Resolving a Catch-22 in Cardiac Gene Regulation

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“You have a big heart.” These five words have almost as many implications. Being “big hearted” can describe a generous and giving nature. In a different context, Secretariat’s heart was unusually large because he carried an X-linked gene \(^1\) conferring on him rare physiological attributes that in 1973 propelled him to thoroughbred horse racing’s Triple Crown. When the same declaration occurs between cardiologist and patient, however, the connotation is overwhelmingly negative. Heart enlargement from cardiac hypertrophy or dilated cardiomyopathy is a powerful positive predictor of morbidity and mortality\(^2,3\). Accordingly, efforts to more fully understand the molecular underpinnings of structural and functional cardiac remodeling have been a major focus of basic cardiovascular research since genetic reprogramming was first mechanistically linked to cardiac hypertrophy \(^4,5\). Over the past 25 years major transcriptional controls for pathological cardiac gene expression have been defined and suggested novel genetic approaches to control or reverse hypertrophy \(^6\).

Our conceptual understanding of the relationship between cardiac gene expression and physiotype derives from the common view that transcriptional regulation of genes determines expression of messenger RNAs (mRNA) that encode myocardial proteins. Recent evidence for epigenetic regulation by microRNAs has revised this linear paradigm. MicroRNAs adjust transcript abundance by binding to complementary sequences of target mRNAs, inducing their destruction, and/or blocking their translation (reviewed in \(^7\)). Clinical and experimental evidence implicates microRNAs as an important component of genetic reprogramming in cardiac hypertrophy and heart failure. Ongoing efforts targeting microRNAs show potential therapeutic efficacy \(^8\).

Based primarily on large-scale microarray analyses, it is estimated that the heart expresses 300-400 microRNAs (of \(~1,000\) known) at meaningful levels. Of these, levels of over
200 cardiac microRNAs are regulated in cardiac pressure overload, ischemia, or failure. The details of these regulatory events will undoubtedly evolve as deep resequencing replaces microarrays to quantify RNA in various disease states. However, the overarching question will remain: “What are the specific roles of genetic/transcriptional versus epigenetic/microRNA regulation in the heart and elsewhere?” The manuscript by Gurha, et al in this issue of Circulation describes consequences of germ-line miR-22 ablation that provide some answers. miR-22 is one of the most abundant cardiac-expressed microRNAs. High baseline abundance is a powerful reason to suspect that a microRNA is important in normal physiology. miR-22 is also reportedly regulated in a variety of cardiovascular diseases; microRNA regulation in disease suggests pathological effects. Thus, there are compelling reasons to interrogate the actions of miR-22 in normal and stressed hearts.

The initial impression from the current report is that miR-22 deletion had little impact on normal hearts. Baseline cardiac structure, function, and histology were all unaffected by miR-22 ablation. Dobutamine administration revealed depressed β-adrenergic inotropic and lusitropic responsiveness that prompted the authors to uncover relatively minor abnormalities in sarcoplasmic reticular (SR) calcium content and reuptake. Given the posited relationship between miR abundance and baseline function, the mildness of the baseline miR-22 null cardiac phenotype is unexpected. It is notable however that fully one half of miR-22 null mice died in utero (some with cardiac defects), revealing an as-yet poorly described embryonic phenotype consistent with an important housekeeping/developmental function for miR-22. The mice that underwent more detailed study represent a subset of miR-22 null mice that, by mechanisms it will be important to define, compensated for lack of miR-22.

An incompletely penetrant but severe cardiac developmental phenotype associated with
modest findings in surviving mice suggests that the principal factor (in this case miR-22) exerts one or more conditional effects linked to cardiac growth. Under these circumstances additional information can sometimes be obtained by provoking cardiac growth, i.e. hypertrophy. Molecular overlap between reactive pressure overload hypertrophy and fetal cardiac development is so strong that the characteristic transcriptional signature of cardiac hypertrophy has become known as the “fetal gene program”. This terminology and its underlying biology reflect shared mechanisms for cardiac growth during embryonic development and under hemodynamic stress. Accordingly, a genetic/epigenetic factor that perturbs fetal cardiac growth may also affect the normal response to a hypertrophic stimulus such as pressure overload.

Consistent with this line of reasoning, Gurha et al performed surgical transverse aortic banding (TAC) on miR-22 deficient mice. The results showed that hypertrophy itself (i.e. heart weight and cardiomyocyte area) was unaffected, but some pathological aspects of hypertrophy that contribute to cardiomyopathic remodeling were exaggerated. In particular, fibrotic replacement of cardiomyocytes was increased and associated with dystrophic calcification that suggested focal myocardial necrosis. Surprisingly, the standard metric of apoptotic cell death, the TUNEL assay, was not correspondingly increased. Thus, the mechanism(s) by which absence of miR-22 intensifies cardiomyocyte loss after TAC is unclear.

A suggested reason for some of the deleterious effects induced by miR-22 ablation is impairment of cardiomyocyte SR calcium storage, release and re-uptake by the SR calcium ATPase, SERCA2a. The relationship between miR-22 and cardiac SERCA2a appears complicated: SERCA2a mRNA, but not protein, levels were depressed in unstressed miR-22 null mice. Phosphorylation of the SERCA inhibitor phospholamban was not altered at baseline, despite apparent insensitivity of miR-22 null hearts to other cardiac adrenergic responses.
Furthermore, SERCA2a mRNA levels were suppressed both by miR-22 gene ablation and transgenic overexpression. Thus, a clear molecular mechanism for observed decreases in SR calcium loading and depressed SR calcium reuptake rate is not obvious. It seems unlikely that observed alterations in SERCA2 and phospholamban are responsible for increased prevalence of cardiomyocyte necrosis after pressure overloading, as this form of programmed cardiomyocyte death has been more commonly associated with cardiomyocyte calcium overload. 

