TIMING AND TARGETING OF TREATMENT IN LEFT VENTRICULAR HYPERTROPHY

Deokhwa Nam, Ph.D.; Erin L. Reineke, Ph.D.
Houston Methodist Research Institute, Houston, Texas

Abstract

In most clinical cases, left ventricular hypertrophy (LVH) occurs over time from persistent cardiac stress. At the molecular level, this results in both transient and long-term changes to metabolic, sarcomeric, ion handling, and stress signaling pathways. Although this is initially an adaptive change, the mechanisms underlying LVH eventually lead to maladaptive changes including fibrosis, decreased cardiac function, and failure. Understanding the regulators of long-term changes, which are largely driven by transcriptional remodeling, is a crucial step in identifying novel therapeutic targets for preventing the downstream negative effects of LVH and treatments that could reverse or prevent it. The development of effective therapeutics, however, will require a critical understanding of what to target, how to modify important pathways, and how to identify the stage of pathology in which a specific treatment should be used.

Introduction

Pathological left ventricular hypertrophy (LVH) is a clinically noticeable indicator of cardiac stress downstream of a number of different conditions that result in decreased cardiac function or capacity. The heart is immensely flexible, able to work under different oxygen conditions, different physical loads, and in the presence of varying metabolic substrates. In many cases, however, comorbidities such as diabetes and hypertension create a persistent change in circulating nutrients, hormones, and cytokines. When these persistent changes accompany altered physical load on the heart, molecular and physical flexibility are hindered and the heart cannot maintain efficient pumping over time.

Cardiomyocytes are the "workhorse" cells of the heart that generate energy in the form of adenosine triphosphate (ATP) and carry out the physical contraction. In the case of LVH, persistent changes force the cardiomyocyte to alter its steady state molecular signature that produces the ATP required for pumping. These alterations can be roughly grouped into two main categories. The first adaptations are transient and cause changes in protein activity or expression, primarily from post-transcriptional regulation. These modifications occur rapidly and can be rapidly reversed as needed. The second set of adaptations are long-term and driven by gene remodeling; they occur when the transient changes are no longer sufficient for maintaining efficiency. Extensive gene remodeling, such as during cardiac stress, generally indicates a long-term adaptation that cannot easily be reversed.

Due to the complexity of LVH, an accurate model requires consideration of all cardiac cell types, each with their own transcription program and regulatory mechanisms. However, to explain the possible role of transcriptional targeting in clinical practice, this review will focus primarily on changes in the cardiomyocyte. The intention is not to provide an inclusive, detailed review of all transcriptional regulators in the cardiomyocyte but to use well-characterized transcriptional control mechanisms of the cardiomyocyte during stress and LVH as examples to highlight treatment strategies and current challenges.

Transcriptional Remodeling During Cardiac Stress

Ultimately, adaptation of the heart is required to maintain cardiomyocyte contraction and is therefore aimed at metabolic efficiency. After birth, the gene expression profile of the heart changes dramatically in terms of metabolic regulators and sarcomeric gene expression. These changes are thought to reflect the drastic shift in cellular environment that occurs at birth, which is driven by the change from low oxygen availability in utero to unrestricted oxygen after birth. This change in oxygen state allows the adult heart to rely heavily on oxidative metabolism of fatty acids for ATP generation.

In the case of pressure overload to the left ventricle, the first observed molecular changes in the heart are metabolic, particularly increased glucose uptake.[1] This increased glucose is initially sufficient to create the ATP required to meet the increased load; however, if the stress persists over time, the cardiomyocyte eventually switches to anaerobic glycolysis as the main metabolic pathway. This switch is accompanied by extensive gene remodeling to the "fetal gene profile," referring to those genes expressed during the embryonic state.[2,3] This gene remodeling involves not only changes in metabolic and sarcomeric genes but also activation of stress regulators such as c-myc and atrial natriuretic peptide. If we consider this massive transcriptional remodeling as an early "point of no return," we must then consider and identify (1) what cellular and extracellular context drives these changes, (2) what are the individual key regulators of the changes, (3) how can they be targeted, and (4) when should they be targeted?

