REVIEW

Hepatic metabolic regulation by nuclear factor E4BP4

Zifeng Zhao^{1,2}, Lei Yin², Feihua Wu¹ and Xin Tong²¹Department of Pharmacology of Chinese Materia Medica, School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu Province, China²Department of Molecular & Integrative Physiology, University of Michigan Medical School, Ann Arbor, Michigan, USACorrespondence should be addressed to F Wu or X Tong: fhwu2000@cpu.edu.cn or xintong@umich.edu

Abstract

Discovered as a b-ZIP transcription repressor 30 years ago, E4 promoter-binding protein 4 (E4BP4) has been shown to play critical roles in immunity, circadian rhythms, and cancer progression. Recent research has highlighted E4BP4 as a novel regulator of metabolisms in various tissues. In this review, we focus on the function and mechanisms of hepatic E4BP4 in regulating lipid and glucose homeostasis, bile metabolism, as well as xenobiotic metabolism. Finally, E4BP4-specific targets will be discussed for the prevention and treatment of metabolic disorders.

Key Words

- ▶ E4BP4
- ▶ insulin
- ▶ lipid
- ▶ glucose
- ▶ metabolism

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Introduction

The PAR proteins, named after the conserved proline- and acid-rich (PAR) domains in a subset of basic leucine zipper (b-ZIP) transcription factors, consist of four family members, TEF/VBP, HLF, DBP, and E4BP4 (Cowell & Hurst 1994). The PAR family proteins form homodimers or hetero-dimerize within other family proteins and function as either transcription activators or repressors (Hai & Hartman 2001). The PAR family proteins have been found to have diverse physiological functions in mammals, including circadian rhythms, immunoregulation, and cancer development (Green 2016). This review will focus on the role of E4 promoter-binding protein 4 (E4BP4), also named as nuclear factor, interleukin 3 regulated (NFIL3), the least known PAR transcription factor, in liver metabolic regulation. NFIL3/E4BP4 was initially cloned as a b-ZIP transcription factor that binds to the consensus sequence (G/A)T(G/T)A(C/T)GTAA(C/T) in the adenoviral E4 promoter DNA (Cowell *et al.* 1992). Further analysis revealed that E4BP4 functions as a transcription

repressor with its repression activity primarily located in its C-terminal region (Cowell & Hurst 1994) (Fig. 1). Although Northern blotting analysis indicates the *E4bp4* mRNA was ubiquitously expressed in various cell lines and mouse tissues, very low abundance of the E4BP4 protein was detected in most cases, suggesting that the E4BP4 protein level is likely controlled at both the translational and post-translational levels (Chen *et al.* 1995).

One of the best-known biological functions of E4BP4 is related to immunomodulation (Male *et al.* 2012). The *E4bp4* promoter was found to be activated by interleukin 3 in T lymphocytes, giving rise to its other name NF-IL3 (Zhang *et al.* 1995). Subsequently, multiple studies have illuminated the essential role of E4BP4 in NK cell development/maturation as well as the development of innate lymphoid cells (ILC) (Geiger *et al.* 2014). E4BP4 also functions as a key regulator of IL 3-dependent pro-B lymphocyte survival and immunoglobulin class switch (Ikushima *et al.* 1997). Moreover, E4BP4 was shown to

Figure 1

Schematic of E4BP4 functional domains. A full color version of this figure is available at <https://doi.org/10.1530/JME-20-0239>.

regulate cytokine production and the effector functions in a subsets of T lymphocytes (Kashiwada *et al.* 2011). Given its broad actions in multiple immune cells, it was speculated that E4BP4 might be relevant in human immune disorders. Indeed, E4BP4 has been reported to be implicated in the pathogenesis of IBD, MS, and systemic lupus erythematosus (Yin *et al.* 2017).

