Histol Histopathol (2015) 30: 141-149
DOI: 10.14670/HH-30.141

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Spatio-temporal expression patterns of microRNAs in remodelling and repair of the infarcted heart

H.H. Chiarella-Redfern[1,3], K.J. Rayner[2,4] and E.J. Suuronen[1,3]

[1]Division of Cardiac Surgery and [2]Atherosclerosis, Genomics and Cell Biology Group University of Ottawa Heart Institute, [3]Department of Cellular and Molecular Medicine and [4]Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa ON, Canada

Summary. MicroRNAs (miRNAs) are small, non-messenger RNAs, 20-22 nucleotides in size, which regulate gene expression at the post-transcriptional level. Typically, miRNAs target the 3' untranslated region (3'UTR) of mRNA transcripts leading to mRNA degradation or translational repression. The known dysregulation of miRNAs during cardiac ischemia and the crucial role of miRNA-dependent regulation of angiogenesis, fibrosis and hypertrophy present interesting therapeutic opportunities for repairing and regenerating the heart after myocardial infarction (MI). An understanding of the expression pattern and localization of deleterious and beneficial miRNAs during cardiac ischemia is necessary for the development of therapeutics designed to specifically treat the affected tissue and cell populations. This review focuses on the role and localization of key miRNAs implicated in MI while highlighting how their manipulation may promote cardiac repair.

Key words: Ischemia, MicroRNA, Myocardial infarction, Regeneration, Tissue remodelling

MicroRNAs - functional concepts

MicroRNAs (miRNAs), a class of small noncoding RNAs, "fine tune" gene expression by negatively regulating the translational output of target messenger RNA (mRNA) in a sequence-dependent manner (Ambros, 2004; Bartel, 2004; Roy et al., 2009; Small et al., 2010). Most miRNA encoding genes are intergenic but can also be generated by the processing of introns of protein-coding genes allowing for the coordinated expression of a miRNA and protein, occasionally leading to the joint modulation of a biological process (e.g. control of cholesterol homeostasis by miR-33 and its host gene) (Najafi-Shoushtari et al., 2010; Small and Olson, 2011). In mammals, the processing of miRNA encoding transcripts by Drosha-DGCR8 and Dicer leads to a miRNA duplex in which one strand is loaded into the RNA-induced silencing complex (RISC), becoming the "mature" miRNA and the other strand, called the star strand, is typically degraded (Fig. 1) (Bartel, 2004; Valencia-Sanchez, 2006). RISC coordinates the binding of the loaded miRNA to the 3' untranslated region (UTR) of target mRNA, which promotes mRNA degradation or translational inhibition, and thus reduces production of the protein (Small et al., 2010; Small and Olson, 2011). Most 3'UTRs contain potential binding sites for numerous miRNAs, allowing redundancy or cooperative interactions between various seemingly unrelated miRNAs (Small et al., 2010). In addition, miRNAs have been shown to regulate multiple genes governing the same biological processes (e.g. the role of miR-29 in fibrosis), thus potentially reducing the importance of single miRNA-mRNA interactions in eliciting a biological response (Berezikov et al., 2005; Small et al., 2010; He et al., 2013). The multi-target and multi-function properties of miRNAs add robustness to gene-regulatory networks making the exploration and regulation of miRNAs extremely therapeutically

Offprint requests to: Erik J. Suuronen, Division of Cardiac Surgery, University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Ontario, K1Y 4W7, Canada. e-mail: esuuronen@ottawaheart.ca

exciting.

