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Pervasive roles of microRNAs in cardiovascular biology

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Abstract

First recognized as regulators of development in worms and fruitflies, microRNAs are emerging as pivotal modulators of mammalian cardiovascular development and disease. Individual microRNAs modulate the expression of collections of messenger RNA targets that often have related functions, thereby governing complex biological processes. The wide-ranging functions of microRNAs in the cardiovascular system have provided new perspectives on disease mechanisms and have revealed intriguing therapeutic targets, as well as diagnostics, for a variety of cardiovascular disorders.

Diseases of the cardiovascular system are the most common congenital birth defects and causes of adult morbidity and mortality[1-3]. Although the cellular mechanisms and gene mutations responsible for numerous cardiovascular disorders have been extensively studied, it has become apparent only recently that microRNAs (miRNAs) have key roles in cardiovascular development and disease[4-8]. The prominent functions of miRNAs in cardiovascular biology probably reflect the sensitivity of the cardiovascular system to relatively subtle perturbations in gene expression, which can result in severe and often fatal abnormalities.

A primary role of miRNAs seems to be the ‘fine-tuning’ of gene expression to control development and tissue homeostasis[8]. However, under conditions of stress, the functions of miRNAs become especially pronounced, underscoring their roles in disease. Highly specific patterns of miRNA expression correlate with different cardiovascular disorders (such as cardiac hypertrophy, heart failure[9-12], post-myocardial infarction remodelling[13,14] and vascular remodelling[15,16]), and gain- and loss-of-function miRNA studies in mice have revealed pathogenic and protective functions of miRNAs *in vivo*[6,17]. Correlation of the cellular targets of miRNA action with cardiovascular phenotypes illuminates new biological pathways and disease mechanisms. Especially intriguing is the ability to manipulate individual miRNAs *in vivo* using oligonucleotide-based inhibitors or miRNA mimics, thereby opening up possibilities for the therapeutic manipulation of miRNAs[18].

In this Review, we describe the biology and mechanisms of action of miRNAs in the cardiovascular system, and consider the opportunities and challenges for the therapeutic modulation of miRNAs in cardiovascular disease. See refs [8, 19] and [20] for more detailed reviews of the biosynthesis and mechanisms of action of miRNAs.

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Functional concepts of miRNA action

MicroRNAs are ~22-nucleotide single-stranded RNAs that inhibit the expression of specific mRNA targets through Watson–Crick base pairing between the miRNA 'seed region' and sequences commonly located in the 3′ untranslated regions (UTRs). The human genome is estimated to encode up to 1,000 miRNAs[21], which are either transcribed as standalone transcripts, frequently encoding several miRNAs, or generated by the processing of introns of protein-coding genes[21]. The integration of miRNAs into introns of protein-coding genes serves to coordinate the expression of the miRNA with the mRNA encoded by that gene, without the necessity for a separate set of *cis*-regulatory elements to drive expression of the miRNA (Fig. 1a). It is not uncommon for intronic miRNAs to modulate the same biological processes as the protein encoded by the host gene[22-26]. The dual functions of such genes, encoding protein and miRNA, provide sophisticated feedback and feedforward regulatory networks, specific examples of which are highlighted throughout this Review.

Genetic deletions of miRNAs in organisms ranging from worms to mice have shown that few developmental processes are absolutely dependent on single miRNAs[8,27]. A recent study using compound mutant worms suggested there was significant redundancy within miRNA families, between unrelated miRNAs, and even between miRNAs and transcription factors, perhaps evolving as a buffer against deleterious variations in gene-expression programs[28,29]. The actions of miRNAs often become pronounced under conditions of physiological or pathological signalling, suggesting conditional activities of miRNAs that necessitate genetic perturbation or sensitizing agents to uncover their functions.

