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# MicroRNAs differentially regulated in cardiac and skeletal muscle in health and disease: Potential drug targets?

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## Abstract

The identification of non-coding RNA species, previously thought of as 'junk' DNA adds a new dimension of complexity to the regulation of DNA, RNA and protein. MicroRNAs are short, non-coding RNA species that control gene expression, are dysregulated in settings of cardiac and skeletal muscle disease, and have emerged as promising therapeutic targets. MicroRNAs specifically enriched in cardiac and skeletal muscle are called myomiRs, and play an important role in cardiac pathology and skeletal muscle biology. Moreover, microRNA profiles are altered in response to exercise and disease, and thus, their potential as therapeutic drug targets is being widely explored. In the cardiovascular field, therapeutic inhibition of microRNAs has been shown to be effective in improving cardiac outcome in preclinical cardiac disease models. MicroRNAs that promote skeletal muscle regeneration are attractive therapeutic targets in muscle wasting conditions where regenerative capacity is compromised.

## Introduction

MicroRNAs (miRNAs) are small, highly conserved non-coding RNAs that target specific complementary sequences in the 3′ untranslated region of target mRNA, leading to mRNA cleavage and/or translational repression.[1,2] MiRNAs are involved in a range of biological processes, including proliferation, apoptosis, and differentiation. Among the miRNAs discovered to date (>1000 human miRNAs), both muscle-specific and ubiquitously expressed miRNAs have been shown to play essential roles in the regulation of cardiac and skeletal muscle function, adaptation and regeneration in both health and disease.[3] The identification of miRNAs dysregulated in cardiac and skeletal muscle-related disorders, taken together with improved methods to specifically target miRNAs in preclinical animal models has led to the development of miRNAs as potential therapeutic drug targets. In this review, we will focus on cardiac and skeletal muscle miRNAs that are regulated in response to exercise, normal growth/development and disease. We will also discuss recent preclinical studies that have targeted these miRNAs as promising therapies for muscle disorders and cardiovascular diseases.

## MicroRNAs

### *MiRNA biology*

MiRNAs are transcribed as long precursor molecules called primary miRNA transcripts in the nucleus. *Drosha* cleaves these transcripts into smaller stem-loop structures called precursor miRNAs.[1] Precursor miRNAs are subsequently exported into the cytoplasm where they are cleaved into mature miRNA strands by *Dicer* and loaded into the RNA-inducing silencing complex (RISC) to regulate the gene expression of target mRNA.[1] MiRNAs are able to recognize target mRNAs by as little as 6-8 nucleotides (called the seed region) to induce gene repression. Perfect or near-perfect base pairing with the target mRNA will result in degradation of target transcripts, whereas miRNAs that are partially complementary to a target mRNA will result in inhibition of protein translation.[1]

***Table 1: Expression of myomiRs in heart and skeletal muscle***

| myomiR | Heart | Skeletal Muscle |
|---|---|---|
| miR-1 | ✔ | ✔ |
| miR-133a | ✔ | ✔ |
| miR-133b | ✘ | ✔ |
| miR-206 | ✘ | ✔ |
| miR-208a | ✔ | ✘ |
| miR-208b | ✔ | ✔ |
| miR-499 | ✔ | ✔ |

### *Muscle-enriched miRNAs - myomiRs*

MiRNAs that are either enriched or selectively expressed in cardiac and/or skeletal muscle are called myomiRs.[3] These include miR-1, miR-133a/b, miR-206, miR-208a/b, and miR-499 (Table 1). miR-1 and miR-133a are abundant in the heart and their transcription is regulated by serum response factor, whereas miR-208a, miR-208b and miR-499 are encoded in the introns of the cardiac muscle myosin heavy chain encoding genes (Myh6, Myh7, Myh7b).[4] Skeletal muscle myomiRs include miR-1, miR-133a, miR-133b and miR-206, and their expression is under the control of myogenic regulatory factors (MRFs) including myogenin, MyoD and Myf5.[5-7] In skeletal muscle, miR-208b is coexpressed with β-myosin heavy chain (β-MHC) and is enriched in the soleus muscle. miR-499 also shows highest expression in the soleus muscle (in addition to cardiac muscle) and is encoded by intron 19 of the mouse Myh7b gene.[4] These, and other ubiquitously expressed miRNAs, have gained notable attention for their roles in the regulation of transcription factors and signalling pathways that play significant roles in cardiac and skeletal muscle biology (Figure 1).

