miR-25 in Heart Failure

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Inhibition of miR-25 Improves Cardiac Contractility in the Failing Heart
Wahlquist et al

Recent work by Wahlquist et al1 showed miR-25 to reduce levels of rco/endoplasmic reticulum calcium ATPase (SERCA) 2a during heart failure, whereas therapeutic inhibition of this microRNA (miRNA) was able to reverse pre-established heart failure in a mouse model of pressure overload. Although these data suggest that miR-25 might be a drug target for the treatment of heart failure, further in vivo analysis is required before we can consider miR-25 inhibition for clinical application.

Recently, Wahlquist et al1 reported on the pathological up-regulation of miR-25 during heart failure and showed that inhibition was able to block and reverse the disease in mice. Although an increase in cardiac miR-25 levels caused a decline in cardiac function, anti–miR-based inhibition of miR-25 halted established heart failure at least in part by increasing the mRNA of SERCA2a. This is intriguing because phase II clinical data from the Calcium Uptregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) trial has demonstrated that gene therapy to increase SERCA2a levels in humans delays the progression of chronic heart failure. This could imply that pharmacological regulation of miR-25 may regulate at least 1 clinically validated target, which may be more straightforward than current gene therapy approaches. However, miRNA biology seems to sketch a somewhat more complex picture than a miRNA functioning through 1 single-gene target. In addition, conflicting data on the cardiac functions of miR-25 highlight the need for further investigation before advancing miR-25 as a clinical target for heart failure.

Promise of miRNAs as Novel Therapeutic Targets

miRNAs are short single-stranded noncoding RNAs that modulate the expression of target proteins by annealing to complementary sequences in their target mRNAs.5 It is thought that a single vertebrate miRNA on average targets a few hundred transcripts.3,4 Because individual miRNAs often target numerous related mRNAs that encode multiple components of a specific signal pathway or cellular process, the modulation of a single miRNA can have a profound impact on cellular phenotypes.

Another interesting aspect of miRNA biology is that although miRNAs are typically moderate regulators under homeostatic conditions (ie, exert modest effects), their function becomes more pronounced under conditions of injury or stress.6 The heightened activity of a miRNA during stress can be explained by the fact that stress, or disease signals, influences numerous aspects of miRNA biogenesis and function, including the abundance of the miRNA itself, changes in expression of the mRNA targets, and differences in RNA-regulating proteins.7

Although the first human miRNA was discovered only a decade ago, therapeutics designed to regulate miRNA function are already entering the clinical arena. The rapid translation of first-generation miRNA-targeting therapies has been facilitated by the prominent role of miRNAs in integrating pathological stress signals, the conserved nature of miRNA sequences, and the growing appreciation that miRNAs, with their specialized role as mediators of systems biology, offer novel therapeutic approaches to diseases that are largely unresponsive to highly selective small molecule drugs.

miRNA-Modulating Drugs

Leveraging lessons learned from antisense technologies, a wealth of recent animal and human studies has rapidly validated the therapeutic benefit of modulating miRNA function and underscore the value of miRNA-targeting oligonucleotides as an emerging class of drugs. Pharmacological regulation of individual miRNAs can be achieved by modified antisense oligonucleotides, referred to as anti-miRs, to inhibit a miRNA, or by a miRNA mimic to replace a miRNA.8

Based on pharmacokinetic studies we now know that anti-miRs can be delivered subcutaneously and are broadly distributed to many tissues, including the heart, with a preferential delivery to the kidney and the liver and that the in vivo half-life of anti-miRs can be in the order of weeks.9 The initial identification and synthesis of active compounds are relatively straightforward; unlike antisense oligos or small molecules, miRNA-targeting oligos are restricted to a highly constrained design space (at least with respect to primary sequence). However, it should be noted that as with small molecule drug development, early miRNA-targeting tool compounds typically require application of substantial chemistry to optimize activity and limit potential toxicity before first-in-man studies.