To answer these questions, one must first appreciate the complexity of transcription (Figure 1). In the simplest view, transcription occurs when a transcription factor binds to the promoter of a single target gene and enhances production of a primary transcript of the target gene by recruiting the general transcription machinery (Figure 1 A). Many people are aware that this simplistic view is not sufficient and envision transcription of a single gene requiring communication between one or more transcription factors with a complex of proteins that have enzymatic activity; these enzymes help modify the chromatin around the target gene promoter before the general transcription machinery can bind and initiate mRNA

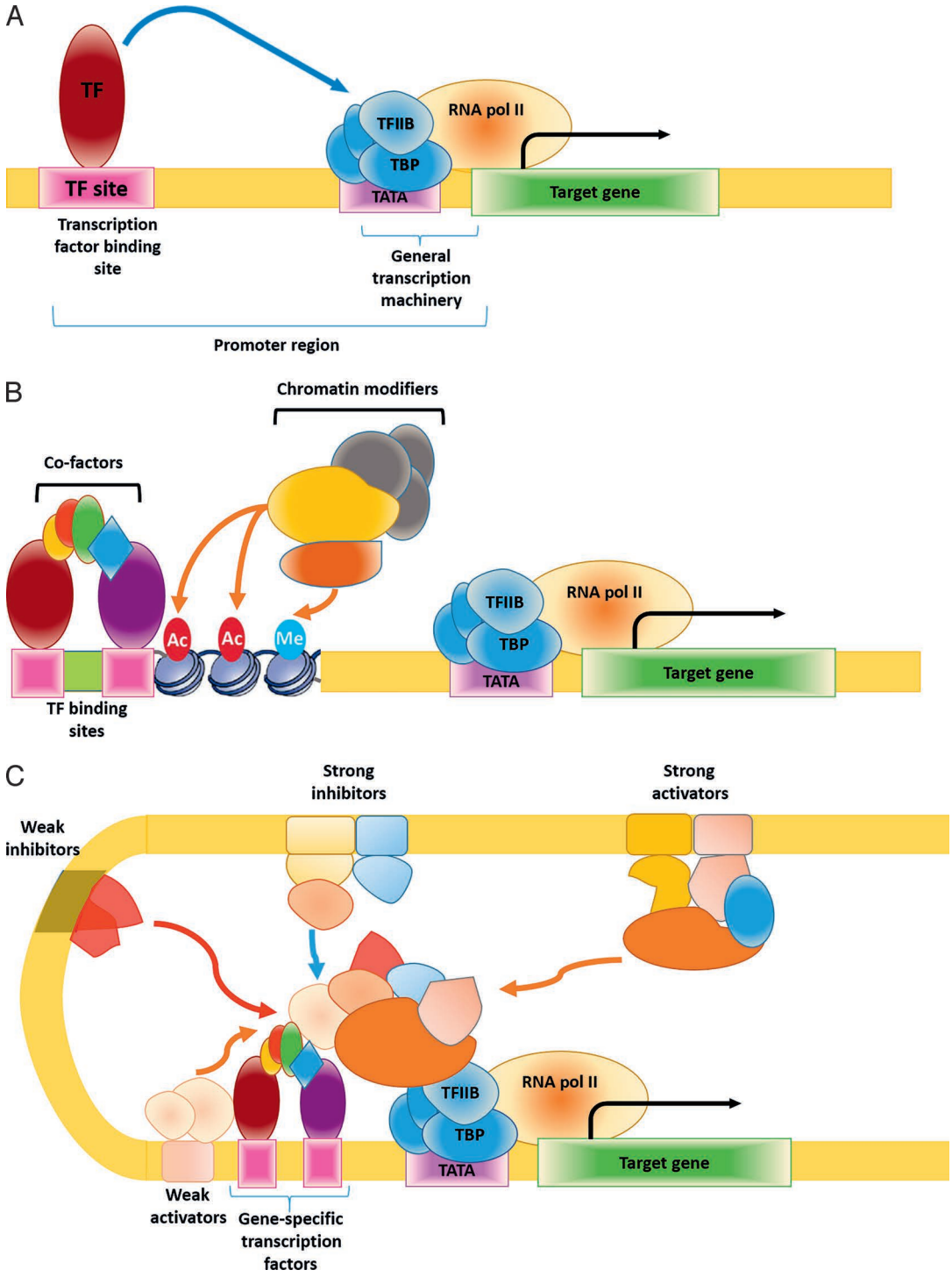

Figure 1. Transcription is a highly complex process subject to numerous modes of regulation. (A) A simplistic view of transcription of a single gene driven by a single transcription factor. (B) A generalized view of the main components involved with regulation of a single gene. The cofactor and chromatin modifiers headings include both positive and negative regulators. (C) A representation of the complexity that can be involved in regulation of a single gene including both cis and trans positive and negative factors. Ac: acetylation; Me: methylation; TF: transcription factor; TBP: TATA-binding protein; RNA pol II: RNA polymerase II; TFIIB: transcription factor IIB