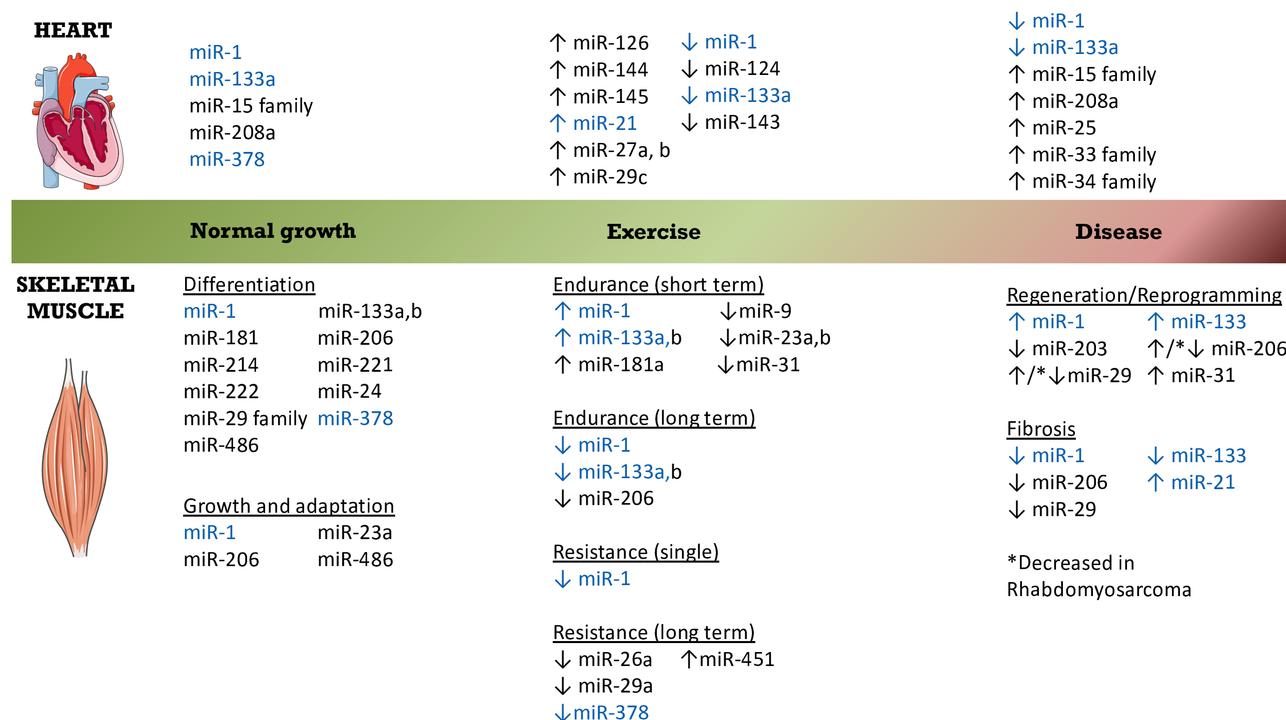


***Figure 1. Summary of miRNAs that are implicated in normal heart and skeletal muscle growth, and those miRNAs that are differentially regulated in the heart and skeletal muscle in response to exercise and disease.*** *miRNAs in blue text highlight common miRNAs in both cardiac and skeletal muscle.*

## Cardiac and skeletal muscle

Cardiac and skeletal muscles are highly specialized structures that perform specific tasks. They are both categorized as striated muscles due to the striated appearance of individual muscle cells and mediate contraction.[8] Skeletal muscle can be further characterized as fast or slow twitch muscle. Both cardiac and skeletal muscle types are able to undergo growth/hypertrophy in response to some common mediators, signalling and mechanisms (*e.g.* exercise, insulin-like growth factor 1 (IGF1) and protein synthesis). However, there are also important differences between cardiac and skeletal muscle including the control of contraction (cardiac muscle contraction is classed as involuntary, whereas skeletal muscle can be made to relax or contract by conscious control, *i.e.* somatic nervous system) and shape of muscle cells.[8]

*Common and distinct signalling pathways regulating cardiac and skeletal muscle growth*

The IGF1-Akt pathway is one of the most recognised pathways responsible for regulating both cardiac and skeletal muscle protein synthesis and hypertrophy[9-11] (Figure 2), whereas myostatin negatively regulates cardiac and skeletal muscle growth[12,13] (Figure 2). In skeletal muscle, transforming growth factor-β (TGF-β) signalling cascade also negatively regulates muscle growth[14] (Figure 2). More recently, new mediators of physiological heart growth have been identified including heat shock transcription factor 1 (Hsf1), CAAT/enhancer binding protein-b (C/EBPb), proline rich AKT substrate of 40 kDa (PRAS40) and hexamethylene-bis-acetamide-inducible protein 1 (HEXIM1) (reviewed in Bernardo *et al.*, 2010;[9] Bernardo, Ooi & McMullen, 2012[15]).

Stimulation of the β-adrenergic signalling pathway in skeletal muscle promotes skeletal muscle hypertrophy[16] in a manner that is dependent of mammalian target of rapamycin (mTOR) signalling.[17] Whilst the targeting of this pathway can potentially yield beneficial effects upon skeletal muscle growth and regeneration,[18-20] activation of this pathway in the heart by ligands such as noradrenaline plays a detrimental role in settings of heart failure and myocardial infarction.[21]

Angiotensin II (Ang II) is the main peptide of the renin-angiotensin system. In skeletal muscle, Ang II causes muscle wasting (*i.e.* atrophy) due to oxidative stress which activates proteasome system-mediated muscle protein degradation.[22] By contrast, in the heart, Ang II is activated in response to hemodynamic overload which contributes to pathological cardiac hypertrophy, fibrosis and dysfunction (reviewed in Bernardo *et al.*, 2010[9]).

The discovery of miRNAs as novel regulators of gene expression has provided new insights on the regulation of these pathways. In this review we have focused on miRNAs which regulate the IGF1-Akt, myostatin and TGF-β signalling pathways (Figure 2).