MicroRNAs in cardiovascular disease

Loss of blood flow and subsequent myocardial infarction (MI) results in cardiomyocyte death. This triggers inflammatory cell recruitment, the removal of cellular debris, and adverse cardiac remodelling leading to impaired cardiac contractility (Fraccarollo et al., 2012). Healing after MI is dependent, in part, on degradation and remodeling of the extracellular matrix and neoangiogenesis, through the secretion of cytokines, proteases, and growth factors (Fraccarollo et al., 2012). The importance of miRNAs in cardiovascular development and function was initially observed after Dicer deletion in myocardial and vascular lineages produced lethal phenotypes four days after birth (Chen et al., 2008; Albinsson et al., 2010). The dynamic regulation of numerous cardiac-enriched miRNAs during cardiac stress indicates their involvement in the regulation of cardiovascular disease (van Rooij et al., 2006; Ikeda et al., 2007). More specifically, several studies indicate a crucial role for miRNA-dependent regulation of cardiac angiogenesis, fibrosis, and cardiomyocyte hypertrophy following MI (as reviewed in (Fiedler and Thum, 2014)). Since miRNAs can be cell- and tissue- dependent, the spatio-temporal expression of miRNAs may provide important insight as to their potential functions (Fig. 2). To this end, in situ hybridization (ISH), involving the hybridization of a labeled nucleic acid probe to a complementary RNA sequence in whole organisms, cells or histological tissue sections is a powerful technique that can be used to localize and specifically detect expression patterns of probe targets in tissues and cell populations. ISH has been applied for the histological analysis of miRNAs and the elucidation of their molecular and biological functions in cardiac tissue (Nielsen, 2012). However, the small size and fragile nature of microRNA can make detection challenging (Jorgensen et al., 2010). To address this issue, techniques such as locked nucleic acids (LNA), modified RNA nucleotides with a methylene bridge connecting the 2' oxygen, and 4'carbon of the ribose backbone have been developed (Jorgensen et al., 2010; Nuovo, 2010). These modifications increase the probe's melting temperature and its target affinity, allowing it to bind more specifically towards complementary miRNAs and are also resistant to many endonucleases (Jorgensen et al., 2010; Nuovo, 2010; Song et al., 2010; Broderick and Zamore, 2011; Nielsen, 2012). This review focuses on the use of such techniques for the localization of key miRNAs implicated in MI and highlight how their manipulation can promote cardiac repair.

