Mired in miR-25

By Lauren Martz, Staff Writer

Preclinical data showing that microRNA-25 inhibition can help treat heart failure directly contradict a 2013 paper suggesting that decreased levels of the miRNA provoke the condition.^{1,2} Although the new study shows that blocking microRNA-25 restores levels of the SERCA2A calcium uptake channel—and thus improves cardiac contractility—the discrepancy with the earlier result needs resolving before a microRNA-25-based therapy can be developed.

The two studies zeroed in on microRNA-25 (miR-25) by different routes.

The new study, headed by Mark Mercola at the **University of California, San Diego**, found miR-25 by focusing on the connection between intracellular calcium and heart muscle contraction and screening for miRNA inhibitors of SERCA2A (*ATPase Ca⁺⁺ transporting cardiac muscle slow twitch 2; ATP2A2*).

The findings were published in *Nature*.

By contrast, last year's study, headed by Leon De Windt at **Maastricht University**, pinpointed miR-25 by searching for post-transcriptional regulators of cardiomyocyte hypertrophy in mouse models of heart failure.

That study found that inhibiting miR-25 in healthy mice led to cardiac dysfunction and increased the animals' susceptibility to heart failure. In addition, inhibiting miR-25 in mice with heart failure exacerbated the disease.

Those data were published in *Nature Cell Biology*.

Mercola is a professor of bioengineering at the University of California, San Diego and a professor and director of the Muscle Development and Regeneration Program at the **Sanford-Burnham Medical Research Institute**. De Windt is a professor of molecular cardiology at Maastricht University.

Mercola's team sought regulators of SERCA2A because previous work from collaborators at the **Icahn School of Medicine at Mount Sinai** and **Celladon Corp.** and from other academic labs tied SERCA2A expression to heart failure. Those studies showed that restoring SERCA2A expression with adeno-associated virus (AAV) vector-mediated gene therapy can improve cardiac function in animal models of heart failure and patients with heart failure.^{3,4}

The patient study was a Phase I/II trial by Celladon and the Icahn School of Medicine on the company's Mydicar (AAV1/SERCA2a), an AAV vector encoding SERCA2A. Mydicar significantly reduced cardiovascular events and met the primary endpoint of reducing a composite of outcome measures in patients with advanced heart failure. The product has breakthrough designation from the FDA for reducing hospitalizations in patients with heart failure and is in Phase IIb testing.

SERCA2A mediates calcium ion uptake in cardiomyocytes, which is necessary for normal contraction of the heart muscle. During heart failure,

decreased SERCA2A activity impairs calcium uptake and prevents normal cardiac contractions.

However, the resulting decrease in cardiac contractility is only one component in the complex process of heart failure; thus, replacing SERCA2A via gene therapy only addresses part of the cause of the heart dysfunction.

In addition, gene therapy allows limited control over the level of protein expression and does not work for all patients because of immunological variability in the population.

Roger Hajjar—the Mount Sinai collaborator on the study—told SciBX, “One of the problems with using AAVs is that half the population has neutralizing antibodies against AAV1 and has to be excluded from the studies.”

William Marshall added that even when patients can be treated with AAV-mediated gene therapy, they are generally limited to one dose. “With a gene therapy, you really only get one shot on goal, and then the immune system is able to prevent the virus from delivering its cargo.”

Hajjar is director of the Cardiovascular Research Center at the Icahn School of Medicine and a professor of medicine in cardiology at **Mount Sinai Hospital**. He is also a cofounder and scientific advisory board member of Celladon. Marshall is CEO of **miRagen Therapeutics Inc.**

miRagen's anti-miR-208, a modified short nucleic acid sequence targeting miR-208, is in preclinical testing for heart failure.

Mercola and colleagues wanted to find an alternative way of controlling SERCA2A that would avoid the drawbacks of gene therapy. Because miRNA dysregulation had been implicated in heart failure pathology,⁵ the researchers thought an miRNA might be responsible for the downregulation of SERCA2A.

miRNAs silence gene expression by binding the mRNA seed region, a 2–8 base pair stretch in the 5' untranslated region, to prevent translation.

The team performed a whole-genome miRNA screen in human embryonic kidney cells and identified miR-25 as the most potent miRNA at targeting and downregulating SERCA2A.

In cardiomyocytes, miR-25 effectively disrupted calcium transport by delaying calcium uptake kinetics in cardiomyocytes.

The researchers examined myocardial samples from five patients with severe heart failure and found higher miR-25 levels in the left ventricles than in tissue from five control hearts from patients without contractile dysfunction.

Next, the team tested the effect of modulating miR-25 levels *in vivo*. In healthy mice, gene transfer of miR-25 decreased Serca2a levels and caused a greater decline in cardiac function compared with what was seen using miR-92a, a miRNA in the same family as miR-25 that shares its seed region.

Conversely, injection of an anti-miR-25 oligonucleotide increased Serca2a levels in wild-type mice compared with injection of a scrambled anti-miRNA. Anti-miR-25 had no effect on Serca2a levels or cardiovascular parameters in *Serca2a^{-/-}* knockout mice.

Finally, the group assessed the effects of the anti-miRNA in a mouse model of heart failure.

In mice with established heart failure induced by transverse aortic constriction for 3 months, anti-miR-25 improved cardiac function

at 4.5 and 5.5 months. The anti-miR-25 also restored left ventricular ejection fraction to normal levels and improved survival, and it increased SUMOylated Serca2a levels and decreased cardiac fibrosis compared with control anti-miRNA.