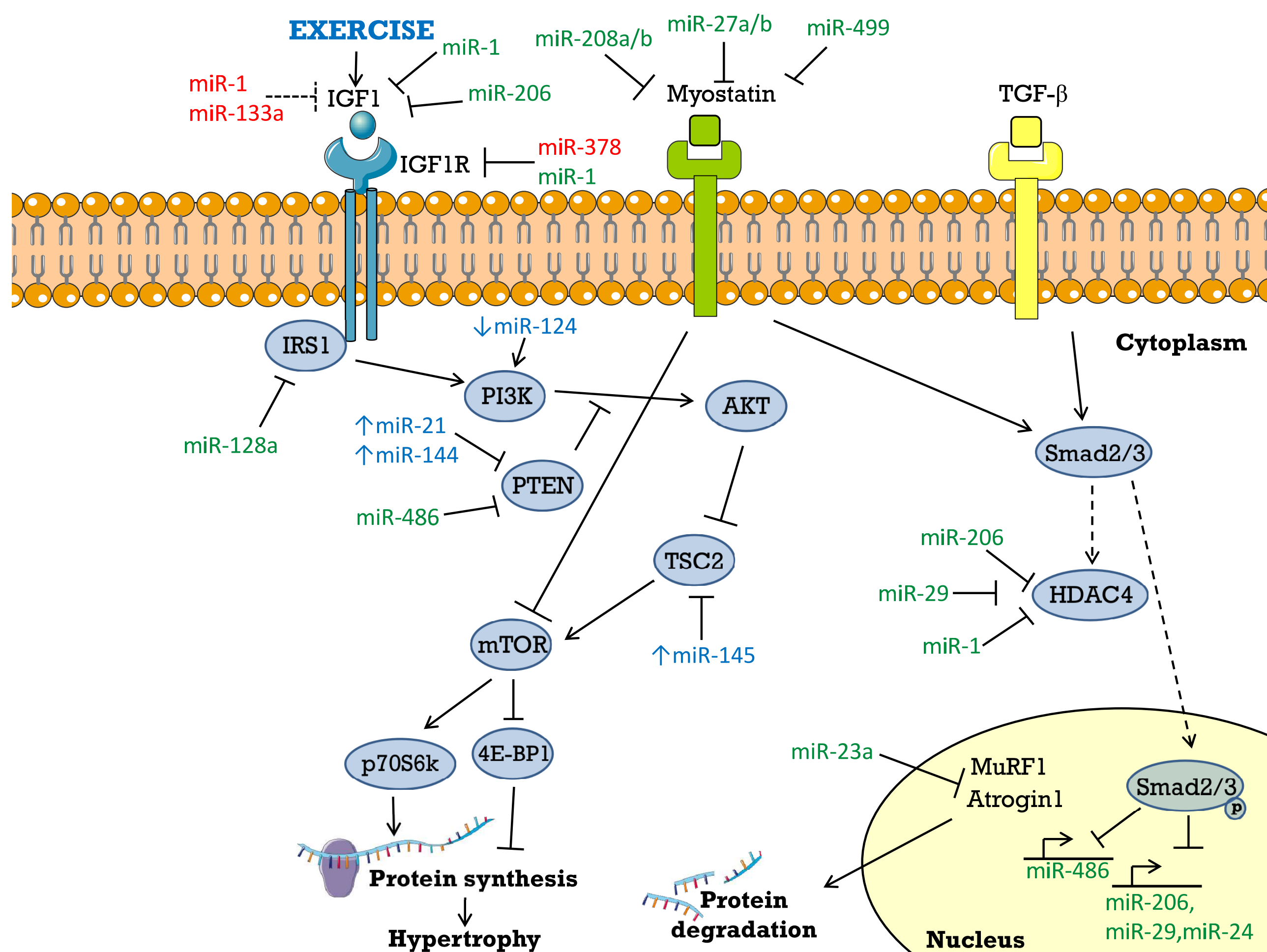


***Figure 2. Schematic representation of miRNAs involved in the IGF1-PI3K-Akt, myostatin, and TGF-β signalling pathways.*** *Shown are those involved in the regulation of normal heart and skeletal muscle growth, and in response to exercise. miRNAs coloured green are involved in skeletal muscle biology, miRNAs coloured in red are involved in cardiac biology, and miRNAs coloured blue are either up or down regulated in response to exercise in the heart. Dashed lines represent proposed inhibitor or activation function. 4E-BP1: Eukaryotic translation initiation factor 4E binding protein 1; HDAC4: histone deacetylase 4; IGF1: insulin-like growth factor 1; IGF1R: IGF1 receptor; IRS1: Insulin receptor substrate 1; mTOR: mammalian target of rapamycin; MuRF1: muscle RING-finger protein-1; p70S6k: p70 ribosomal protein S6 kinase; PI3K: phosphoinositide-3-kinase; PTEN: phosphatase and tensin homolog; TGF-β: transforming growth factor-β; TSC2: tuberous sclerosis complex 2.*

## MicroRNAs regulated in normal cardiac and skeletal muscle development and disease

### *MiRNAs associated with cardiac contractile function and normal heart growth*

MiRNAs play fundamental roles in the regulation of heart biology.[2,23] Since the identification that cardiac-specific deletion of *Dicer* (essential enzyme for miRNA biosynthesis) led to embryonic lethality due to heart failure,[24] it has been shown that miRNAs regulate proliferation, differentiation and cardiac conductivity.[24-27] Tamoxifen-inducible cardiac-specific deletion of *Dicer* caused a decline in cardiac contraction and a downregulation of miR-1 expression.[28] Inhibition of miR-1 in non-transgenic mice recapitulated the phenotype of tamoxifen-inducible *Dicer* mice.[28] The role of miR-1 in cardiac contractility is due to increased expression of its predicted mRNA targets, Sorcin and the $Na^+/Ca^{2+}$ exchanger (NCX), key regulators of calcium signalling and electrical conduction.[28,29] Overexpression of miR-208a resulted in cardiac hypertrophy and defects in electrical conduction *via* repression of negative regulators of muscle hypertrophy such as thyroid hormone receptor-associated protein 1 (Thrap1) and myostatin.[27,30] In addition, miR-25 is a critical repressor of the sarcoplasmic reticulum uptake pump (SERCA2a) and acts to suppress intracellular calcium handling.[31] SERCA2a expression and activity is impaired in heart failure, and inhibition of miR-25 improved cardiac performance which was associated with increased SERCA2a protein expression following pressure overload

in mice.[31]

MiR-1 and miR-133a have also been implicated in the regulation of cell proliferation and differentiation[24,25] whilst members of the miR-15 family are dynamically regulated shortly after birth and regulate the cardiac cell cycle.[26] MiRNAs including miR-1 and -133a have been shown to abrogate IGF1-induced hypertrophy suggesting a requirement for miR-1 and miR-133a downregulation in this mode of hypertrophy[32] (Figure 2). Whilst levels of miR-378 are normally suppressed by IGF1, increases to its expression led to degradation of the IGF1 receptor (IGF1R), suggesting that targeting of this miRNA may be beneficial in settings of cardiac stress[33] (Figure 2).

