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Negative Elongation Factor Controls Energy Homeostasis in Cardiomyocytes

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SUMMARY

Negative elongation factor (NELF) is known to enforce promoter-proximal pausing of RNA polymerase II (Pol II), a pervasive phenomenon observed across multicellular genomes. However, the physiological impact of NELF on tissue homeostasis remains unclear. Here, we show that whole-body conditional deletion of the B subunit of NELF (*NELF-B*) in adult mice results in cardiomyopathy and impaired response to cardiac stress. Tissue-specific knockout of *NELF-B* confirms its cell-autonomous function in cardiomyocytes. NELF directly supports transcription of those genes encoding rate-limiting enzymes in fatty acid oxidation (FAO) and the tricarboxylic acid (TCA) cycle. NELF also shares extensively transcriptional target genes with peroxisome proliferator-activated receptor α (PPARα), a master regulator of energy metabolism in the myocardium. Mechanistically, NELF helps stabilize the transcription initiation complex at the metabolism-related genes. Our findings strongly indicate that NELF is part of the PPARα-mediated transcription regulatory network that maintains metabolic homeostasis in cardiomyocytes.

INTRODUCTION

RNA polymerase II (Pol II) is preferentially accumulated at transcription start sites (TSSs) of a large number of genes in multicellular organisms (Adelman and Lis, 2012; Levine, 2011). Whereas the enrichment of Pol II density at TSS alone is not necessarily indicative of a distinct mode of regulation, whole-genome analysis of nascent transcripts clearly demonstrates that Pol II indeed pauses at a position downstream of TSS after the synthesis of short stretches of RNA (Core et al., 2008). Furthermore, negative elongation factor (NELF) in metazoan is an important regulator of Pol II pausing (Kwak and Lis, 2013; Yamaguchi et al., 1999). Depletion of any of the four NELF subunits results in disintegration of the entire complex and global reduction of Pol II pausing. NELF-mediated Pol II pausing is antagonized by the positive transcription elongation factor, P-TEFb, a cyclin-dependent kinase (Zhou et al., 2012). Whereas NELF was first identified biochemically as an inhibitor of transcription elongation, subsequent studies indicate that NELF-mediated Pol II pausing can lead to both decreased and increased transcription (Adelman and Lis, 2012). The underlying mechanism by which NELF facilitates transcription is not fully understood. However, it has been shown that NELF-mediated Pol II pausing can prevent the encroachment of nucleosomes at the promoter-proximal region, suggesting that NELF may support multiple rounds of transcription in vivo by maintaining a nucleosome-free region at the promoter (Gilchrist et al., 2010; Sun and Li, 2010). In contrast to the extensive in vitro studies, there is a significant gap of knowledge concerning the physiological roles of NELF in mammals.

Cardiomyopathy is characterized by a rigid, thick, and enlarged heart muscle (Cahill et al., 2013). As cardiomyopathy deteriorates, normal cardiac functions (e.g., blood pumping and maintenance of electrical rhythm) are significantly compromised due to myocyte loss and increased fibrosis. This can ultimately result in heart failure, a prevalent and debilitating disease with high morbidity and mortality. At the histological level, hearts with cardiomyopathy manifest with infiltrating inflammatory cells and interstitial collagen accumulation. One of the major causes of cardiomyopathy is inefficient energy production in cardiomyocytes, which results in failure to meet the high demands of energy consumption and compromised intracellular Ca^{2+} homeostasis for contraction (Frey et al., 2012). In the normal myocardium, cardiomyocytes alternate between carbohydrates and fatty acids as sources of energy, with the latter contributing up to 70% of the energy requirement for an adult heart (Stanley et al., 2005). Energy metabolism is regulated by both acute mechanisms (e.g., allosteric controls and posttranslational modifications) and long-term transcriptional regulation that renders