Transcriptional profiling by mRNA expression arrays suggested that absence of miR-22 coordinately dysregulated sarcomeric protein genes. This finding is similar to knocking out miR-1 or miR-133. Like miR-22, these two microRNAs are highly expressed in myocardium, they regulate muscle gene expression, and their genetic ablation induced severe cardiac developmental phenotypes. Gurha et al used bioinformatic algorithms to select candidate miR-22 targets and implicate the widely expressed transcription factor purine-rich element binding protein B (PURB) as a miR-22 targeted regulator of muscle gene expression. There are two predicted miR-22 binding sites in murine PURB, both of which are conserved in human and other mammalian PURB 3′UTRs. Although cross-species conservation of nucleotide sequence for mature microRNAs is the rule, much more variability is typically seen in mRNA binding sites; conservation therefore suggests broad biological importance. The miRDB database lists 200 murine mRNAs as predicted targets of miR-22 (3p); PURB is 32nd from the top (ranked by best fit). Of 257 human mRNAs predicted to be targeted by miR-22, PURB is #115. Thus, bioinformatics and biology agree on a central role for microRNA targeting of this transcriptional regulator. Serum response factor (SRF), which like PURB is a regulator of muscle gene transcription and was indirectly implicated (via regulation of myocardin) in miR-22 effects, is regulated in parallel by another cardiac hypertrophy-associated microRNA, miR-133.
Interestingly, SRF can also regulate miR-1 and miR-133, revealing microRNA-mediated feed-forward and feedback orchestration of muscle transcription (Figure 1) 16.

The complex effects of miR-22 described by Gurha et al are characteristic of microRNAs. It is somewhat ironic (given its name) that miR-22 is the current example. Catch-22 is a term originated in Joseph Heller’s 1961 novel 17 describing "a problematic situation for which the only solution is denied by a circumstance inherent in the problem or by a rule," in other words a no-win situation. The Catch-22 encountered when using reductionist approaches to define microRNA effect is that the full spectrum of effects can only be understood in a completely integrated system: each microRNA has multiple mRNA targets, and each target mRNA can exert secondary and tertiary effects. The observed phenotypes represent the cumulative impact of all these events and the compensatory processes they, in turn, invoke. One microRNA is therefore like a domino within a matrix where any domino can knock down two others, downstream or upstream. The small initial effect of pushing over a domino at any point within the network is amplified and spreads throughout the system. The net effects of perturbing a given domino (or microRNA) are unforeseeable without a comprehensive understanding of the higher-order systemic interactions: On one hand, the ultimate role/function of a microRNA is difficult to infer based only on the identity of its direct mRNA targets. On the other hand, the identities of a microRNA’s direct targets may be unpredictable from the end-organ phenotype induced by its overexpression or ablation. The prototypical example in the heart is the small myomiR family of miR-208a, -208b, and -499. Each of the myomiRs is encoded within a different myosin heavy chain gene, and their major effect in the heart is to direct myosin heavy chain isoform expression 18. Yet, myosin heavy chain mRNAs that are so strikingly impacted by these microRNAs are not their direct targets. Instead, myomiRs modulate mRNAs of
transcription factors that, in turn, control Myh6 and Myh7 mRNA expression.

Targeting transcription factors, then, is a unifying mechanism for cardiac muscle gene regulation by miR-22, miR-1 and miR-133, and the family of myomiRs. The paradigm that emerges from studies of individual microRNAs is that each can modify cardiac development and muscle gene expression by adjusting the levels of a few critical transcription factors. In other words, a dominant mechanism by which microRNAs epigenetically regulate cardiac mRNA levels is by targeting the trans-activating factors that drive (or suppress) their transcription. It is worth noting that typical microRNA-mRNA interactions have modest effects (50% suppression is a large in vivo biological effect; 19, 20), and that microRNAs and the RNA-induced silencing complexes to which they recruit complementary mRNAs are limited in their capacity for mRNA degradation. Accordingly, the laws of mass-action dictate that a given % or fold-change of microRNA-mediated mRNA degradation should have greatest systemic impact on mRNA targets expressed at lower levels in the cardiac myocyte; mRNAs of transcription factors are typically expressed at only a few copies per cell 10. Thus, the most biologically efficient means for transcript suppression would be achieved when a highly abundant microRNA senses an alteration in physiological demand and responds by fine-tuning the levels of lower abundance mRNAs encoding transcription factor mRNAs whose downstream effects are then magnified through regulation of primary cardiac gene transcription. This concept resolves the apparent paradox posed when genetic manipulation of a microRNA having modest effects on direct mRNA targets nevertheless induces a striking phenotype 21.

Conflict of Interest Disclosures: None
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**Figure Legend:**

**Figure 1.** Mechanisms of microRNA-mediated heart gene reprogramming. Greatly simplified schematic depiction of highly abundant cardiac-expressed microRNAs targeting low-abundance transcription factor mRNAs to coordinately regulate muscle gene expression. Transcriptional regulators are in the box. Arrows indicate direct gain of function, crossed lines are direct loss of function, and ball-lines are indirect and/or complex effects.
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