*MiRNAs controlling physiological and pathological hypertrophy of cardiac muscle*

Cardiac hypertrophy is defined as enlargement of cardiac muscle and is classified as physiological or pathological hypertrophy.[9] Activation of phosphoinositide-3-kinase [PI3K(p110α)], a downstream target of IGF1R, is essential for postnatal heart growth and exercise-induced heart growth (*i.e.* physiological heart growth) through its ability to regulate cell size.[34,35] To better understand the mechanisms responsible for PI3K-induced physiological heart growth and cardiac protection, we identified miRNAs that correlated with PI3K activity and cardiac stress and protection in the heart, suggesting a potential role of these miRNAs in physiological heart growth.[36] Pathological hypertrophy occurs in response to disease (*e.g.* in response to hypertension or myocardial infarction) and is associated with accumulation of collagen in the extracellular matrix (fibrosis), cardiomyocyte loss (apoptosis), impaired cardiac function and, ultimately, heart failure (reviewed in Bernardo *et al.*, 2010;[9] Bernardo, Ooi & McMullen, 2012[15]). A number of studies have shown a signature pattern of miRNAs that are differentially expressed in pathological cardiac hypertrophy and heart failure[37-39] and miRNAs associated with the regulation of pathophysiological processes of the heart including apoptosis, fibrosis and pressure overload-induced remodelling where they have been implicated as either causative or protective in settings of cardiac stress (reviewed in Hata, 2013;[2] Smaii & Olsen, 2007[40]). MiR-1, the most abundant miRNA in the heart, expression is decreased in mouse models of pathological cardiac hypertrophy.[41,42] Through its ability to repress calmodulin (a crucial mediator of calcium signalling), myocyte enhancer factor-2a (MEF2a) and GATA binding protein 4 (transcription factors important for the regulation of cardiomyocyte hypertrophy and activation of hypertrophic gene expression), miR-1 is able to antagonize cardiomyocyte hypertrophy following chronic isoproterenol infusion.[43] Inhibition of miR-133 (using antisense oligonucleotides) in mice induced a marked and sustained cardiac pathological hypertrophy, suggesting miR-133 as a key regulator of cardiac hypertrophy and potential cardioprotective role. However, adding to the complexity of miRNA actions, miR-133a null mice have a normal hypertrophic response[25] highlighting differences between genetic miRNA mouse models and chemical inhibition of miRNAs *in vivo*.

The myomiRs (miR-208a, miR-208b and miR-499) are encoded within the introns of myosin heavy chain genes and control myosin gene expression during adaptation to pathological signalling. miR-208a is required for cardiomyocyte hypertrophy, fibrosis and expression of β-myosin heavy chain (β-MHC) in response to stress by negatively regulating thyroid hormone receptor associated protein 1 (THRAP1).[27] miR-208a knockout mice display a blunted cardiac hypertrophy response and diminished fibrosis following pressure overload.[27] In contrast, results of mouse cardiac miR-499 overexpression are inconsistent. Elevated miR-499 expression has been implicated in both cardioprotection from ischemic injury and induction and exacerbation of heart failure following pressure overload.[44-46]

These studies provide clear evidence that miRNAs regulate a diverse spectrum of processes in the heart related to development, proliferation, hypertrophy and cardiac conduction. However, the contrasting findings in some of these studies emphasize the gaps in our understanding of the mechanisms of miRNA actions and regulation. Understanding miRNA functions and identifying targets through which they regulate biological processes will further increase our knowledge of cardiac biology.

*MiRNAs controlling skeletal muscle differentiation*

The commitment of progenitor cells to a program of differentiation that facilitates the formation of skeletal muscle fibres (termed myogenesis) is a process that is finely regulated by a vast number of miRNAs (Figures 1 & 2). Their importance to the development of skeletal muscle in particular is highlighted by the generation of skeletal muscle-specific *Dicer* knock-out mice, which display reduced skeletal muscle mass and perinatal lethality.[8] The myomiRs were the first to be characterized as regulators of muscle cell differentiation. Through the ability of miR-206 to directly target a number of genes that regulate myoblast proliferation including utrophin, follistatin-like1, connexin43 and Pax3/7, miR-206 promotes muscle cell differentiation.[6,47-50] Whilst miR-1 also promotes differentiation,[51] MyoD dependent transcription of miR-1 is regulated by mTOR in the control of differentiation and regeneration where it targets histone deacetylase 4 (HDAC4) and controls the expression of follistatin.[52] Whilst a role for miR-133a, and miR-133b in promoting muscle cell differentiation by controlling the extracellular signal-regulated kinases (ERK) signalling pathway has been identified[53] this miRNA was originally characterized as an enhancer of myoblast proliferation by repressing serum response factor.[54] Despite the role of myomiRs as regulators of muscle cell differentiation *in vitro*, mice with genetic ablation of either miR-1, miR-133a, and miR-206 have no obvious skeletal muscle phenotype under basal conditions[24,25,54] suggesting potentially redundant roles of miRNAs or regulatory network buffering.[55]

