bZIP transmembrane transcription factor CREBH: Potential role in non-alcoholic fatty liver disease (Review)

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Abstract. The cyclic adenosine monophosphate (cAMP)-responsive element-binding protein H (CREBH) is a transcription factor localized to the endoplasmic reticulum (ER) membrane. Previous studies have demonstrated that CREBH is activated by ER stress, hepatic glucose and lipid metabolism signaling, and inflammation. Thus, it may be critical in the regulation of various physiological functions associated with the development of non-alcoholic fatty liver disease (NAFLD), which results from inflammation and disorder of hepatic glucose and lipid metabolism. Therefore, CREBH may have potential as a pharmacological target for NAFLD. This review summarizes recent scientific developments and the biological actions of CREBH with a particular focus on its involvement in NAFLD.

Contents

1. Introduction

The endoplasmic reticulum (ER) is important in maintaining intracellular calcium stores, steroid and lipid biosynthesis, membrane regeneration, gluconeogenesis and the folding and assembly of newly synthesized proteins (1,2). Accumulation of misfolded or unfolded proteins in the ER induces ER stress and results in the unfolded protein response (UPR). Activation of the UPR is primarily a protective mechanism for cells under stress, that results in the degradation of unfolded proteins accumulated in the ER by ER-associated degradation. The UPR system involves three major pathways dependent on inositol-requiring enzyme-1, activating transcription factor 6 (ATF6) and double-stranded RNA-dependent protein kinase-like endoplasmic reticulum kinase to accelerate unfolded protein degradation, increase the synthesis of chaperones and other proteins required for processing and suppress the synthesis of new proteins, respectively (3). UPR has been observed to be activated in numerous liver diseases, such as chronic viral hepatitis (4), insulin resistance (5), alcoholic liver disease (6), ischemia-reperfusion injury (7,8) and acute liver toxin insults (9). However, when these responses are activated to a high level and/or persistently, cells or organs undergo ER stress-induced injury (10-12).

The cyclic adenosine monophosphate (cAMP)-responsive element-binding protein H (CREBH), encoded by the CREB3L3 gene, is a type of ER-residing transcription factor that has a region of high sequence similarity with ATF6. It belongs to the CREB/ATF family (13), which also includes the following proteins: cAMP-responsive element-binding protein 3 (CREB3) also known as Luman or LZIP (14,15); cAMP-responsive element binding protein 3-like 1 (CREB3L1) also known as OASIS (16); cAMP-responsive element binding protein 3-like 2 (CREB3L2) also known as BBF2H7 (17); and cAMP-responsive element binding protein 3-like 4 (CREB3L4) also known as CREB4, AIBZIP or Tisp40 (18,19). Although CREBH is robustly expressed in the liver, it is also expressed at lower levels in the small intestine and stomach (13,20). CREBH contains an ER transmembrane domain, a transcription-activation domain and a basic leucine zipper (bZIP) domain. In response to ER stress, CREBH is activated by regulated intramembrane proteolysis and translocates from the ER to the Golgi apparatus, where it is cleaved by the site-specific proteases, site-1 protease and site-2 protease, releasing bZIP (13). This forms the transcriptionally active form of CREBH, CREBH(N), which translocates to the nucleus (21,22), similar to sterol regulatory element-binding transcription factor 1 (SREBP) and ATF6 (23,24). Chan *et al* (25) observed that CREBH was modified at three N-linked glycosylation sites in the luminal domain. Disruption of all three sites by site-directed mutagenesis abrogated

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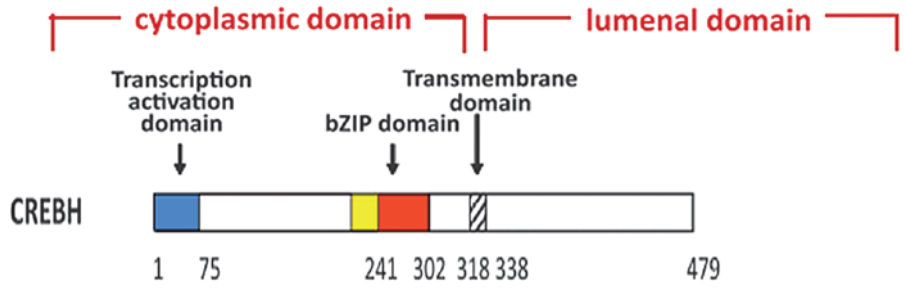

