Long Noncoding RNAs in Pathological Cardiac Remodeling

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The long noncoding RNA *Chaer* defines an epigenetic checkpoint in cardiac hypertrophy
Wang et al
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A novel long noncoding RNA *Chaer* acts as noncoding epigenetic regulator at the onset of cardiac hypertrophy and enables an improved understanding about the complex mechanisms in cardiovascular disease.

DNA makes RNA, RNA makes protein, and protein makes us. This central dogma of life provides a simple description how an organism develops: DNA serves as a blueprint of the genetic information, and proteins are its functional and structural manifestation, whereas RNA mediates this process as a plotted construction plan. However, the relation between the genetic code and its biological implementation is much more complicated than originally supposed. Exemplarily, regulation on the epigenetic levels and functional transcripts derived from the non–protein-coding dark matter of the genome step out of the classical flow of genetic information. This information, by large, changed our view of traditional biology and opened a new avenue of research and development of next-generation mechanism-orientated therapeutic concepts.

The function of the heart is profoundly influenced by the organization of the chromatin, an epigenetic landscape that modulates the activity of the cardiac transcription network. Reorganization of epigenetic marks involving aberrant gene expression is crucial for the dysfunction of the myocardium and leads to cardiac hypertrophy or progresses to heart failure.[1] This process encompasses a complex interplay between various regulators that arise from the transcriptome and retain as functional transcripts, namely, noncoding RNAs including the subclass of long noncoding RNAs (lncRNAs).[2,3] Despite a broad interest in lncRNAs, only a handful of transcripts have been well studied including regulators of cardiomyocyte differentiation like *Braveheart*[4] and *Fendrr*[5] or noncoding actors in cardiac remodeling like *Myheart*[6] or *Chast*.[7] We still know little about how lncRNAs act at the molecular level and their exact role in cardiac development and disease.

In a recent article, Wang *et al*[8] identified a novel, cardiac-enriched, and partially functionally conserved lncRNA that is involved in the pathophysiological reprogramming of the heart. This transcript, named cardiac hypertrophy–associated epigenetic regulator (*Chaer*), acts as an epigenetic regulator promoting the development of cardiac hypertrophy. Accordingly, genomic deletion of this transcript attenuated pathological cardiac remodeling, whereas its overexpression induced hypertrophic gene expression. *Chaer* activity is mediated at the onset of cardiac hypertrophy through a stress-induced transient interaction between the lncRNA and the polycomb repressor complex 2 (PRC2), which depends on mTOR (mechanistic target of rapamycin) signaling. *Chaer* sequesters this epigenetic modulator from genomic loci, facilitating prohypertrophic genes to escape PRC2-dependent histone H3 lysine 27 trimethylation and likewise suppression. Pharmacological inhibition of *Chaer* before the establishment of cardiac hypertrophy reduced pathological remodeling and cardiac dysfunction.

This novel lncRNA study provides a comprehensive and novel understanding of the cardiovascular pathophysiology unwinding its complexity by linking the noncoding to the epigenetic landscape. In addition, *Chaer*'s functionality indicates that not only major changes in expression levels of a lncRNA affects disease development and progression in the heart but also the spatial and temporal distribution of a lncRNA's activity alters the outcome of cardiac remodeling. Importantly, future therapies will use better spatiotemporal control for optimized outcomes.

Chaer—A Noncoding and Epigenetic Functionality With Pathophysiological Relevance

Wang et al provide detailed insight into *Chaer*'s mode of action. Many studies in various research fields have uncovered the function of lncRNAs, but only a few have investigated the (patho)physiological relevance of a noncoding transcript trough genetic ablation. To unravel the role of *Chaer* in cardiac disease development, Wang et al depleted *Chaer* from the heart by CRISPR-Cas9–mediated knockout and by application of pharmacological inhibitors and characterized the outcome on morphometric and functional cardiac parameters. In accordance with other druggable transcripts such as *Malat1*[9] and *Chast*,[7] the approach of Wang et al provides a new access to dissect a lncRNA's role in the pathophysiology of cardiac hypertrophy and in other cardiovascular conditions, presumably enabling the translation of lncRNA research into future therapeutic strategies. This is based on in-depth knowledge on mechanisms behind the

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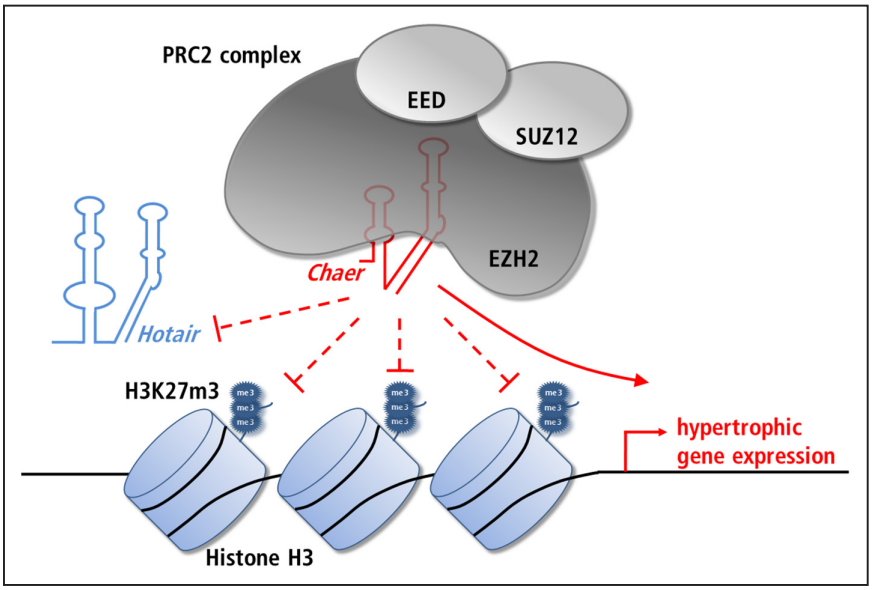