Ubiquitously expressed miRNAs also play roles in controlling muscle cell differentiation. Together with miR-206, the miR-29 family targets and inhibits the expression of HDAC4, a known-repressor of muscle cell differentiation, in a TGF-β dependent manner.[56] Whilst the TGF-β pathway is a well-established inhibitor of skeletal myogenesis, only recently have studies identified that the TGF-β pathway controls the expression of miR-206 and the miR-29 family, and miR-24, which normally act to promote muscle cell differentiation[57] (Figure 2). Taken together with roles for miR-486, miR-181, miR-221, miR-222, miR-214 and miR-378 in the differentiation program[58-62] these studies collectively underscore the integral function that miRNAs play in regulating differentiation (Figure 1).

*MiRNAs that regulate skeletal muscle hypertrophy*

The TGF-β pathway is a dominant regulator of skeletal muscle catabolism.[63] Whilst the TGF-β pathway can control mTOR mediated protein synthesis,[64] only recently have studies established mechanisms underlying the cross-talk between the two pathways. Hitachi and colleagues demonstrated that *via* the ability of myostatin to inhibit miR-486 promoter activity, which subsequently leads to derepression of PI3K/Akt signalling, myostatin can negatively regulate mTOR signalling[65] (Figure 2). It is intriguing to speculate what role miR-486 plays in the regulation of hypertrophy *in vivo*. Myostatin expression is also regulated by miR-27a/b,[66] miR-208a/b[30,67] and miR-499.[68]

In *Texel* sheep bearing a mutation that creates an "illegitimate" miR-206 target site in the 3′ untranslated region of the myostatin gene, it has been demonstrated that miR-206 can also promote increased muscularity *via* myostatin repression during development and maturation.[69] Whilst studies have identified adaptive changes to miR-206 in settings of atrophy and hypertrophy,[70,71] and that miR-206 can negatively regulate IGF1 in the teleost tilapia (a cichlid fish),[72] our study established that the over-expression or inhibition of miR-206 levels in adult murine skeletal muscle did not affect basal skeletal muscle growth or adaptation.[71] Of the other myomiRs, miR-1 and miR-133a are differentially expressed during functional overload and dexamethasone-induced atrophy[73,74] however, transgenic over-expression of miR-133a yielded normal skeletal muscle development and function[75] despite studies demonstrating that miR-133 can target IGF1R.[76] A compelling role for miR-1 in adaptation is supported by studies that demonstrate that miR-1 can target IGF1 and IGF1R[77] and that the suppression of miR-1 expression prevents dexamethasone-induced atrophy.[78] Of those miRNAs that can target the IGF1/Akt pathway, miR-128a, which is highly expressed in skeletal muscle, targets insulin receptor substrate 1 (IRS1) and significantly, antisense-mediated inhibition of miR-128a *in vivo* led to skeletal muscle hypertrophy following four weeks of treatment.[79] miR-23a can specifically suppress the expression of the key ligases atrogin1 and muscle RING-finger protein-1 (MuRF1) that promote proteasomal degradation of skeletal muscle proteins[80] (Figure 2). Importantly, the over-expression of miR-23a can protect skeletal muscle from atrophy *in vivo*[80] and it is thought that the loss of miR-23a in atrophic conditions can be manifested by selective packaging into exosomes.[81]

In summary, miRNAs expressed in skeletal muscle play fundamental roles in the regulation of muscle cell differentiation and miRNAs including miR-206 and the miR-29 family present themselves as promising therapeutic targets where regeneration is desirable. Whilst a key role for miR-128 has been attributed to the regulation of skeletal muscle growth *in vivo*, the role of other miRNAs *in vivo* is still to be elucidated. These studies will be particularly important because as demonstrated by the disparate role of miR-206 *in vitro* and *in vivo*, miRNAs can play differential roles in particular contexts.

*Response and adaptation of miRNAs to exercise*

Increases in muscular activity, such as those occurring during exercise, are associated with adaptations of the cardiovascular and musculoskeletal system including cardiac hypertrophy, skeletal muscle oxidative capacity and changes in skeletal muscle metabolic and contractile protein expression.[82] Exercise training has been shown to alter miRNA expression in the heart and skeletal muscle.

*Cardiac muscle response of miRNAs to exercise*

The majority of studies performed in the heart have focused on endurance exercise after chronic training. The myomiRs, miR-1 and miR-133a, are downregulated in rat hearts following endurance treadmill training.[41] Using a microarray approach, Soci and colleagues identified miRNAs that are differentially expressed in hearts of rats that underwent 10 weeks low intensity swim training. The expression of miR-29c was upregulated in swim-trained rats, which corresponded to the decrease in fibrotic genes such as collagen type I and type III (validated targets of miR-29).[83] The observation that exercise training reduced fibrosis *via* miR-29c is consistent with previous findings as the miR-29 family has been shown to regulate myocardial infarction-induced fibrosis.[84] Furthermore, swim training induced downregulation of miR-143 and upregulated the expression of miR-27a and miR-27b.[85] Swimming also increased the expression of miR-126 and decreased the expression of two members of the vascular endothelial growth factor (VEGF) family, Sprouty related protein 1 (Spred1) and PI3K regulatory subunit 2 (PI3KR2) (87) (Figure 1).

