



Role of microRNAs in stem/progenitor cells and cardiovascular repair

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Abstract

MicroRNAs (miRNAs), small non-coding RNAs, play a critical role in differentiation and self-renewal of pluripotent stem cells, as well as in differentiation of cardiovascular lineage cells. Several miRNAs have been demonstrated to repress stemness factors such as Oct4, Nanog, Sox2 and Klf4 in embryonic stem cells, thereby promoting embryonic stem cell differentiation. Furthermore, targeting of different miRNAs promotes reprogramming towards induced pluripotent stem cells. MicroRNAs are critical for vascular smooth muscle cell differentiation and phenotype regulation, and miR-143 and miR-145 play a particularly important role in this respect. Notably, these miRNAs are down-regulated in several cardiovascular disease states, such as in atherosclerotic lesions and vascular neointima formation. MicroRNAs are critical regulators of endothelial cell differentiation and ischaemia-induced neovascularization. miR-126 is important for vascular integrity, endothelial cell proliferation and neovascularization. miR-1 and miR-133 are highly expressed in cardiomyocytes and their precursors and regulate cardiomyogenesis. In addition, miR-499 promotes differentiation of cardiomyocyte progenitor cells. Notably, miRNA expression is altered in cardiovascular disease states, and recent studies suggest that dysregulated miRNAs may limit cardiovascular repair responses. Dysregulation of miRNAs may lead to an altered function and differentiation of cardiovascular progenitor cells, which is also likely to represent a limitation of autologous cell-based treatment approaches in these patients. These findings suggest that targeting of specific miRNAs may represent an interesting novel opportunity to impact on endogenous cardiovascular repair responses, including effects on stem/progenitor cell differentiation and functions. This approach may also serve to optimize cell-based treatment approaches in patients with cardiovascular disease.

Keywords

MicroRNA • Stem cell • Progenitor cell • Cardiovascular repair • Endothelium

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1. Introduction

MicroRNAs (miRNAs) are small, non-coding RNAs regulating gene expression at the post-transcriptional level by mRNA degradation or translational repression.¹ The human genome has been estimated to contain up to 1000 miRNAs.¹ The regulation and processing of miRNAs is still incompletely understood; however, important progress has been made in unravelling of these pathways over the past few years. In the first part of this review article, we summarize some recent insights into the biogenesis and processing of miRNAs (see also *Figure 1*) that are also important for the discussion of studies evaluating the role of miRNAs in stem/progenitor cell differentiation and function.

Notably, miRNAs have been found to target transcription factors critically regulating embryonic stem cell (ESC) self-renewal and

differentiation.^{2–5} In addition, several miRNAs have been shown to facilitate reprogramming towards induced pluripotent stem cells (iPSCs).^{6–10} Moreover, specific miRNAs have been identified to regulate differentiation and function of cardiac, vascular smooth muscle, and endothelial progenitor cells, which are discussed in the present article.

Furthermore, emerging data suggest that in cardiovascular disease altered miRNA expression may limit and impair cardiovascular repair responses, including differentiation and function of stem/progenitor cells. Hence, understanding of the physiological and pathophysiological regulation and role of miRNAs in stem and progenitor cells will lead to an improved understanding of the pathophysiology of cardiovascular disease and is likely to provide interesting novel potential therapeutic targets to promote endogenous cardiovascular repair processes.

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2. Biogenesis and processing of miRNA

Most miRNAs are located within introns, and are processed from the primary transcript of their host genes.¹¹ This co-transcription of miRNAs is thought to aid in the fine-tuning of biological processes regulated by the host genes.^{12–14} miRNAs are mostly transcribed by RNA polymerase II,¹⁵ which generates the primary transcript with a stem–loop structure, called pri-miRNA (Figure 1).

Pri-miRNAs are further processed by the microprocessor, i.e. the complex of the nuclear RNase III protein Drosha and the associated co-factor DGCR8 (DiGeorge syndrome critical region 8), which releases a shorter hairpin of about 65 nucleotides (pre-miRNA)

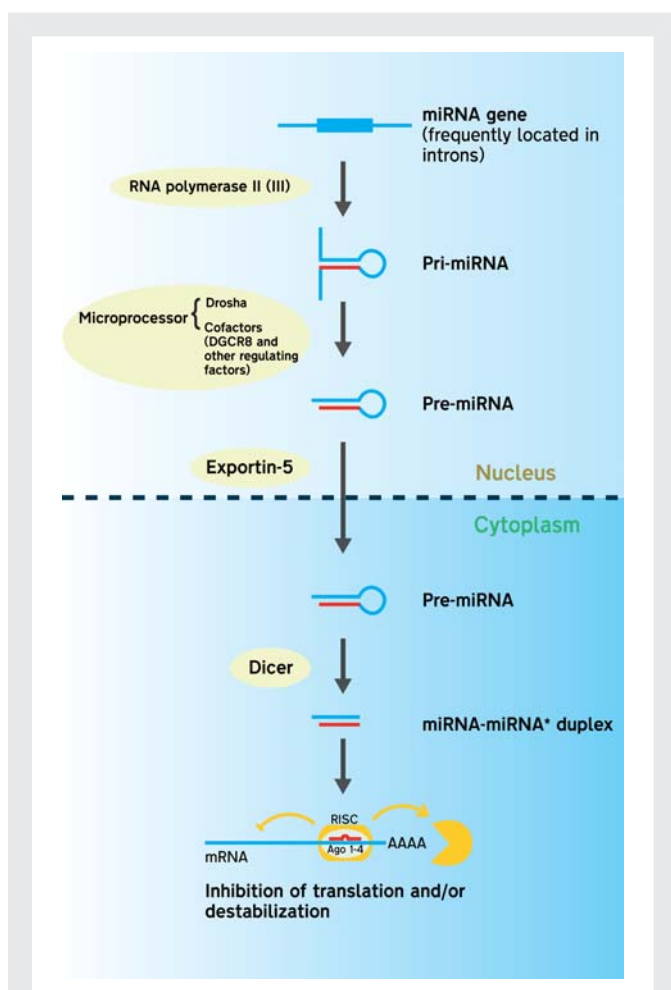


Figure 1 Biogenesis of microRNA (miRNA). Schematic diagram of the canonical pathway of miRNA transcription and processing. miRNAs are transcribed mainly by RNA polymerase II, leading to the primary transcript (pri-miRNA). In the nucleus, pri-miRNAs are cleaved by the RNA-specific endoribonuclease Drosha (and its cofactor DGCR8), resulting in the pre-miRNA. Exportin-5 transports the pre-miRNA into the cytoplasm. In the cytoplasm, pre-miRNAs are processed by Dicer, leading to the miRNA–miRNA* duplex, where the miRNA is the active component and miRNA* gets degraded. In the RNA-induced silencing complex (RISC), which contains a member of the Argonaute family, the miRNA strand leads to mRNA destabilization or translational repression of mRNA. Ago1-4, Argonaute 1-4.

after cleavage.^{16,17} Pre-miRNAs are transported via exportin-5 from the nucleus to the cytoplasm,¹⁸ where they are cleaved by Dicer, an RNase III enzyme.^{19–23} One strand of the unwound miRNA helix is preferentially chosen and loaded into the RNA-induced silencing complex (RISC), whereas the other strand, miRNA*, is degraded.^{24,25} In the RISC, miRNAs interact mainly with the 3' untranslated regions (UTRs) of mRNAs, leading to mRNA destabilization or translational repression, or both.²⁶ Recent data suggest that in humans miRNAs largely lead to mRNA destabilization.²⁷ Of note, more recently miRNAs have also been suggested to increase protein expression of some targets;^{28,29} however, the underlying mechanisms need to be investigated further.