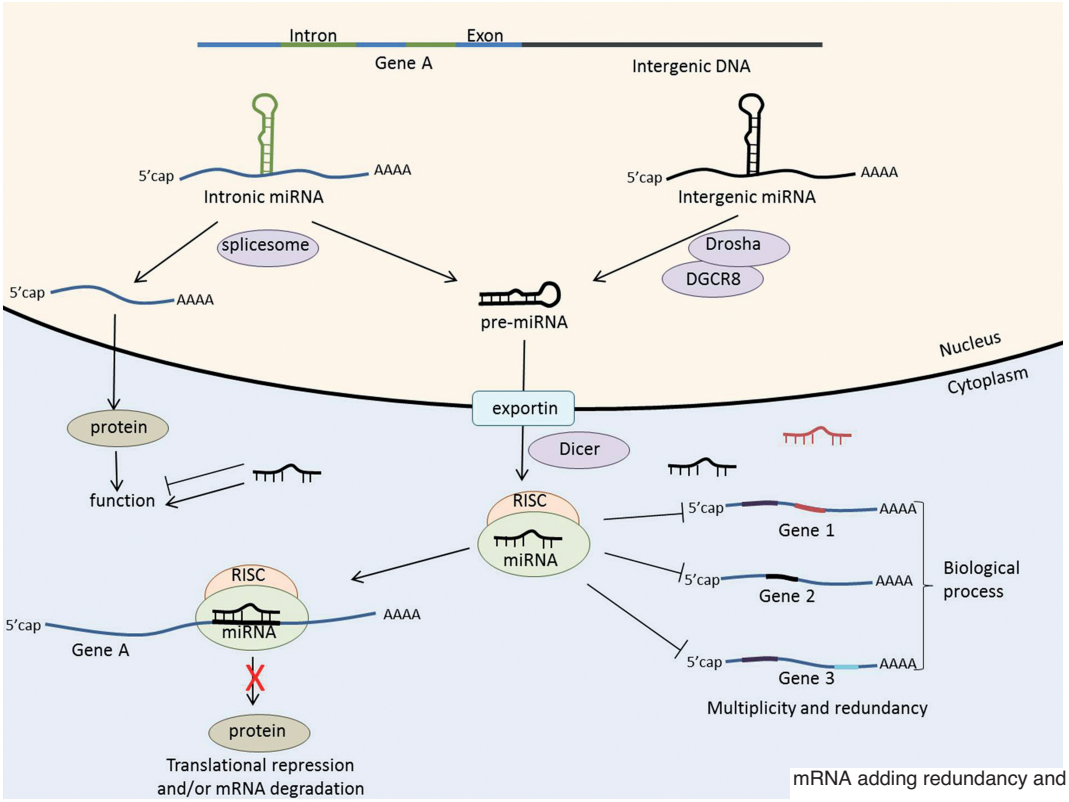

Fig. 1. miRNA biogenesis and functional concepts. From the introns of pre-mRNA or the non-coding regions of the genome, miRNAs are transcribed as long precursors which are cleaved by Drosha and DGCR8 into pre-microRNAs and are actively transported to the cytoplasm to be further cleaved by dicer into a miRNA duplex. Subsequently, one strand of the miRNA is assembled into the RNA-induced silencing complex (RISC) to guide the sequence-specific recognition of its target mRNA leading to mRNA degradation or translational repression (gene A). Since intronic miRNAs are transcribed at the same time as its host gene, it is common for the miRNA to regulate a similar process as the protein it was transcribed from. A characteristic of miRNAs is the ability of a single miRNA to regulate multiple mRNAs in a biological process (gene 1, 2 and 3). In addition, multiple miRNAs can target the same mRNA adding redundancy and cooperation between miRNAs.

MicroRNAs implicated in myocardial ischemia and cardiac repair

MicroRNA-21

To date, the role of miR-21 in cardiovascular disease is controversial. Thum et al. observed a robust up-regulation of miR-21 in cardiac fibroblasts in a mouse model of cardiac failure (Thum et al., 2008). Within fibroblasts, sprouty homologue 1 (Spry1) was identified as a direct target of miR-21; and Spry1 is a potent inhibitor of the Ras/MEK/ERK pathway that regulates fibroblast survival and growth factor secretion (Casci et al., 1999; Hanafusa et al., 2002; Thum et al., 2008). *In vivo* silencing with antimiR-21 in a mouse pressure-overload-induced cardiac hypertrophy model reduced cardiac ERK–MAP kinase activity, inhibited interstitial fibrosis and attenuated cardiac dysfunction, thus highlighting the therapeutic potential of targeting miR-21 (Thum et al., 2008). In a model of ischemia/reperfusion (IR) injury, it was demonstrated by ISH that miR-21 up-regulation in the mouse heart occurred at 2 and 7 days after injury and was found to be localized specifically within fibroblasts of the infarct zone (Roy et al., 2009). It was also shown that miR-21 regulates matrix metalloprotease-2 (MMP-2) expression in cardiac fibroblasts via a phosphatase and tensin homologue (PTEN) pathway, thus identifying another miR-21 mechanism (Roy et al., 2009). Another study using lateral LV wall samples from patients with severe aortic stenosis (AS) undergoing aortic valve replacement therapy showed higher myocardial expression levels of primary transcripts as well as mature miR-21 in comparison to a group of surgical controls with no pressure or volume overload and normal left ventricular mass (Villar et al., 2013). ISH using digoxigenin-double-labelled specific miR-21 probes showed that the signal in AS patient samples was restricted to cells in the interstitial space, presumably fibroblasts, with only a very week hybridization signal in the LV myocardium of the control patients, thus reinforcing the specific localization and up-regulation of miR-21 in fibroblasts (Villar et al., 2013). In contrast, Patrick et al. demonstrated that miR-21-null mice and LNA-antimiR-21 treated mice displayed cardiac hypertrophy, fibrosis and loss of cardiac contractility similar to wild-type littermates after various cardiac stresses, indicating that miR-21 does not play an important role in pathological cardiac remodelling (Patrick et al., 2010). The opposing evidence for the involvement of miR-21 in cardiac disease could be, in part, due to a compensatory mechanism activated in the persistent absence of miR-21, which may be different from pharmacological inhibition of a miRNA in an adult organism. Another factor could be attributed to the differences in chemistry and oligonucleotide length of the short LNA-modified antimiR-21 and longer cholesterol conjugated antagomiR, but still there is no consensus (Patrick et al., 2011; Thum et al., 2011; Tu et al., 2013; Bang et al., 2014).