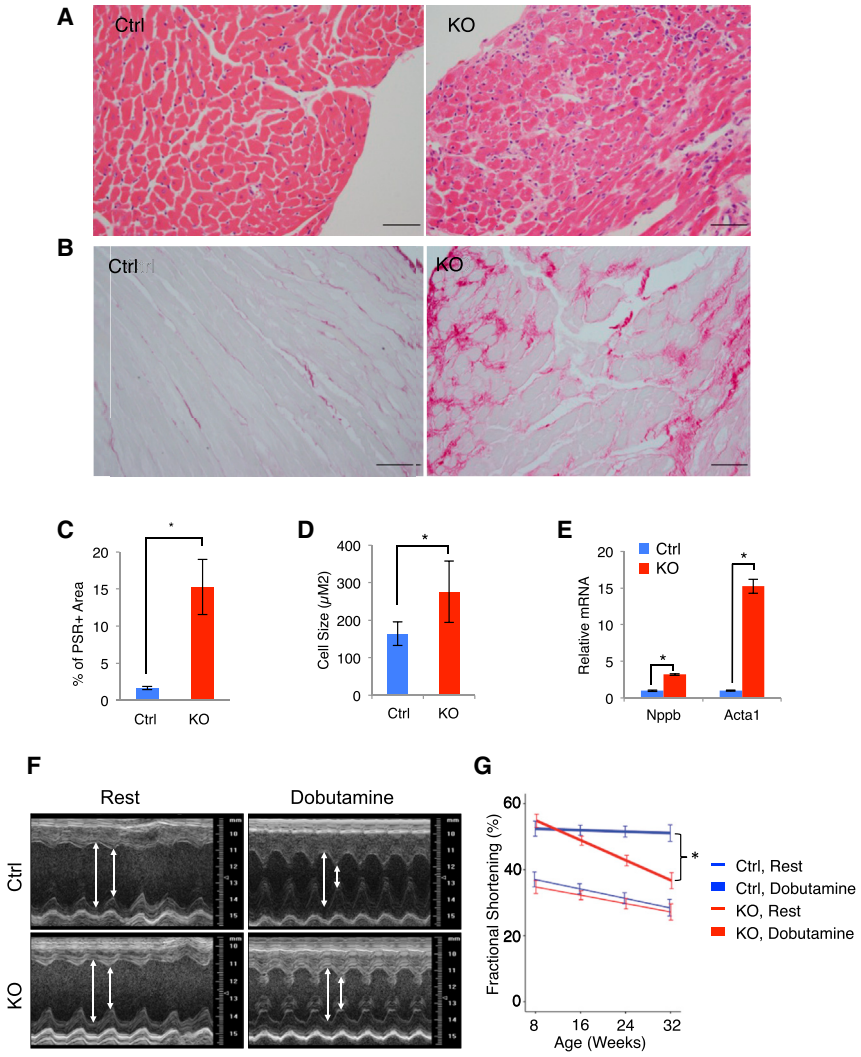

Figure 1. *NELF-B* KO Leads to Cardiomyopathy

(A) Hematoxylin and eosin staining of left ventricles from control (Ctrl) and *NELF-B* knockout (KO) mice. The scale bar represents 50 μm.

(B) Picrosirius red staining for collagen deposition. The scale bar represents 50 μm.

(C) Quantification of picrosirius red staining (n = 5). Here and elsewhere in the figures, *p < 0.05. Error bars represent SEM.

(D) Quantification of cell size of over 100 individual cardiomyocytes (n = 3).

(E) Relative mRNA levels of two hypertrophy markers (n = 6).

(F) Representative left ventricular M-mode echocardiography. The double-headed arrows indicate the left ventricular dimension at diastole (longer arrow) and systole (shorter arrow).

(G) Fractional shortening from the left ventricle (LV) at rest (thin lines) and following dobutamine injection (thick lines). Ctrl: n = 11; KO: n = 8.

more sustained changes in metabolic rates. Reduced transcription of rate-limiting enzymes involved in cardiac fatty acid metabolism is often associated with heart failure, forcing the cardiac switch to carbohydrates as the main source of energy (Hue and Taegtmeyer, 2009).

Several members of the nuclear receptor superfamily and their coactivators, in particular peroxisome proliferator-activated receptors (PPARs), PPAR gamma coactivator 1 (PGC-1), and estrogen-related receptors (ERRs), are known to play critical roles in controlling energy-metabolism-related transcription in cardiomyocytes (Giguère, 2008; Madrazo and Kelly, 2008; Rowe et al., 2010). Impairment of the transcriptional programs dictated by these critical regulators in humans is often associated with heart failure and myocardial ischemia (Sihag et al., 2009). Consistent with the clinical observations, heart-specific disruption of the mouse counterparts of these transcription factors results in cardiac dysfunction with reduced capacity in fatty acid oxidation (FAO) and mitochondrial ATP production (Huss et al., 2007; Smeets et al., 2008).

To elucidate the physiological role of NELF in homeostasis of adult tissues, we generated both whole-body and cardiomyocyte-specific knockout (KO) mouse models that deleted the B subunit of mouse NELF (*NELF-B*). By combining transcriptomics and metabolomics approaches, we further investigated the underlying mechanism by which NELF contributes to cardiac functions.

RESULTS AND DISCUSSION

Inducible NELF-B Knockout Animals Develop Cardiomyopathy

Our previous work indicates that mouse NELF-B is essential for early embryogenesis (Amleh et al., 2009). To determine the role of NELF-B in adult tissue, we circumvented embryonic lethality of conventional *NELF-B* KO by crossing the *NELF-B*$^{f/f}$ mice with a whole-body tamoxifen (TAM)-inducible Cre-ER strain (Figure S1A). The resulting *NELF-B*$^{f/f}$,*Cre-ER* mice were born with the expected Mendelian ratio. *NELF-B*$^{f/f}$,*Cre-ER* and their control littermates were injected with TAM at 8 weeks of age and confirmed for TAM-induced deletion of the floxed NELF-B allele (Figure S1B). Compared to TAM-treated control mice, *NELF-B*$^{f/f}$,*Cre-ER* mice with inducible KO of NELF-B had a shortened life span (11 months), resulting from increased incidences of sudden death with no outward signs of gross abnormalities. For this reason, we selected 10 months (40 weeks) as our primary endpoint for the following histological study.

Histological analysis of KO myocardium revealed obvious signs of cardiomyopathy accompanied by infiltrating inflammatory cells including neutrophils (Figures 1A, S1C, and S1D). This was in sharp contrast to the myocardium from age-matched animals carrying one or two alleles of wild-type NELF-B, either with or without Cre-ER (*NELF-B*$^{+/+}$, *Cre-ER*; *NELF-B*$^{f/f}$; and