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Review

Exploiting the hypoxia sensitive non-coding genome for organ-specific physiologic reprogramming☆

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ABSTRACT

In this review we highlight the role of non-coding RNAs in the development and progression of cardiac pathology and explore the possibility of disease-associated RNAs serving as targets for cardiac-directed therapeutics. Contextually, we focus on the role of stress-induced hypoxia as a driver of disease development and progression through activation of hypoxia inducible factor 1α (HIF1α) and explore mechanisms underlying HIFα function as an enforcer of cardiac pathology through direct transcriptional coupling with the non-coding transcriptome. In the interest of clarity, we will confine our analysis to cardiac pathology and focus on three defining features of the diseased state, namely metabolic, growth and functional reprogramming. It is the aim of this review to explore possible mechanisms through which HIF1α regulation of the non-coding transcriptome connects to spatiotemporal control of gene expression to drive establishment of the diseased state, and to propose strategies for the exploitation of these unique RNAs as targets for clinical therapy. This article is part of a Special Issue entitled: Cardiomyocyte Biology: Integration of Developmental and Environmental Cues in the Heart edited by Marcus Schaub and Hughes Abriel.

1. The evolution of oxygen signaling

The rise of photosynthesis and the ensuing oxygenation of the planet's atmosphere represent a pivotal event in the evolution of our ecosystem. While many organisms retreated to anoxic environments upon introduction of oxygen into the atmosphere, others adapted and evolved complex biochemical and metabolic networks to utilize oxygen as a high-potential redox coupler [1]. This led to the establishment of a highly efficient and robust system of oxidative phosphorylation to transfer energy stored in glucose and fatty acids to the high-energy phosphate bond of ATP and reducing equivalents such as NADH, NADPH and $FADPH_2$ [2]. While early metazoan species were sufficiently small that oxygen could diffuse from the atmosphere to all of the organism's cells, oxygenation by diffusion alone imposed an inherent limitation to organismal growth and complexity. To overcome this constraint, the nervous, circulatory and respiratory systems, were formed to efficiently sense, capture and distribute oxygen to cells deep within the body of larger organisms [1,3,4].

At the cellular level a central mediator of oxygen sensitization and adaptation is hypoxia inducible factor 1α (HIF1α) [5], a heterodimeric transcription factor composed of HIF1α and HIFβ/ARNT subunits. The HIF1α subunits are under exquisite oxygen control in that they accumulate and translocate to the nucleus rapidly under conditions of reduced oxygen (hypoxia) to direct the transcription of genes regulating aspects of oxygen homeostasis including genes involved in oxygen uptake [6], erythrocyte maturation [7,8], angiogenesis [9] and mitochondrial oxygen utilization [10]. In conditions of oxygen sufficiency (normoxia), HIFα is bound by the von Hippel–Lindau (VHL) protein, which recruits the ubiquitin ligase system that targets HIFα for proteasomal degradation. VHL binding is dependent on hydroxylation of proline residues in HIF1α by the α-ketoglutarate-dependent dioxygenases prolyl hydroxylases (PHD) and the asparaginyl hydroxylase, factor-inhibiting HIF (FIH) [11,12]. Complete deficiency of Hif1α in mice results in embryonic lethality at day 10.0 post-coitus (pc), characterized by repressed cell, tissue and organismal growth, leading to a broad spectrum of abnormalities encompassing retarded embryonic development, neural tube defects, dysfunctional vasculogenesis and angiogenesis, a reduction in somite number and cardiovascular malformations [13]. When Hif1α inactivation is confined to the embryonic cardiac ventricle, development of other tissues occurs normally but cardiac development is aborted at looping morphogenesis coinciding with reduced expression of key cardiogenic transcription factors including Mef2c, Tbx5 and titin, a giant protein that serves as template for the assembly and

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organization of the cardiac sarcomere [14]. In the adult mouse, conditional deletion of Hif1α in various tissues including skeletal muscle [15], cartilage [16], heart [17–19], liver [20] and myeloid [21] cells through Cre-lox methodologies reveals a consensus function in regulating cellular metabolism and cell proliferation or growth [16,19,21].

As a result of gene mutation, stenosis, hypertension, smoking or obesity, increased workload is imposed on the heart, precipitating the formation of hypoxic foci in cardiac tissue as a result of a mismatch between myocardial oxygen demand and supply during stress-induced hypertrophy [22–25]. The resulting accumulation and activation of Hif1α facilitates adaptation to a new oxygen equilibrium through remodeling of the cellular RNA landscape, resulting in preferential enrichment of disease-associated coding and non-coding genes leading to increased cardiac growth, the reprogramming of cardiac metabolism from predominantly glucose and fatty acid oxidation to glycolysis, and contractile dysfunction. Consequently, healthy adult variants of contractile proteins, ion channels and metabolic components are gradually replaced by pathologic variants. However, we and other have observed a markedly attenuated hypertrophic response to increased workload demand in mice deficient for ventricular Hif1α, concomitant with the maintenance of oxidative metabolism and normal contractile function [18,19,26,27].

2. The oxygen-regulated non-coding transcriptome

Non-coding RNAs have for a long time been ignored in favor of coding genes and proteins as principal regulators of cell physiology and function. However, the recent finding that coding genes account only for about 1.5% of RNA transcripts produced by the human and mouse genome, is suggestive of a possible relevance for non-coding RNAs in the maintenance and control of cellular homeostasis [28,29]. Unlike most protein coding genes, non-coding RNA function remains for the most part elusive. However, our unpublished data and evidence from other groups points towards a greater degree of tissue-specific and spatio-temporally restricted pattern of non-coding RNA expression, in comparison to the largely ubiquitous pattern of coding gene expression [28,29]. This defining characteristic of non-coding RNAs has made them appealing targets for tissue- and context-dependent therapeutics [30]. We outline below key non-coding RNA mediators of cardiac pathology, highlight a subset of direct transcriptional targets of the hypoxia-induced Hif1α pathway, and explore how anti-sense RNA technology can and has been utilized to regulate these RNAs particularly in the context of pathologic hypertrophy and heart failure (Fig. 1).