miRNAs typically exert modest inhibitory effects on many mRNAs, which often encode proteins that govern the same biological process — for example, the fibrotic response is inhibited by miR-29 (ref. [14]), cardiac conduction by miR-1 (refs [30-32]), actin cytoskeletal dynamics by miR-145 (ref. [16]), the phosphatidylinositol-3-OH kinase (PI(3)K)–AKT pathway by miR-486 (ref. [33]) and stem-cell pluripotency by miR-145 (ref. [34]). The cumulative reduction in expression of several components of a molecular pathway reduces the importance of a single miRNA-mRNA interaction to elicit a biological response, and adds robustness to gene-regulatory networks (Fig. 1b). The multiplicity of miRNA targets may also promote combinatorial regulation by miRNAs that individually target various mRNAs whose protein products contribute to one particular regulatory axis (Fig. 1c). In this model, a biological response would be expected only after co-expression of several miRNAs that cooperatively target various components of a functional network or are all required to sufficiently repress a single target. By contrast, some miRNAs seem to reinforce an appropriately 'balanced' pathway by targeting both positive and negative regulatory components (for example, agonism and antagonism of Nodal signalling by miR-430)[35] (Fig. 1d). This mode of action allows buffering against minor physiological variations. Clearly, miRNA biology is a complex and highly orchestrated mode of gene regulation, potentially impinging on nearly all biological processes in mammals and having particularly important roles in disease states.

Oligonucleotide modulation of miRNA function

The ability of miRNAs to modulate important biological pathways offers opportunities for the manipulation of miRNA function using oligonucleotide inhibitors (antimiRs) or miRNA mimics (Fig. 2). Antisense oligonucleotides directed against specific miRNA sequences are efficiently taken up by a variety of tissues and block miRNA function in the heart and vasculature[18].

Other oligonucleotide-based techniques involve 'target protectors' or 'masks', which block individual miRNAs from binding to their mRNA targets, thereby rescuing the mRNA from

inhibition. Target protectors have been validated in zebrafish[35] and in cultured cardiac myocytes[36]. miRNA 'sponges' or 'decoys' containing several miRNA-binding sites also act as competitive inhibitors for miRNA binding[37,38]. Although the results obtained from pharmacological knockdown or overexpression of miRNAs sometimes differ from those obtained using genetic mouse models, the development of these various technologies has greatly accelerated the rate at which basic biological questions can be answered in an experimental setting.

miRNAs in cardiovascular development

The requirement of miRNAs for cardiovascular development and function was initially demonstrated by tissue-specific deletion in mice of the *Dicer* gene, which encodes an enzyme that is essential for miRNA processing. Lethal phenotypes were observed after *Dicer* deletion in myocardial and vascular lineages[39,40]. Although these findings highlight the crucial roles of miRNAs in the cardiovascular system, no specific miRNA deletion has yet been found to cause fully penetrant embryonic lethality in mice, indicating significant redundancy of miRNA function[28] and suggesting that the lethal consequences of *Dicer* deletion reflect the collective functions of many miRNAs rather than any single miRNA. The rapidly expanding number of miRNAs implicated in various aspects of cardiovascular biology precludes an in-depth review of all of them, so general principles of miRNA regulation and function are considered throughout this Review.

Roles of miRNAs in heart development

Heart formation requires precise and complex interactions among diverse cell types from several lineages — cardiomyocytes, endocardial, epicardial and vascular cells, fibroblasts and cells of the conduction system. Specific miRNAs are enriched in different cardiac cell types and, in some cases, have been found to participate in the specification of cell identity. Genetic ablation and antisense oligonucleotide-mediated knockdown studies have shown miRNA contributions to developmental processes as diverse as embryonic stem (ES)-cell differentiation, cardiomyocyte proliferation, contractility, ion-channel regulation and cardiac conduction (Fig. 3).

Expression profiling has shown that the 18 most abundant miRNAs in the heart account for more than 90% of all cardiac miRNAs[41]. Because a threshold level of miRNA expression seems to be required for the efficient repression of target gene expression (typically >100 copies per cell)[37,42], the regulation of heart development may depend on either a relatively discrete set of miRNAs or the combinatorial function of a larger array of miRNAs expressed at a low level. So far, a functional role in heart development has been ascribed to only the most enriched miRNAs, reflecting a dosage requirement, functional redundancy or both.

