

# MicroRNA Based Treatment of Cardiomyopathy: Not all Dystrophies are Created Equal

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Dilated cardiomyopathy (DCM) represents the leading cause of mortality in patients with muscular dystrophy (MD). Standard pharmacological treatments are unable to combat the underlying genetic defects that result in chronic myocardial wasting and fibrotic infiltration. Alternative strategies to treat DCM are under intense investigation including gene and cell therapy approaches. Although currently the most promising, gene therapy approaches directed at treating the heart must carefully consider the choice of vector, gene replaced, and delivery method. Challenges may include accommodating transgene size (eg, Dystrophin), and avoiding potential immunity to the transgene and/or viral capsid. As a target for therapy, microRNAs (miRNAs) offer advantages that evade both of these challenges. MicroRNAs are short noncoding RNAs that modify gene expression by regulating mRNA stability or translation during various developmental or disease processes.<sup>1</sup> Within the past decade miRNAs have been shown to play a key role in regulating the progression of cardiomyopathy and as a result have become genetic targets for therapy.<sup>1</sup> Encouraging results in preclinical studies have led to the integration of miRNAs into clinical trials. To date, the majority of this inclusion has focused on miRNA expression signatures that are used as biomarkers for disease progression and readouts of therapeutic efficacy for other therapies, as opposed to a miRNA-targeted therapy.<sup>2</sup> There has been one miRNA product, Miravirsen, which has reached the clinic for treatment of hepatitis C virus (HCV) infection as an anti-miR of miR-122, a hepatic-specific miRNA that directly

targets HCV.<sup>3</sup> Miravirsen showed dose-dependent efficacy in chronic HCV-infected patients without dose-dependent adverse events.<sup>3</sup> This trial, along with impending trials using miRNA-based therapies for cancer,<sup>2</sup> provide valuable safety data that can be applied to future trials for DCM.

The potential for a common miRNA therapy that can be applied to multiple forms of muscular dystrophy (MD) with a severe cardiomyopathy component has become an attractive option. The degenerative process in muscle indicative of MDs is often thought of as the result of a common origin; however, caution must be taken when considering a universal approach to treat DCM. This is evidenced in this issue of *JAHA* by Quattrocchi et al<sup>4</sup> and in previous studies from the Sampaollesi group showing that not all dystrophies complicated by DCM arise from a common pathway or follow the same progression. Nonetheless, the potential power of a miRNA-based approach is indisputable as demonstrated by Quattrocchi et al.<sup>4</sup> This sets the stage for long-term treatments for DCM particularly when the miRNA target is well defined and preclinical safety and efficacy is established in the model for the disease it is intended to treat.

## Muscular Dystrophy Associated Cardiomyopathy

MDs are a heterogeneous group of muscle wasting genetic disorders. Those associated with cardiomyopathy primarily include the dystrophinopathies, both Duchenne (DMD) and Becker (BMD) forms, and a subset of recessive limb girdle MDs (LGMD) called the sarcoglycanopathies. Dystrophin, which is mutated or absent in DMD and BMD, and the sarcoglycans ( $\alpha, \beta, \delta, \gamma$  and  $\epsilon$ ) are the primary components of the dystrophin-associated protein complex (DAP). The disruption of the DAP that occurs in MD leads to instability and calcium influx in muscle fibers, which then leads to chronic muscle fiber degeneration followed by fibrosis and fat replacement.<sup>5</sup> Cardiomyopathy is seen in almost all DMD patients who survive into their third decade and in approximately 30% to 70% of LGMD2 patients.<sup>6-8</sup> The same histopathological features that affect skeletal muscle in MD (eg, necrosis and fibrosis) also affect cardiac muscle leading to myocardial ischemia and arrhythmias. As a result, the

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myocardium undergoes hypertrophic remodeling and ventricular dilation leading to progressive dilated cardiomyopathy.<sup>9,10</sup>