synthesis (Figure 1 B). However, even this view does not account for the true complexity of transcriptional control. In reality, transcription of a single target involves a dynamic process of many proteins, including both the departure of the gene's negative regulators and entry of positive regulators that can bind both in close proximity to the transcription start site or several thousand kilobases away due to chromatin looping.[4] These primary DNA-bound factors are subject to regulation of their own activity and recruit several more proteins, which collectively leads to initiation of transcription (Figure 1 C). This multigene, multifactorial remodeling that occurs during cardiac stress is clearly an overwhelmingly complex process. Although this realistic view of transcription may

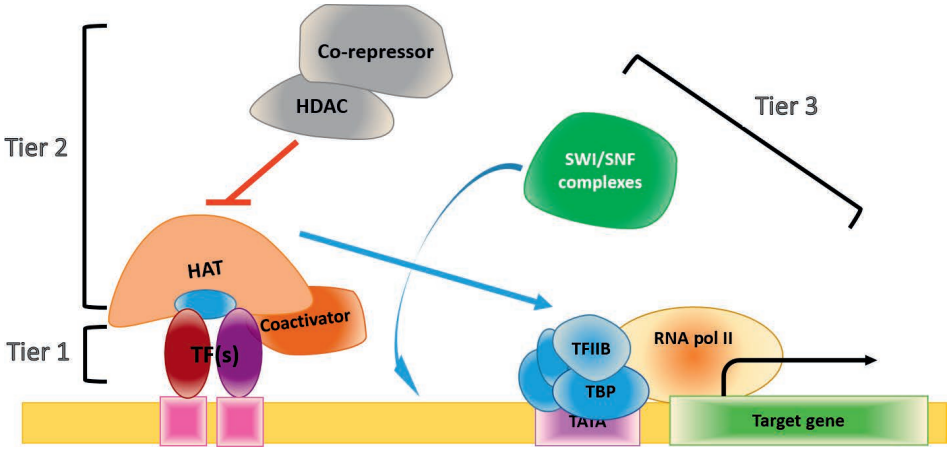

Figure 2. Transcription regulators vary in specificity for target gene regulation. In general, each gene has a promoter with specific binding sites for a few key transcription factors that result in the highest level of specificity of gene regulation (Tier 1). Bound to these transcription factors are protein complexes involving both transcription factor-specific and more pleiotropic coactivators and corepressors (Tier 2). The general transcription machinery and proteins required to open the chromatin and initiate transcription are the least specific class of transcription regulators (Tier 3). HDAC: histone deactylases; SWI/SNF: SWItch/Sucrose NonFermentable; HAT: histone acetyltransferase; TFIIB: transcription factor IIB; TBP: TATA-binding protein; RNA pol II: RNA polymerase II

make its manipulation appear nearly impossible or impractical, there are actually several tiers of transcriptional regulators that can be considered as therapeutic targets.