miR-1 is the most abundant miRNA in cardiac myocytes, and it was the first miRNA implicated in heart development[30]. miR-1 and the related miRNA miR-133 arise from a common precursor RNA, the expression of which in the embryonic heart is mediated by two separate enhancers that are regulated by the transcription factors SRF and MEF2 (refs [43,44]), integrating these miRNAs into well-characterized transcriptional networks. miR-1 and miR-133 seem to function cooperatively to promote mesoderm differentiation of ES cells and suppress endodermal and ectodermal cell fates[45]. By contrast, they have opposing roles later in the cardiac lineage when miR-1 promotes and miR-133 inhibits cardiomyocyte differentiation[45]. Neither miR-1 nor miR-133 is absolutely required for the specification of cardiac cell fates *in vivo*, as 50% of mice lacking either miRNA are viable[30,46]. This disparity between the functions of miRNAs as determined by *in vitro* assays versus *in vivo* loss-of-function studies is a common theme, and suggests that compensatory mechanisms that account for the unexpectedly mild phenotypes may be activated in genetic knockout

mice. Zebrafish seem to be particularly sensitive to miRNA regulation, such that inhibition of specific miRNAs evokes more dramatic phenotypes than those seen in mutant mice. For example, antisense-mediated knockdown of miRNAs in zebrafish has revealed roles for miRNAs in the formation of the cardiac chambers and the atrioventricular canal[47,48].

Roles of miRNAs in vascular and blood development

The formation and function of the vascular system requires the establishment and remodelling of a contiguous series of lumenized tubes made of endothelial cells. Concurrent recruitment of vascular smooth muscle cells (SMCs) to the endothelial plexus during vessel maturation imparts the necessary tone and contractility for proper blood flow. Numerous miRNAs have been shown to govern these processes during vascular development and disease (Fig. 4).

The endothelial-cell-specific miRNA miR-126 is encoded by an intron of the epidermal growth factor-like domain 7 (*Egfl7*) gene, which encodes an endothelial-cell-enriched growth factor involved in the control of cell migration[49]. miR-126 is induced by blood flow and controls angiogenic sprouting of aortic arch vessels by the stimulation of vascular endothelial growth factor signalling[50]. Mice lacking miR-126 are partially viable but have fragile and leaky blood vessels and defects in angiogenesis[51,52]. Antisense-oligonucleotide-mediated knockdown of miR-126 in zebrafish causes complete embryonic lethality owing to the loss of vascular integrity and haemorrhaging[53]. Vascular patterning in mouse retinas is also modulated by miR-218, which is encoded by an intron of the *Slit1* and *Slit2* genes and inhibits several components of the SLIT–ROBO signalling pathway[54]. This study is an important demonstration of the coordinated regulation of a biological process by an miRNA and its host gene.

miR-143 and miR-145, encoded by a bicistronic pre-miRNA, are expressed specifically in SMCs under the control of SRF and members of the myocardin family of co-activators. These miRNAs target numerous regulators of actin signalling, including Rho GTPases, sling-shot homologue 2, adducin, cofilin and actin itself[16]. miR-145 has been reported to be necessary and sufficient for SMC differentiation *in vitro*[55]. However, mice lacking both miR-143 and miR-145 are viable, suggesting that further mechanisms modulate their functions *in vivo*[16,56,57]. miR-145-mutant mice have reduced vascular tone, which contributes to a reduction in blood pressure[16,57]. Vascular SMCs from these mutant mice show diminished sensitivity to mechanical injury and an abnormality in phenotypic switching in response to injury that seems to reflect perturbations in actin signalling and SRF activation. Collectively, these studies demonstrate that miRNAs function as sensors of mechanical and environmental changes, thus linking dynamic physiological processes with the regulation of gene expression.

The differentiation of blood cells is also dependent on miRNA activity[58]. An miRNA expression signature has been described for haematopoietic stem-cell progenitors, which show dynamic regulation during differentiation[59]. Furthermore, the *Ago2*-(also known as *Eif2c2*-) null mouse has erythroid lineage defects[60], and modulation of miRNA expression in erythroid progenitors suggests a role for miRNAs in their differentiation[61,62]. Indeed, loss-of-function studies in mice have also implicated miRNAs, including miR-223 and miR-451, in erythroid proliferation and differentiation[63-66]. For example, gene targeting and pharmacological knockdown of miR-451, which is enriched in erythroid cells, results in reduced baseline haematocrit levels and impaired erythroid expansion in response to oxidative stress[63,65,66]. Further analysis of miRNAs in circulating cells may reveal roles in functions such as oxygen delivery, angiogenesis and the inflammatory process.

miRNAs in cardiovascular disease

Heart failure and several cardiovascular diseases are associated with the re-expression of the fetal cardiac gene program, which may have causative or adaptive roles[1] and includes a signature pattern of miRNAs[10,12]. Indeed, numerous cardiac-enriched miRNAs show dynamic regulation in human heart disease, suggesting their involvement in the regulation of cardiovascular disease[9,11,12].