MicroRNA biogenesis may also differ from the above canonical stepwise processing pathway. For example, mirtrons are very short introns of genes that form pre-miRNAs directly, by splicing, and are therefore not processed by Drosha.^{30,31} In addition, there are miRNAs that are processed independent of Dicer that require the Argonaute protein Ago2 for miRNA maturation.^{32,33}

3. MicroRNAs regulate embryonic stem cell differentiation and self-renewal

3.1 Dicer and DGCR8 knockout inhibit ESC differentiation

The contribution of miRNAs to differentiation and function of ESCs has initially been elucidated using knockout models of parts of the miRNA-processing pathways, i.e. deletion of Dicer or the Drosha co-factor, DGCR8. Notably, Dicer deficiency was lethal at an early stage of development in mouse embryos.³⁴ *In vitro*, Dicer-null ESCs failed to differentiate and remained in a self-renewal state, indicating a critical role of miRNAs for ESC differentiation.³⁵ Likewise, DGCR8-knockout ESCs failed to undergo cell lineage commitment, and had an incomplete silencing of the pluripotency factors Oct4, Sox2, Nanog and Rex1.³⁶ These findings underscore the critical role of miRNAs for ESC differentiation into specific cell lineages (Figure 2).

3.2 MicroRNAs regulating ESC self-renewal and differentiation

Subsequently, several miRNAs have been observed to be expressed preferentially in embryonic stem cells. By sequencing short RNAs, the miRNA 290–295 cluster has been found to be highly expressed in embryonic stem cells.² These miRNAs promote ESC proliferation by facilitating the G1 to S transition in the cell cycle,^{37,38} and are therefore considered to have a role in maintaining ESC pluripotency (Figure 2).

Several miRNAs have been shown to be part of the ESC differentiation process and to repress multiple pluripotency factors involved in self-renewal of ESCs, as described below. miR-134, miR-296, and miR-470, which are up-regulated in the differentiation of mouse embryonic stem cells, target the transcription factors Nanog, Sox2, and Oct4.⁴ miR-21 suppresses self-renewal of mouse ESCs, at least in part by decreasing expression of the pluripotency factors Oct4, Nanog, Sox2, and c-Myc.³ miR-145 expression is low in self-renewing human ESCs and is highly up-regulated during human ESC differentiation. miR-145 directly targets the pluripotency factors Oct4, Sox2, and Klf4, and increased

