miRNAs to Regenerate the Heart

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Study Hypothesis
MicroRNAs (miRNAs) are small nucleic acid products that simultaneously modulate expression levels of several different genes by partial complementation, leading to posttranscriptional regulation. Among their functions, miRNAs have been shown to regulate cardiac development and differentiation. During heart development, immature cardiomyocytes proliferate actively to accommodate increasing heart size and function; however, this proliferation is abruptly abrogated shortly after birth, leaving the heart with a limited regenerative capacity insufficient to replace substantial amounts of tissue lost after injury. In this article, Eulalio et al hypothesize that neonatal proliferation capacity can be reactivated in adult cardiomyocytes by the exogenous administration of selected miRNAs, leading to cardiac repair.

How Was the Hypothesis Tested?
The authors used a synthetic whole-genome human miRNA library to induce individual expression of miRNAs in neonatal cardiomyocytes. To assess the effect of the miRNA mimics, they immunostained transfected cells for cardiac-specific proteins together with markers of DNA synthesis and proliferation. High-content fluorescence microscopy enabled quantification of proliferating cardiomyocytes and selection of the miRNAs with the highest potential to induce cell cycle reentry. To ensure that DNA replication led to nuclear division (karyokinesis) and cytoplasm division (cytokinesis), characteristic markers for late mitosis and midbodies were tested by immunostaining in neonatal cardiomyocytes after treatment with the respective top 10 proliferation-inducing miRNAs for mouse and rat. This allowed for selection of the best-performing miRNAs to be used in subsequent studies. Selected candidates were then transfected onto 7-day-old and adult rat cardiomyocytes to validate their proliferation-inducing properties in nonspontaneously dividing cells. Immunostaining and gene-expression analysis were used to characterize proliferation, dedifferentiation, and cell cycle reentry.

Mechanistic studies aimed at identifying the molecular targets involved in triggering cardiomyocyte replication were then done using the 2 most effective miRNAs. Genes implicated in the reactivation of the proliferative potential of treated cells were uncovered using deep sequencing of neonatal mouse cardiomyocytes transfected with each of the candidates and comparing them with untransfected controls. Functional analysis of transcripts upregulated or downregulated after treatment allowed identification of prioritized functions activated or repressed by the candidate miRNAs. Using short interfering RNA technology, the authors knocked down genes originally downregulated by miRNA treatment and measured proliferation, looking to identify relevant hubs limiting cell cycle reentry.

Finally, in vivo activity of the 2 selected candidate miRNAs was tested, first in a physiological model and later in the setting of a myocardial infarction. For this, miRNAs were delivered via direct injection into the hearts of neonatal rats for short-term studies or as part of highly cardiotropic adeno-associated virus serotype 9 for longer follow-up in healthy neonatal or adult mice. Induction of proliferation (determined by immunostaining), cardiac hypertrophy, and induction of fibrosis were characterized. As the final test for this study, the authors evaluated the effect of miRNA therapy in an adult mouse model of myocardial infarction. For this, immunostaining allowed again monitoring of cardiomyocyte proliferation, and 2-dimensional echocardiography and histological analysis were used to characterize functional consequences and structural effects of adenoviral delivery of candidate miRNAs.

Principal Findings
The authors found that miRNAs have the ability of selectively induce cardiomyocyte proliferation in vitro and in vivo. According to their results, >200 miRNAs had the capacity to increase neonatal rat cardiomyocyte proliferation in vitro. From this panel, only 40 conserved their functions across species when applied to mouse neonatal cardiomyocytes. Focusing on the top-performing miRNAs, the authors demonstrated induction of complete cell division in a cardiomyocyte-specific manner, with surrounding cardiac fibroblasts not being induced to proliferate. In fact, the top 2 candidates for mouse and for rat were tested (4 miRNAs total), showing increased proliferation of postmitotic 7-day-old and adult cardiomyocytes, as well as dedifferentiation.
of adult cardiomyocytes during this process. From these experiments, 2 miRNAs were postulated as most promising (hsa-miR-590-3p and hsa-miR-199a-3p) and used in subsequent experiments.

Transcriptional analysis of miRNA-treated versus non-treated cardiomyocytes identified upregulation of genes associated with cell cycle and proliferation, whereas factors related to differentiation and function were downregulated. Individual knockdown of genes repressed by miRNA treatment failed to induce cardiomyocyte proliferation, highlighting the synergistic effect of the multiple targets of each miRNA.

Moving on to in vivo experiments, the authors demonstrated induction of cardiomyocyte proliferation without increased fibrosis after only 4 days of miRNA treatment in healthy neonatal hearts. This was recapitulated in an adult model, where heart enlargement was observed 12 days after adenoviral delivery of selected miRNAs, in the absence of collagen increase. In both cases, proliferation was detected predominantly in cardiomyocytes. Lastly, adenoviral delivery of the 2-candidate miRNAs in a murine myocardial infarction model led to improved cardiac function and reduction of the infarcted area, presumably as a result of the increase in cardiomyocyte proliferation and the integration of the new cardiomyocytes into the contractile tissue.

Implications
This study shows for the first time the reactivation of the dormant proliferative capacity of adult cardiomyocytes as a direct effect of miRNA delivery. This proliferation-induction effect could not be recapitulated by single-gene manipulation, highlighting the combined effects of individual miRNAs on multiple target genes to stimulate intrinsic regeneration, which is here leveraged for the treatment of a complex disease. miRNA delivery to the infarcted heart notably results in structural and functional recovery. Therefore, the broader action of miRNAs impacting multiple pathways opens up a new translational perspective for the treatment of complex cardiac disease as a stand-alone therapy or in combination with other regenerative resources.

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