Roles of miRNAs in heart disease

The importance of individual miRNAs in the setting of heart disease has been shown by genetic deletion in mice subjected to various cardiovascular insults (Fig. 3). miRNAs are implicated in pathologies as diverse as arrhythmias (miR-1 (ref. [31]), miR-133 (ref. [32]) and miR-208a (ref. [67])), fibrosis (miR-21 (ref. [68]) and miR-29 (ref. [14])), pressure-overload-induced remodelling (miR-208 (refs [67, 69]) and miR-133 (ref. [70])), and metabolic disorders (miR-33 (ref. [24])).

One of the best-characterized examples of stress-dependent gene regulation by an miRNA involves a family of miRNAs encoded by myosin heavy chain (*MHC*) genes, referred to as MyomiRs[67,69,71]. This is also an example of intronic miRNAs participating in a process related to host gene function. Three members of this miRNA family, miR-208a, miR-208b and miR-499, are encoded by the α-*MHC* (also known as *Myh6*), β-*MHC* (*Myh7*) and *Myh7b* genes, respectively. These MyomiRs regulate a collection of transcriptional repressors and signalling molecules that govern MHC expression, as well as thyroid hormone activity and the stress-responsiveness of cardiac muscle cells. Deletion of *Mir208a* in mice abrogates the re-activation of the fetal β-*MHC* gene in response to haemodynamic cardiac stress, and protects the heart from pathological remodelling[67,69]. The MyomiR family thus constitutes an intricate regulatory circuit that controls myosin gene expression and cardiac stress responsiveness during adaptation to pathological signalling.

In addition to the miR-208 family, several other miRNAs have been implicated as either causative or protective in heart disease. NFATc3 is a transcriptional mediator of cardiac stress signalling that promotes pathological hypertrophy[72]. NFATc3 was recently shown to induce miR-23a expression in cardiomyocytes, and antagomir-based knockdown of miR-23a in mice abrogates isoproterenol-induced cardiac hypertrophy[73]. Conversely, acute knockdown of miR-133 was shown to induce pathological cardiac hypertrophy in mice[70], suggesting a potential cardioprotective role for endogenous miR-133. However, these findings contrast with the phenotype of *Mir133*-null mice, which undergo a normal hypertrophic response[46], highlighting the difference between pharmacological modulation of miRNA expression and genetic deletion studies.

The miR-29 family, which is downregulated after myocardial infarction, inhibits the expression of several collagens and extracellular matrix proteins, thereby contributing to scar formation and fibrosis[14]. Similarly, the miR-199 family is rapidly downregulated in cardiac myocytes under hypoxic conditions, relieving the repression of sirtuin 1 and hypoxia-inducible factor 1-α in a model of hypoxia preconditioning[74].

The miRNA that repeatedly shows dynamic regulation after cellular stress is miR-21, which was shown to promote cardiac hypertrophy and fibrosis in response to pressure overload. Knockdown of miR-21 with a cholesterol-modified antagomir attenuated cardiac remodelling after thoracic aortic constriction[68]. This response was attributed to the derepression of the protein sprouty, which negatively regulates the profibrotic extracellular signal-regulated kinase-mitogen-activated protein kinase (ERK–MAPK) cascade in cardiac fibroblasts[68]. Paradoxically, however, neither genetic deletion nor tiny locked-nucleic-acid

(LNA)-mediated knockdown of miR-21 in mice alters fibrosis or hypertrophy in response to thoracic aortic constriction or other cardiac stresses[75]. The contrasting conclusions of these studies emphasize the gaps in our understanding of the mechanisms of miRNA action and oligonucleotide-based targeting strategies for their inhibition.

Roles of miRNAs in vascular disease

The vessel wall is composed of endothelial cells and SMCs that must maintain a sealed barrier yet allow the exchange of oxygen and nutrients with adjacent tissues. Vessels can respond to injury or changes in the environment by undergoing phenotypic changes that promote endothelial cell migration or fragility, as well as SMC de-differentiation, proliferation and migration. Numerous miRNAs show marked alterations in expression during vascular injury and disease, and expression signatures have now been correlated with pathologies such as ischaemia, tumour angiogenesis, atherosclerosis and a proliferative thickening and obstruction of the vessel known as restenosis[15]. Some miRNAs have been shown to have causal roles in these disorders (Fig. 4).

