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The Long Noncoding RNA CAREL Controls Cardiac Regeneration

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ABSTRACT

BACKGROUND Adult mammalian heart loses regeneration ability following ischemic injury due to the loss of cardiomyocyte mitosis. However, the molecular mechanisms underlying the post-mitotic nature of cardiomyocytes remain largely unknown.

OBJECTIVES The purpose of this study was to define the essential role of long noncoding ribonucleic acids (lncRNAs) in heart regeneration during postnatal and adult injury.

METHODS Myh6-driving cardiomyocyte-specific lncRNA-CAREL transgenic mice and adenovirus-mediated in vivo silencing of endogenous CAREL were used in this study. The effect of CAREL on cardiomyocyte replication and heart regeneration after apical resection or myocardial infarction was assessed by detecting mitosis and cytokinesis.

RESULTS An lncRNA CAREL was found significantly up-regulated in cardiomyocytes from neonatal mice (P7) in parallel with loss of regenerative capacity. Cardiac-specific overexpression of CAREL in mice reduced cardiomyocyte division and proliferation and blunted neonatal heart regeneration after injury. Conversely, silencing of CAREL in vivo markedly promoted cardiac regeneration and improved heart functions after myocardial infarction in neonatal and adult mice. CAREL acted as a competing endogenous ribonucleic acid for miR-296 to derepress the expression of Trp53inp1 and Itm2a, the target genes of miR-296. Consistently, overexpression of miR-296 significantly increased cardiomyocyte replication and cardiac regeneration after injury. Decline of cardiac regenerative ability in CAREL transgenic mice was also rescued by miR-296. A short fragment containing the conserved sequence of CAREL reduced the proliferation of human induced pluripotent stem cell-derived cardiomyocytes as the full-length CAREL.

CONCLUSIONS LncRNA CAREL regulates cardiomyocyte proliferation and heart regeneration in postnatal and adult heart after injury by acting as a competing endogenous ribonucleic acid on miR-296 that targets Trp53inp1 and Itm2a. (J Am Coll Cardiol 2018;■:■-■) © 2018 by the American College of Cardiology Foundation.

Myocardial infarction (MI) remains the leading cause of human death worldwide, with compromised cardiac function and heart failure partly due to cardiomyocyte loss (1). Although stem cell transplantation has been proposed as a therapeutic approach to replace apoptotic cardiomyocyte after injury, efficient retention and differentiation of engrafted stem cells in the host

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ABBREVIATIONS AND ACRONYMS

CAREL = cardiac regeneration-related long noncoding ribonucleic acid

ceRNA = competing endogenous ribonucleic acid

EF = ejection fraction

FS = fractional shortening

lncRNA = long noncoding ribonucleic acid

iPS = induced pluripotent stem cells

MI = myocardial infarction

pH3 = phosphohistone H3

myocardium remain a major challenge (2,3). It has long been believed that cardiomyocytes are terminally differentiated cells that have minimal capability and capacity to proliferate or regenerate. Recently, however, several seminal studies have overturned this conception and provided convincing evidence for the presence of a proliferative potential of cardiomyocytes from fetal or neonatal hearts that is expected to be sufficient for efficient repair of injured myocardium (4-6). However, this regenerative ability for the heart is gradually diminished with time after birth to adulthood, corresponding to the stage of decline of cardiomyocyte proliferation during postnatal development (7,8). Although evidence has been presented for the preservation of a rather low level of proliferation activity in adult cardiomyocytes after injury, the capacity is insufficient to replenish lost cardiomyocytes and to recover the proper heart function (9,10). Thus, the reactivation of cardiomyocyte proliferation has emerged as a promising strategy to regenerate new myocardium and to prevent heart failure following cardiac damages (11). However, despite the growing attention paid to cardiomyocyte proliferation in cardiac repair, the molecular mechanisms that govern regenerative capacity during the neonatal period are incompletely understood and remain a central subject in the field of cardiac biology research.

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The long noncoding ribonucleic acids (lncRNAs) are a set of ribonucleic acids (RNAs) longer than 200 nucleotides in length, which regulate the expression of genes and/or function of gene products/proteins by diverse molecular mechanisms at the transcriptional or post-transcriptional level (12). The lncRNAs participate in a variety of biological processes, such as cellular proliferation, apoptosis, genomic imprinting, cell fate determination, RNA alternative splice, and chromatin modification (12,13). Furthermore, increasing evidence has shown that lncRNAs are importantly implicated in pathological conditions, such as cancer, cardiac diseases, and Alzheimer's disease, and may be novel molecular targets for the treatment of these disorders (13,14). Recently, Braveheart has been suggested to play an important role in cardiomyocyte differentiation of pluripotent stem cells (15). Fendrr was shown to modulate cardiomyogenesis and maintain the expression of muscle genes during development (16). Nevertheless, the biological roles of lncRNAs in cardiomyocyte proliferation and cardiac regeneration are yet to be determined.

To shed light on this issue, we conducted the present study to investigate if lncRNA deregulation participates in the regulation of mammalian cardiac regeneration through transient activation of cardiomyocyte proliferation.

METHODS

Expanded information about materials and methods are available in the Online Appendix.

ADENOVIRAL CONSTRUCTION AND INFECTION. The adenoviruses harboring lncRNA CAREL and adenoviral CAREL short hairpin ribonucleic acid (shRNA) were designed and produced by GenePharma (Shanghai, China). The target sequence of adenoviral CAREL shRNA is 5′-GCTCTTAGGATTCAGCTCT-3′. An unrelated shRNA without any match with the mouse genomic sequence was used as a control (5′-TTCTCCGAACGTGTCACGT-3′).

TRANSFECTION OF MICRORIBONUCLEIC ACID INHIBITOR AND MIMICS. The chemically modified microribonucleic acids (miRNAs) were obtained from GenePharma. Cells were transfected with miRNA mimics and inhibitor using Lipofectamine 2000 (Invitrogen, Carlsbad, California) according to the manufacturer's instruction. The miRNA mimics and inhibitor sequences are shown in Online Table 1.

ANIMAL EXPERIMENTS. All experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee of Harbin Medical University. C57BL/6 mice were purchased from the Experimental Animal Center of the Affiliated Second Hospital of Harbin Medical University (Harbin, China). Myh6-CAREL transgenic mice were purchased from Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China). Neonatal mice were subjected to apical resection surgeries as previously described (6). The MI surgeries performed on P7 and adult mice were performed as described previously (11). Cardiac function was evaluated by histological, morphological, and echocardiographic analysis.

IMMUNOCYTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY. Hearts and cells were harvested and incubated with antibodies against phosphohistone H3 (pH3), Aurora B, and α-actinin, as described previously (10).

HISTOLOGY. Harvested hearts were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Global heart architecture was determined