JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 9, 2023

EDITORIAL COMMENT

Circadian-Regulated Cardiac Metabolism Involves Transcription Factor E4BP4*

Inna Rabinovich-Nikitin, PhD,[a,b] Lorrie A. Kirshenbaum, PhD[a,b,c]

E4BP4 is a nuclear factor that binds to consensus activating transcription factor elements on various gene promoters involved in cell immunity; tumorigenesis; lipid and glucose metabolism; and, more recently, circadian rhythm.[1] At the molecular level, the circadian clock is regulated by a series of interwoven positive and negative transcriptional-translational feedback loops that are responsible for the daily oscillations of circadian-regulated biological processes such as metabolism. In this regard, E4BP4 was shown to be an important circadian output gene with a robust oscillatory pattern that impinged on the regulation of genes associated with the negative inhibitory feedback loop of the molecular clock through its effects on the expression of the clock regulatory proteins period 1/2 (Per1/2) and cryptochrome 1/2, (Cry1/2). In this context, E4BP4 was found to repress the expression levels of Per1 and Per2, as well as interact with Cry2.[1] In addition, E4BP4 was also found to be a direct target of REV-ERB in the heart, which, in addition to its role as regulator of the circadian clock activity, has also been found to regulate other vital cellular processes, such as metabolism and growth. Specifically in the context of cardiac metabolism, functional loss of REV-ERB led to E4BP4-induced down-regulation of fatty acid oxidation, resulting in dilated cardiomyopathy.[2] Furthermore, E4BP4 was also shown to regulate the expression levels of nicotinamide phosphoribosyltransferase (Nampt), which is an enzyme involved in the NAD^+ salvage pathway. When REV-ERB was inhibited, it led to inhibition of Nampt and up-regulation of E4BP4, highlighting the important role of E4BP4 in the regulation of Nampt and NAD^+ pathways.[2]

In this issue of *JACC: Basic to Translational Science*, Mia et al[3] provide a novel insight into the circadian regulation of the metabolism and electrophysiologic properties of cardiac myocytes by E4BP4. To begin to elucidate the role of E4BP4 in the development of cardiomyopathy, Mia et al first tested in a mouse model whether cardiomyocyte-specific gene ablation of E4BP4 ($E4bp4^{flox/flox}$/MHCαCre$^{+/-}$ [CEK]) in $Bmal1^{flox/flox}$/MHCαCre$^{+/-}$ (CBK) mice would prevent age-onset cardiomyopathy. The notion for these experiments was based on previous reports showing that cardiac-specific deletion of BMAL1 or REV-ERBα/β results in up-regulation of E4BP4 levels, which may contribute to age-dependent development of cardiomyopathy.[2,4] Consistent with this study, Mia et al showed that hearts from CBK mice exhibited increased levels of E4BP4, presumably from genetically induced down-regulation of BMAL1, resulting in age-related cardiomyopathy, whereas CBK/CEK mice had no change in cardiac outcomes. Furthermore, the age-dependent increase in E4BP4 expression coincided with the inhibition of several genes required for cell metabolism, cell signaling, and electrophysiologic function. Based on these findings, together with the fact that E4BP4 is a circadian-dependent gene, Mia et al next tested whether E4BP4 contributes to circadian-dependent changes in cardiac transcriptome. For this purpose, they analyzed transcriptomic changes in hearts collected from CEK

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

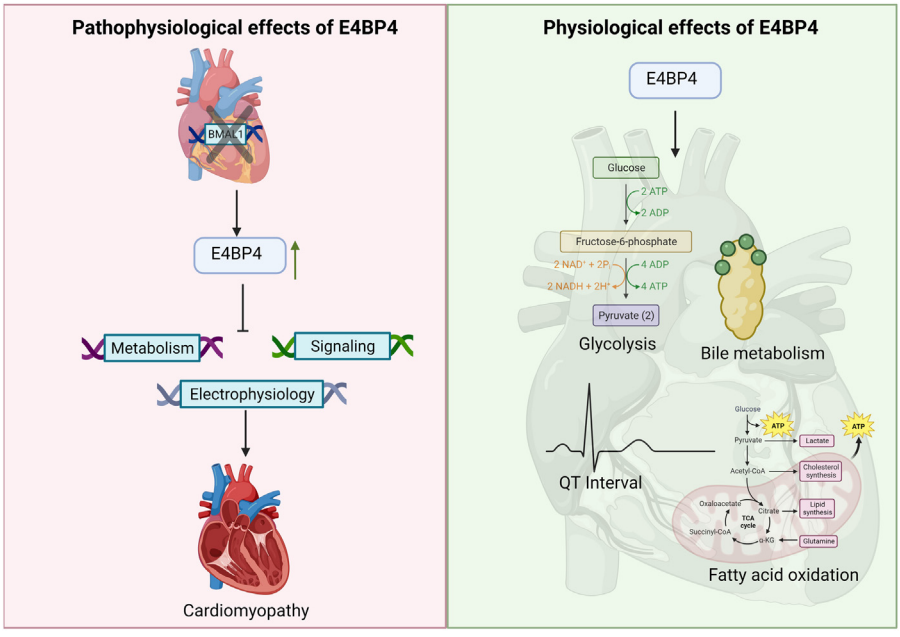
From [a]The Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, Winnipeg, Manitoba, Canada; [b]Department of Physiology and Pathophysiology, Rady College of Medicine, Max Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada; and the [c]Department of Pharmacology and Therapeutics, Rady College of Medicine, Max Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.06.011

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 9, 2023
SEPTEMBER 2023:1157–1159