Regulators of Cardiomyocyte Transcription

To better understand these levels of transcription regulation, we will illustrate a very well-characterized metabolic switch in the stressed heart and its effects on hypertrophy. In the adult heart, peroxisome proliferator-activated receptor α (PPARα) promotes transcription of many genes, several of which encode enzymes for fatty acid metabolism. PPARα binds a specific agonist that allows association with a transcription coactivator complex, which includes the well-characterized peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1α).[5,6] Together, these associate with several general histone acetyltransferases (HATs), such as p300 and CBP, that help promote association with other required chromatin modifiers, such as the SWItch/Sucrose NonFermentable (SWI/SNF) complex, and recruitment of the general transcription machinery.

As mentioned above, the heart must adjust its metabolism during pressure overload to create enough ATP in an environment that is less oxygen abundant.[1] Therefore, there is decreased oxidative metabolism and increased anaerobic metabolism of glucose. When this switch in metabolic pathways occurs, there is decreased activation of PPARα and simultaneous increased transcription of glycolytic target genes through a separate set of transcription factors such as c-myc and hypoxia-inducible factor-1α (HIF-1α).[7,8] These new transcription factors driving glycolysis recruit their own coactivator complexes, which have both unique and common components compared to those recruited by PPARα. For this activation to occur, there is also loss of corepressor complexes at the promoter-containing histone deactylases (HDACs) and transcription factor-specific negative regulators. Additionally, PGC-1α controls mitochondrial biogenesis in the heart by binding to a different set of transcription factors that includes the ligand-inducible estrogen-related receptor (ERR) family.[5,9]

Genetic models that prevent this remodeling often control hypertrophic responses and result in cardiac dysfunction under stress. For example, MHC-PPARα mice, which have cardiac-specific overexpression of PPARα, have expected changes in metabolic rates—i.e., increased fatty acid uptake and oxidation along with decreased glucose uptake and use—but develop ventricular hypertrophy and systolic dysfunction over time. However, PPARα knockout (KO) mice, which have the expected increased glucose metabolism, also cannot maintain function in response to stress.[10] Similarly, both PGC-1α KO and cardiac-specific overexpression mice are unable to efficiently adapt to stress; pressure overload to the left ventricle through transverse aortic constriction (TAC) induces rapid left ventricular dilation in PGC-1α KO mice compared to the normal hypertrophic response of wild-type mice.[11]

The "fetal gene" expression profile of the stressed heart also involves sarcomeric gene expression changes.[3] Another group of cardiac transcription factors are responsible for controlling sarcomeric and calcium handling gene expression, both integral components of cardiomyocyte contraction. These transcription factors include GATA4, MEF2, TBX5, SRF, and the muscle-specific coactivator myocardin. Genetic manipulation of many of these factors also controls the heart's ability to respond to TAC with hypertrophy.[2,12] For example, cardiac overexpression of GATA4 induces hypertrophic gene expression changes,[13] whereas mice harboring a mutant of *gata4* with impaired transcriptional activity have a similarly impaired hypertrophic response to pressure overload.[14] We have shown that loss of another transcription coactivator, steroid receptor coactivator-2 (SRC-2), is another factor important for adult expression of fatty acid-focused metabolic genes. SRC-2 also controls the transcription activity of GATA4, MEF2, and TBX5, which results in accelerated cardiac decline in response to pressure overload, in this case in the absence of hypertrophy.[15,16]

Targeting Transcription in Left Ventricular Hypertrophy

Two ultimate themes emerge from studies on transcription changes during heart failure. First, metabolic and hypertrophic changes, while not fully dependent on one another, are connected through shared transcriptional regulators. Therefore, long-term changes to one ultimately affect the degree of the other. Second, loss of cardiac flexibility to respond to stress, such as loss of a transcription factor requiring use of a specific metabolic pathway, usually results in decreased function and impaired stress response. Therefore, an effective treatment for heart failure would consider timing and target choice. For example, it could be beneficial to either prevent extensive long-term transcriptional remodeling during early stages of cardiovascular disease or to promote greater flexibility at later stages.