Figure 1. Protein structure of CREBH. CREBH has a transmembrane domain, a bZIP domain and a transcription activation domain. Adjacent to the N-terminal end of the bZIP region, ~30 amino acids are conserved in CREBH and this is termed the ATB domain (indicated by the yellow box). CREBH, cyclic adenosine monophosphate-responsive element-binding protein H; bZIP, basic leucine zipper.

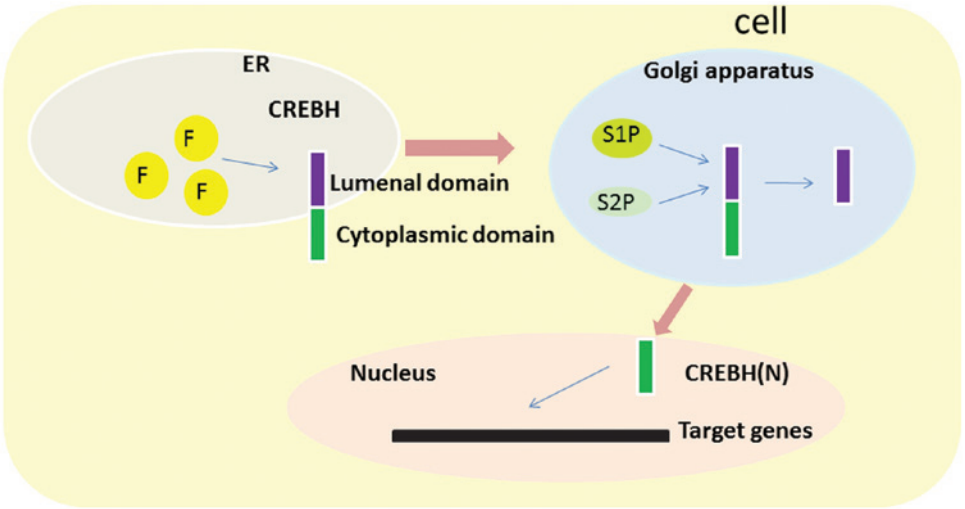

Figure 2. CREBH processing. Upstream factors activate CREBH in the ER. CREBH translocates to the Golgi apparatus and cleaved by S1P and S2P. The cleaved N-terminal fragment of CREBH, CREBH(N), translocates to the nucleus and regulates the expression of target genes. CREBH, cyclic adenosine monophosphate-responsive element-binding protein H; ER, endoplasmic reticulum; S1P, site-1 protease; S2P, site-2 protease; F, upstream factors.

N-linked glycosylation of CREBH, and it was observed that N-linked glycosylation is necessary for the activation of CREBH via intramembrane proteolysis (25). Unglycosylated CREBH is largely uncleaved and retained in an inactive form in the ER even if treated with an activator of ER stress and the UPR, such as brefeldin A, which induces proteolytic activation of CREBH (25). Unglycosylated CREBH is less capable of activating the transcription driven by the UPR element (25). Furthermore, Barbosa *et al* (26) reported that the CREB3 class of proteins has a highly conserved region designated the ATB domain, immediately adjacent to the N-terminal end of the bZIP region and ~30 residues in length. It is observed only in the CREB3 class of proteins, however not in ATF6. The conserved ATB domain is required for CREBH in the context of physiological activation of target secretory pathway genes in human tissue culture cells and in *Drosophila* embryos (Figs. 1 and 2) (26).

Previous studies have demonstrated that following translocation to the nucleus, CREBH(N) binds to the CREBH-responsive element (CRE), box B and ATF6-binding element (13,27,28) in the promoter of target genes involved in the regulation of the hepatic iron metabolism (27,29), gluconeogenesis (30-32), lipid metabolism (33,34) and inflammation (22,35), and the pathophysiological procedure of NAFLD (33) and associated conditions. The present review summarizes physiological functions of CREBH in NAFLD development and progression.