Angiogenesis is a process of endothelial cell proliferation and vascular tube sprouting that is promoted in adulthood by various stimuli, including tumour growth, retinal damage and ischaemia. miR-21 can influence the function and migration of angiogenic progenitor cells during coronary artery disease[76]. Likewise, ischaemia-induced angiogenesis in adult tissues can be promoted or inhibited by antisense oligonucleotides directed against miR-92a (ref. [77]) or miR-126 (ref. [78]), respectively. miR-126 may also influence susceptibility to atherosclerosis, through the modification of endothelial cell function[79,80].

Vessel injury, instigated by diverse factors such as atherosclerosis, hypertension and damage due to a mechanical stenting, results in SMC phenotypic changes indicative of a de-differentiated state. Such SMCs become proliferative and migratory, entering the vessel lumen and causing restenosis. Recent studies have implicated miRNAs as mediators of SMC phenotypic modulation and vessel remodelling. The expression of miR-21 and the miR-143/145 cluster are up- and downregulated, respectively, after mechanical injury of large vessels[15], and restoration of miR-21 and miR-145 to normal levels prevents restenosis[15,56,81].

miRNA mutations as the basis of disease

The pervasive influence of miRNAs on cardiovascular function and disease raises questions as to whether polymorphisms in miRNAs or their target sequences in mRNA transcripts affect human disease. Although mutations within the seed regions of evolutionarily conserved miRNAs are not common, single nucleotide polymorphisms (SNPs) within miRNA-binding sites in the 3′ UTRs of target mRNAs have been observed at a higher frequency[82,83]. A notable example is in the Texel breed of sheep, which develops extreme skeletal muscle hypertrophy owing to an SNP in the 3′ UTR of the mRNA encoding myostatin, a negative regulator of muscle growth[84]. This mutation creates a binding site for miR-1, resulting in repression of myostatin expression and unrestricted muscle growth. SNPs within potential miRNA-binding sites have also been identified in mRNAs associated with hypertension and cardiovascular disease[85]. SNPs in miRNAs or their targets that cause significant phenotypes seem unlikely to be a widespread occurrence, however, because of the substantial degeneracy allowed in miRNA-mRNA interactions and the redundant regulation of an individual mRNA by several unrelated miRNAs. It remains to be determined whether a causative link can be made between miRNA SNPs and human disease.

Clinical perspectives

Identifying the signature patterns of miRNAs associated with different cardiovascular disorders has opened up opportunities for miRNA diagnostics. miRNA profiling can discriminate between specific forms of heart disease[10,12], such as dilated cardiomyopathy, ischaemic cardiomyopathy and heart failure, and disease-associated miRNA expression patterns in failing human hearts can be normalized by the stabilization of cardiac output[11,86]. Recently, several miRNAs have been detected in plasma and reported to be diagnostic for heart failure and myocardial infarction[87-89]. Whether circulating miRNAs are functionally relevant or are simply released from injured tissues remains to be determined. However, the cellular secretion of particular miRNAs by exosomes suggests specificity in the process of miRNA secretion.

In contrast to many cellular mediators of disease, which are difficult (or impossible) to modulate therapeutically, it is unquestionable that drugs can target miRNAs. Thus, the involvement of miRNAs in almost every aspect of cardiovascular disease raises exciting possibilities for the therapeutic manipulation of miRNA-regulated processes. Therapies based on antimiRs or miRNA mimics are now being developed to repress pathological miRNAs or overexpress protective miRNAs, respectively. Indeed, antimiR-based studies demonstrating efficacy in non-human primates have already been reported[90,91] and have been advanced to human clinical trials.

The ability of individual miRNAs to modulate complex disease pathways through the targeting of several components of regulatory networks enables miRNAs to modulate tissue stress responses in a manner that is distinct from that of classical drugs. The multiplicity of miRNA targets also enables miRNAs to bypass mechanisms that render cells or tissues insensitive to certain drugs. For example, cells can develop insensitivity to single drugs through rare mutations in drug targets or desensitization of cell-surface receptors. Such mechanisms are unlikely to diminish sensitivity to miRNA inhibitors, which target several steps in a disease pathway.

