molecular processes regulated by microRNAs and the identification of novel microRNA targets in the pathogenesis of hypertension are valuable and exciting strategies that may eventually lead to the development of novel treatments to prevent and reverse the consequences of hypertension.

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- Of importance
- Of major importance

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