## Cardiac Gene Transfer

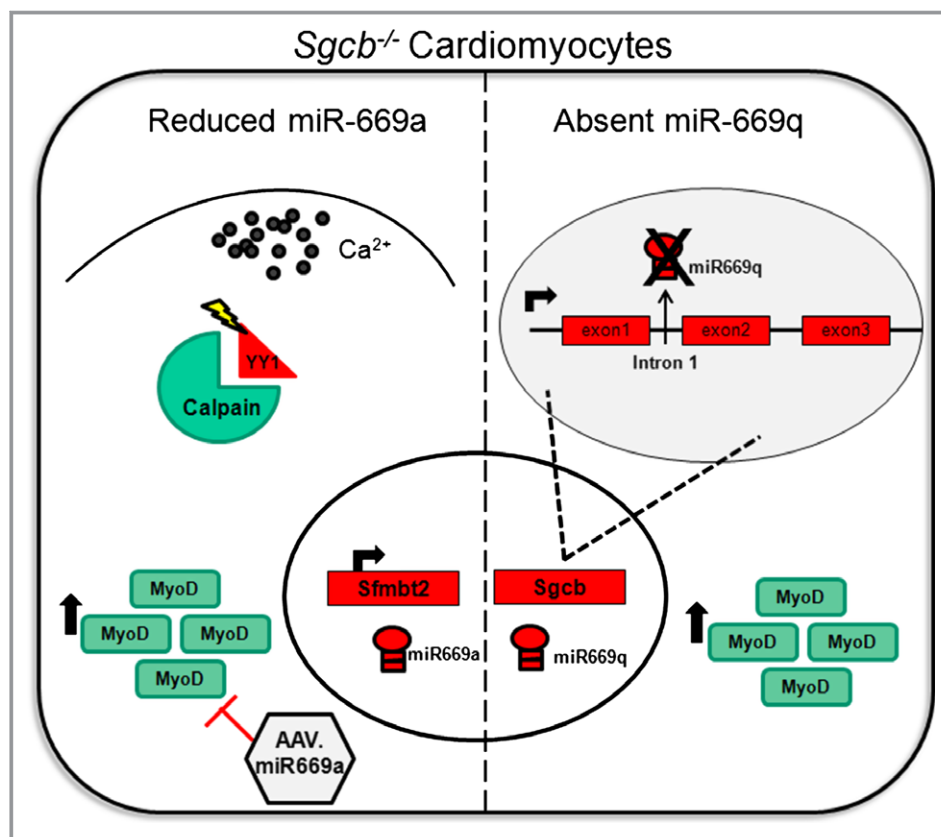
Gene transfer using adeno-associated virus (AAV) appears to be a promising method for gene delivery to the heart. There are multiple serotypes including AAV8 and AAV9 that lead to efficient transduction of cardiomyocytes.<sup>11,12</sup> The benefits of delivering a gene therapy vector directly to the heart rather than through the systemic circulation include dose reduction, minimizing off-target gene expression, and maximizing transduction levels in the heart. Challenges for delivery directly to the heart still pose some translational hurdles due to the protected internal location; however, intracoronary delivery has shown promise in preclinical studies<sup>13,14</sup> and importantly has now been shown to be safe in a gene therapy trial with patients with advanced heart failure.<sup>15</sup> This phase 2 study tested safety and efficacy of intracoronary infusion of the sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA2a) delivered using AAV1. AAV1.SERCA2a intracoronary delivery was shown to be safe and well-tolerated in the 12-month study with a trend towards clinical significance in the functional capacity of patients in the high-dose group.<sup>15</sup> This sentinel study has laid the foundation for treatment of DCM using AAV-mediated intracoronary delivery.