As a protein wearing multiple hats, E4BP4 has also been identified as a key circadian output oscillator in the molecular circadian clock system. In its core, the circadian clock comprises a transcription-translational feedback loop driven by key circadian proteins including BMAL1, CLOCK, PERIOD, CRY, and nuclear receptors (NR1D1 and RORs) (Partch *et al.* 2014). The *E4bp4* mRNA expression was shown to display a classical circadian oscillation pattern that was blunted in the *Bmal1*^{-/-} mice, supporting that *E4bp4* is a direct output gene of the molecular circadian clock (Chen *et al.* 2019b). E4BP4 was also found to interact with the core clock protein CRY2 in cultured cells (Ohno *et al.* 2007). However, deletion of *E4bp4* showed little impact on the oscillations of core clock genes. Most recently, the Lazar group has shown that the rhythmic expression of *E4bp4* was disrupted in Kupffer cells from the liver of adult hepatocyte-specific *Rev-erba* and *Rev-erbb* double knockout mice, highlighting an essential role of the hepatic clock in coordinating metabolic events in the liver (Guan *et al.* 2020). Given the extensive expression of E4BP4 in a variety of tissues, it is likely that E4BP4 may control a subset of circadian output genes in a tissue-specific manner.

E4BP4 in liver metabolism

Liver is the central organ in charge of metabolic homeostasis in mammals. During the cycles of food intake, liver turns on anabolic metabolism upon feeding and switches to catabolic metabolism during fasting (Rui 2014). This switch between different metabolic pathways is tightly controlled at both the transcriptional and post-translational levels. A panel of transcription factors sensitive to the nutrient status have been identified as critical regulators in maintaining metabolic homeostasis in the liver (Wang *et al.* 2016, Hatting *et al.* 2018).

For instance, SREBP-1c, FXR, and ChREBP have been identified as critical transcription factors in lipid and bile acid biosynthesis during feeding (Chiang 2013, Wang *et al.* 2015), whereas FOXO1, CREB, and PPAR α are required in gluconeogenesis and lipid oxidation during fasting (Rui 2014, Wang *et al.* 2016). Although the *E4bp4* expression was shown to be most abundant in the liver, the regulation of its mRNA by nutrients and hormone signaling has been largely uncharacterized. Our lab has discovered that refeeding induces the *E4bp4* mRNA and protein in the mouse liver (Tong *et al.* 2010). We also found that insulin potently induces *E4bp4* expression through the AKT/mTORC1/SREBP-1c pathway (Tong *et al.* 2016). Most recently, we reported that chemical or diet-induced ER stress potently induces hepatic E4BP4 to promote lipid accumulation via the suppression of AMPK pathway (Yang *et al.* 2020). Altogether, these data suggest that E4BP4 could be a critical player during anabolic metabolism.

E4BP4 in lipid metabolism

E4BP4 and FGF21-promoted fatty acid oxidation

During fasting, liver turns on lipolysis to break down triglycerides to release free fatty acids (FFAs) for fatty acid oxidation and ATP production inside the mitochondria (Rui 2014). The nuclear receptor PPAR α plays a dominant role in activating fatty acid oxidation genes in the liver during fasting (Bougarne *et al.* 2018). *Ppara*^{-/-} mice were shown to develop liver steatosis along with the suppression of FAO gene expression under food deprivation (Sugden *et al.* 2002, Lee *et al.* 2004, Bougarne *et al.* 2018). One of the PPAR α targets is FGF21, a hepatic hormone critical for energy mobilization during fasting (Badman *et al.* 2007, Inagaki *et al.* 2007). Once released from liver, FGF21 can stimulate adipocyte lipolysis in white and brown adipose tissues and increase the levels of FFAs in circulation. We observed that E4BP4 represses the *Fgf21* expression in a circadian fashion in hepatocytes. The *Fgf21* mRNA oscillations were anti-phase to those of *E4bp4* during a circadian cycle in the mouse liver (Tong *et al.* 2010). We also detected a drastic decrease in the level of FGF21 in the medium from primary mouse hepatocytes transduced with Ad-E4bp4. Our chromatin immunoprecipitation (CHIP) assay uncovered the direct binding of E4BP4 to the *Fgf21* promoter, consistent with its potent repression of the *Fgf21* expression in hepatocytes. We further confirmed the physiological role of E4BP4 as a suppressor of FGF21 in hepatocytes in response to insulin. Later on, we reported that G9a,

a histone methyl-transferase, is required to mediate the suppression of FGF21 by E4BP4 in hepatocytes (Tong *et al.* 2013). In contrast, CREBH, an ER-sensitive transcription factor, has been shown to facilitate the PPAR α -stimulated *Fgf21* expression in the liver during fasting (Zheng *et al.* 2016). The Zhang group reported that E4BP4 interacts with CREBH and antagonizes the CREBH-mediated activation of FGF21 in the liver (Zheng *et al.* 2016, Bhattacharya *et al.* 2018). Collectively, these results suggest that E4BP4 might contribute to catabolism of lipids by suppressing hepatic FGF21 in response to insulin during feeding.