Recently, a microarray approach was used to identify differentially PI3K-regulated miRNAs in the hearts of a rat model of chronic swim training.[87] They identified a decrease in miR-124, which could upregulate the PI3K(p110α) gene and an increase in miR-21 and miR-144, which could inhibit phosphatase and tensin homolog (PTEN; a negative regulator of the PI3K pathway) (Figure 2). In addition, they also showed an increase in miR-145 which suppressed the expression of tuberous sclerosis complex 2 (TSC2).[87] Collectively, these results suggest that

exercise training alters the expression of miRNAs that target collagen deposition/fibrosis (miR-29c), RAS (miR-143, miR-27a, miR-27b), angiogenesis (miR-126) and the PI3K pathway (miR-124, miR-21, miR-144, miR-145). Alterations to these pathways could explain the beneficial effects of exercise on the heart (Figures 1 & 2).

*Skeletal muscle response of miRNAs to exercise*

The characterization of miRNAs that are differentially regulated following exercise may lead to the identification of miRNAs that are novel regulators of skeletal muscle mass and/or metabolism. For instance in response to 10 days of endurance training, miR-1, miR-133a, miR-133b and miR-181a were all increased whilst miR-9, miR-23a, miR-23b and miR-31 were decreased.[88] Longer term endurance training decreased expression of miR-1, miR-133a, miR-133b and miR-206 after 12 weeks suggesting that temporal changes to myomiRs may be modality and context dependent.[89] Resistance training has also been shown to induce changes to the expression of the miRNA profile. A single bout of resistance training reduced expression of miR-1.[90] Longer term resistance training (12 weeks in this case) did not induce changes to those they described as "high responders" and only yielded changes to "low responders," including increases to miR-451 and decreases to miR-26a, miR-29a and miR-378.[91]

Interestingly, changes to circulating miRNAs have been identified in response to various exercise regimes. Resistance exercise has been shown to induce decreases to circulating levels of miR-146a and miR-221 and increases to miR-149.[92] It is currently unclear as to their role in muscle adaptation and the source of these miRNAs. Marathon running has also been shown to induce increases to the circulating levels of miRNAs including miR-1, miR-133a, miR-206, miR-499, and miR-208a, as well as miR-146a.[93,94] The potential for circulating miRNAs such as these to be used as biomarkers of aerobic capacity is highlighted by Mooren *et al.*[94] who demonstrated that miR-1, miR-133a and miR-206 correlated with key performance indicators including maximum oxygen uptake ($VO_2$max).

Whilst these studies have characterized key changes to skeletal muscle and circulating miRNAs following various exercise regimes (Figure 1), their exact molecular functions remain to be established, as do the targets that they may regulate during exercise.

**Clinical applications – potential drug targets?**

*Cardiac muscle: clinical applications*

An overwhelming number of studies have illustrated that the aberrant expression of miRNAs contributes to various aspects of cardiovascular disease (*e.g.* adverse cardiac remodelling such as hypertrophy, fibrosis and apoptosis), suggesting that their targeting in disease may provide an efficient and effective basis for the development as novel therapies and diagnostic tools (reviewed in Dangwal & Thum, 2014[95]). MiRNAs can be therapeutically manipulated by systemic or local delivery of inhibitors (referred to as 'antimiRs'). The first example of the therapeutic potential of targeting miRNAs in humans was recently reported in hepatitis C patients in a phase 2a clinical trial with miravirsen, a miR-122 locked nucleic acid (LNA)-antimiR.[96] Results from the clinical study indicate that the treatment was effective, well tolerated in patients and resulted in long lasting viral suppression.[96]

Moreover, inhibition of miRNAs by LNA-antimiRs in preclinical animal models has also demonstrated that their long term use is effective, with no evidence of toxicity.[97-103] We have demonstrated that inhibition of the miR-34 family attenuated cardiac remodelling and improved cardiac function in a mouse model of pressure overload (*i.e.* hypertension) and chronic MI (*i.e.* heart attack), however inhibition of miR-34a alone provided no significant benefit in the MI setting.[97] In contrast, a separate group reported that inhibition of miR-34a prevented cardiac contractile dysfunction, and reduced apoptosis and fibrosis following acute MI[99] (Figure 3A). Consistent with the ability of miR-34a to provide protection in early settings of disease, our recent studies demonstrated that inhibition of miR-34a was also beneficial in a setting of moderate cardiac pathology, but not severe pathology.[98] Our studies therefore suggest that drugs that target the entire miR-34 family are likely to have greater therapeutic benefit in settings of severe pathology than inhibition of miR-34a alone.

Other studies have shown that pharmacologic inhibition of miR-208a prevented pathological cardiac remodelling, improved cardiac function and prolonged survival in a rat model of hypertension-induced heart failure.[102] More recently, miR-208a in the heart has been shown to control whole body metabolism and miR-208a inhibition in mice conferred resistance to high fat diet-induced obesity.[100] Thus, not only is miR-208a a potential therapeutic target for heart failure, but also for metabolic disorders. In obese insulin resistant non-human primates, pharmacological inhibition of the miR-33 family (helps regulate cholesterol and lipid homeostasis, which is associated with cardiovascular disease) increased high density lipoprotein cholesterol (*i.e.* 'good' cholesterol) to levels associated with protection against cardiovascular disease.[103] Inhibition of the miR-15 family was shown to protect cardiomyocytes from apoptosis and increase proliferation, resulting in an improvement in cardiac function following MI.[101,104] Heart failure is characterized by progressive loss of contractile function and boosting intracellular calcium handling has been shown to be a promising treatment for heart failure.[105] Recently, increasing levels of miR-25 has been shown to contribute to declining cardiac function by inhibiting calcium uptake.[31] Therapeutic inhibition of miR-25 in a mouse model of pressure overload was shown to improve cardiac function through restoration of SERCA2a activity (a calcium handling protein important for cardiac contractility).[31] The ability of the adult heart to repair itself following injury, such as MI or heart failure, is very limited, thus there is