## Modeling Cardiomyopathy and the Role of MicroRNAs

MD animal models often fall short in recapitulating disease severity in skeletal muscle, and the same holds true for cardiomyopathy in the *mdx* mouse model for DMD. These mice fail to exhibit signs of cardiomyopathy until after 20 months of age.<sup>16</sup> Conversely, *sgcb*-null mice, deficient for  $\beta$ -sarcoglycan, and a model for LGMD2E, exhibit early and progressive signs of cardiomyopathy starting with necrotic lesions at 9 weeks and fully developed DCM at 20 weeks.<sup>16</sup> This parallels the progression and severity in limb girdle muscular dystrophies 2E (LGMD2E) patients who have the highest incidence of cardiac involvement of the sarcoglycanopathies with estimates of 50% to 67% of patients.<sup>2,3,5</sup> As Quattrocchi et al compellingly demonstrated in this issue of *JAHA*, the *sgcb*-null mouse serves as an ideal model for assessing AAV-based treatment of MD-associated cardiomyopathy due to its histopathological and functional correlates to DCM in LGMD2E patients.<sup>4</sup> Moreover, the miRNA expression signature that has now become a standard measure of cardiomyopathy progression is also recapitulated in the model and restoration of that signature can be used as an outcome for efficacy.<sup>1,18</sup>

The impetus for this current study began with a 2011 report from the Sampaolesi group where they elegantly demonstrated that miR-669a and the novel miR-669q prevent skeletal muscle differentiation in postnatal cardiac progenitors.<sup>18</sup> The authors were investigating the potential for developing an ex vivo gene therapy approach for LGMD2E using cardiac progenitor cells. Unexpectedly, they found that the cardiac progenitors isolated from *sgcb*-null mice spontaneously differentiated into skeletal muscle regardless of whether they were cultured in vitro or delivered in vivo. Through a series of expression studies comparing *sgcb*-null clones to wild-type clones, Crippa et al found that the aberrant skeletal muscle activation correlated with upregulation of MyoD, an activator of skeletal muscle myogenesis. This unique feature was recapitulated in degenerating 9-month-old *sgcb*-null hearts and not in normal, *mdx*, or  $\alpha$ -sarcoglycan-null mice. Further studies to assess differential miRNA expression revealed that miRNA-669a, highly expressed in normal cardiac progenitor cells, was nearly absent in *sgcb*-null clones as well as the novel miR-669q. Interestingly, miR-669q is located within the first intron of the *sgcb* gene and, therefore, lacking in *sgcb*-null mice. MiR-669a and its closely related homolog miR-669q are involved in the epigenetic silencing of skeletal myogenesis by directly inhibited the 3' UTR of MyoD. The *sgcb* mouse model for LGMD2E presents a unique situation where both miR-669a and miR-669q are absent; thus there is no functional redundancy to inhibit skeletal myogenesis in cardiac tissue as is typically the case following injury or chronic cardiomyocyte wasting (Figure). MiR-669a is a member of the MiR-669 cluster that is encoded and cotranscribed with the *Sfmbt2* gene.<sup>20,21</sup> The *Sfmbt2* promoter is regulated by the transcription factor Yingyang 1 (Yy1). In the absence of SGCB protein, intracellular calcium levels are increased and so is the cleavage of YY1 by the calcium-dependent protease Calpain. In the absence of YY1, *Sfmbt2* and MiR-669a are not transcribed and therefore MyoD is upregulated, resulting in aberrant skeletal muscle activation (Figure, left). MiR-669q is located in intron 1 of the *sgcb* gene. When *sgcb* is deleted, MiR-669q is also absent, which contributes to the upregulation of MyoD and aberrant skeletal myogenesis (Figure, right).

In this issue of the *JAHA*, Quattrocchi et al<sup>4</sup> investigate the potential of viral delivery of miR-669a as a long-term treatment for cardiomyopathy in the *sgcb*-null mouse, hypothesizing that restoration of normal *MyoD* expression and aberrant calcium leak will delay or prevent DCM. The authors packaged miR-669a and the nuclear *LacZ* gene as a tracer into an AAV2/9 vector with tropism for cardiac and skeletal muscle. A randomized placebo-controlled study was performed in which newborn *sgcb*-null mice were treated intraventricularly with AAV2/9-miR-669a, a scrambled miR control, or PBS. Survival was monitored for 18 months at