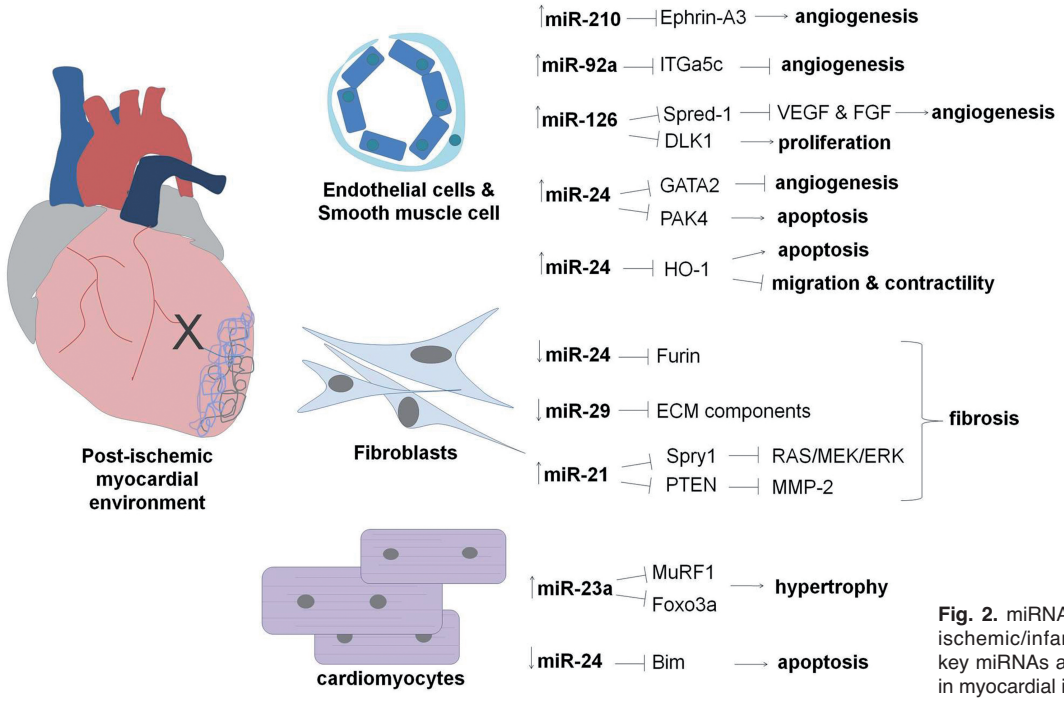

Fig. 2. miRNA regulation and function in the ischemic/infarcted myocardium. Targets of key miRNAs and the processes they regulate in myocardial ischemia/infarction are shown.

MicroRNA-23a

Hypertrophic growth as an adaptive process post-cardiac injury has been shown to be regulated by a number of miRNAs including miR-23a (van Rooij et al., 2006; Care et al., 2007; Cheng et al., 2007; Callis et al., 2009). Van Rooij et al. identified miR-23a as a pro-hypertrophic miRNA after observing its up-regulation in cardiomyocytes upon inducing cardiac hypertrophy (Van Rooij et al., 2006). The same group demonstrated that miR-23a is regulated by the binding of nuclear factor of activated T cells (NFATc3) to the promoter region of miR-23a, and it may convey a hypertrophic signal by directly targeting the anti-hypertrophic muscle specific finger protein 1 (MuRF1) (Lin et al., 2009). *In vivo* inhibition of miR-23a inhibited isoproterenol-induced hypertrophy and restored MuRF1 expression, highlighting the therapeutic potential of antimiR-23a therapies (Lin et al., 2009). In another study, the involvement of miR-23a in hypertrophy was confirmed using transgenic miR-23a mice, and Foxo3a, which is involved in the regulation of cardiac hypertrophy, has been identified as a direct target of miR-23a (Skurk et al., 2005; Wang et al., 2012b). Up-regulation of miR-23a was also shown in a rat model of pressure overload-induced hypertrophy 14 days after aortic banding when compared to sham-operated controls, highlighting conservation of the role of miR-23a in cardiac hypertrophy across species (Busk and Cirera, 2010). In addition to being expressed in adult mouse and rat hearts, miR-23a was shown to be abundantly expressed in embryonic mouse hearts (E12.5, E14.5, E16.5 and E18.5), suggestive of its importance in cardiac development (Cao et al., 2012). Ruan et al. identified a role for miR-23a in endothelial cells; specifically, endothelial cell apoptosis stimulated by 24 hours of exposure to TNF-α caused the down-regulation of the highly expressed miR-23a, which when reversed by pharmacological up-regulation of miR-23a, a significant decrease in endothelial cell apoptosis was observed (Ruan et al., 2012). In addition, TNF-α treatment increased the expression of the miR-23a targets caspase-7 and STK4 (Ruan et al., 2012). Similarly, a 24 hour TNF-α treatment of bone marrow- mesenchymal stem cells (BM-MSC) induced apoptosis, reduced miR-23a levels and increased capase-7 expression (Mao et al., 2013). When BM-MSCs over-expressing miR-23a were injected into the infarct zone 14 days after MI, a significant improvement in left ventricular ejection fraction (LVEF) and reduced infarct size were observed (Mao et al., 2013). Variability in miR-23a expression in cardiomyocytes, endothelial cells and mesenchymal cells highlights the importance of understanding the spatio-temporal expression pattern of miR-23a in order to develop therapeutics that efficiently target the miRNA for specific treatment applications.