FIGURE 1 Schematic Diagram Depicts the Effects of E4BP4 Under Basal Conditions and Age-Onset Cardiomyopathy



(Left) Physiologic effects of E4BP4. E4BP4 regulates glycolysis, bile metabolism, fatty acid oxidation, and QT interval. **(Right)** Pathophysiologic effects of E4BP4. Disruption of the circadian clock gene *BMAL1* in the heart results in elevated levels of E4BP4, which negatively regulates key genes involved in cellular processes, including metabolism, cell signaling, and electrophysiology, which leads to age-onset cardiomyopathy. Created with BioRender.com. α-KG = alpha ketoglutarate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; CoA = coenzyme; TCA = tricarboxylic acid cycle.

mice and showed that cardiac-specific deletion of E4BP4 impaired diurnal transcriptome changes in key metabolic pathways. Mia et al next analyzed the metabolomics profile of CEK mice over 24 hours and found that, in contrast to control, metabolic products associated with bile production, glycolysis, lipids, and fatty acid metabolism were changing in a time-dependent manner, supporting the notion that E4BP4 plays a critical role in the regulation of cardiac metabolism. Diurnal variations in oxidative metabolism were also evaluated by using radiolabeled tracers in isolated working mouse heart perfusions. This analysis revealed that higher rates of oleate oxidation occurred during the light phase, in accordance with increased levels of fatty acyl intermediates in CEK hearts. These findings suggest that E4BP4 inhibits fatty acid metabolism by reducing β-oxidation during the light phase. Finally, Mia et al also found certain ion channels associated with increased heart rate and decreased QT and QTc intervals to be altered in the CEK mice. This phenotype was rescued by administration of E4bp4-Adeno associated virus, which not only up-regulated cardiac E4BP4 levels by approximately 10-fold but also restored QT and QTc intervals comparable to control—highlighting the important role of E4BP4 as a regulator of ion channel expression and QT interval.

Taken together, the work by Mia et al[3] (**Figure 1**) demonstrates an indispensable role for E4BP4 in cardiac metabolism and age-related cardiomyopathy through direct regulation of a subset of gene transcripts involved in cardiac metabolism, such as fatty acid oxidation. Furthermore, the study also highlights a novel link between E4BP4 and regulation ion channels important for maintaining normal QT intervals.

Although the findings by Mia et al[3] strongly support E4BP4 as a regulator of cardiac metabolism under normal physiologic and pathologic conditions, it also raises some important questions that need to be considered. In particular, despite the investigators having demonstrated a putative link between BMAL1, E4BP4, and more than 50 important metabolites that may be regulated by E4BP4, several questions remain. For example, it is unknown whether the regulation of E4BP4 by BMAL1 is direct or indirect or

whether it impinges on other signaling pathways independent of circadian clock regulation. Another important question relates to the pathophysiology of age-dependent onset cardiomyopathy. One of the key cellular processes associated with the development of cardiac impairment is cell death, which can result with age from impaired cellular quality control mechanisms, such as autophagy. Because autophagy and cell death of cardiomyocytes are known to be circadian regulated,[5] it would be important to know the relationship between E4BP4 cell death and autophagy regulation during associated cardiomyopathy. In addition, because E4BP4 is highly expressed in a variety of tissues, it is also unknown whether the effects of E4BP4 on metabolic gene expression are a feature restricted to cardiac muscle or are universal in other cell types that comprise the heart, such as fibroblasts, endothelial cells, and so on. Additionally, it is unknown whether the circadian and age-related changes by E4BP4 are sex specific. For this reason, it would be important to assess the effects of E4BP4 in female and male patients to establish whether the severity of age-onset cardiomyopathy is affected by sex, as has been reported for the occurrence of heart failure with preserved ejection fraction and heart failure with reduced ejection fraction, respectively.

Nevertheless, the study by Mia et al[3] provides new important insight into the role of the circadian-regulated transcription factor E4BP4 in the pathogenesis of aging-induced cardiac dysfunction. Further, the study highlights the importance of the body's biological clock as a potential therapeutic target in age-onset-induced cardiomyopathy.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Lorrie A. Kirshenbaum, Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, Room 3016, 351 Taché Avenue, Winnipeg, Manitoba R2H 2A6, Canada. E-mail: LKirshenbaum@sbrc.ca.

REFERENCES

1. Zhao Z, Yin L, Wu F, Tong X. Hepatic metabolic regulation by nuclear factor E4BP4. *J Mol Endocrinol*. 2021;66:R15–R21.

2. Dierickx P, Zhu K, Carpenter BJ, et al. Circadian REV-ERBs repress E4bp4 to activate NAMPT-dependent NAD+ biosynthesis and sustain cardiac function. *Nat Cardiovasc Res*. 2022;1:45–58.

3. Mia S, Sonkar R, Williams L, et al. Novel roles for the transcriptional repressor E4BP4 in both cardiac physiology and pathophysiology. *J Am Coll Cardiol Basic Trans Science*. 2023;8:1141–1156.

4. Young ME, Brewer RA, Peliciari-Garcia RA, et al. Cardiomyocyte-specific BMAL1 plays critical roles in metabolism, signaling, and maintenance of contractile function of the heart. *J Biol Rhythms*. 2014;29:257–276.

5. Rabinovich-Nikitin I, Rasouli M, Reitz CJ, et al. Mitochondrial autophagy and cell survival is regulated by the circadian clock gene in cardiac myocytes during ischemic stress. *Autophagy*. 2021;17:3794–3812.

KEY WORDS BMAL1, circadian rhythm, E4BP4, heart failure, metabolism