Several chemical modifications are used to increase nuclease resistance, facilitate cellular uptake, and to reduce clearance by glomerular filtration and urinary excretion.10–12 The first anti-miR drug has now entered the clinical arena as Santaris Pharma recently reported on both the safety and the efficacy of
their anti-miR against miR-122, miravirsen, in humans. These data indicated that miravirsen given as a 4-week monotherapy to hepatitis C virus patients provides long-lasting suppression of viremia and provides a high barrier to viral infection.13

Restoring the function of lost or downregulated miRNAs can be achieved by therapeutic mimicry or replacement of a miRNA using synthetic RNA duplexes designed to mimic the endogenous functions of the miRNA of interest. These miRNA mimics harbor chemical modifications that improve their stability and cellular uptake, without interfering with their function.9 The first phase 1 study of a liposome-formulated miR-34 mimic-based drug was initiated recently in patients with primary liver cancer or metastatic cancer with liver involvement by Mirna Therapeutics. This is the first miRNA mimic to advance into the clinic and thus an important milestone for the development of miRNA-based replacement therapeutics.14 Translation of these pioneering miRNA drugs into clinical trials has helped validate this new therapeutic paradigm and has generated enthusiasm for exploring opportunities in other disease areas.

In cardiovascular diseases, numerous miRNAs have been shown to influence cardiomyocyte hypertrophy, cardiomyocyte survival, changes in cardiac metabolism, and other processes associated with the progression of heart disease.15 Our rapidly increasing understanding of the contribution of miRNAs to the pathogenesis of cardiovascular disease, the shortage of effective therapies, and the ability to potently and specifically regulate miRNAs in vivo has catalyzed efforts to explore pharmacological manipulation of miRNAs for treating heart disease. Preclinical efficacy studies using oligonucleotide chemistries to modulate miRNA levels have proven to be effective in targeting pathological miRNAs and produce therapeutic benefit in a variety of disease settings.6

Inhibition of MiR-25 as Therapy for Heart Failure

With heart failure reaching epidemic proportions, the need for effective therapies is urgent. Although the pathogenesis and pathology of heart failure are heterogeneous, a common denominator seems to be a decline in cardiomyocyte calcium handling and contractility.16 Contraction of the cardiomyocyte is regulated by extracellular calcium (Ca2+) uptake via the L-type Ca2+-channel, which in turn drives the release of intracellular Ca2+ from the sarcoplasmic reticulum via ryanodine receptors, a process also known as Ca2+-induced calcium release. Subsequent relaxation occurs by the reuptake of calcium back into the sarcoplasmic reticulum by the calcium transporting ATPase SERCA2a. During heart failure there is a progressive decline in SERCA2a levels, which contributes to the decrease in cardiac function.16

Recent clinical studies have shown that gene therapy approaches to restore SERCA2a levels in patients with heart failure are able to attenuate the decline in cardiac function.17 Although single-gene replacement strategies to directly enhance cardiac SERCA2a expression seem to help normalize aberrant Ca2+ handling in the failing heart, miRNAs represent attractive next-generation therapeutic targets because they have evolved to regulate networks of genes that contribute to pathogenesis rather of a single target. Furthermore, miRNA-targeting drugs are unlikely be limited by the immunologic issues that accompany adeno associated virus-mediated gene therapy.

Seeking to identify key miRNAs that control pathological suppression of SERCA2a in the failing heart, Wahlquist et al performed a high-content screen for miRNAs capable of interacting and thereby regulating the levels of SERCA2a. Of the 875 miRNAs screened, 144 miRNAs were able to interact with a recognition element in the SERCA2a message, of which miR-25 was the most promising lead. This miRNA was upregulated in failing human cardiomyocytes and prolonged the decay of calcium transient in a cardiomyocyte cell line. After confirming that AAV9-mediated overexpression of cardiac miR-25 reduced SERCA2a expression and produced contractile dysfunction, the authors showed that therapeutic administration of anti–miR-25 oligonucleotide was capable of reversing pre-established heart failure in mice subjected to thoracic aortic constriction (TAC) despite the continued presence of constant pressure overload. The protective effect of miR-25 inhibition appeared at least partially because of the regulation of SERCA2a and potentially inositol 1,4,5 trisphosphate receptor type 1 (IP3R1), with no effect on other key calcium-handling proteins (eg, phospholamban or the sodium–calcium exchanger).1 Taken together, these findings suggest that increased expression of cardiac miR-25 directly reduces SERCA2a expression and contributes to reduced contractility during progression of human heart failure. This proposed function of miR-25 suggests that inhibition of miR-25 may be a novel therapeutic strategy for the treatment of heart failure.

