MicroRNA Therapy for the Failing Heart

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Study Hypothesis

Heart failure (HF) may result from diverse pathological processes leading to progressive organ decompensation. Improper functionality of the calcium-handling machinery is a frequent component of the pathobiology underlying this debilitating process. Case in point, loss of intracellular calcium homeostasis because of decreased function of sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase 2a (SERCA2a) is characteristic of HF, and modulation of calcium transients occurs through repression of contractility might be involved in HF and could, therefore, be used as therapeutic targets. Specifically, the authors focus their efforts on miRNAs involved in calcium dynamics regulation.

How Was the Hypothesis Tested?

To search for miRNAs capable of downregulating SERCA2a, a whole-genome collection of miRNAs was tested on Human Embryonic Kidney 293 cells cotransfected with an enhanced green fluorescent protein reporter fused to the 3' untranslated region of the SERCA2A gene, enabling functional detection of miRNAs capable of decreasing SERCA2a through fluorescence (enhanced green fluorescent protein) readout. miRNAs with a predicted downregulating effect were subsequently tested for their capacity to affect calcium transient duration (from 75% to 25% of maximal calcium transient amplitude, Ca\(^{2+}\)-ATPase in patients with advanced heart failure. Circulation. 2011;124:304–313.

Wahlquist et al\(^2\) hypothesize that miRNAs capable of repressing contractility might be involved in HF and could, therefore, be used as therapeutic targets. Specifically, the authors focus their efforts on miRNAs involved in calcium dynamics regulation.

Principal Findings

Through functional screening in cells containing a target sensor, 144 miRNAs capable of decreasing enhanced green fluorescent protein fluorescence representative of the SERCA2a levels in vitro were identified. This list was narrowed down to evolutionarily conserved miRNAs known to be upregulated in HF (15 candidates) and further reduced to 4 miRNAs capable of affecting calcium dynamics as shown by Ca\(^{2+}\)-ATPase delay in HL-1 cells. Of these candidates, miR-25 stood out for its potent effects; moreover, its presence could be verified in mouse cardiomyocytes from failing left ventricles, and significant upregulation was found in human failing hearts, postulating it as a candidate to be involved in the pathophysiology of HF.

Next, several miR-25 targets within calcium-handling pathways were identified in silico from which direct regulation of SERCA2a and inositol-1,4,5-trisphosphate receptor 1 could be verified. Selective interaction of miR-25 with the untranslated regions of these candidate miRNAs was demonstrated by mutation of the miRNA recognition sites resulting in loss of reporter inhibition. However, only downregulation of SERCA2a (using siRNA) reproduced the observed effect of miR-25 on calcium dynamics, suggesting that miR-25 modulation of calcium transients occurs through repression.
of SERCA2a. To extrapolate the interaction between miR-25 and SERCA2a to the in vivo setting, the authors used an adenoviral system to induce expression of miR-25 in mice. As expected, this resulted in decreased ventricular SERCA2a levels and depressed cardiac function.

Moving on to the physiological evaluation of miR-25 inhibition as a potential therapy, anti–miR-25 was found to restore parameters of calcium kinetics altered in the presence of miR-25 in transfected cardiomyocytes (both HL-1 and rat neonatal). At the whole animal level, intravenous injection of this miR-25 antagonist resulted in reduced levels of miR-25 in wild-type and SERCA2a knockout myocardium. To test the therapeutic potential of this intervention, the authors used a HF model based on chronic transaortic constriction. Intravenous treatment with anti–miR-25 was applied 3 months after transaortic constriction surgery, once HF was established. Monitoring 2.5 months after treatment initiation revealed recovery of cardiac function as shown by parameters, such as ejection fraction, fractional shortening, heart:body weight ratio, and survival. Structurally, ventricular fibrosis was reduced and cardiomyocyte cross-sectional area was normalized. At the molecular level, miR-25 decreased in treated animals, leading to increased SERCA2a.

Implications

In this study, Wahlquist et al. establish that miR-25 modulates SERCA2a levels affecting calcium dynamics and impairing cardiac function. Once presence of this miRNA was verified in the failing heart, targeted downregulation through anti–miR-25 injection resulted in amelioration of HF parameters at the structural, functional, and molecular levels. Therapeutic targeting of miR-25 offers a new approach to restore SERCA2a function in failing myocardium bypassing the potential risks of current gene therapy strategies. However, the fact that miRNAs may affect multiple cellular pathways simultaneously poses a challenge for this novel platform. To increase the translational value of anti–miR therapies, organ-specific delivery strategies aimed at minimizing off-target effects need to be developed. Overall, this proof-of-concept study sets the stage for a new chapter in the management of HF.

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Disclosures

None.

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