2. Regulation of CREBH expression

CREBH is activated by ER stress. An initial study demonstrated that CREBH expression was upregulated and the levels of CREBH(N) increased when cells were treated with ER stress inducers such as tunicamycin (TM), thapsigargin and dithiothreitol (22). However, subsequent studies did not observe the proteolytic activation of CREBH by ER stress inducer (21,25,36). Xu *et al* (36) demonstrated that TM had no effect on CREBH mRNA expression levels and suppressed the processing of CREBH to CREBH(N) *in vitro* and *in vivo*, suggesting that CREBH processing is not increased by chemical ER stress-inducers. As mentioned above, the activation of CREBH via intramembrane proteolysis requires N-linked glycosylation; TM treatment inhibits CREBH glycosylation, thus, it is likely to result in the degradation of the unglycosylated CREBH protein (21). However, CREBH has been demonstrated to regulate the expression of hepcidin and proinflammatory and acute phase response genes, thus, linking ER stress to inflammation and iron metabolism (20,27,37) and

suggesting that the activation of CREBH in the context of ER stress is dependent on the specific agent and experimental conditions utilized. It remains to be determined whether CREBH processing is regulated under any physiologically relevant conditions in the liver.

CREBH is regulated by cannabinoid receptor type 1 (CB1R) signaling. Previous studies investigating the underlying mechanisms of CB1R signaling in the regulation of hepatic gluconeogenesis demonstrated that CREBH is a downstream target gene of CB1R signaling, and is key in regulating hepatic gluconeogenesis (31), disrupting hepatic insulin receptor signaling (32) and mediating alcohol-induced regulation of bile acid enzyme gene expression (38). Following treatment with AM251 (a CB1R-specific antagonist), CB1R-mediated induction, by 2-AG (a CB1R agonist) of CREBH was reversed in rat hepatocytes (31). By pretreating primary hepatocytes with multiple specific inhibitors of cell signaling pathways prior to 2-AG treatment, Chanda *et al* (31) observed that CB1R signaling induces CREBH gene expression via the extracellular signal-regulated kinase 1/2 and c-Jun N-terminal kinase (JNK) pathways. Furthermore, it was also demonstrated that an activator protein 1 binding site renders 2-AG responsive to the CREBH promoter. Administration of 2-AG to cells with multiple serial deletion constructs of the CREBH promoter demonstrated a kinase-dead mutant of c-Jun (c-Jun KD; S63/73A) cotransfection significantly inhibited 2-AG-mediated activation of the CREBH gene promoter (31). Chanda *et al* (38) additionally observed that JNK phosphorylation and CREBH activation were significantly reduced in CB1R knockout mice challenged with alcohol. Overall, these data suggest that the CB1R is a critical component for induction of CREBH via the JNK signaling transduction pathway.

CREBH is regulated by estrogen-related receptor-γ (ERRγ). The orphan nuclear receptor, ERRγ is a constitutively active transcription factor that regulates genes involved in the hepatic glucose metabolism, alcohol metabolism and the ER stress response (39-41). Misra *et al* (35) reported that ERRγ directly regulated CREBH gene expression in response to ER stress by binding to the ERRγ response element in the CREBH promoter. Overexpression or knockdown of ERRγ significantly increased or reduced, respectively, the expression of CREBH and C-reactive protein (CRP) (35). It also demonstrated the transcriptional coactivator, peroxisome proliferator-activated receptor γ coactivator 1-α (PGC1α) was required for ERRγ-mediated induction of the CREBH gene by binding the CREBH promoter with ERRγ. The two proteins increased template-associated H3 and histone H4 acetylation to facilitate CREBH gene transcription (35). Previous studies have demonstrated that SHP-interacting leucine zipper protein (SMILE) inhibited the transactivation of ERRγ by competition with transcriptional co-activator, PGC1α (42,43). SMILE belongs to the bZIP family (44,45), and the gene produces two isoforms, SMILE-L (long isoform, also termed CREBZF) and SMILE-S (short isoform, previously termed Zhangfei) (45). Misra *et al* (46) reported that curcumin and SMILE significantly inhibit the transcriptional activity of CREBH, however did not repress ATF6 transactivity. Following knockdown of endogenous SMILE, curcumin no longer inhibited the transcriptional activity of CREBH on the reporter gene, indicating that SMILE was a repressor of the transcription factor, CREBH45. They also demonstrated that SMILE interacted with CREBH via its bZIP domain, without being homodimerized, and competed with PGC1α to inhibit CREBH transcriptional activity, similar to ERRγ (46).