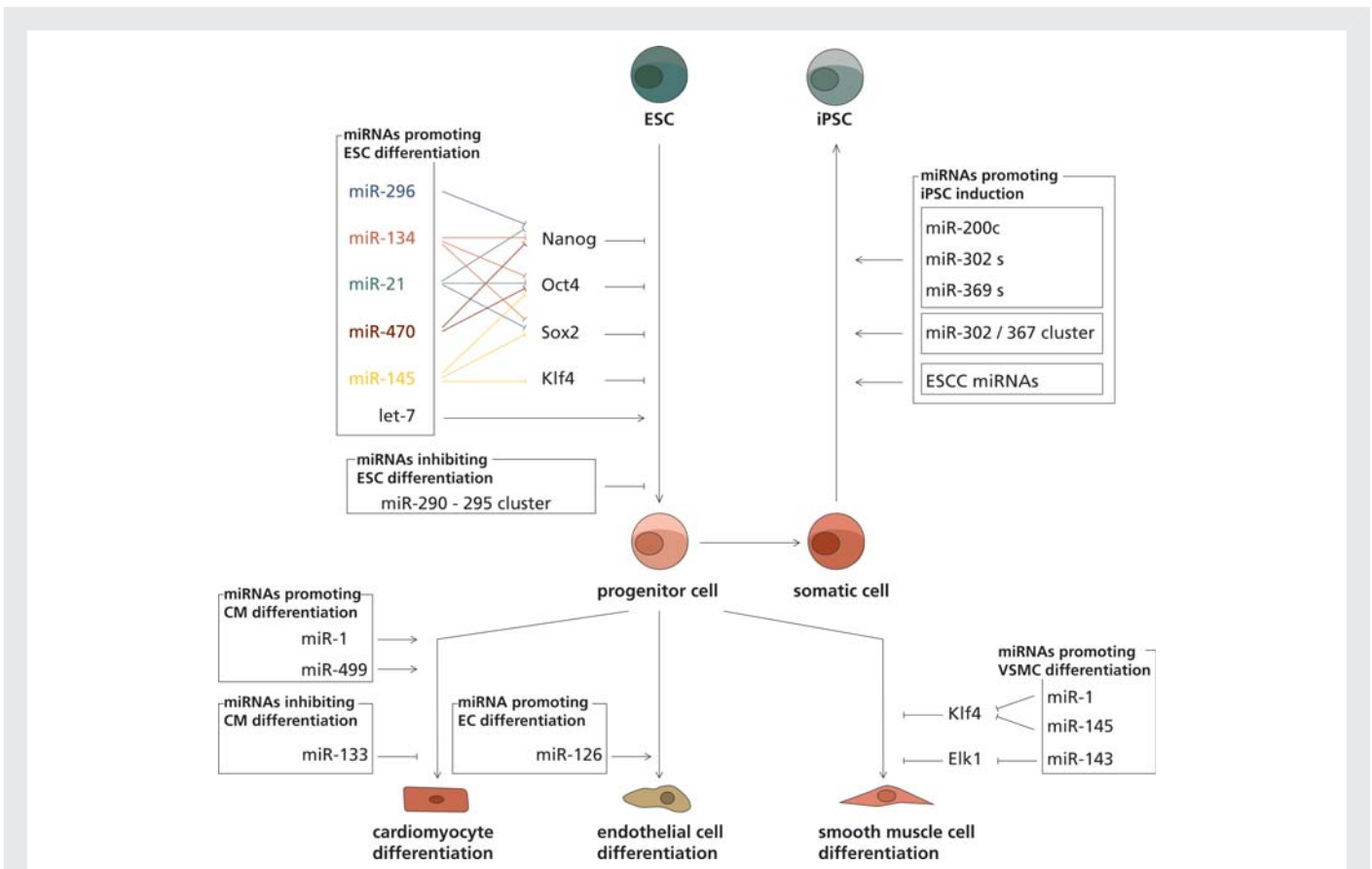


Figure 2 Role of miRNAs in self-renewal and differentiation of pluripotent stem cells and in the differentiation of stem/progenitor cells into cardiovascular lineage cells. Various miRNAs promote embryonic stem cell (ESC) differentiation, mostly by targeting stemness factors. In contrast, the miR-290–295 cluster inhibits ESC differentiation. A subset of the miR-290–295 cluster (miR-291a-3p, miR-291b-3p, miR-294, and miR-295) plus miR-302 are termed embryonic stem cell-specific cell cycle-regulating miRNAs (ESCCs) because they promote cell cycle progression in ESCs. These miRNAs have also been used to increase efficacy of induced pluripotent stem cell (iPSC) formation. Furthermore, several miRNAs play an important role in cardiovascular cell lineage differentiation and in the regulation of the phenotype of these cells. CM, cardiomyocyte; EC, endothelial cell; VSMC, vascular smooth muscle cell.

miR-145 expression inhibits ESC self-renewal, represses expression of the above pluripotency genes, and induces lineage-restricted differentiation.⁵

4. Targeting miRNAs to promote reprogramming towards induced pluripotent stem cells

Mouse fibroblasts were the first differentiated somatic cells to be reprogrammed into iPSCs by viral transduction of Oct3/4, Sox2, c-Myc, and Klf4.³⁹ Human fibroblasts could subsequently be converted into iPSCs by transduction of the same four transcription factors⁴⁰ or by introduction of Oct4, Sox2, Nanog, and Lin28.⁴¹ As mentioned above, stemness factors are regulated by miRNAs, which renders them highly interesting for promoting nuclear reprogramming processes of somatic cells, given the advantage of a non-viral transfection procedure. The role of miRNAs in promoting reprogramming of iPSCs has therefore been intensely examined.⁴²

4.1 Enhancing transcription factor-induced reprogramming towards iPSCs using miRNAs

Notably, silencing of members of the let-7 miRNA family in murine somatic cells has been shown to promote reprogramming towards iPSCs.⁸ Introduction of miR-291-3p, miR-294, and miR-295, together with the transcription factors Oct4, Sox2, and Klf4, substantially enhanced the efficacy of reprogramming towards iPSCs.⁷ These miRNAs have been termed embryonic stem cell-specific cell cycle-regulating miRNAs (ESCCs) because of their abundance and role in proliferation of mouse ESCs.³⁷ Similar results have been reported using the human ESCC miRNA orthologues miR-302b and miR-372.¹⁰

4.2 Induction of reprogramming towards iPSCs using miRNAs without stemness factors

Recently, a non-transcription factor-mediated methodology using miRNAs to reset mouse embryonic fibroblasts into a pluripotent state has been reported.⁶ By transducing the miR-302/367 cluster,

as well as the histone deacetylase inhibitor valproic acid (VPA), into mouse embryonic fibroblasts, an increased efficacy of iPSC formation was observed in comparison with transduction using stemness factors (with VPA).⁶

Moreover, it has been shown that it is possible to reprogramme cells to pluripotency without viral transfection by direct transfection of mature miRNAs (miR-200c, miR-302s, and miR-369s family members) into adipose stromal cells.⁹ However, the efficacy of dedifferentiation was reduced in comparison with the viral transfection procedures, especially in human dermal fibroblasts.⁹

The above studies demonstrate that miRNAs are strongly involved in the regulation of pluripotency and differentiation of stem cells. Lineage commitment steps are frequently regulated by multiple miRNAs. Targeting miRNAs has therefore become an interesting novel approach to modulate stem cell differentiation. A further refined understanding of these miRNA-regulated pathways will lead to novel tools to guide stem cell differentiation and function that is particularly important for regenerative medicine. Moreover, direct reprogramming of adult fibroblasts into induced cardiomyocytes has recently been reported,⁴³ and it will be very interesting to see whether this can also be enhanced or even achieved alone by targeting or introduction of miRNAs.

5. MicroRNAs regulating vascular smooth muscle cell differentiation and phenotype

5.1 Vascular smooth muscle cell-specific knockout of Dicer reveals a critical role of miRNAs in vascular smooth muscle cell differentiation

Recently, the role of miRNAs in differentiation and function of vascular smooth muscle cells (VSMCs) has been elucidated by generating a VSMC-specific Dicer knockout mouse. Mutant mice died between embryonic day 16 and 17 due to extensive haemorrhage and a thinned wall of the aorta caused by a decreased proliferation of VSMCs.⁴⁴ Furthermore, a loss of contractile function and differentiation of VSMCs could be observed, whereas transfection with mimic miR-145 could partly rescue the impaired contractile function and differentiation of VSMCs.⁴⁴

5.2 miR-143 and miR-145 regulate VSMC differentiation and phenotype

miR-143 and miR-145 are co-transcribed and are crucial for the fate of VSMCs, regulating their differentiation, phenotype, and function.²⁹ These miRNAs are co-transcribed and are controlled by Nkx2-5 (NK2 transcription factor related, locus 5) and the serum response factor with its co-activator myocardin. miR-143 promotes VSMC differentiation by repressing Elk-1,²⁹ thereby preventing Elk-1-dependent suppression of VSMC differentiation marker gene expression.⁴⁵ The potent properties of miR-145 to promote VSMC differentiation were underlined by the observation that overexpression of miR-145 in neural crest stem cells resulted in differentiation towards VSMCs *in vitro*.²⁹ Mice lacking either miR-143 or miR-145, or both express a less differentiated VSMC phenotype, indicating that miR-143/145 modulate the fine-tuning of the plasticity of

VSMCs.⁴⁶ Consistently, a decrease of contractile VSMCs and an accumulation of synthetic VSMCs were found in the aorta and the thinned femoral artery in another study examining a miR-143/miR-145 knockout mouse model.⁴⁷ Moreover, agonist-mediated contractility in arterial rings and blood pressure *in vivo* were reduced in miR-143/145 knockout mice.⁴⁷

Inducible depletion of smooth muscle cell-specific Dicer in adult mice led to a similar, although substantially more severe phenotype as observed in miR-143/135 knockout mice, suggesting that additional miRNAs are involved in maintaining post-natal VSMCs in a differentiated state.⁴⁸