E4BP4 and insulin-induced *de novo* lipogenesis

During food intake, liver activates anabolic lipid metabolism via insulin to upregulate *de novo* lipogenesis and cholesterol biosynthesis (Rui 2014). Once bound to the insulin receptor on hepatocytes, insulin activates the PI3K-AKT-mTORC1 signaling cascade. The Goldstein lab discovered that insulin potently induces the lipogenic master sterol regulatory element-binding protein 1 (SREBP-1) downstream of AKT-mTORC1, which in turn upregulates the mRNA levels of key enzymes for lipogenesis such as *Fasn*, *Acc1*, *Atp-cl*, and *Scd1* (Brown & Goldstein 1997, Horton *et al.* 2002). SREBP-1 is a member of the basic-helix-loop-helix (bHLH) leucine zipper transcription factors (Shimano 2001). SREBP-1 consists of both SREBP-1a and SREBP-1c isoforms. SREBP-1a is mainly expressed in the intestine and spleen, whereas SREBP-1c is the predominant isoform in the liver (Shimano 2001). The factors that regulate the SREBP-1c transcription, processing, and stability have been shown to impact the degree of hepatic lipid biosynthesis and diet-induced liver steatosis.

We recently reported that insulin stimulates both the mRNA and protein abundance of E4BP4 in mouse hepatocytes via the classical AKT-mTORC1-SREBP-1c pathway (Tong *et al.* 2016). *E4bp4*-deleted hepatocytes display a more than 50% reduction in the rate of *de novo* lipogenesis and the expression of lipogenic enzymes. We detected a marked reduction of nuclear SREBP-1c in the *E4bp4*-deleted primary mouse hepatocytes. Adenoviral overexpression of E4BP4 enhances the nuclear abundance of SREBP-1c in a feed-forward feedback loop. More interestingly, the pro-lipogenic action of E4BP4 relies on its ability to enhance the SREBP-1c acetylation via protein-protein interaction. These findings strongly indicate that hepatic E4BP4 is a prolipogenic factor downstream of the insulin signaling.

E4BP4 in glucose metabolism

Liver is also critical for maintaining glucose homeostasis (Rui 2014). After food intake, liver synthesizes glycogen and inhibits gluconeogenesis. In contrast, liver undergoes glycogenolysis and gluconeogenesis to maintain blood glucose level during fasting. These metabolic processes are tightly controlled by a panel of hormonal and nutritional signals (Rui 2014, Petersen *et al.* 2017). Insulin promotes glycogen synthesis mainly by stimulating the phosphorylation of GSK3 and inhibits gluconeogenesis by enhancing the FOXO1 phosphorylation (Lee & Dong 2017, Bergman *et al.* 2019). During fasting, the glucagon signaling activates the PKA-CREB pathway and triggers glycogenolysis and gluconeogenesis in the liver (Ravnskjaer *et al.* 2016). Many factors that influence either the insulin-AKT-FOXO axis or the glucagon-PKA-CREB signaling could impact liver glucose metabolism and consequently systemic glucose homeostasis.

Recently, the Koo group has reported the *in vivo* role of E4BP4 in glucose metabolism, suggesting that E4BP4 may down-regulate gluconeogenesis in the liver (Kang *et al.* 2017). They found that E4BP4 reduces glucose production and suppresses the gluconeogenic genes including *G6pase* and *Pepck* in part through competing with CREB. Ectopic expression of E4BP4 in the liver of *ob/ob* mice ameliorated hyperglycemia and glucose intolerance. On the flip side, acute depletion of *E4bp4* in the mouse liver elevated blood glucose and the expression of hepatic gluconeogenic genes. These findings are in line with our own unpublished data supporting the role of E4BP4 as an insulin-induced gene and the ability of E4BP4 to suppress the *Pgc-1 α* transcription.