CREBH is regulated by nutrition. Hepatic CREBH is activated in a fasting state and markedly suppressed following refeeding (36). Long-term intake of a high-fat diet impairs the fasting/refeeding regulation (33) and these nutritional alterations in CREBH expression levels were markedly associated with plasma levels of free fatty acids (FFAs). Danno *et al* (47) demonstrated that CREBH expression increased in primary hepatocytes with the administration of various fatty acids (FAs). This is in agreement with the observations of Gentile *et al* (48), who also demonstrated that FAs upregulate CREBH via the activation of gene transcription. This processing is blocked by inhibitors of proteasome activity, suggesting that the upregulation of CREBH mRNA expression levels by FAs requires proteasome activity. Notably, insulin was indicated to prevent FFA-mediated upregulation of CREBH and the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitors affected the insulin-mediated suppression of the upregulation of CREBH47 by FFAs. This suggests that insulin and PI3K signaling are key determinants of the FFA-mediated regulation of CREBH in liver cells. The present review hypothesized that the suppression of CREBH mRNA expression levels in the fed state may be due to postprandial hyperinsulinemia. Glucocorticoids promote gluconeogenesis in an antagonistic effect to insulin by binding to the glucocorticoid receptor (GR) (49). Notably, dexamethasone, a synthetic corticosteroid, induces CREBH gene transcription by activating the binding of GR to the glucocorticoid transcriptional response element in the proximal promoter region (30). In addition, Danno *et al* (47) observed that the CREBH gene promoter contains a peroxisome proliferator responsive element for peroxisome proliferator-activated receptor (PPAR)α transactivation and indicated that administration of fenofibrate, a PPARα agonist, increased CREBH expression. This induction was blocked by the further addition of a PPARα inhibitor, MK886, suggesting that CREBH was regulated by PPARα. As FAs are endogenous ligands for PPARα, PPARα is key in the adaptive response to fasting and increased concentrations of FAs, which may partially explain CREBH activation by fasting and FAs. However, another study (48) observed that the regulation of CREBH mRNA expression levels by FAs did not involve PPARα signaling. Thus, it remains to be elucidated whether PPARα is essential for FA- and fasting-induced upregulation of CREBH mRNA and protein expression levels. Toll-like receptor 4 (TLR4), similarly to PPARα, has emerged as an important mediator of the biological effects of FAs (50,51). In H4IIE liver cells incubated with lipopolysaccharide (LPS), which activates TLR4, CREBH mRNA expression levels were observed to be significantly increased, suggesting that TLR4 signaling is also involved in the regulation of CREBH mRNA expression (48). Via TLR4-dependent pathways, gut microbiota, which increase serum LPS, have been suggested to be

involved in the pathogenesis of NAFLD, due to the induction of liver inflammation (52).

CREBH is regulated by hepatocyte nuclear factor 4α (HNF4α). PPARα has also been demonstrated to be regulated by HNF4α (53), which is critical in hepatocyte differentiation and liver function (54). Luebke-Wheeler *et al* (20) demonstrated that HNF-4α regulated CREBH directly via binding an HNF4α recognition element site lying 3.7 kb upstream of CREBH exon 1, and that HNF4α was important for the expression of CREBH in the liver, however not the small intestine (20). A possible explanation is that intestinal transcription factors, such as the HNF4γ protein that is predominantly expressed in the gut, regulate CREBH with no requirement for HNF4α. Further research to elucidate this disparity in expression is required.