5.3 miR-1 regulates VSMC differentiation

Recently, miR-1, which mediates cardiac muscle differentiation after activation of myocardin, was shown to be involved in the differentiation process of VSMCs.^{49,50} Overexpression of myocardin in human VSMCs induces miR-1 and represses proliferation. This process can be reversed by inhibition of miR-1.⁴⁹ Furthermore, ESC differentiation into VSMCs depends on miR-1. Expression of miR-1 was increased during differentiation of ESCs into VSMCs, whereas inhibition of miR-1 repressed VSMC differentiation.⁵⁰ miR-1 shares an identical target with miR-145, the transcription factor Klf4.⁵⁰

5.4 miR-221 and miR-222 regulate VSMC phenotype

miR-221 and miR-222 target the cyclin-dependent kinase inhibitors, p27 and p57, in VSMCs, thereby promoting cell cycle progression and proliferation of these cells.^{51,52} Notably, knockdown of miR-221 and miR-222 suppressed VSMC proliferation *in vivo* and neointimal lesion formation after carotid angioplasty,^{52,53} and these miRNAs may therefore provide a potential therapeutic target to suppress formation of neointima.^{51,52}

6. MicroRNAs regulate vascular integrity, endothelial cell proliferation, and function

6.1 Dicer knockdown reveals critical role of miRNAs in vascular development and endothelial cell functions

Several miRNAs have been identified to play an important role in vascular growth, endothelial cell differentiation, proliferation, and functions. First attempts to gain insight into the role of miRNAs in vascular development/angiogenesis were made by engineering vertebrates lacking Dicer.²⁰ Dicer deficiency resulted in lethality in early development.^{34,53} Morphological analysis revealed less developed blood vessels in embryos and fewer blood vessels in yolk sacs, suggesting that compromised vessel formation/maintenance is leading to embryonic lethality.⁵³ Consistent with this observation, mice with a hypomorphic Dicer expression are infertile owing to impaired angiogenesis in the corpus luteum.⁵⁴ Moreover, a reduction of endothelial miRNAs by Cre-dependent knockout of Dicer in endothelial cells resulted in impaired angiogenesis in distinct models of post-natal angiogenesis.⁵⁵

Subsequently, *in vitro* studies using Dicer-deficient endothelial cells were performed. Silencing of Dicer in endothelial cells caused an impairment of capillary sprouting, tube formation, and migration capacity.^{56,57} This was, at least in part, related to down-regulation of

pro-angiogenic miRNAs, i.e. let-7f and miR-27b, and subsequently an increased expression of several anti-angiogenic factors.^{56,57}

6.2 miR-126 regulates vascular integrity, endothelial cell proliferation, and neovascularization

The most abundant miRNA in CD31⁺ cells is miR-126.⁵⁸ Morpholino-induced knockdown of miR-126 in zebrafish caused compromised vascular integrity and led to haemorrhages.⁵⁸ Likewise, defects in vascular maintenance were also shown in mice lacking miR-126.⁵⁹ Forty per cent of miR-126-deficient mice died embryonically or perinatally owing to defects in endothelial cell proliferation, migration, and angiogenesis causing leaky vessels and haemorrhages.⁵⁹ The surviving subset of mutant mice had a markedly increased mortality and reduced infarct borderzone neovascularization after myocardial infarction.⁵⁹ In summary, miR-126 is important for maintaining vessel integrity during embryogenesis. This was further highlighted in a study investigating aortic arch development in zebrafish, where blood flow activated miR-126, which in turn enhanced vascular endothelial growth factor signalling, thereby facilitating angiogenic sprouting.⁶⁰ However, miR-126 overexpression in ESCs failed to up-regulate endothelial cell-specific markers *in vitro*, hence it is not sufficient to induce endothelial lineage commitment in ESCs.⁵⁸

6.3 miR-17-92 cluster regulates neovascularization

As first shown in several studies in the oncology field, the miR-17-92 cluster, which is induced by the transcription factor c-Myc, regulates distinct angiogenic functions.^{61,62} miR-92a, a member of the miR-17-92 cluster, exerts antiangiogenic functions.⁶³ Antagomir-based silencing of miR-92a increases infarct borderzone neovascularization after myocardial infarction.⁶³ miR-17, miR-18a, miR-19a, and miR-20a inhibit angiogenic activity of mature endothelial cells *in vitro*, and knockdown of miR-17 and miR-20a increased vessel formation as observed in a matrigel plug assay *in vivo*.⁶⁴ In contrast, miR-17-5p, miR-18a, and miR-20a promote proliferation and cord formation in Dicer-deficient human endothelial cells⁵⁵ and enhance neovascularization in tumour cells transduced with the miR-17-92 cluster,⁶⁵ indicating that the regulatory mechanisms of the miR-17-92 cluster differ in distinct tissues and are dependent on the cellular context. Therefore, the effect of miRNAs from the 17-92 cluster differs for ischaemia-induced and tumour-associated angiogenesis, which is important if one considers these miRNAs as potential therapeutic targets.

7. MicroRNAs and cardiomyocyte differentiation

7.1 miR-1 and miR-133 control cardiomyocyte differentiation

miR-1 and miR-133 are highly expressed in cardiac and skeletal muscle cells, and their precursors belong to the same transcriptional unit and play a critical role in myogenesis.^{66–69} miR-1, consisting of two miRNA genes (miR-1-1 and miR-1-2) is co-transcribed with two (miR-133a-2 and miR-133a-1) of the three existing miR-133 genes.^{66,69} These miRNAs are induced by the muscle differentiation factors serum response factor, Mef2, and myocardin.^{66,70}

miR-1 (and to a lesser extent miR-133) promotes mesodermal progenitor formation and suppresses non-muscle gene expression in mouse and human ESCs.⁷¹ The miR-1-dependent adoption of a myogenic phenotype is supported by the repression of Hdac4, which negatively regulates Mef2, an essential transcription factor for the differentiation pathway of muscle cells.^{70,72} Furthermore, miR-1 down-regulates Notch ligand Delta-like 1, which is involved in cardiomyocyte differentiation of ESCs and cardiac progenitor cell division.⁷¹ miR-1 also modulates the fine-tuning of cell polarity.⁷³ Fly embryos deficient in dmiR-1 had misaligned cardioblasts.⁷³

In vivo, deletion of dmiR-1, the single orthologue of miR-1 in *Drosophila*, is lethal due to a loss of muscle differentiation.⁶⁷ miR-1-2 mutant mice revealed ventricle septum defects, cardiac hyperplasia, and abnormal conduction.⁷⁴ In this study, miR-1-1, the second member of the miR-1 family, could not compensate for the loss of its redundant miR-1-2.⁷⁴ Overexpression of dmiR-1 decreased cardiac cell number,⁶⁷ while cardiac-specific excess of miR-1 in mice decreased proliferation by targeting Hand2, a transcription factor regulating ventricular cardiomyocyte expansion.⁶⁶

miR-133 plays a distinct role after mesodermal differentiation. In contrast to miR-1, miR-133 represses cardiac markers, promotes proliferation of myoblasts, and inhibits differentiation through repression of serum response factor.⁷¹ Depletion of the miR-133 family resulted in defects of cardiac morphogenesis and increased proliferation of cardiomyocytes, whereas single depletion resulted in a normal phenotype.⁷⁵

