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Review article

BET-ting on chromatin-based therapeutics for heart failure

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ABSTRACT

Studies of transcriptional mechanisms in heart failure have focused heavily on roles of sequence-specific DNA-binding factors such as NFAT, MEF2 and GATA4. Recent findings have illuminated crucial functions for epigenetic regulators in the control of cardiac structural remodeling and mechanical dysfunction in response to pathological stress. Here, we review the current understanding of chromatin-dependent signal transduction in cardiac gene control, and highlight the potential for pharmacologic regulation of BET acetyl-lysine binding proteins as a means of treating heart failure.

Contents

1. Introduction

Abnormalities in the control of gene expression are central to the pathogenesis of heart failure (HF). Transcript expression profiling in animal models [1] and diseased human hearts [2] consistently demonstrates stereotypical patterns of aberrant myocardial gene control. The most cogent evidence that implicates transcriptional misregulation in HF pathogenesis comes from a large body of work using murine gene-targeting and transgenesis. Collectively, these studies have clearly demonstrated that activation of specific DNA-binding transcription factors (TFs), such as NFAT, MEF2, NF-κB, GATA4, and C-MYC, is critical for pathological cardiac remodeling *in vivo* [3]. However, the precise molecular mechanisms by which these potent TF signal downstream to trigger pathologic gene expression in the heart has remained poorly understood. To unravel these mechanisms, one must consider that TF function in the context of chromatin to drive cell state-specific gene expression programs [4]. In this article, we review current concepts in eukaryotic transcription, and highlight recent studies that explore the role of chromatin-dependent signal transduction in cardiac gene control and HF pathogenesis. As drugs that target chromatin-dependent signaling effectors are being developed as anti-cancer agents [5], a deeper

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understanding of these epigenetic pathways in the myocardium may provide novel therapeutic opportunities.

2. Current concepts in eukaryotic gene control

Chromatin refers to a dynamic macromolecular complex of genomic DNA complexed with a diverse array of RNA and proteins [6]. The fundamental unit of chromatin is the nucleosome, comprised of 147 base pairs of double-stranded DNA wrapped in approximately 1.7 superhelical turns around a histone octamer consisting of two copies each of the core histones H2A, H2B, H3 and H4 [7]. Histones within nucleosomes can be post-translationally modified and/or exchanged with variants to alter the primary chromatin structure [8]. Primary chromatin, in turn, is arrayed into higher order three-dimensional configurations that permit local accessibility of the genome and participate in signaling. By vastly expanding the signaling repertoire of the primary DNA template, a higher order chromatin structure endows eukaryotes with the ability to generate remarkable cellular plasticity from a single genome [4,9].

We will first briefly review some fundamental features of eukaryotic gene regulation, as these concepts are the necessary framework for understanding cardiac gene control in physiology and disease. Eukaryotic cell identity or more broadly, "cellular state", is largely governed by precise spatiotemporal coordination of gene expression programs [4]. While the concept of "cell state transformation" is obviously pertinent to the study of organogenesis and developmental specification (e.g., the differentiation of a pluripotent stem cell into a cardiomyocyte), we emphasize here that activation of pathologic transcriptional programs in the stressed heart (e.g., transformation of a healthy cardiomyocyte into one that is hypertrophied and hypo-contractile) also represents an equally robust cell state transition that is driven by defined molecular events. Control of these gene expression programs is orchestrated by dynamic interplay between activity of DNA-binding TFs and changes in the higher-order chromatin structure. Accumulating evidence demonstrates that a limited number of TFs are capable of controlling the selective transcription of genes by RNA Polymerase II (Pol II), thereby governing any given cell state [4]. TFs typically regulate gene expression by binding regulatory DNA elements called enhancers, an event which recruits cofactors and facilitates assembly of the general transcriptional machinery (e.g. Pol II) to the transcriptional start sites of target genes [10,11]. An active enhancer typically binds multiple TFs in a cooperative fashion and regulates transcription from core promoters, often via long-range genomic interactions that involve looping of DNA [12,13]. In addition, TFs can also bind to core promoter elements in proximity to transcriptional start sites to recruit transcriptional machinery and regulate cellular state [14].