MicroRNA-29

The miR-29 family is dramatically down-regulated

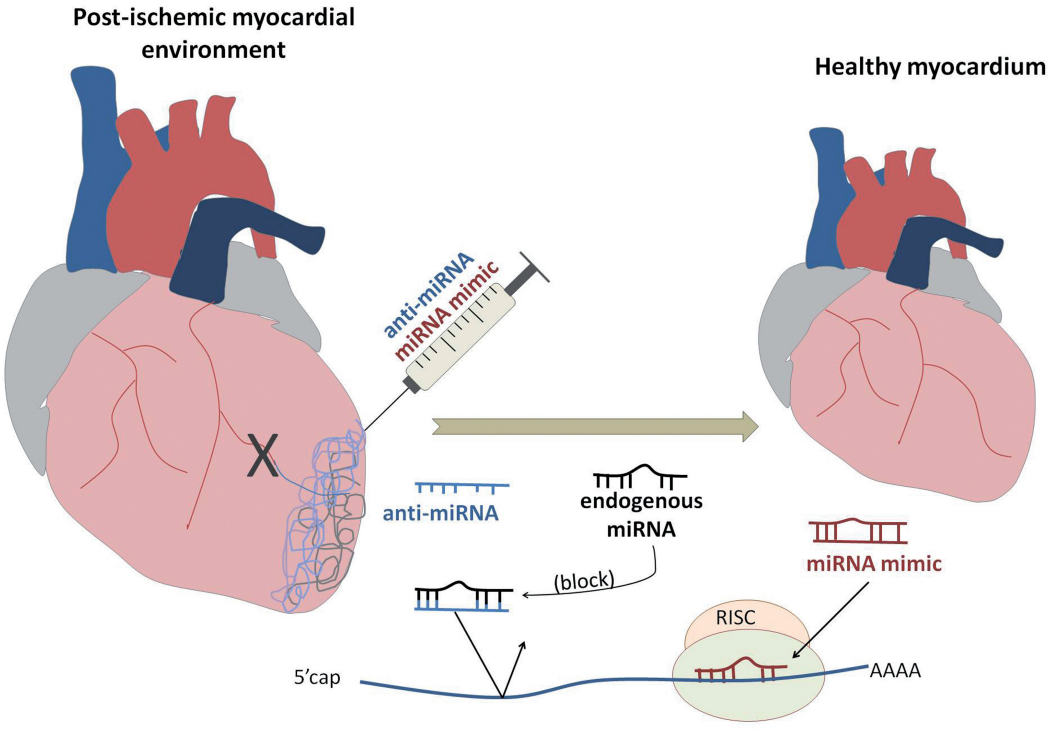

Fig. 3. Clinical perspectives for cardiac repair using miRNA-based therapies. The ease by which miRNAs can be manipulated pharmacologically provides a therapeutic opportunity to alter miRNA levels dysregulated during ischemia/infarction to promote cardiac repair. Anti-miRNAs bind to endogenous miRNAs preventing them from binding to their target, thus increasing target gene expression. In contrast, miRNA mimics increase endogenous miRNA levels and reduce target gene expression through translational repression or mRNA degradation.

in human and mouse hearts after myocardial infarction through signaling involving transforming growth factor-β (TGF-β), a major regulator of cardiac fibrosis (Border and Noble, 1994; van Rooij et al., 2008b). Specifically, miR-29 was shown to be down-regulated in the infarct border zone 3 days after MI and continued to be lowly expressed 11 days later in comparison to sham operated mice (van Rooij et al., 2008b). Its localization within cardiac fibroblasts in pressure overloaded hypertrophic hearts has been identified by ISH (Abonnenc et al., 2013). This correlates nicely with the findings that the miR-29 family plays a role in attenuating cardiac fibrosis through the targeting of multiple ECM proteins such as collagens, fibrillin and elastin; however, the specific mechanism(s) involved are not very clear and require further investigation (van Rooij et al., 2008b; Abonnenc et al., 2013; He et al., 2013). In cardiac fibroblasts, the over-expression of miR-29 was shown to inhibit collagen and other extracellular protein synthesis *in vivo* and *in vitro*, indicating its potential as an anti-fibrotic therapy (van Rooij et al., 2008b). The expression patterns of cardiac miR-29b in both normal and hypertrophic hearts were examined by ISH, and its presence was found in all cardiac tissues including vascular smooth muscle cells, endothelial cells, fibroblasts and cardiomyocytes in the normal mouse heart, while its expression was reduced at 14 and 28 days after induction of hypertrophy, particularly in the areas with severe cardiac fibrosis (Zhang et al., 2014). The ability of miR-29 to simultaneously regulate multiple mRNAs with roles in fibrosis and ECM remodelling illustrates the potential and power of miRNAs to control complex cellular processes.