3. miRNAs implicated in heart disease

Specific miRNAs are dysregulated in cardiomyopathy, and gain- and loss-of-function studies in mice have revealed a critical sufficiency and requirement for miRNA function in the development of heart disease. In Table 1 we list miRNAs implicated in the development and progression of heart disease and highlight a subset of these miRNAs below.

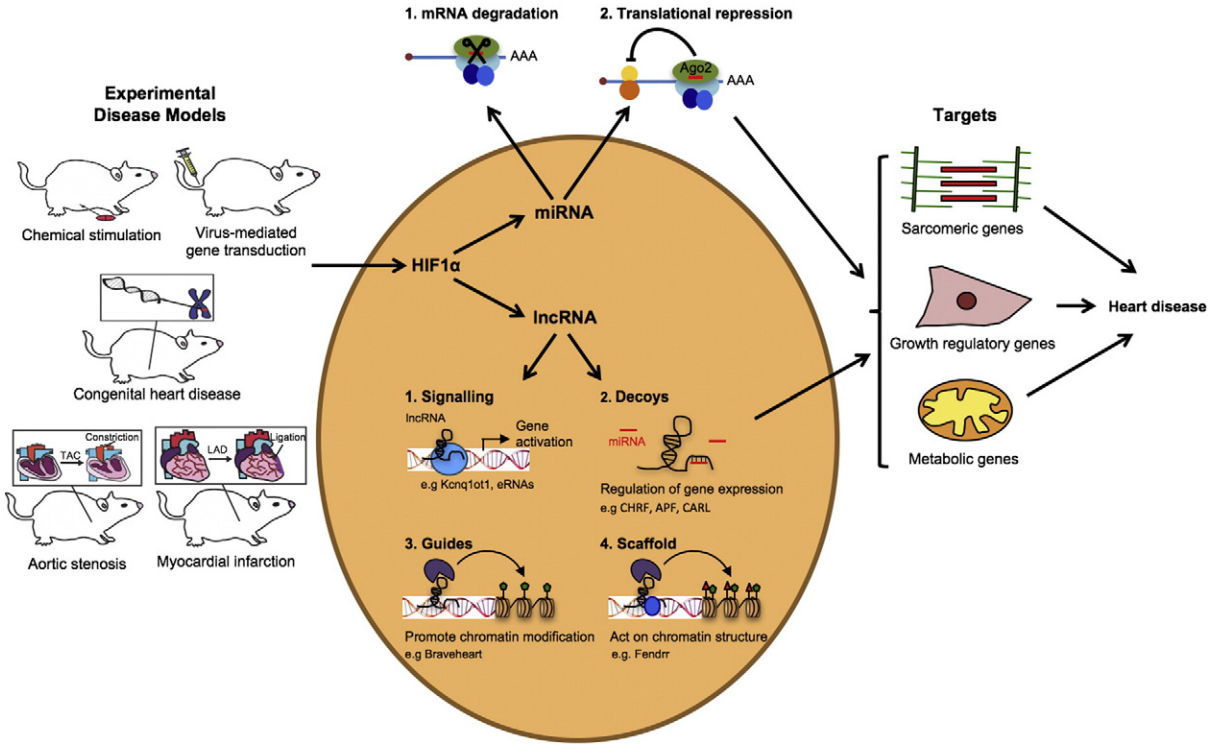

Fig. 1. Schematic of miRNA and lncRNA regulation by Hif1α and their mechanisms. Pathologic cardiac growth in experimental disease models such as chemical stimulation with isoproterenol or phenylephrine, virus-mediated gene transduction, genetic and surgical mouse models of heart disease (aortic stenosis and myocardial infarction) leads to an accumulation of Hif1α and subsequent transcriptional activation of miRNAs and lncRNAs. miRNAs bind to complementary sites in the 3'UTR of target mRNAs and degrade the mRNA or inhibit translation. Four different mechanisms have been described for regulation of gene expression by lncRNAs. lncRNAs can function as 1) signals, combined action of lncRNAs and transcription factors or signaling pathways can regulate gene expression spatio-temporally. Kcnq1ot1 and eRNAs act as signals by inhibiting the expression of some neighboring genes in development [79] and inducing flanking gene expression [80], respectively. 2) Decoys, lncRNAs can bind miRNAs thereby titrating them away and regulating gene expression post-transcriptionally. Competing endogenous RNAs such as CHRF, APF and CARL function as decoys by sequestering miRNAs and limiting their interaction with target mRNAs [68,81,82]. 3) Guides, lncRNAs can direct chromatin-modifying enzymes to proximal or distant loci. LncRNA HOTAIR functions as a guide by regulating or altering the epigenetic state of a cell through the targeting of chromatin modifying complexes [83]. 4) Scaffolds, lncRNAs can bind multiple proteins to form ribonucleoprotein complexes and modify the histones. Fendrr functions as a scaffold by interacting with both the PRC2 and TrxG/MLL complexes thereby bringing the effectors together and directing the chromatin state [84]. The Hif1α-mediated expression of miRNA and lncRNAs can alter the expression levels of genes that are important to the transition to heart disease, such as genes involved in cell growth, metabolism or contractility which ultimately leads to heart disease.