As is often the case with emerging therapeutic targets, contradictory data add complexity and cloud interpretation. A different picture of cardiac miR-25 function had been presented previously by Dirkx et al.18 In examining the contribution of the transcription factor HAND2 to pathological cardiac hypertrophy and heart failure, the authors established that Hand2 mRNA is a direct target of miR-25. In pressure-overloaded TAC mouse hearts, increased HAND2 expression correlated with reduced miR-25 expression. Critically, administration of an antagoniR oligonucleotide targeting miR-25 to pressure-overloaded TAC mice markedly increased cardiac HAND2 expression and significantly reduced cardiac function.

How can these 2 disparate findings be explained and if possible, reconciled? From a translational standpoint, it is perhaps easiest to focus on the authors’ in vivo pharmacology results with miR-25 anti-miRs. First, it is important to recognize that although the 2 studies targeted the same miR-25 sequence, they used significantly different chemistries. Although Wahlquist used a commercially available 2′-O-methyl–modified anti-miR oligonucleotide design, Dirkx used a phosphorothioate-stabilized cholesterol conjugate antagoniR. These differences in oligonucleotide chemistries alone could produce distinct in vivo pharmacology profiles (both on- and off-target). Second, although Dirkx administered 80 mg/kg IP doses of antagoniR, Wahlquist used an 10-fold lower dose level of anti-miR formulated in a cationic polymer vehicle delivered intravenously. Third, the 2 studies examined the effects of miR-25 inhibition at different times after the initiation of pressure overload stress: Wahlquist began anti–miR-25 treatment 3 months after TAC (functional assessments at 3.5, 4.5, and 5.5 months post TAC).
and Dirkx began anti–miR-25 at day 3 post TAC (functional assessments at 2 and 4 weeks). Until a more complete picture of miR-25 biology is available, it is conceivable that miR-25 could play a beneficial role acutely by helping the heart adapt to pressure stress but produce longer term maladaptive effects. Future studies with expanded group sizes will be vitally important to further explore the therapeutic relevance of miR-25 inhibition in the setting of heart failure.

Looking to the Future

MiRNA research has unveiled an unconventional disease mechanism that provides a unique opportunity to exploit an entirely new area of biology. Enhancing cardiac contractility by regulating miR-25 is one of the many potentially exciting opportunities that have surfaced in the past few years. Growing support for miRNAs as key drivers of diverse human disease has generated great enthusiasm for their continued exploration as new drug targets. Although miRNA-based cardiovascular therapeutics have yet to enter the clinic, we anticipate that this critical milestone will be reached in the near future. Although miRNA-targeting chemistries have advanced significantly, some inherent hurdles remain that will need to be overcome for their successful development as long-term therapies for cardiac indications. Safety studies in humans have shown a good tolerability for this novel class of drugs,13 but the effects of chronic treatment regimens in the setting of heart failure, as for anti–miR-25, will need to be evaluated carefully, especially because oligonucleotide drugs typically accumulate in the kidney.19

As is true for most miRNAs, miR-25 is expressed in a broad range of cell types and likely targets many more genes besides SERCA2a and inositol 1,4,5 trisphosphate receptor type 1 (IP3R1). Potential sources of toxicity after administration of a miRNA inhibitor not only can result from off-target toxicities induced by the chemistry or unwanted gene changes but can also arise from unintended on-target effects of the anti-miR in nontargeted, nondiseased tissues. Hence, before moving forward with anti–miR-25 as a clinical candidate, it will be important to learn about the functions of miR-25 outside of the heart and its effects not related to myocyte Ca2+ handling. Several questions still surround the mode of action of different anti-miR chemistries. Current data suggest that specific chemistries influence cellular uptake and ability to inhibit the function of a miRNA. The precise mechanism for these pharmacokinetic differences is still unclear and more in-depth biochemistry will be required to gain additional insight. Despite these unknowns, there is high interest surrounding miRNAs as novel therapeutic entities and the anticipated success of the early forerunners will likely trigger additional innovation of miRNA therapeutics and will advance the search for techniques for efficient in vivo delivery of these therapeutics. In the case of anti–miR-25, we are eagerly awaiting more efficacy data in additional models of heart failure and how this translates into larger animals. Undoubtedly, the next few years promise to provide many more insights into the therapeutic use of these new therapeutic agents and will hopefully even further strengthen the enthusiasm for these drugs as a treatment for heart failure.

Disclosures

E. Bush is an employee of miRagen Therapeutics, Inc, and E. van Rooij is a scientific co-founder and member of the Scientific Advisory Board of miRagen Therapeutics, Inc.

References

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