MicroRNA-24

After MI, miR-24 expression is altered in a number of cardiac cells. Down-regulation of miR-24 is seen in cardiomyocytes and fibroblasts within the peri-infarct tissue, whereas it is up-regulated in endothelial cells at 1 and 3 days post-MI (Fiedler et al., 2011; Qian et al., 2011; Wang et al., 2012a; Meloni et al., 2013). MiR-24, which can suppress cardiomyocyte apoptosis by targeting the BH3-only domain–containing protein Bim, was shown to be down-regulated in the ischemic border zone after MI but not in the distant myocardium (Qian et al., 2011). In this same study, the spatio-temporal expression pattern was specifically identified using qPCR and ISH techniques, demonstrating that the down-regulation was present at 24 hours, 3 days and 1 week post-MI in the border zone, which was attenuated by 4 weeks (Qian et al., 2011). In addition, the down-regulation was temporally correlated with increased apoptosis peaking at 24 hours post-MI (Qian et al., 2011). Over-expression of miR-24 *in vivo* inhibited cardiomyocyte apoptosis, attenuated infarct size, and reduced cardiac dysfunction after MI (Qian et al., 2011). Another group showed that down-regulation of miR-24 after MI was closely linked with ECM remodelling and that its over-expression *in vivo* could attenuate fibrosis and improve heart function, potentially through a furin-TGF-β pathway in cardiac fibroblasts (Wang et al., 2012a). Furthermore, the expression of miR-24 was measured and its location was visualized histologically in cardiac sections at different time-points after MI; it was found to be down-regulated in the infarct zone, border zone and remote myocardium at 1 and 2 weeks post-MI, which was attenuated by 4 weeks and was closely associated with fibrotic remodeling (Wang et al., 2012a). *In vitro*, miR-24 up-regulation in endothelial cells induces apoptosis and abolishes capillary network formation via GATA2 and PAK4 targeting, and *in vivo* inhibition of miR-24 after MI reduces endothelial cells apoptosis leading to enhanced vascularity and cardiac function (Fiedler et al., 2011). A spatio-temporal analysis of miR-24 expression in fractionated cardiac cell types demonstrated selectively strong induction of miR-24 in endothelial cells isolated from ischemic but not remote myocardium early after MI (Fiedler et al., 2011). Using ISH co-staining methods, it was demonstrated that the miR-24 enrichment was specific to endothelial but not smooth muscle cells in the infarcted tissue (Fiedler et al., 2011). A role of miR-24 as a master regulator of smooth muscle cell proliferation was also recently identified (Fiedler et al., 2014). *In vitro* over-expression of miR-24 in human arterial smooth muscle cells (HASMC) induced apoptosis, migration defects, enhanced autophagy and loss of contractile marker genes, which was mediated in part by heme-oxygenase 1 (Fiedler et al., 2013). The anti-proliferative effect of miR-24 transfection in HASMCs was shown to be time-dependent, with the largest effects seen 72h after transfection (Fiedler et al., 2014). Like the other miRNAs already discussed, the altered expression (+ or -) and function of miR-24 within different cell types and at different time-points post-MI highlights the importance of identifying the cell-, location-, and time-specific expression and regulation of miRNAs in order to optimize cardiac repair.