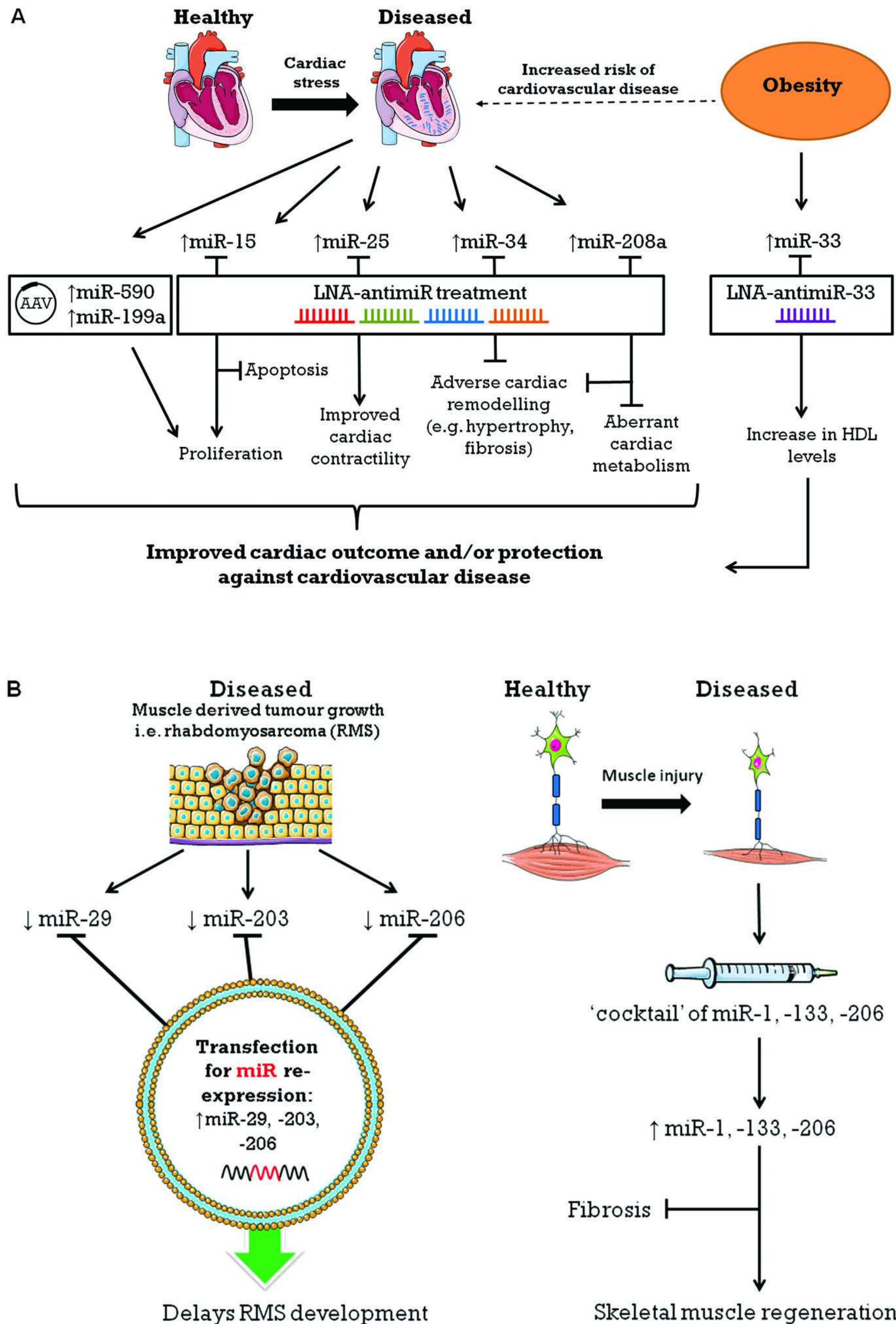


***Figure 3. Translational potential of targeting miRNAs in cardiovascular and skeletal muscle diseases. A:*** *Schematic representation of miRNA-based therapies (inhibition of miRNA-15, -25, -34, and -208a using LNA-antimiRs; or overexpression of miR-590 and -199a using an adeno-associated viral [AAV] vector) and their mechanisms that have had a favourable outcome in preclinical animal models for the treatment of cardiovascular disease. As obesity is a risk factor for cardiovascular disease and associated with an increase in miR-33 expression, inhibition of miR-33 increases HDL levels and leads to cardiac protection.* ***B:*** *Schematic representation of miRNA-based targets for therapeutic intervention for skeletal muscle disease. Reexpession of miR-29, -203 and -206 attenuates development of rhabdomyosarcoma (RMS, left panel) while administration of a cocktail mix of miR-1, -133 and -206 promotes muscle regeneration (right panel).*

great interest in developing therapies that can restore the proliferative capacity of the damaged heart. For instance, miR-590 and miR-199a was recently shown to promote cardiomyocyte proliferation and preserve cardiac function following MI in mice.[106]

The studies outlined above demonstrate that miRNA mediated intervention is a promising avenue for the development of novel therapeutics for cardiovascular disease through inhibition of pathological remodelling, preservation of cardiac function or promoting regeneration of cardiomyocytes following injury (Figure 3A).