A critical mechanism by which enhancer-bound TFs set the stage for gene control is via the recruitment of co-factors that alter the local chromatin structure. Two major categories of cofactors are those that mobilize nucleosomes (e.g. the ATP-dependent chromatin remodeling complexes) [6] and those that enzymatically modify histones via post-translational modifications (e.g. acetylation, methylation, phosphorylation, and ubiquitylation) [15]. With regard to the latter, there are enzymes that add or remove post-translational modifications, which have been dubbed epigenetic "writers" and "erasers", respectively. Consequently, there are proteins harboring recognition motifs for each of these histone modifications, termed epigenetic "readers", which facilitate protein complex formation and signal propagation. Together, these modifications to DNA and DNA-associated proteins alter the local chromatin structure in a stereotypical fashion across the regulatory and coding regions of the genome in a manner that correlates with transcriptional activity [4,15]. For example, H3K27ac is found at active promoters and enhancers, H3K36me3 marks actively transcribed gene bodies, and H3K27me3 marks heterochromatic or transcriptionally repressed regions [16]. In addition to histone proteins, DNA itself can be covalently modified, including methylation of carbon-5 of cytosine (5mC), a mark that has been associated with transcriptional repression [16]. Collectively, the dynamic interplay between TFs and alterations in the local chromatin structure dictate higher order genomic architecture and allow genomic signal transduction to occur with spatiotemporal precision [4,9].

The main cellular machinery for transcribing protein-coding genes is the Pol II complex. Phases of Pol II-dependent transcription include formation of initiation complexes at transcriptional start sites, promoter-proximal pausing, pause release, elongation and termination. The dynamics of Pol II function are tightly regulated in a locus-specific and signal-dependent manner. In general, TFs and recruited co-regulatory proteins signal via chromatin to control both initiation and elongation of Pol II [17–19]. Once a recruited Pol II molecule initiates transcription, it generally travels a short distance (generating a nascent mRNA of 20–50 nucleotides), and then pauses. Pausing is, in part, mediated by the physical association of Pol II with pause control factors such as NELF and DSIF [17]. Release of this paused state and subsequent transcriptional elongation is mediated by recruitment and activation of kinase complexes, such as positive transcription elongation factor b (P-TEFb). P-TEFb phosphorylates Pol II and its associated pausing factors, thereby allowing pause release and productive transcriptional elongation [20]. It is now increasingly appreciated that a major mechanism by which many transcription factors affect gene expression is via downstream control of pause release and transcriptional elongation. Factors that regulate this latter step of pause release can have profound effects on cell state [4], some of which have been shown to be critically involved in pathologic cardiac remodeling [21,22] (see Section 5 below).

3. Probing the epigenome using rapidly evolving technologies

The last decade has witnessed unprecedented growth in epigenomic technologies. Rapidly evolving technical advances in our ability to probe TF enrichment, histone modifications, chromatin structure, and transcriptional dynamics on a genome-wide scale have transformed our understanding of how cell state-specific gene expression is orchestrated. Previous to the advent of next generation sequencing (NGS), assays of chromatin structure (e.g., chromatin immunoprecipitation [ChIP] and 5mC detection) were used to specifically probe loci of interest one at a time. However, NGS platforms now allow for massively parallel short reads of DNA sequence in an increasingly high-throughput, fast, and cost effective manner [16]. Therefore, the epigenetic assays previously used to measure a single locus are now integrated with NGS technology to provide unbiased, genome-wide chromatin state maps and transcript expression profiles. These state maps include genome-wide assessment of nucleosome structure, histone modifications, DNA modifications, transcription factor cistromes, enrichment of chromatin-associated proteins and nucleic acids, long-range chromatin interactions, and transcriptional dynamics [16,23]. Much of our knowledge of chromatin state across a wide variety of cell and tissue types has come from the NIH/NHGRI sponsored ENCODE consortium (Encyclopedia of DNA Elements). Data from ENCODE has significantly advanced the identification of functional elements within the human genome and has provided essential insight into cell-state specific gene control [24]. The elucidation of thousands of previously unappreciated regulatory elements (e.g. enhancers) and non-coding transcripts (e.g. lnc-RNAs) is rapidly changing our understanding of cellular differentiation, development and disease pathogenesis [16]. As human genome-wide association studies have demonstrated that the vast majority of disease-associated loci map to non-protein coding regions of DNA [25], the mechanistic insight gleaned from epigenomic maps is likely to provide a more detailed understanding of disease susceptibility and pathogenesis. These genome-wide assays of chromatin state, which are just beginning to be applied to cardiovascular biology [21,26,27], have the potential to transform our understanding of cardiac hypertrophy and HF.

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