MicroRNA-92a

A member of the highly conserved miR-17~92a cluster, miR-92a is expressed in human endothelial cells and plays a role in controlling the growth of new blood vessels (Ventura et al., 2008; Bonauer et al., 2009). Forced over-expression in human endothelial cells blocks sprout formation, inhibits vascular network formation and reduces cell migration and adhesion (Bonauer et al., 2009). In a mouse MI model, miR-92a was significantly up-regulated after injury, but when levels were reduced by the systemic administration of antagomiR-92a, enhanced blood vessel growth and functional recovery of the damaged tissue was observed, and this was believed to be mediated in part through effects on its direct target, integrin α5 (Itgα5) (Bonauer et al., 2009). Interestingly, the group used ISH with DIG-labelled probes to identify the cells that were being

targeted by antagomiR-92 treatment and found that miR-92a was inhibited in the endothelium and in cardiomyocytes (Bonauer et al., 2009). Catheter-based delivery of LNA-92a after ischemia/reperfusion in pigs, increased capillary density in the border zone of the infarcted hearts and increased Itgα5 expression (Hinkel et al., 2013). LNA-92a treatment also reduced the number of leukocytes in the infarcted region, improved left ventricular end-diastolic pressure and conferred direct cardiomyocyte protection, demonstrating a secondary role for miR-92a independent of angiogenesis (Hinkel et al., 2013). Studies have also linked miR-92a to a role in inflammatory endothelial dysfunction, which can lead to atherosclerosis. Endothelial cells exposed to "athero-prone" low sheer stress (SS) conditions and oxidized low-density lipoproteins had increased miR-92a levels in comparison to cells exposed to "athero-protective" high SS conditions (Ventura et al., 2008). In corroboration, miR-92a was highly expressed in the aortic arch of atherogenic $Ldlr^{-/-}$ mice compared to normal animals, and *in vivo* miR-92a inhibition altered the development of atherosclerosis, decreasing plaque size and promoting a more stable lesion phenotype in $Ldlr^{-/-}$ mice (Ventura et al., 2008). ISH in aortic arches of these animals demonstrated that miR-92a was confined to the endothelium and showed stronger expression in the inner curvature where low and oscillatory SS prevail compared to the outer curvature (Ventura et al., 2008). Therefore, it appears that miR-92a also plays a role in regulating multiple functions in different cells, and may provide a therapeutic target for cardiac regeneration.

MicroRNA-126

MiR-126 is an endothelial cell-restricted miRNA that mediates developmental angiogenesis (Wang et al., 2008). Targeted deletion of miR-126 in mice results in reduced angiogenic growth factor signalling that causes defects in endothelial cell proliferation, migration and angiogenesis, ultimately leading to vascular leakage, hemorrhaging, and partial embryonic lethality (Wang et al., 2008; van Solingen et al., 2009). The endothelial cell-specific expression of miR-126 *in vivo* was examined in human renal sections by miRNA ISH and co-staining for endothelial cells using the CD31 endothelial marker (van Solingen et al., 2009). Co-localization of miR-126 and CD31 in the endothelium of glomerular and peritubular capillaries and the endothelium of larger vessels confirmed the cell specific expression of the miRNA in humans (van Solingen et al., 2009). Endothelial cell-specific expression of miR-126 spanning from embryonic day 7.5 to adulthood was also confirmed in mice by ISH, confirming its co-localization with intron 7 of the EDF-like domain 7 (Egfl7) gene, an endothelial cell-specific protein that encodes miR-126 (Wang et al., 2008). Myocardial infarction was lethal in the majority of miR-126-/- mice, with surviving mice displaying defective cardiac neovascularization and Spred-1 up-regulation in endothelial cells, compared to their wild-type littermates (Wang et al., 2008). Spred-1, a direct target of miR-126, negatively controls intracellular VEGF, FGF and MAP kinase signalling, which is required for neoangiogenesis during cardiac repair (Cross and Claesson-Welsh, 2001; Kutryk and Stewart, 2003; Fish et al., 2008). Recent studies revealed that a loss of miR-126 in the circulating angiogenic cells of ischemic cardiomyopathy patients with cardiac heart failure (CHF) limited the cells' capacity to improve cardiac neovascularization and function; such information may be useful in the diagnosis and treatment of CHF patients (Jakob et al., 2012; Qiang et al., 2013). Notably, a recent study identified a preventative role for the star strand of miR-126, miR-126-5p, in atherosclerotic lesions. Specifically, miR-126-5p is responsible for reduced lesion formation after hyperlipidemic stress in regions of the atrial tree protected from atherosclerosis by preserving EC proliferation through the targeting of DLK1, a negative regulator of EC replication (Schober et al., 2014). In contrast, miR-126-5p is suppressed at predilection sites leading to the promotion of lesion formation due to inadequate EC proliferation. As a result, the administration of miR-126-5p mimics offers promising therapeutic potential as it rescues EC proliferation and reduces lesion formation at predilection sites (Schober et al., 2014).

