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Preclinical Development of a MicroRNA-Based Therapy for Elderly Patients With Myocardial Infarction

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ABSTRACT

BACKGROUND Aging populations show higher incidences of myocardial infarction (MI) and heart failure (HF). Cardiac remodeling post-MI leads to progressive impaired cardiac function caused by a disarray of several processes including derailed autophagy. Microribonucleic acids (miRNAs) are known to be key players in cardiovascular disease but their involvement in cardiac autophagy and aging is not well understood.

OBJECTIVES This study sought to identify new miRNA candidates that regulate cardiac autophagy and aging.

METHODS We exploited a high-throughput, fluorescence-activated cell sorting-based green fluorescent protein-LC3 detection method to measure the autophagic flux in cardiomyocytes after transfection of a precursor miRNA library consisting of 380 miRNAs. This was followed by a series of molecular and in vivo studies.

RESULTS Together with additional expression screenings, we identified miR-22 as an abundant and strong inhibitor of the cardiac autophagy process. Cardiac miR-22 expression levels increased during aging of mice as well as in aging neonatal cardiomyocytes in vitro by a P53-dependent mechanism. Inhibition of miR-22 in aging cardiomyocytes in vitro activated autophagy and inhibited cellular hypertrophy. Pharmacological inhibition of miR-22 post-MI in older mice activated cardiac autophagy, prevented post-infarction remodeling, and improved cardiac function compared with control subjects. Interestingly, similar effects were less pronounced in younger mice with significantly lower cardiac miR-22 expression levels. In addition, circulating levels of miR-22 in 154 patients with systolic HF were highly associated with early mortality.

CONCLUSIONS We concluded that miR-22 is an important regulator of cardiac autophagy and a potential therapeutic target, especially in the older myocardium. Finally, circulating miR-22 provides prognostic information for HF patients, highlighting miR-22 as a promising therapeutic and biomarker candidate for cardiovascular disorders. (J Am Coll Cardiol 2016;68:1557–71) © 2016 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

Atg = autophagy-related gene

FACS = fluorescence-activated cell sorting

GFP = green fluorescent protein

LNA = locked nucleic acid

MI = myocardial infarction

Noncommunicable diseases, notably cancer, diabetes, cardiovascular and neurological disorders, account for 65.5% of deaths (approximately 34.5 million) worldwide (1). Nearly 45% of these deaths are due to cardiovascular disease (CVD), which remains the leading cause of mortality worldwide (1). Myocardial infarction (MI) leading to chronic heart failure (HF) is the most common form of CVD that is prevalent in our society, and its incidence increases dramatically with age. The loss of cardiac tissue and ensuing impaired contractile function due to MI results in cardiac remodeling and cardiac hypertrophy, fibrosis, and altered autophagic activity. In older patients, all these processes are more pronounced (2), although the molecular rationale remains unclear.

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Autophagy, a process that recycles cellular components to maintain cellular homeostasis, is deregulated in age-related disease, like HF (3). Indeed, cardiac-specific deletion of the autophagy gene *Atg5* in mice leads to a progressive decline in function with age, together with early appearance of other cardiac aging hallmarks (cardiac hypertrophy and fibrosis) eventually leading to premature death (4). Temporally controlled deletion of *Atg5* in adult mice also results in cardiac dysfunction, hypertrophy, disorganized sarcomere, mitochondria aggregation, and accumulation of ubiquitinated proteins (5). Importantly, in the case of MI, accumulation of p62 colocalized aggresomes has been observed in the infarct border zone and in remote areas (6). Mammalian sterile 20-like kinase 1($Mst1^{-/-}$) mice do not show aggresome accumulation and pathological left ventricular remodeling post-MI. This phenotype is lost when $Mst1^{-/-}$ are crossed with $Beclin^{+/-}$ mice, which show lower autophagic activity (6). Therefore, the identification of therapeutic targets that modulate autophagy holds great promise as a new treatment approach to cardiac remodeling and HF, especially in older age.

Microribonucleic acids (miRNAs) are a class of noncoding regulatory ribonucleic acid (RNA) molecules of around 22 nucleotides in length; they exert their function by complementary binding via their seed sequence. In the past decade, miRNAs have been identified as critical regulators of cardiac pathophysiology (7–9), but only a few miRNAs regulating cardiac autophagy have been studied in detail (10). miRNA-212/132 was demonstrated to have prohypertrophic and antiautophagic effects in cardiomyocytes via regulation of FoxO3a (8). Additionally, the miRNAs miR-199a and -221 have been reported to be antiautophagic, thus promoting cardiac hypertrophy (11,12).

We conducted a detailed functional study of 380 miRNAs to gain further insight into miRNA-dependent regulation of cardiac autophagy. We identified miR-22 as a strong inhibitor of cardiac autophagy using in vitro and preclinical in vivo models of MI in young and older mice. Likewise, the prognostic importance of circulating miR-22 was tested in a clinical cohort of 198 patients with systolic heart failure.

METHODS

FLUORESCENCE-ACTIVATED CELL SORTING–BASED AUTOPHAGY MEASUREMENTS AND LIBRARY SCREENING. To measure autophagic flux by a fluorescence-activated cell sorting (FACS)-based method, HL-1 cells were seeded in 48 well cell culture plates. The next day, cells were transfected with precursor miRNAs together with Lipofectamine 2000 (Thermo Fisher Scientific, Inc., Waltham, Massachusetts) and transduced together with a green fluorescent protein (GFP)-LC3 adenovirus at a multiplicity of infection of 10; 48 h later, cells were trypsinized and fixed, and GFP intensity was measured with a Guava Easycyte Flow Cytometer (EMD Millipore Corporation, Darmstadt, Germany). Data were analyzed with the FlowJo software (FlowJo LLC, Ashland, Oregon), and mean fluorescence intensity (MFI) was calculated and normalized to the control subjects. miRNA library screening was performed with transfection of an miRNA precursor library (4391437 AMO01T9H Mouse - Pre-miR, Ambion, Thermo Fisher Scientific). Normal medium was changed to starvation medium 8 h prior to the endpoint, and thus flux was measured in response to starvation. Screening was performed in single wells, whereas validation of the screen was done in triplicates.

A detailed Methods section can be found in the Online Appendix.

RESULTS

A FACS-based high-throughput assay measuring GFP-LC3 was used to determine the autophagic flux in the HL-1 cardiac cell line (13). A lower GFP intensity after starvation indicates activation of autophagy, and an accumulation of GFP with thapsigargin, chloroquine, and bafilomycin A1 treatment indicates autophagy inhibition (**Figures 1A and 1B**, Online Figures 1A to 1D). We performed a functional screen of a miRNA precursor library (380 miRNAs) by using this FACS-based strategy to identify miRNAs that serve as autophagic regulators. We identified several miRNAs that were functioning as inhibitors or