Conversely, the targeting of large collections of mRNAs raises possibilities for off-target effects or even opposing effects of miRNAs in different tissues. Because the mechanistic basis of miRNA-based therapeutics is not clear, the possibility exists that modulating such a diverse set of target mRNAs will affect beneficial processes as well as the pathological condition. The heart takes up globally administered antimiR oligonucleotides less efficiently than the kidneys and liver, and the pharmacokinetics of miRNA-based therapies remain a hurdle. This issue may necessitate the development of new cardiovascular delivery systems for miRNA-based therapeutics, to limit uptake in healthy tissue. These methods would not be required for strategies involving knockdown of cardiac-specific miRNAs. Conjugation of antimiRs or miRNA mimics to homing molecules such as peptides, antibodies or other bioactive molecules might enrich uptake in cardiac tissue. This technology has not yet been successfully translated to the clinic, however, and other methods may improve tissue-specific uptake, such as direct administration by cardiac catheterization or a drug-coated stent.

Looking to the future

Despite recent advances in identifying miRNA contributions to cardiovascular development and disease, as well as in developing miRNA diagnostics and miRNA inhibitors, many gaps remain in our knowledge of miRNA-based regulation of gene expression in the normal and diseased heart and cardiovascular system. For example, the many potential target mRNAs for each miRNA pose significant challenges to the identification of those mRNAs that are relevant to a particular miRNA-regulated process. Compounding this difficulty is the

apparent variability in miRNA function based on physiological context or cell type, making it necessary to define the potential disparate functions of individual miRNAs in different settings. Another important consideration is that combinatorial interactions between multiple miRNAs with common or coordinated target mRNAs are likely to have a major role in gene regulation and the control of physiological pathways. Thus, it will be crucial to identify sets of miRNAs acting cooperatively within the cardiovascular system. This information will be particularly relevant to the development of miRNA-based therapeutics, as cocktails of miRNA inhibitors may prove more efficacious than targeting a single miRNA.

With the current pace of advancements in deciphering the basic principles of miRNA action in cardiovascular development and disease, we foresee new therapeutic applications for the prevention and treatment of human pathologies based on miRNA biology in the relatively near future.

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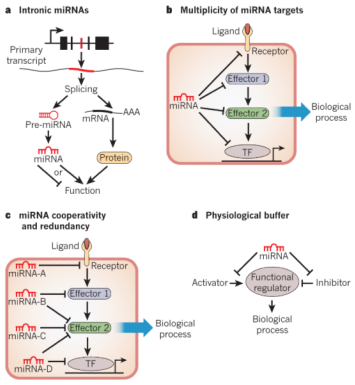

Figure 1. Concepts of miRNA function

The potential modes of miRNA-based regulation of gene expression are shown. **a**, Intronic miRNAs are encoded within an intron of a host gene. mRNA splicing generates a protein-coding transcript and an miRNA stem–loop. Intronic miRNAs often regulate similar processes to that of the protein encoded by the host gene. AAA, polyadenylated tail of the transcript; pre-miRNA, precursor miRNA. **b**, A common mechanism of miRNA function involves the modest repression of several mRNAs in a common biological process by a single miRNA. This mechanism reduces the dependence on a single miRNA-mRNA interaction and increases the robustness of the gene-regulatory network. TF, transcription factor. **c**, Many miRNAs may cooperatively or redundantly regulate a single biological process, by individually targeting many components of that process or by synergistically repressing a crucial component of a pathway. **d**, miRNAs may act as a ‘buffer’ against minor perturbations in a biological pathway. This is accomplished by the targeting of factors that positively and negatively influence a particular process, thereby insulating that process from environmental fluctuations.

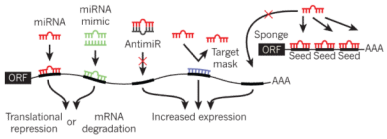

Figure 2. Oligonucleotide manipulation of miRNA function

The various methods of artificially modulating miRNA expression or activity are shown. Endogenous miRNA (red) binds to complementary sequences in the 3′ UTR of a target gene, resulting in translational repression or mRNA degradation. An miRNA mimic (green) consists of an oligonucleotide duplex of the miRNA and a passenger strand. The miRNA mimic comprises the same nucleotide sequence as an endogenous miRNA, and is designed to target the same mRNAs as that miRNA. An antimiR (grey) is an oligonucleotide that is complementary to an endogenous miRNA, thereby designed to bind and inhibit its function. A target mask (blue) is an oligonucleotide designed to bind to a portion of an endogenous miRNA target without initiating mRNA degradation or translational inhibition. This strategy rescues one particular mRNA from miRNA-mediated repression. miRNA sponges consist of an open reading frame (ORF) linked to a 3′ UTR that contains several binding sites for a particular miRNA, acting as competitive inhibitors for miRNA binding.