Together, the data suggest that miR-25 upregulation during heart failure causes SERCA2A suppression and, consequently, contractile dysfunction. The team also concluded that blocking miR-25 can reverse the decline in heart function.

The group included researchers from the **University Medical Center Utrecht, ICIN Netherlands Heart Institute** and **Gwangju Institute of Science and Technology**.

miR-25 conflict

Several researchers told *SciBX* that the first step should be to reconcile the results with the conflicting data from the Maastricht study.

According to Mercola, the discrepancies may be due to the different disease stages analyzed by the two groups. Whereas his team examined miR-25 in samples from patients with advanced heart failure, De Windt's team investigated changes during the early stages of pressure overload.

Mercola added that data from the Maastricht team suggest that "we might not want to block miR-25 in people who do not yet have clear signs of heart failure."

"Since heart failure results from various diseases, we will need to learn more about what types and states of disease are likely to benefit," he said.

Marshall suggested that the conflicting data could be due to the different anti-miR-25 molecules used by each team. "miR-25 is part of a family of molecules with conserved seed regions. This means that multiple miRNAs may be binding to the same regulatory motif on *SERCA2A*, and the selectivity of the molecules for miR-25 over other family members could affect the results."

De Windt told *SciBX* that his team plans to repeat the mouse experiments. "This happens all the time in science, and it is our responsibility to sort it out."

He also pointed out some aspects of the work in the *Nature* paper that may contribute to the inconsistent results.

"First, the [Sanford-Burnham] team screened for miRNAs targeting *SERCA2A* in human embryonic kidney cells. While it was a smart idea to screen for miRNAs against the target, miRNAs behave completely differently in different cell types. Most of the miRNA candidates identified in their screen are not even present in the heart," he said.

De Windt added that Mercola's team drew conclusions from a small number of human hearts and animals and suggested that replication in larger cohorts would strengthen the results.

Despite the tissue data from patients with advanced heart failure, De Windt commented that the mouse model used in the *Nature* paper was not very severe. "The left ventricular ejection fraction only decreases by 6% during heart failure relative to healthy mice. In healthy human hearts, the ejection fraction is about 60% of the heart's blood volume per pump, and this fraction is reduced by about half in heart failure. The experiments should be repeated in a model that more closely mimics the extent of heart failure in patients," he said.

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**— William Marshall,
miRagen Therapeutics Inc.**

Translational resolution

De Windt told *SciBX* that his team now plans to develop a cardiac-specific *miR-25* knockout mouse model. He said that would give a definitive answer about how the miRNA is involved in mouse heart failure.

But Krisztina Zsebo, president and CEO of Celladon, said that mouse models of heart disease and the effects of polynucleotide therapeutics including viral vectors in mice do not generally translate well to humans.

"In mice, the effects of i.v. injection of antisense oligonucleotides are unlikely to translate to effects in large animal models due to the difference in blood volume. The small blood volume in mice allows a high concentration of the nucleic acid that you just can't achieve in large animal models," she said.

Indeed, Mercola's next step is to evaluate miR-25 in heart failure models in larger animals such as pigs, dogs, sheep or nonhuman primates.

However, delivery of oligonucleotides to the heart in larger species presents its own challenges.

Neil Gibson, CSO of **Regulus Therapeutics Inc.**, said that delivering oligonucleotides to specific tissues presents an opportunity for advancement in the field.

"Single-stranded oligonucleotides in saline can be used as therapeutics to target miR-25 to treat heart failure, for example, but the majority of the oligonucleotide tends to go to

limited places in the body such as the liver or macrophages. Delivery to the heart or other less-accessible cell types is a challenge that still needs a lot of work," he said.

"Active investigation is ongoing to determine the best type of anti-miRNA delivery strategy for the heart. Our company has found that conjugating oligomers to a ligand that engages cell-specific receptors effectively targets hepatocytes. It may be possible to identify receptors with cardiac-specific expression to enrich oligomer accumulation in the heart to treat heart failure," he added.

Regulus' RG-101, a ligand-conjugated anti-miRNA targeting miR-122, is in Phase I testing to treat HCV.

Antisense vs. AAV

Celladon's Zsebo is not convinced that an oligonucleotide-based approach will upregulate *SERCA2A* as effectively as gene therapy, despite the fact that anti-miR-25 may affect multiple dysregulated pathways in heart failure, including *SERCA2A*.

"*SERCA2A* is downregulated at both the transcriptional and translational levels in heart failure. Antisense oligonucleotides only block the translational downregulation," she said.

She added that the effects of antisense oligonucleotides are very short lived compared with the effects of gene therapy as they have half-lives of only 10–12 hours. The effects of AAV-mediated gene therapy may last longer than four years.

However, Marshall said that antisense therapeutic formulation methods can achieve high stability and extend half-lives *in vivo*. Although miRagen's antisense molecules have not been tested in humans, he expects that, based on rat studies, patients could be dosed every few weeks or every month.

Mercola told *SciBX* that there are two principal advantages to an miR-

25 approach. “Pharmacological intervention can be titrated or even halted depending on the needs for managing a patient’s disease, and miR-25 might block expression of other proteins besides SERCA2A that control calcium handling and contractility.”

Sanford-Burnham has filed a patent application covering the work. The IP is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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