So far, whether E4BP4 contributes to impaired glucose metabolism during obesity remains controversial. The Koo group found that the protein but not mRNA level of E4BP4 was reduced in the liver of *ob/ob* mice after 27-week high-fat diet feeding, possibly due to the impaired E4BP4 protein stability in the insulin-resistant mouse liver (Kang *et al.* 2017). K. Hofmann *et al.* found that streptozotocin (STZ)-induced diabetes did not affect the *E4bp4* mRNA level. Their data showed a loss of diurnal rhythm in the expression of *E4bp4* in spontaneous type 1 diabetic male rats (Hofmann *et al.* 2013). In addition, the Gimble group reported that the *E4BP4* mRNA level displays a positive correlation with BMI in the overweight young (age < 36 years) people (Wu *et al.* 2009). More research using a variety of animal models is needed to clarify the role of E4BP4 in glucose metabolism in the liver.

E4BP4 in bile acid metabolism

Liver is also the primary organ for the biosynthesis of bile acids from cholesterol. Conversion of cholesterol into bile acids requires a complex biosynthetic pathway involving up to 17 enzymatic steps (Chiang 2013). CYP7A1 represents the rate-limiting enzyme of the bile acid biosynthesis. One of the major regulators of bile acid metabolism is the bile acid-activated nuclear receptor farnesoid X receptor (FXR) (Urizar *et al.* 2000, Huang *et al.* 2006, Preidis *et al.* 2017). Extensive research from multiple groups has established the model by which FXR inhibits hepatic CYP7A1 expression. FXR was shown to indirectly suppress the *Cyp7a1* transcription by inducing both small heterodimer partner (SHP) in the liver and fibroblast growth factor-15 in the intestine (Goodwin *et al.* 2000, Inagaki *et al.* 2005, Huang *et al.* 2006, Shin & Osborne 2009, Kliewer & Mangelsdorf 2015, Byun *et al.* 2017, Preidis *et al.* 2017). In a luciferase reporter assay, E4BP4 overexpression potentially suppressed the *Cyp7A1* promoter-driven luciferase activity (Noshiro *et al.* 2007). Other studies have provided indirect evidence for E4BP4 as a physiological regulator of *Cyp7a1* expression and bile acid metabolism. In a mouse model with increased hypoxia signaling in the liver, elevated E4BP4 leads to the suppression of hepatic *Cyp7a1* (Ramakrishnan *et al.* 2014). In another study, E4BP4 was found to directly bind to the *Cyp7A1* promoter by ChIP assay (Yoshitane *et al.* 2019). As of now, whether E4BP4 is required for controlling the diurnal expression of hepatic *Cyp7A1* remains to be established.

E4BP4 in xenobiotic metabolism

Liver is the primary organ to carry out xenobiotic metabolism of drugs, pollutants, and toxins (Zhang *et al.* 2018). Xenobiotic metabolism depends on three types of enzymes: Phase I enzymes modify the substrates, Phase II enzymes conjugate the substrates, and Phase III enzymes excrete the end products. P450 (CYP) enzymes are the most abundant among Phase I enzymes responsible for clearing drugs in the liver. In the mouse liver, several cytochrome P450 enzymes including *Cyp17*, *Cyp2a4*, and *Cyp2e1* were shown to display a diurnal oscillation in their mRNA expression (Manikandan & Nagini 2018, Tornio & Backman 2018, Zhang *et al.* 2018, Chen *et al.* 2019a). E4BP4 was also reported to control the expression of *CYP3A4* (*Cyp3a11* in mice), one of the most important enzymes for drug metabolism and detoxification (Tong *et al.* 2019). Takiguchi *et al.* found

that in synchronized human hepatoma cells HepG2, the mRNA levels of *Cyp3A4*, *Dbp*, and *E4bp4* exhibited a 24-h oscillation (Takiguchi *et al.* 2007). They also found that DBP overexpression activates the promoter of *Cyp3A4*, whereas E4BP4 does the opposite in an *in vitro* luciferase reporter assay. Moreover, the CYP3A4 protein and activity were found to increase in the liver of *E4bp4*^{-/-} mice, resulting in altered pharmacokinetics of Midazolam (Takiguchi *et al.* 2007).

