Molecular Therapy
Nucleic Acids
Commentary

Non-coding sabotage: How Gadlor lncRNAs hijack heart function

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Non-coding RNAs (ncRNAs) are now widely accepted as orchestrators of cellular signaling and function in homeostasis and disease. Given the vast number of ncRNAs and their often cell- or disease-specific expression, they have great potential as targets for novel therapeutic interventions. In cardiovascular disease, several successful pre-clinical studies have been conducted, and the first clinical studies are currently underway.[1] One subclass of ncRNAs is long ncRNAs (lncRNAs), defined by a length at least 200 nt, which have been demonstrated to orchestrate gene expression at all levels.[2] In this regard, several lncRNAs were reported to influence cardiac function and remodeling in pathological and physiological contexts.[3] Despite the rapid advancement of research elucidating the function of several lncRNAs in the heart, the biological relevance and mode of action of the majority of lncRNA transcripts remain unknown.

In addition to intra-cellular communication, the transmission of a stress stimulus or other biological information from one cell(-type) to another is vital for the heart.[4] Among other pathways, intercellular communication is mediated by extracellular vesicles, which carry signaling molecules including lncRNAs.[5] Indeed, several ncRNAs were described to be transported between cardiac cells, resulting in complex disease phenotypes.[5]

In their current research, Keles and colleagues investigated the role of the endothelial-derived and secreted GATA-dependent lncRNAs, Gadlor1 and Gadlor2, previously identified by the same research group, as potential intercellular modulators of cardiomyocyte stress responses.[6] The intergenic lncRNAs derive from the *Lsamp* (limbic system-associated membrane protein) locus, which exhibits minimal expression in the myocardium. However, Gadlor1/2 were strongly upregulated during pressure-overload-induced heart failure by transverse aortic constriction (TAC) in cardiac tissue. Utilizing a range of assays, the authors could unequivocally demonstrate that the lncRNAs were secreted from endothelial cells within extracellular vesicles, which were taken up by the surrounding tissue, including cardiomyocytes and fibroblasts. A global Gadlor1/2 knockout protected mice from pressure-overload-induced remodeling characterized by decreased hypertrophy and fibrosis, while increased angiogenesis was observed in the knockout animals. It is noteworthy that in the long term (8 weeks after TAC surgery), the knockout mice exhibited a higher mortality rate, i.e., sudden death, which the authors hypothesize may be attributable to cardiac arrhythmia. To prove that the beneficial effects in the Gadlor1/2 knockout mice are based on the two lncRNAs, Gadlor1/2 were overexpressed in mice with Gadlor-loaded extracellular vesicles. To this end, generation of those vesicles was achieved in the dish in murine endothelial cells by the adenoviral overexpression of Gadlor1/2. Intramyocardial injection of Gadlor1/2-loaded extracellular vesicles prior to TAC surgery resulted in exacerbated cardiac remodeling with increased hypertrophy and fibrosis.

At the transcriptome level, endothelial cells derived from knockout mice exhibited enhanced angiogenesis and proliferation, while the expression of inflammatory genes was decreased. This was validated by the authors by immunofluorescence staining of $Ki67^+$ endothelial cells and *in vitro* with a sprouting assay after Gadlor1/2 overexpression. Additionally, isolated fibroblasts from the knockout mouse hearts after TAC surgery exhibited a less fibrotic phenotype, with an increase in angiogenesis-related pathways. Conversely, the overexpression of Gadlor1/2 in fibroblasts *in vitro* resulted in the activation of pro-fibrotic gene sets. Further mono- and co-culture experiments on cardiomyocytes, fibroblasts, and endothelial cells revealed that Gadlor-loaded extracellular vesicles were predominantly taken up by cardiomyocytes.

To understand the mode of action of Gadlor1 and Gadlor2 in cardiomyocytes, they were overexpressed and captured with streptavidin beads, and bound proteins were identified via mass spectrometry. Notably, one of the binding partners was calcium/calmodulin-dependent kinase type II (CaMKII). And indeed, calcium transients and the contractility were influenced in Gadlor-deficient cardiomyocytes with prolonged calcium transients and increased relaxation, while treatment with Gadlor-loaded extracellular vesicles reversed the phenotype in cardiomyocytes.