Several clinical studies have attempted to target LVH and cardiovascular disease through modulation of transcription. If we consider the possible tiers of transcriptional regulation, there are multiple ways to target this remodeling that may be context specific (Figure 2). For the highest specificity, the best targets are the

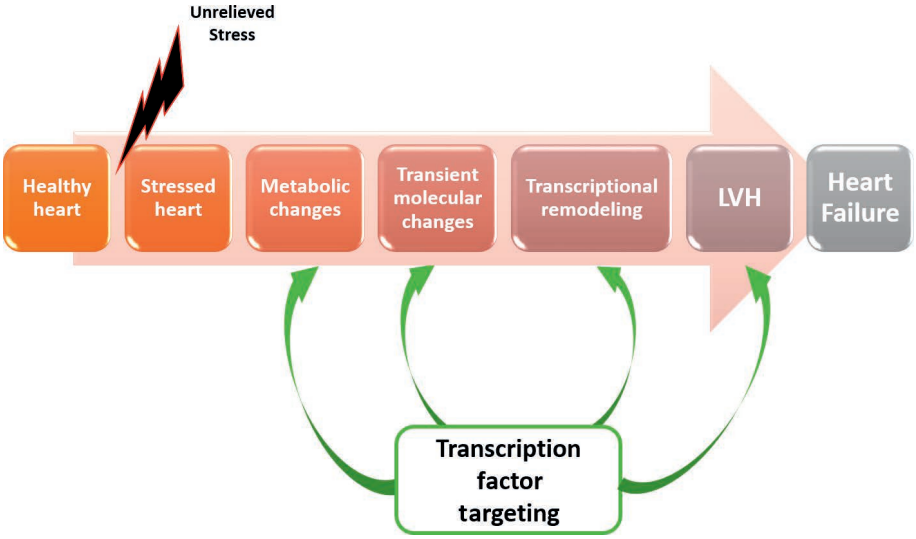

Figure 3. Beneficial treatment of left ventricular hypotrophy (LVH) through regulation of transcription will likely depend on timing and target choice. In general, once a heart receives a persistent or detrimental stress that will eventually cause LVH, there are transient molecular changes that give way to long-term transcriptional changes. At each of these steps, a different class or set of transcription factors is likely the optimal target for treatment. Early detection and treatment would ideally be able to reverse or prevent long-term remodeling.

transcription factors themselves (Figure 2, Tier 1); however, these may be difficult to drug. Each transcription factor controls a specific set of genes and often responds to one or a few specific signals. For ligand-inducible targets, such as PPARα and the nuclear hormone receptors, controlling their activity can be accomplished with synthetic agonists or antagonists. In the case of the PPAR family, and especially for PPARγ, this has been tried with thiazolidinediones in type 2 diabetes mellitus; however, treatment with this specific class of drugs resulted in increased events of heart failure.[17] This could be because the drugs are used in patients who already have adverse cardiovascular remodeling from previously undiagnosed diabetes and/or other circumstances, or because the drugs can cause edema, which can further increase load on the heart.[18]

Thyroid hormone, which activates thyroid hormone receptors to control transcription, has also been suggested as an attractive target and has shown some promise in small-scale clinical trials. However, clinical success with synthetic thyroid hormones appears to depend heavily on timing, concentration, and receptor affinity since general thyroid hormone treatment can lead to tachycardia.[19,20] Specific targeting of other non-ligand-induced transcription factors is slightly more difficult, as their activity often depends on recruitment of several other proteins. Previously, this type of targeting was done through modulation of upstream signaling, such as the use of cardiac glycosides that effect downstream NFkappaB signaling,[21] but such treatments will likely affect several other pathways and lead to side effects or decreased efficacy. Some studies recently tested direct control of several transcription factors in treating cancers using small molecules, immunotherapy, or other techniques[22]; these studies indicated that although direct control of transcription factors may be difficult, it is not impossible and should be considered for use in the treatment of LVH.