7.2 miR-17-92 cluster regulates cardiac development

The miR-17-92 cluster contributes to regulation of heart development, as indicated by ventricular septal defects of the hearts in a loss-of-function model.⁷⁶ Furthermore, miR-92 controls endoderm development.⁷⁷ Overexpression of miR-92 in zebrafish results in a suppression of endoderm formation, which leads to cardia bifida.⁷⁷

7.3 miR-499 promotes cardiomyocyte differentiation

During cardiomyocyte differentiation of human cardiomyocyte progenitor cells,⁷⁸ human cardiac stem cells,⁷⁹ and human ESCs,⁸⁰ miR-499 is highly up-regulated. Overexpression of miR-499 in human cardiomyocyte progenitor cells indirectly promotes cardiac muscle differentiation by targeting sex determining region Y-box 6 (Sox6) and regulator of differentiation 1 (Rod1) *in vitro*.^{78,79} Furthermore, overexpression of miR-499 in hESCs and human cardiomyocyte progenitor cells up-regulated cardiac marker expression, suggesting a role for miR-499 to control cardiomyocyte cell fate.^{78,80} However, in adult mice cardiac miR-499 overexpression resulted in cellular cardiac hypertrophy and cardiac dysfunction.⁸¹

8. MicroRNA alterations in human cardiovascular disease

Dysregulated expression of miRNAs has been observed in human cardiovascular disease, including altered regulation in progenitor cells.⁸² miRNA tissue profiling approaches in cardiomyopathies⁸³ and in progenitor cells⁸⁴ revealed an altered regulation of miRNAs in different cardiovascular disease states. Subsequently, circulating human miRNA expression profiles in plasma samples were investigated in

distinct cardiovascular diseases, such as stable coronary artery disease⁸⁵ and essential hypertension,⁸⁶ as well as in patients with cardiovascular risk factors, such as diabetes mellitus.^{87,88}

Recently, miRNAs have been implicated in the functional cardiovascular repair capacity of progenitor cells (Table 1). Thus, differential miRNA regulation may play a role in cardiovascular disease both by altering the repair potential of progenitor cells, as well as by impacting on the function and phenotype of differentiated cells. Here we will discuss in particular the miRNAs that may also play a role in progenitor cells.

9. MicroRNA regulation in early endothelial progenitor cells in cardiovascular disease

Early endothelial progenitor cells (EPCs) have been suggested to promote endothelial cell growth (largely in a paracrine manner) and may thereby promote endothelial repair responses and improve cardiac function. As described in detail below, several recent studies have described alterations in miRNAs in early EPCs derived from patients with cardiovascular disease, which may critically limit the endogenous repair response.

9.1 miR-21 is increased in early EPCs in coronary artery disease

In circulating EPCs, miR-21 has been reported to be up-regulated after treatment with asymmetrical dimethylarginine.⁸⁴ Expression of miR-21 was increased in early EPCs from patients with coronary

artery disease.⁸⁴ Moreover, overexpression of miR-21 in early EPCs has been shown to inhibit their migratory capacity through repression of superoxide dismutase 2, a key protection protein against oxidative damage, and the endogenous mitogen-activated protein kinase inhibitor, sprouty-2.⁸⁴

9.2 miR-221 and miR-222 are increased in early EPCs in coronary artery disease

miR-221/222 levels have been observed to be increased in early EPCs derived from patients with coronary artery disease in comparison with healthy subjects and were inversely correlated with EPC number in these patients.⁸⁹ After treatment with atorvastatin, miRNA-221/222 expression decreased and early EPC number increased in patients with coronary artery disease, but no causal relationship has been examined.⁸⁹ miR-221 and miR-222 are also expressed in VSMCs and endothelial cells. Endothelial cells overexpressing miR-222 showed a diminished proliferation and vessel formation in matrigel plugs.⁹⁰

In VSMCs, platelet-derived growth factor, a major growth factor to promote neointimal formation, induces miR-221/222 expression *in vitro*.⁵² Knockdown of miR-221/222 decreases cell proliferation in cultured VSMCs, as well as neointimal formation in balloon-injured rat arteries.⁵²

9.3 miR-126 and miR-130a are reduced in early EPCs in chronic heart failure

Early EPCs from patients with chronic heart failure due to ischaemic cardiomyopathy showed a markedly reduced expression of miR-126 in comparison with early EPCs from healthy subjects, and the *in vivo*

Table 1 MicroRNAs (miRNAs) differentially regulated in early endothelial progenitor cells or miRNAs mediating preconditioning and regulating viability of stem/progenitor cells

Cell type	Differentially expressed miRNA	Disease/modulator	Targets	Function	Reference
A: miRNA regulation in early endothelial progenitor cells					
Early endothelial progenitor cells	miR-21 ↑	CAD/ADMA	SOD2 ↓; SPRY2 ↓	Migration ↓ ROS formation ↑	84
Early endothelial progenitor cells	miR-221/222 ↑	CAD		Early EPC number ↓	89
Early endothelial progenitor cells	miR-126 ↓	CHF	Spred1 ↑	Angiogenesis ↓	91
Early endothelial progenitor cells	miR-130a ↓	CHF	HoxA5 ↑	Angiogenesis ↓	91
Early endothelial progenitor cells	miR-34a ↑	miRNA-overexpression	Sirt1 ↓	Senescence ↑ Angiogenesis ↓	95
B: miRNAs mediating preconditioning and regulating viability of stem/progenitor cells					
Mesenchymal stem cells	miR-210 ↑	IP	Flash/Casp8ap2 ↓	Apoptosis ↓	100
Skeletal myoblast	miR-21 ↑	PP (diazoxide)		Apoptosis ↓	96
Mesenchymal stem cells	miR-146a ↑	PP (diazoxide)	Fas (CD95) ↓	Apoptosis ↓	98
Cardiomyocyte progenitor cells	miR-155 ↑	H ₂ O ₂	RIP1 ↓	Necrosis ↓	99
Cardiac progenitor cells	miR-21, miR-24, miR-221 ↑ (cocktail)	miRNA overexpression	Bim ↓	Viability ↑	101

ADMA, asymmetrical dimethylarginine; Bim, Bcl211 (Bcl-2 protein family); CAD, coronary artery disease; CHF, chronic heart failure; EPC, endothelial progenitor cell; Flash/Casp8ap2, FLICE-associated huge protein/caspase-8-associated protein-2; IP, ischaemic preconditioning; PP, pharmacological preconditioning; RIP1, receptor interacting protein 1; Sirt1, Silent information regulator 1; ROS, reactive oxygen species; SOD2, superoxide dismutase type II; Spred1, Sprouty-related EVH-1 domain containing-1; SPRY2, Sprouty2.