Carboxylesterases (CES) are a family of Phase I enzymes that play an important role in xenobiotic clearance and lipid metabolism. One of CES proteins, CES2, was found to be reduced in the liver of *E4bp4*^{-/-} mice. This reduction led to slowed metabolism of its main substrate CPT-11 (irinotecan) (Zhao *et al.* 2018). Flavin-containing monooxygenase 5 (FMO5) is another important Phase I enzyme in xenobiotic metabolism in the liver. Hepatic Fmo5 expression was found to be upregulated and its circadian rhythm was attenuated in *E4bp4*^{-/-} mice. All these findings support E4BP4 as a novel regulator of the pharmacokinetics of detoxification enzymes. Identification of the underlying mechanisms could lead to a better understanding of how the molecular circadian clock system influences drug metabolism in the liver (Chen *et al.* 2019b).

E4BP4 in obesity and metabolic syndrome

The ever-increasing prevalence of obesity leads to type 2 diabetes, cardiovascular-renal complications, and many types of cancers worldwide. Obesity is likely to be a result of both altered lifestyles and genetic susceptibilities. Nowadays, circadian disruption/misalignment has garnered a lot of attention as one of the major contributors to metabolic dysfunction and obesity (Rana *et al.* 2003, Zvonic *et al.* 2006, Wu *et al.* 2009). Several studies have found that high-fat diet feeding dampens the amplitude of circadian genes while altering the period of molecular clock system in multiple tissues of rodents (Kohsaka *et al.* 2007, Kaneko *et al.* 2009, Hatori *et al.* 2012). Specifically, the circadian oscillations of *Dbp* and *E4bp4*, two direct circadian output genes, were largely lost in both the liver and kidney of those high-fat diet-fed mice (Hsieh *et al.* 2010).

So far, whether E4BP4 could be involved in metabolic dysfunction after high-fat diet feeding remains largely undetermined. Several studies suggest that E4BP4 could regulate obesity in a tissue-specific manner. The Lee group reported that E4BP4 is required for the accumulation of NK cells in the adipose tissue upon high-fat diet feeding

Figure 2

Overview of metabolic actions of E4BP4 in the liver. Hepatic E4BP4 expression and activity are likely to be sensitive to the cycle of fasting/refeeding, hormones, stress signals, and the circadian clock. Hepatic E4BP4 regulates lipid metabolism, glucose metabolism, bile acid metabolism, and xenobiotic metabolism to impact the whole body homeostasis. A full color version of this figure is available at <https://doi.org/10.1530/JME-20-0239>.

(Lee *et al.* 2016). *E4bp4*^{-/-} mice were shown to be resistant to diet-induced adipose inflammation along with improved insulin resistance (Lee *et al.* 2016). The Hoover group discovered that E4BP4 in intestinal epithelial cells controls the expression of a circadian lipid metabolic program and regulates lipid absorption and export. Intriguingly, they found that microbiota regulates body composition by modulating the circadian oscillations of *E4bp4* within enterocytes (Wang *et al.* 2017). In our most recent study, we discovered that both ER stress inducer tunicamycin and high-fat low methionine and choline-deficient (HFLMCD) diet induce E4BP4 and promote lipid accumulation in the liver. Using hepatocyte-specific *E4bp4* knockout mice, we have found that loss of hepatic *E4bp4* protects mice against HFLMCD diet-induced liver steatosis and hepatocyte injury. Mechanistically, we show that *E4bp4* is most likely to promote lipid droplet formation by suppressing the AMPK pathway in hepatocytes (Z Zhao, L Yin, F Wu & X Tong, unpublished observations).

Conclusion and future directions

In summary, recent findings have highlighted the novel role of E4BP4 in regulating metabolism and metabolic diseases, supporting that E4BP4 could be an important regulator of glucose, triglycerides, cholesterol, and xenobiotic metabolism in the liver. It has become increasingly clear that E4BP4 impacts various metabolic pathways via its specific regulation of a subset of genes in different tissues. Given the complexity of its functions,

E4BP4 can either repress or activate the gene expression of metabolic pathways through completely different mechanisms (Fig. 2). Currently, the biochemical basis for this functional switch remains unknown. Therefore, an in-depth understanding of how E4BP4 controls the expression of its targets in different tissues is crucial to identifying unique drug targets for the prevention and treatment metabolic disorders such as diabetes and NAFLD.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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