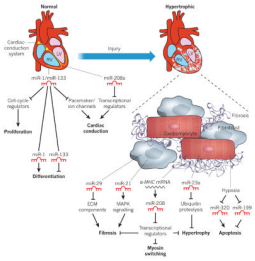

Figure 3. Functional role of miRNAs in the normal and diseased heart

A normal and a hypertrophic heart are shown in schematic form, depicting miRNAs that contribute to normal function or pathological remodelling. The expression of selected miRNAs within the heart is shown, along with their corresponding functions. All arrows denote the normal action of each component or process. miR-1 and miR-133 are involved in the development of a normal heart (left) by regulating proliferation, differentiation and cardiac conduction. For example, proliferation is promoted by cell-cycle regulators, but miR-1 and miR-133 block these regulators, thus blocking proliferation. miR-208a also contributes to the regulation of the conduction system. After cardiac injury (right), various miRNAs contribute to pathological remodelling and the progression to heart failure. miR-29 and miR-21 block and promote cardiac fibrosis, respectively. miR-29 blocks fibrosis by inhibiting the expression of ECM components, whereas miR-21 promotes fibrosis by stimulating mitogen-activated protein kinase (MAPK) signalling. miR-208 controls myosin isoform switching, cardiac hypertrophy and fibrosis. miR-23a promotes cardiac hypertrophy by inhibiting ubiquitin proteolysis, which itself inhibits hypertrophy. Hypoxia results in the repression of miR-320 and miR-199, which promote and block apoptosis, respectively. ECM, extracellular matrix; LV, left ventricle; MHC, myosin heavy chain; RV, right ventricle.

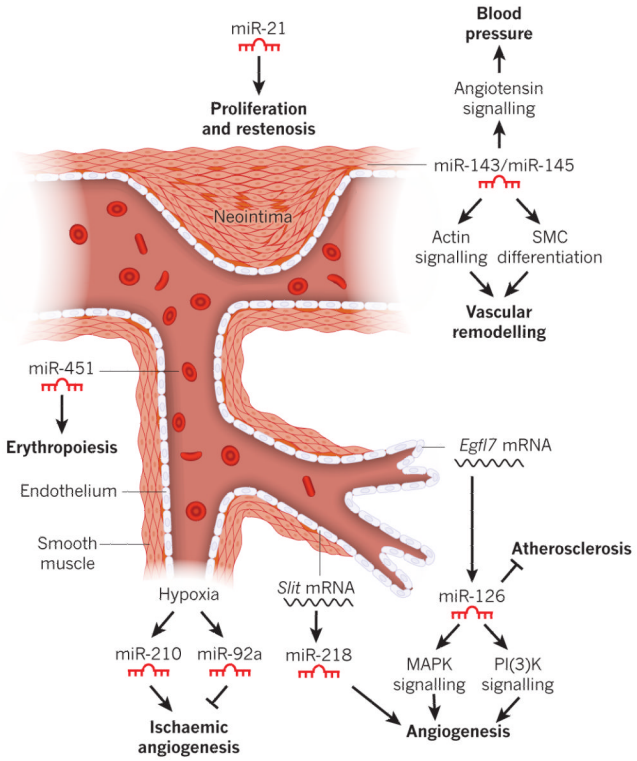

Figure 4. Functional role of miRNAs in the vascular system

Blood vessel schematic showing the endothelial and smooth muscle layers, red blood cells and the proliferating SMCs of a neointimal lesion. The expression of select miRNAs is shown, along with their observed functional role. Hypoxia results in the activation of miR-210 and miR-92a, which promote and inhibit angiogenesis, respectively. miR-126, an endothelial-cell-enriched miRNA encoded by an intron of the *Egfl7* gene, modulates atherosclerosis and angiogenesis by regulating MAPK and PI(3)K signalling. Angiogenesis is also regulated by miR-218, which is encoded by an intron of the *Slit* genes. miR-143 and miR-145 are expressed in SMCs and control blood pressure and vascular tone, and contribute to vascular remodelling. miR-21 is induced in SMCs after vascular injury, and promotes proliferation and neointima formation. miR-451 regulates the proliferation and differentiation of erythroid cells.