Figure. *Chaer* acts as an early epigenetic checkpoint in cardiac hypertrophic reprogramming. At the onset of cardiac hypertrophy, stress signaling induces a transient interaction between *Chaer* and EZH2 (Enhancer of zeste homolog 2) presumably sequestering this catalytic subunit of PRC2 (Polycomb Repressive Complex 2) from its target sites and from other competing lncRNAs like *Hotair*. The interaction is mediated by a bitetra-loop motif within the 5' end of *Chaer*, hampers repressive PRC2-depedent H3 lysine 27 trimethylation and finally leads to an activation of the hypertrophic gene program.

function of a noncoding transcript. Upstream regulatory mechanisms that direct the temporally restricted activity of *Chaer* and the fact that this prohypertrophic transcript is mainly suppressed over the time course of cardiac hypertrophy remains unexplained. Nevertheless, the strength of the *Chaer* study relies an in-depth analysis of the downstream mechanism. Wang et al identified a new regulatory pathway linking a noncoding factor to epigenetic events in cardiovascular disease and directing the attention to a core aspect of the noncoding functionality, the RNA structure. Interestingly, *Chaer* acts via a conserved structural motif enabling the recognition of the epigenetic modulator EZH2 by simultaneous displacement of competing transcripts finally provoking pathological epigenetic patterns in the heart (Figure). Thus, RNA structure appears as a crucial feature of individual lncRNA's function that unfortunately has been rarely addressed to date. LncRNAs exhibit a broad variety regarding their length, internal organization, and biological activity,[3] suggesting that a general modus operandi for this class of transcripts is unlikely. However, common activities or features of noncoding transcripts seem to be dependent on their overall or local architecture, probably according to the principle form follows function. This is supported by the fact that the secondary structure of a lncRNA is usually stronger conserved than the primary sequence[3] and is critical for cardiomyocyte differentiation, although the lncRNA itself is a low-abundance transcript.[4,10] Thus, it would be desirable that future efforts are directed to the recognition and evaluation of functional domains and structural characteristics that may be shared among noncoding transcripts. Accordingly, detailed knowledge about the lncRNAs architecture would facilitate a specific and efficient targeting of lncRNAs in cardiovascular disease.

Chaer—A Promising Therapeutic Target?

From a translational perspective, *Chaer* appears as an interesting therapeutic target because it meets important criteria for further clinical development; for example, using inhibitors suppressing the activity of this lncRNA: *Chaer* is a regulator of cardiovascular disease, functionally conserved, and its expression is mainly restricted to the heart, minimizing the risk of off-target effects in other organs. However, potential clinical applications of *Chaer* inhibitors remain challenging. Silencing of this transcript has been achieved by chemically modified siRNAs, and beneficial therapeutic are based on the delivery before, but not after, the onset of cardiac hypertrophy. In general, therapeutic application of synthetic siRNAs and their chemical derivatives are limited by their low efficacy, potency, and stability.[11] The siRNA directed against *Chaer* efficiently silenced this transcript in the heart. Unfortunately, this effect seems to last for a short period, only. For nuclear-restricted lncRNAs like *Chaer* chemistries that trigger RNase H-dependent degradation in the nucleus, thus directly at the locus where the mature transcript is produced or active, have been shown to be more efficient[12] and might be the better choice for therapeutic applications. For further development of *Chaer* inhibitors, potential side effects or toxicological parameters should be addressed. Wang et al, namely, underline the nature of *Chaer* activity as an early checkpoint in epigenetic reprogramming of hypertrophic gene expression. However, patients are (as yet) rarely presented to the clinician before the onset of cardiovascular disease and heart failure. According to the transient and early activity of *Chaer*, its pharmacological inhibition might be of interest for the treatment of acute cardiovascular events rather than for the therapy of the chronic failing heart. After myocardial infarction, cardiac remodeling is a consequence of cardiac repair mechanisms but occurs in a later phase. This might open a window for *Chaer* inhibitor delivery before the onset of cardiac hypertrophy, presumably enabling therapeutic and beneficial effects that have been observed for the preventative strategy of the present study.

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Disclosures

J. Viereck and T. Thum have filed patents for the use of ncRNAs in cardiovascular disease. T. Thum is founder of a ncRNA-based company (Cardior Pharmaceuticals).

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