The next class of druggable targets are the coactivators and/or corepressors, which have slightly less specificity but can hit a concerted pathway or function (Figure 2, Tier 2). For example, during cardiac stress, PGC-1α generally controls mitochondrial biogenesis and fatty acid regulation via a small group of transcription factors.[11] Another attractive target is SRC-2, which controls both metabolism and hypertrophic targets.[15] Currently, these are more difficult to drug because they do not have specific enzymatic activity and therefore active sites. However, recent work in cancer cells has shown that small molecules can be used to both inhibit or activate other members of the SRC family (SRC-1 and SRC-3), suggesting that targeting is feasible.[23,24]

The coactivators, such as SRC-2 and PGC-1α, and parallel corepressors recruit more general transcription regulators, the HATs and HDACs (Figure 2, Tier 2). Although some of these, such as the coactivators p300/CBP, are pleiotropic and would likely fall more closely into Tier 3 for target specificity, others show some target specificity (e.g., HDAC4 and 5 regulation of MEF2 in the heart).[25] Furthermore, some HDACs are constitutively nuclear while others shuttle between the nucleus and cytoplasm and/or require different cofactors for activity, therefore allowing for some targeting specificity in drug development. Both HAT and HDAC inhibitors are being tested for control of cancer cells in clinical trials, but they have also been shown to play a role in controlling cardiac hypertrophy in animal models.[26,27] The evidence that inhibiting both global activators and repressors has beneficial effects in cardiac stress strongly supports the hypothesis that control of transcription flexibility, and not the exact gene expression per se, may be an effective therapy to manage already progressing cardiac stress.

The final druggable targets are pleiotropic targets, including chromatin modifiers such as SWI/SNF and the general transcription machinery. These are more generally used by all genes and therefore lack specificity, but they have intrinsic enzymatic activity that can be targeted. Furthermore, SWI/SNF regulators have been used in clinical trials for cancer treatment,[28] and cancer cells show much of the same metabolic dynamics and gene remodeling as cardiomyocytes in response to stress.[29] Additionally, one recent study reported that selective inhibition of TFIIB, a general transcription factor, resulted in decreased hypertrophy and fibrosis and prevented decreased ejection fraction in response to TAC in mice.[30] The authors attributed these effects to their ability to exploit the different kinetics of TFIIB action on genes with paused RNA polymerase II complexes versus those that require de novo binding of RNA polymerase II. Targeting these two groups through TFIIB allowed inhibition of specialized genes responsible for hypertrophy during cardiac stress without inhibiting general housekeeping genes. These data strongly support the idea that stress-induced changes and remodeling during cardiac stress can allow for unique sets of genes to be manipulated as groups; however, timing of this treatment is crucial to its success.

Early Detection and Temporal Control of Treatment

The cardiac stressors that result in LVH eventually lead to transcriptional remodeling that ultimately dictates the function of each cell during stress. Targeting these changes could prove to be an effective therapeutic strategy; however, careful consideration must be paid to identifying the correct target and the kinetics involved (Figure 3). As such, personalized treatments targeting transcriptional control in the early stages of cardiovascular stress may be different than treatments needed in late stages. This type of treatment strategy would greatly benefit from the development of imaging modalities that could detect cardiovascular stress earlier. For example, imaging that could identify metabolic substrate shifts before long-term transcription remodeling could allow for prevention of maladaptive gene changes. It is also conceivable that there are systemic signals being sent from the heart such as hormones, exosomes, cytokines, or noncoding RNA that could help to identify early signs of stress.

In order to effectively target transcription for therapeutic use, we must better understand when these transcription changes start and how reversing them during cardiac stress, and not just before onset, affects the response. Understanding these events can also lead to the development of beneficial treatment "cocktails" that could, for example, target a transcriptional response and metabolism[31] together during early heart failure to reverse or prevent long-term remodeling.

Key Points:

- Once left heart hypertrophy is observed clinically, the cardiomyocyte has likely already gone through major stress-induced transcription remodeling, which may be adaptive or maladaptive depending on the degree of disease progression.
- Targeting stress-induced transcriptional changes in the heart could be an effective new therapeutic strategy.
- Target choice in transcriptional control-based treatments requires consideration of both timing and target specificity and should be aimed at increasing molecular flexibility in the heart.

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