As Gadlor1/2 were not only present in the cytosol of cardiomyocytes but also shuttled to the nucleus, Gadlor1/2 might have several functions in cardiomyocytes and impacts on the transcription. Therefore, transcriptomic changes were analyzed in cardiomyocytes isolated from knockout animals after TAC surgery and control mice. Pathways associated with vasculature development, extracellular matrix organization, and cytokine production were upregulated,

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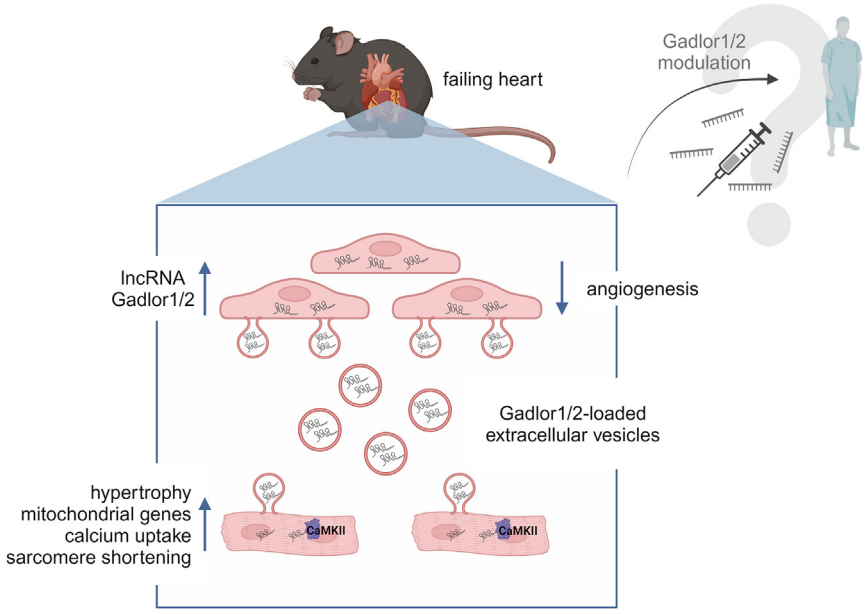

Figure 1. Endothelial cell-derived Gadlor1/2-loaded extracellular vesicles induce cardiac remodeling
Under conditions of pressure-overload-induced heart failure, Gadlor1/2 are upregulated in endothelial cells, resulting in a reduced angiogenesis. These lncRNAs are then secreted into extracellular vesicles, which are subsequently taken up by cardiomyocytes. In cardiomyocytes, Gadlor1/2 exacerbate hypertrophy and upregulate mitochondrial gene expression. By binding to calcium/calmodulin-dependent kinase type II (CaMKII), calcium uptake to the sarcoplasmic reticulum and sarcomere shortening are modulated. The discovery of Gadlor1/2's role in cardiovascular disease has significant implications for the development of novel therapeutic strategies, offering a new avenue for the treatment of heart failure and related conditions.

while mitochondria- and contractility-related genes were reduced. The overexpression of Gadlor1/2 resulted in a hypertrophic phenotype in cardiomyocytes, which was mitigated by the inhibition of CaMKII.

The study findings collectively suggest that the lncRNAs Gadlor1 and Gadlor2 exert a role in the development and progression of cardiac remodeling across different cardiac cell types. They are secreted from endothelial cells in extracellular vesicles and predominantly taken up by cardiomyocytes. Loss of Gadlor1/2 was observed to mitigate hypertrophy and fibrosis in mice, while it may potentially induce arrhythmogenic events via the modulation of CaMKII (Figure 1).

Despite the challenges associated with lncRNA conservation, Keles and colleagues have successfully identified and characterized the conserved human lncRNAs Gadlor1 and Gadlor2, indicating their likely essential function. The authors demonstrated that the human Gadlor1/2 transcripts are similarly regulated in failing human hearts and that the abundance of Gadlor2 is increased in the serum of patients with hypertrophic disease. These findings support the translatability of the results from the pre-clinical mouse models to human cells or culture systems and, subsequently, to clinical applications.

In summary, the laboratory of Prof. Heineke conducted a comprehensive investigation into the communication between cardiac cells via lncRNA-loaded extracellular vesicles, which significantly adds to our understanding of intercellular communication in failing hearts. In a pre-clinical mouse model of cardiac remodeling, the mode of action of Gadlor1/2 was elucidated through the utilization of cutting-edge techniques, both *in vivo* and *in vitro*, encompassing the analysis of primary cells and cell lines. At the same time, this investigation also prompted the emergence of new inquiries, including the mechanisms underlying the activation of Gadlor1/2 in response to stress cues and the nature of their packaging, which may be either passive or active recruitment into extracellular vesicles. The recent development of more complex, multicellular, and three-dimensional human *in vitro* and *ex vivo* platforms will facilitate the further translation of the promising results presented here.[7] It is anticipated that these platforms will help to address the therapeutic potential and potential arrhythmic side effects in the human context in the forthcoming years.

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The figure accompanying this commentary was created in BioRender. BioRender.com/x21o292.

DECLARATION OF INTERESTS

C.B. filed patents claiming the therapeutic use of ncRNAs in cardiovascular disease.

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