MicroRNA-210

MiR-210 is strongly induced in endothelial cells and ischemic skeletal muscle upon exposure to hypoxia through a hypoxia-inducible factor-1 mediated mechanism (Fasanaro et al., 2008; Pulkkinen et al., 2008). In endothelial cells, up-regulation can be detected as early as 4 hours after the onset of hypoxia, which increases further at 72 hours and declines shortly after re-oxygenation, indicating an adaptive response (Fasanaro et al., 2008). MiR-210 over-expression in normoxic endothelial cells *in vitro* stimulates the formation of capillary-like structures and VEGF-induced chemotaxis, whereas miR-210 blockade has the opposite effect, and also inhibits cell growth and apoptosis in both normoxia and hypoxia (Fasanaro et al., 2008). Ephrin-A3 has been identified as a miR-210 target, with down-regulation being necessary for tubulogenesis and chemotaxis of endothelial cells to occur after exposure to hypoxia for 24 and 48 hours (Fasanaro et al., 2008; Pulkkinen et al., 2008). In addition to endothelial cells, the expression pattern of miR-210 has been examined in ischemic cardiomyocytes and found to exhibit a similar trend with significant miR-210 up-regulation detected at 24 hours, which increased further after 72 hours (Hu, 2010). Ischemic preconditioning of mesenchymal stem cells resulted in increased miR-210 expression and improved cell survival engraftment (via caspase8-associated protein-2) when transplanted into the infarcted heart; and the transplanted cells were shown to transfer miR-210 to host cardiomyocytes, leading to

increased functional recovery (Kim et al., 2009, 2012). The therapeutic potential of miR-210 for MI has been further demonstrated in a study whereby miR-210 over-expression lead to decreased apoptosis, increased neovascularization, and significant improvements in left ventricular fractional shortening (Hu, 2010).

Clinical perspectives

With the relative ease by which miRNAs can be manipulated pharmacologically, and the fact that miRNAs are involved in almost every aspect of cardiovascular health and disease, we are presented with interesting therapeutic opportunities for the treatment of MI (van Rooij, 2011). *In vivo*, pharmacological intervention can be accomplished with the use of miRNA mimics and/or anti-miRNAs (Fig. 3). Anti-miRNAs are modified antisense oligonucleotides harbouring the full or partial complementary reverse sequence of a mature miRNA. The application of an anti-miRNA leads to a reduction in endogenous miRNA levels. In therapy, they would be applied to activate gene expression by binding to the pathological miRNA, de-repressing the miRNAs inhibitory effects on target gene expression (Hutvagner, 2004; Krutzfeldt et al., 2005; van Rooij, 2011). To increase cellular uptake, anti-miRNAs can be conjugated to cholesterol (termed antagomiRs) and often contain a modified sugar backbone to increase stability (Small et al., 2010). Systemic delivery of anti-miRNAs has proven to be sufficient for the reduction of miRNAs levels in cardiac tissue for an extended period of time (van Rooij et al., 2008a). In contrast, miRNA mimics are synthetic double-stranded RNA which have been modified for cellular uptake and stability. Similar to endogenous miRNAs, miRNA mimics are loaded into RISC by the cell, and function to elevate endogenous protective miRNA levels by further inhibiting target mRNA expression (van Rooij et al., 2008a). However, double stranded RNAs are removed from the bloodstream by phagocytic cells that filter and excrete molecules of less than 50kDa; and this has challenged the efficacy of systemic delivery of miRNA duplexes (Broderick and Zamore, 2011). It is also important to note that therapies using miRNA mimics and anti-miRNAs, particularly with systemic administration, can lead to unwanted side effects since they can be taken up by non-target tissues. This highlights the potential need to develop strategies for cell- or tissue-specific delivery of miRNA therapeutics (van Rooij, 2011).

MiRNA therapeutics offer the potential to treat cardiovascular disease in a distinct manner compared to classical drugs because they may be capable of simultaneously targeting several components of a regulatory network, rather than simply one member of a pathway. This target multiplicity also provides a stronger and cumulative effect on a set of related proteins within the same biological pathway. However, this also raises the possibilities of off-target effects or opposing effects of miRNAs in different tissues/cells, as seen in miR-24, which could affect beneficial and pathological processes. The understanding of miRNA spatio-temporal patterns and the development of new cardiovascular therapy delivery systems with the ability to target specific cell populations are thus necessary to better control miRNA therapy strategies and cardiac repair.

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Accepted September 3, 2014