cardiac repair capacity of EPCs from healthy subjects was markedly reduced after anti-miR-126 transfection.⁹¹ Moreover, a reduced expression of miR-130a was observed in early EPCs from patients with chronic heart failure.⁹¹ This miRNA is known to promote angiogenesis by targeting the homeobox genes *GAX* and *HOXA5* in endothelial cells.⁹²

The role of miR-126 in ischaemia-induced angiogenesis has been further shown by an antagomir-based silencing of miR-126 in a hind-limb ischaemia model, in which capillary vessel formation was reduced.⁹³

9.4 miR-34a limits pro-angiogenic capacity of early EPCs

miR-34a has been suggested as an anti-angiogenic and pro-apoptotic miRNA expressed in early EPCs, and has been shown to target silent information regulator 1 (Sirt1) in colorectal carcinoma cells.⁹⁴ Sirt1 is a regulator of apoptosis, the cell cycle, and senescence and acts as a mediator of angiogenesis in endothelial cells. Introduction of mimic miR-34a into rat early EPCs inhibited angiogenesis and induced senescence *in vitro*.⁹⁵ This was suggested to be mediated by Sirt1 targeting and partly by up-regulation of a downstream target of Sirt1, forkhead box O transcription factor 1.⁹⁵

10. MicroRNAs mediate preconditioning and regulate viability of stem/progenitor cells

Recent studies, as described below, have explored the role of miRNAs in mediating preconditioning effects on stem/progenitor cells. These findings may provide interesting novel opportunities for optimization of cell-based treatment approaches for cardiac repair.

10.1 miR-21 exerts cytoprotective effects in skeletal myoblasts

In skeletal myoblasts, miR-21 was up-regulated after pharmacological preconditioning with diazoxide.⁹⁶ The observed cytoprotective effects (with respect to oxidative stress challenge) were reduced after anti-miR-21 treatment, suggesting a critical role of miR-21 in this process.⁹⁶ miR-21 was also up-regulated in left ventricles of rats after ischaemic preconditioning *in vivo* and has been proposed to exert anti-apoptotic effects through inhibition of programmed cell death 4.⁹⁷

10.2 miR-146a protects mesenchymal stem cells from apoptosis

Preconditioning of mesenchymal stem cells with diazoxide prevented these cells from apoptosis through a nuclear factor- κ B-dependent induction of miR-146a *in vitro*.⁹⁸ Up-regulation of miR-146a has cytoprotective effects by repression of Fas (CD 95), a tumour necrosis factor receptor family member, which is required for cell death programming.⁹⁸

10.3 miR-155 may improve survival of cardiomyocyte progenitor cells

miR-155 is up-regulated in human cardiomyocyte progenitor cells after exposure to hydrogen peroxide and has been suggested to improve their survival and inhibit necrosis through repression of

receptor interacting protein 1, a death domain receptor-associated protein.⁹⁹

10.4 miR-210 improves survival of transplanted mesenchymal stem cells in the heart

Ischaemic preconditioning of mesenchymal stem cells up-regulated miR-210 expression in a hypoxia-inducible factor-1 α -dependent fashion and improved their survival after engraftment in the rat infarcted heart.¹⁰⁰ This was mediated by targeting FLICE-associated huge protein/caspase-8-associated protein-2, a pro-apoptotic regulator.¹⁰⁰

10.5 miRNA cocktail (miR-21, miR-24, and miR-221) improves viability of transplanted cardiac progenitor cells

As miRNAs can improve the functional behaviour of progenitor cells, transfection with multiple miRNAs is an emerging approach in order to target the same deleterious transcript more efficiently or to repress multiple mRNAs in order to influence a pathophysiological process. In this respect, Hu *et al.* recently used an miRNA cocktail consisting of miR-21, miR-24, and miR-221 to transfect mouse cardiac progenitor cells, thereby showing that the viability of the transfected cells is enhanced after intramyocardial delivery, accompanied by an enhanced cardiac function after myocardial infarction.¹⁰¹

11. Conclusions

MicroRNAs have been identified as critical regulators of embryonic stem cell self-renewal and differentiation. Several miRNAs have also been reported to promote iPSC reprogramming, which is particularly important for regenerative medicine. Specific miRNAs have been suggested to play a critical role in the differentiation of cardiovascular lineage cells, i.e. in vascular smooth muscle, endothelial cell, and cardiomyocyte differentiation and function.

Notably, miRNAs are differentially regulated in different cardiovascular diseases, including miRNAs expressed in stem/progenitor cells. Altered expression profiles in circulating progenitor or resident tissue stem/progenitor cells may impair the endogenous cardiovascular repair capacity. As miRNA-targeted therapies are entering the clinical arena, a detailed understanding of the role of miRNAs in stem/progenitor cell differentiation and functions, as well as the alterations in cardiovascular disease, is therefore required. A further understanding of the role of specific miRNAs in regulating or limiting endogenous cardiovascular repair responses involving stem/progenitor cells and cardiovascular lineage differentiation may lead to interesting novel therapeutic strategies to promote cardiovascular repair.

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References

1. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. *Nature* 2011;**469**:336–342.