**Figure.** Dual deficiency in miR-669a and miR-669q *Sgcb*-null cardiac progenitor cells display an aberrant activation of skeletal muscle genes. MiR-669a and its closely related homolog miR-669q are involved in the epigenetic silencing of skeletal myogenesis by directly inhibited the 3' UTR of *MyoD*. The *sgcb* model for LGMD2E presents a unique situation where both miR669a and miR669q are absent and thus there is no functional redundancy to inhibit skeletal myogenesis in cardiac tissue as is typically the case following injury or chronic cardiomyocyte wasting. (Left) miR-669a is a member of the miR-669 cluster that is encoded and cotranscribed with the *Sfmtb2* gene. The *Sfmtb2* promoter is regulated by the transcription factor Yy1. In the absence of SGCB protein, intracellular calcium levels are increased and so is the cleavage of YY1 by the calcium dependent protease Calpain. In the absence of YY1, *Sfmtb2* and miR-669a are not transcribed and therefore *MyoD* is upregulated resulting in aberrant skeletal muscle activation. (Right) miR-669q is located in intron 1 of the *sgcb* gene. In the absence of *sgcb*, miR-669q is also absent which contributes to the upregulation of *MyoD* and aberrant skeletal myogenesis. AAV mediated delivery of miR-669a will inhibit and restore normal levels of *MyoD* expression in cardiomyocytes. AAV indicates adeno-associated virus; LGMD2E, limb girdle muscular dystrophies 2E.

which time the remaining animals were analyzed for miR expression and reversal of histologic and functional measures of cardiomyopathy. Impressively, the miR-669a treatment significantly increased survival over the 18 month study compared to sham-treated animals. Quantitative PCR showed a significant increase in *mir-669a* levels in treated mice and corresponding ubiquitous expression of the *nLacZ* marker. Histological outcomes revealed that miR-669a treatment normalized cardiomyocyte cross-sectional area (CSA), reduced fibrosis, and reduced apoptotic nuclei. In addition, analysis of the ventricular myocardium showed partial restoration of sarcomere organization and the presence of de novo-forming myofibrils. There are multiple miRNAs that have been implicated with the progression of DCM and cardiac degeneration including *miR-1*, *miR-133a*, *miR208a*, *miR-21*, *miR-378*, and *miR-208a*.<sup>1,18,22-24</sup> Sustained expression of

miR-669a in *sgcb*-null hearts was sufficient to largely normalize this DCM miR signature. Importantly, the functional consequences of DCM were also significantly alleviated following miR-669a treatment. The authors found significant reduction of the end-diastolic long axis dimension of the left ventricle and posterior wall thickness. Moreover, the left ventricle internal diameter was decreased in diastole and systole leading to a significant increase in systolic fractional shortening compared to sham-treated animals. Lastly, the authors looked for off-target effects in skeletal muscle because of the inhibitory role of *miR-669a* on *MyoD*. Significant levels of miR-669a were not noted and there was no evidence of toxicity or indication of a defective regenerative capacity of the skeletal muscle.

In summary, the study by Quattrocchi et al provides proof-of-principle for the use of viral-mediated delivery of

miR-669a to treat DCM in patients with LGMD2E.<sup>4</sup> The authors demonstrated robust results including extension of lifespan, histological correction of hypertrophic remodeling, and correction of functional measures. Although this study focused primarily on prevention, additional studies to test whether initiating treatment following the onset of DCM will be crucial to predicting success in MD patients. The impact of this work extends beyond LGMD2E patients and provides a platform for which to test additional miRNA targets and efficacy in other forms of MD. The authors' carefully executed preliminary characterization studies to show the unique profile of miR-669a and miR-669q in the *sgcb*-null model versus other forms of MD should be a role model for others in the field. Although the potential power of miRNA-based therapies for DCM is apparent, caution should be taken to fully characterize the miRNA profile in each disease.

## Disclosures

None.

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