*Skeletal muscle: clinical applications*

MiRNAs present themselves as targets for therapeutic intervention in a number of skeletal muscle diseases. The development of the muscle derived rhabdomyosarcoma (RMS) can be prevented by the re-expression of repressed miRNAs including miR-206, miR-203 and miR-29, which act by reprogramming the profile of the RMS cell toward one of terminal myogenic differentiation[107-109] (Figure 3B). The targeting of miR-206 may also provide therapeutic benefit in amyotrophic lateral sclerosis (ALS) which is characterized by the loss of motor neuron supply to skeletal muscle and severe skeletal muscle atrophy[54] (Figure 3B).

MiRNAs that regulate regeneration are also attractive therapeutic targets in skeletal muscle wasting conditions where muscle regeneration is compromised, such as in the various muscular dystrophies. Demonstrating the fundamental role of myomiRs in promoting regeneration of skeletal muscle, the administration of a double-stranded "cocktail" of miR-1, miR-133 and miR-206 promoted rat skeletal muscle regeneration and prevented fibrosis[110] (Figure 3B). Other miRNAs also play pivotal roles in promoting regeneration through the repression of key pathways that normally inhibit regeneration. Through the regulation of the canonical TGF-β pathway, miR-26a promotes myoblast differentiation and its inhibition *in vivo* delays regeneration.[111] Consistent with the role that miR-206 plays in promoting regeneration, the genetic deletion of miR-206 led to exacerbated disease progression in murine muscular dystrophy.[112] It is intriguing to note that under basal conditions, mice with genetic ablation of miR-206 have no obvious phenotype, yet in conditions of injury or stress, obvious abnormalities in regenerative capacity are evident.[112] Skeletal muscles of dystrophin deficient mice display reduced miR-29 family expression and re-expression ameliorated disease progression.[113] The targeting of miR-31 has also been postulated to act to restore dystrophin expression in Duchenne muscular dystrophy (DMD) and *via* an exon skipping strategy, inhibition of miR-31 expression in human DMD myoblasts led to rescuing of dystrophin expression.[114] This suggests that strategies that can utilize miRNA-based therapies to restore dystrophin may be viable therapeutic options to treat the devastating symptoms associated with DMD.

MiRNAs have also been shown to drive the fibrogenic pathogenesis in skeletal muscle diseases and because fibrosis is often a key indicator of declining muscle function, they may be particularly amenable to therapeutic intervention in this context. miR-21 is dysregulated in fibroblasts and inhibition of miR-21 could prevent fibrosis in a PAI-1-dependent manner, whereas forced miR-21 expression promoted fibrosis.[115] Previous studies have identified that the miR-29 family is a regulator of muscle cell differentiation and fibrosis.[56,84] Identifying that restoration of miR-29 expression can promote regeneration of skeletal muscle and prevent fibrosis suggests that the miR-29 family is an important potential therapeutic target for the treatment of DMD.[113]

Whilst the potential for targeting miRNAs in skeletal muscle disease states is high, further elucidation of their roles *in vivo* will undoubtedly lead to the identification of novel targets to prevent skeletal muscle disease progression.

*Current limitations of miRNA-based drug therapy*

Studies in mice, non-human primates and results from clinical trials in humans clearly demonstrate that there is potential for miRNAs to be developed into valuable therapeutics. MiRNA delivery systems include antisense modified oligonucleotides (AMOs), viral vectors, mimics and nanoparticle-based delivery.[116] Of these, AMOs (such as those modified with LNA chemistry) are the most promising targeted therapy approach in relation to safety, stability and efficacy, and have successfully been used in human clinical trials,[96] although the cost to develop antimiR-based therapies is high.[116] However, miRNAs may have diverse effects by targeting hundreds of mRNAs, further *in vivo* studies will be critical to assess potential off-target effects in animal models and humans. Conversely, the therapeutic benefit of targeting miRNA families or multiple miRNAs simultaneously that function cooperatively to regulate multiple biological networks may actually be necessary and more efficacious than targeting individual miRNAs in particular disease settings.[98] Another challenge in translating miRNA-based therapies to the clinic is achieving organ/cell type specificity. miRNAs are ubiquitously expressed and miRNA-based therapies are taken up by various organs upon systemic delivery.[116] Targeted inhibition of miRNAs has been achieved using adeno-associated viral (AAV) vectors and specificity can be attained based on choice of AAV serotype and promoter.[31,71,117] Safety and efficacy of AAV based delivery in clinical trials is promising, particularly with the development of strategies to overcome immune responses.[105,118] Our understanding of miRNA biology and therapeutics has expanded at a rapid rate, however further research is required to facilitate the development of miRNA-targeting therapies for human cardiovascular disease and skeletal muscle disorders.

## Conclusion

The discovery of miRNAs over a decade ago has added another layer of complexity to the regulation of gene expression in mammalian tissues, including in cardiac and skeletal muscle. Given the interplay between cardiac and skeletal muscle signalling cascades, it is not surprising that

a number of miRNAs are expressed in both cardiac and skeletal muscle in response to growth and exercise (Figures 1 & 2). Many of the cardiac remodelling processes following a cardiac insult are orchestrated by miRNAs which has led to the development of novel therapeutic strategies for the treatment of cardiovascular disease, as illustrated by promising results in preclinical heart failure animal models. Given many miRNAs are ubiquitously expressed, future therapeutics may consider a targeted delivery approach with the use of viral vectors. Whilst the role of miRNAs in the cardiovascular system has been comprehensively studied, our understanding of the molecular mechanisms underlying the regulation of skeletal muscle development, regeneration and mass by miRNAs is still limited. Future studies that can manipulate miRNAs *in vivo* will further define the role of miRNAs in skeletal muscle.

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