2. Houbaviy HB, Murray MF, Sharp PA. Embryonic stem cell-specific microRNAs. *Dev Cell* 2003;**5**:351–358.
3. Singh SK, Kagalwala MN, Parker-Thornburg J, Adams H, Majumder S. REST maintains self-renewal and pluripotency of embryonic stem cells. *Nature* 2008;**453**:223–227.
4. Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I. MicroRNAs to *Nanog*, *Oct4* and *Sox2* coding regions modulate embryonic stem cell differentiation. *Nature* 2008;**455**:1124–1128.
5. Xu N, Papagiannakopoulos T, Pan G, Thomson JA, Kosik KS. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell* 2009;**137**:647–658.
6. Anokye-Danso F, Trivedi CM, Jühr D, Gupta M, Cui Z, Tian Y et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell Stem Cell* 2011;**8**:376–388.
7. Judson RL, Babiarz JE, Venere M, Blleloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. *Nat Biotechnol* 2009;**27**:459–461.
8. Melton C, Judson RL, Blleloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature* 2010;**463**:621–626.
9. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y et al. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. *Cell Stem Cell* 2011;**8**:633–638.
10. Subramanyam D, Lamouille S, Judson RL, Liu JY, Bucay N, Derynck R et al. Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. *Nat Biotechnol* 2011;**29**:443–448.
11. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. *Genome Res* 2004;**14**:1902–1910.
12. Truscott M, Islam AB, Lopez-Bigas N, Frolow MV. *mir-11* limits the proapoptotic function of its host gene, *dE2f1*. *Genes Dev* 2011;**25**:1820–1834.
13. Barik S. An intronic microRNA silences genes that are functionally antagonistic to its host gene. *Nucleic Acids Res* 2008;**36**:5232–5241.
14. Lutter D, Marr C, Krumsiek J, Lang EW, Theis FJ. Intronic microRNAs support their host genes by mediating synergistic and antagonistic regulatory effects. *BMC Genomics* 2010;**11**:224.
15. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH et al. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 2004;**23**:4051–4060.
16. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003;**425**:415–419.
17. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N et al. The Microprocessor complex mediates the genesis of microRNAs. *Nature* 2004;**432**:235–240.
18. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 2003;**17**:3011–3016.
19. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 2001;**409**:363–366.
20. Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 2001;**293**:834–838.
21. Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I et al. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. *Cell* 2001;**106**:23–34.
22. Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. *Genes Dev* 2001;**15**:2654–2659.
23. Knight SW, Bass BL. A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. *Science* 2001;**293**:2269–2271.
24. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* 2000;**404**:293–296.
25. Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ. Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science* 2001;**293**:1146–1150.
26. Lim LP, Lau NC, Garrett-Engle P, Grimson A, Schelter JM, Castle J et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005;**433**:769–773.
27. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 2010;**466**:835–840.
28. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science* 2007;**318**:1931–1934.
29. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* 2009;**460**:705–710.
30. Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass Drosha processing. *Nature* 2007;**448**:83–86.
31. Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The mirtron pathway generates microRNA-class regulatory RNAs in *Drosophila*. *Cell* 2007;**130**:89–100.
32. Cheloufi S, Dos Santos CO, Chong MM, Hannon GJ. A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. *Nature* 2010;**465**:584–589.
33. Cifuentes D, Xue H, Taylor DW, Patnode H, Mishima Y, Cheloufi S et al. A novel miRNA processing pathway independent of Dicer requires Argonaute2 catalytic activity. *Science* 2010;**328**:1694–1698.
34. Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ et al. Dicer is essential for mouse development. *Nat Genet* 2003;**35**:215–217.
35. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T et al. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev* 2005;**19**:489–501.
36. Wang Y, Medvid R, Melton C, Jaenisch R, Blleloch R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat Genet* 2007;**39**:380–385.
37. Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L, Blleloch R. Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. *Nat Genet* 2008;**40**:1478–1483.
38. Lichner Z, Pall E, Kerekes A, Pallinger E, Maraghechi P, Bosze Z et al. The miR-290–295 cluster promotes pluripotency maintenance by regulating cell cycle phase distribution in mouse embryonic stem cells. *Differentiation* 2011;**81**:11–24.
39. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–676.
40. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;**131**:861–872.
41. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007;**318**:1917–1920.
42. Maherali N, Hochedlinger K. Guidelines and techniques for the generation of induced pluripotent stem cells. *Cell Stem Cell* 2008;**3**:595–605.
43. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 2010;**142**:375–386.
44. Albinsson S, Suarez Y, Skoura A, Offermanns S, Miano JM, Sessa WC. MicroRNAs are necessary for vascular smooth muscle growth, differentiation, and function. *Arterioscler Thromb Vasc Biol* 2010;**30**:1118–1126.
45. Wang Z, Wang DZ, Hockemeyer D, McAnally J, Nordheim A, Olson EN. Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. *Nature* 2004;**428**:185–189.
46. Elia L, Quintavalle M, Zhang J, Contu R, Cossu L, Latronico MV et al. The knockout of miR-143 and –145 alters smooth muscle cell maintenance and vascular homeostasis in mice: correlates with human disease. *Cell Death Differ* 2009;**16**:1590–1598.
47. Boettger T, Beetz N, Kostin S, Schneider J, Kruger M, Hein L et al. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the *Mir143/145* gene cluster. *J Clin Invest* 2009;**119**:2634–2647.
48. Albinsson S, Skoura A, Yu J, DiLorenzo A, Fernandez-Hernando C, Offermanns S et al. Smooth muscle miRNAs are critical for post-natal regulation of blood pressure and vascular function. *PLoS One* 2011;**6**:e18869.
49. Chen J, Yin H, Jiang Y, Radhakrishnan SK, Huang ZP, Li J et al. Induction of microRNA-1 by myocardin in smooth muscle cells inhibits cell proliferation. *Arterioscler Thromb Vasc Biol* 2011;**31**:368–375.
50. Xie C, Huang H, Sun X, Guo Y, Hamblin M, Ritchie RP et al. MicroRNA-1 regulates smooth muscle cell differentiation by repressing Kruppel-like factor 4. *Stem Cells Dev* 2011;**20**:205–210.
51. Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A. Induction of microRNA-221 by platelet-derived growth factor signaling is critical for modulation of vascular smooth muscle phenotype. *J Biol Chem* 2009;**284**:3728–3738.
52. Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. *Circ Res* 2009;**104**:476–487.
53. Yang WJ, Yang DD, Na S, Sandusky GE, Zhang Q, Zhao G. Dicer is required for embryonic angiogenesis during mouse development. *J Biol Chem* 2005;**280**:9330–9335.
54. Otsuka M, Zheng M, Hayashi M, Lee JD, Yoshino O, Lin S et al. Impaired microRNA processing causes corpus luteum insufficiency and infertility in mice. *J Clin Invest* 2008;**118**:1944–1954.
55. Suarez Y, Fernandez-Hernando C, Yu J, Gerber SA, Harrison KD, Pober JS et al. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. *Proc Natl Acad Sci USA* 2008;**105**:14082–14087.
56. Kuehbach A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circ Res* 2007;**101**:59–68.
57. Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. *Circ Res* 2007;**100**:1164–1173.
58. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD et al. miR-126 regulates angiogenic signaling and vascular integrity. *Dev Cell* 2008;**15**:272–284.
59. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. *Dev Cell* 2008;**15**:261–271.
60. Nicoli S, Standley C, Walker P, Hurlstone A, Fogarty KE, Lawson ND. MicroRNA-mediated integration of haemodynamics and Vegf signalling during angiogenesis. *Nature* 2010;**464**:1196–1200.
61. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* 2005;**435**:839–843.

62. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S *et al.* A microRNA polycistron as a potential human oncogene. *Nature* 2005;**435**:828–833.
63. Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A *et al.* MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. *Science* 2009;**324**:1710–1713.
64. Doebele C, Bonauer A, Fischer A, Scholz A, Reiss Y, Urbich C *et al.* Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. *Blood* 2010;**115**:4944–4950.
65. Dews M, Homayouni A, Yu D, Murphy D, Seignani C, Wentzel E *et al.* Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat Genet* 2006;**38**:1060–1065.
66. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets *Hand2* during cardiogenesis. *Nature* 2005;**436**:214–220.
67. Kwon C, Han Z, Olson EN, Srivastava D. MicroRNA1 influences cardiac differentiation in *Drosophila* and regulates Notch signaling. *Proc Natl Acad Sci USA* 2005;**102**:18986–18991.
68. Rao PK, Kumar RM, Farkhondeh M, Baskerville S, Lodish HF. Myogenic factors that regulate expression of muscle-specific microRNAs. *Proc Natl Acad Sci USA* 2006;**103**:8721–8726.
69. Liu N, Williams AH, Kim Y, McAnally J, Bezprozvannaya S, Sutherland LB *et al.* An intragenic MEF2-dependent enhancer directs muscle-specific expression of microRNAs 1 and 133. *Proc Natl Acad Sci USA* 2007;**104**:20844–20849.
70. Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM *et al.* The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 2006;**38**:228–233.
71. Ivey KN, Muth A, Arnold J, King FW, Yeh RF, Fish JE *et al.* MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. *Cell Stem Cell* 2008;**2**:219–229.
72. Lu J, McKinsey TA, Zhang CL, Olson EN. Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. *Mol Cell* 2000;**6**:233–244.
73. King IN, Qian L, Liang J, Huang Y, Shieh JT, Kwon C *et al.* A genome-wide screen reveals a role for microRNA-1 in modulating cardiac cell polarity. *Dev Cell* 2011;**20**:497–510.
74. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN *et al.* Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell* 2007;**129**:303–317.
75. Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R *et al.* microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev* 2008;**22**:3242–3254.
76. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. *Cell* 2008;**133**:217–222.
77. Li N, Wei C, Olena AF, Patton JG. Regulation of endoderm formation and left-right asymmetry by miR-92 during early zebrafish development. *Development* 2011;**138**:1817–1826.
78. Sluijter JP, van Mil A, van Vliet P, Metz CH, Liu J, Doevendans PA *et al.* MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. *Arterioscler Thromb Vasc Biol* 2010;**30**:859–868.
79. Hosoda T, Zheng H, Cabral-da-Silva M, Sanada F, Ide-Iwata N, Ogorek B *et al.* Human cardiac stem cell differentiation is regulated by a mircrine mechanism. *Circulation* 2011;**123**:1287–1296.
80. Wilson KD, Hu S, Venkatasubrahmanyam S, Fu JD, Sun N, Abilez OJ *et al.* Dynamic microRNA expression programs during cardiac differentiation of human embryonic stem cells: role for miR-499. *Circ Cardiovasc Genet* 2010;**3**:426–435.
81. Shieh JT, Huang Y, Gilmore J, Srivastava D. Elevated miR-499 levels blunt the cardiac stress response. *PLoS One* 2011;**6**:e19481.
82. Bauersachs J, Thum T. Biogenesis and regulation of cardiovascular microRNAs. *Circ Res* 2011;**109**:334–347.
83. Ikeda S, Kong SW, Lu J, Bisping E, Zhang H, Allen PD *et al.* Altered microRNA expression in human heart disease. *Physiol Genomics* 2007;**31**:367–373.
84. Fleissner F, Jazbutyte V, Fiedler J, Gupta SK, Yin X, Xu Q *et al.* Short communication: asymmetric dimethylarginine impairs angiogenic progenitor cell function in patients with coronary artery disease through a microRNA-21-dependent mechanism. *Circ Res* 2010;**107**:138–143.
85. Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C *et al.* Circulating microRNAs in patients with coronary artery disease. *Circ Res* 2010;**107**:677–684.
86. Li S, Zhu J, Zhang W, Chen Y, Zhang K, Popescu LM *et al.* Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. *Circulation* 2011;**124**:175–184.
87. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M *et al.* Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res* 2010;**107**:810–817.
88. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K *et al.* Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008;**18**:997–1006.
89. Minami Y, Satoh M, Maesawa C, Takahashi Y, Tabuchi T, Itoh T *et al.* Effect of atorvastatin on microRNA 221/222 expression in endothelial progenitor cells obtained from patients with coronary artery disease. *Eur J Clin Invest* 2009;**39**:359–367.
90. Dentelli P, Rosso A, Orso F, Olgasi C, Taverna D, Brizzi MF. microRNA-222 controls neovascularization by regulating signal transducer and activator of transcription 5A expression. *Arterioscler Thromb Vasc Biol* 2010;**30**:1562–1568.
91. Doerries C, Briand S, Besler C, Mocharla P, Mueller M, Manes C *et al.* Antimir treatment reveals a critical role of miR-126 and miR-130a for in vivo cardiac and endothelial repair capacity of early endothelial progenitor cells: marked down-regulation in patients with ischemic cardiomyopathy. *Circulation* 2010;**122**:A21339.
92. Chen Y, Gorski DH. Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes *GAX* and *HOXA5*. *Blood* 2008;**111**:1217–1226.
93. van Solingen C, Seghers L, Bijkker R, Duijs JM, Roeten MK, van Oeveren-Rietdijk AM *et al.* Antagomir-mediated silencing of endothelial cell specific microRNA-126 impairs ischemia-induced angiogenesis. *J Cell Mol Med* 2009;**13**:1577–1585.
94. Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. *Proc Natl Acad Sci USA* 2008;**105**:13421–13426.
95. Zhao T, Li J, Chen AF. MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1. *Am J Physiol Endocrinol Metab* 2010;**299**:E110–E116.
96. Haider KH, Idris NM, Kim HW, Ahmed RP, Shujia J, Ashraf M. MicroRNA-21 is a key determinant in IL-11/Stat3 anti-apoptotic signalling pathway in preconditioning of skeletal myoblasts. *Cardiovasc Res* 2010;**88**:168–178.
97. Cheng Y, Zhu P, Yang J, Liu X, Dong S, Wang X *et al.* Ischaemic preconditioning-regulated miR-21 protects heart against ischaemia/reperfusion injury via anti-apoptosis through its target PDCD4. *Cardiovasc Res* 2010;**87**:431–439.
98. Suzuki Y, Kim HW, Ashraf M, Haider H. Diazoxide potentiates mesenchymal stem cell survival via NF- κ B-dependent miR-146a expression by targeting Fas. *Am J Physiol Heart Circ Physiol* 2010;**299**:H1077–H1082.
99. Liu J, van Mil A, Vrijssen K, Zhao J, Gao L, Metz CH *et al.* MicroRNA-155 prevents necrotic cell death in human cardiomyocyte progenitor cells via targeting RIP1. *J Cell Mol Med* 2011;**15**:1474–1482.
100. Kim HW, Haider HK, Jiang S, Ashraf M. Ischemic preconditioning augments survival of stem cells via miR-210 expression by targeting caspase-8-associated protein 2. *J Biol Chem* 2009;**284**:33161–33168.
101. Hu S, Huang M, Nguyen PK, Gong Y, Li Z, Jia F *et al.* Novel microRNA pro-survival cocktail for improving engraftment and function of cardiac progenitor cell transplantation. *Circulation* 2011;**124**:S27–S34.