3. CREBH and hepatic lipid metabolism

CREBH is a transcription factor that is key in the regulation of hepatic lipid accumulation. Following feeding with an atherogenic high-fat (AHF) diet, greater accumulation of hepatic lipid contents, such as hepatic triglycerides (TGs), plasma cholesterol, high-density lipoproteins, and low-density lipoproteins and an increase in plasma TG was observed in CREBH knockout mice than in control wild-type (WT) mice. Notably, the levels of plasma TG increased when feeding with a normal chow diet (33). To further investigate the involvement of CREBH in maintaining lipid homeostasis, CREBH null and WT control mice were fasted for 16 h, and it was observed that the levels of plasma TG in the CREBH null mice were markedly increased compared with those of the control mice. Under nutrient starvation, a condition that stimulates lipolysis, the levels of ketone, 3-hydroxybutyric acid, a product of FA oxidation in the plasma, were slightly reduced in the CREBH-null mice, compared with the control mice, suggesting that CREBH deletion leads to a defect in TG lipolysis resulting in higher levels of plasma TG (33). Zhang *et al* (33) and Lee *et al* (34) demonstrated that plasma TG levels were significantly lower in CREBH(N) overexpression mediated by adenoviral infection or transgenic mice than in WT littermates. Zhang *et al* (33) demonstrated adenoviral delivery of activated CREBH resulted in a major increase in hepatic TGs.

CREBH regulates hepatic lipid accumulation predominantly by impacting the genes encoding the key enzymes involved in lipid metabolism. Gene microarray analysis and quantitative polymerase chain reaction analysis indicated that the deletion of CREBH in the liver reduced expression levels of five groups of genes involved in lipid metabolism, including lipogenic regulators, TG synthesis enzymes, enzymes or regulators in lipolysis and lipid transport, FA elongation enzymes and FA oxidation or cholesterol biosynthesis enzymes (33). Decreased expression levels of the lipogenic regulators and TG synthesis enzymes resulted in reduced *de novo* lipogenesis, and reduced expression levels of enzymes or regulators involved in FA elongation. Oxidation or cholesterol biosynthesis may be responsible for abnormal accumulation of hepatic lipid metabolites, and defective expression of enzymes required for lipolysis and lipid transport may account for hypertriglyceridemia, reduced fat mass and body weight gain, and massive steatosis in the CREBH null mice fed the AHF diet. The genes involved in regulation of CREBH in lipid metabolism in the liver include apolipoprotein (APO)C2, APOA4, APOA5, APOC3, fibroblast growth factor 21 (FGF21), fat-specific protein 27 (FSP27) and lipin 132 (34). APOC3 is a lipoprotein lipase (LPL) inhibitor, while APOA5 and APOC2 activate LPL. The activity of LPL is further increased by APOA4, facilitating the delivery of hydrolyzed FAs to peripheral cells, thus, lowering plasma levels of TG (55,56). Patients with genetic defects in APOC2, APOA5 or LPL have high circulating TG levels due to impaired clearance (56-59). FGF21 is predominantly produced by the liver (60). Studies indicate that its concentration is correlated with hypertriglyceridemia, hepatic steatosis and insulin resistance (61-63). FSP27 is a lipid droplet (LD)-associated protein that promotes LD growth and TG storage in white adipocytes; it is also highly expressed in the steatotic liver and contributes to TG accumulation (64-66). Lipin 1 is a cytosolic phosphatidic acid phosphatase that generates diacylglycerol (DAG) in response to an increased intracellular FFA level, and is also crucial in lipid metabolism in the liver (67). CREBH regulates the transcriptional activation of APOC2, APOA4, APOA5, FGF21, FSP27 and lipin 1 directly by binding the CRE binding motifs in their gene promoters (32,34,36,68-70). It remains unclear whether the APOC3 promoter contains a CRE binding site, however, the very low density lipoprotein (VLDL)-associated APOC3 expression level is markedly higher in CREBH$^{-/-}$ mice compared with WT mice, with no significant alterations in mRNA expression level (34), suggesting post-transcriptional control of APOC3 by CREBH. Therefore, CREBH has a crucial role in the maintenance of hepatic TG homeostasis. Multiple nonsynonymous mutations in CREBH, including W46X, 245fs, E240K, V180M, G105R and P166L that produced nonfunctional or hypomorphic CREBH protein were identified in patients with extreme hypertriglyceridemia (34), further demonstrating a critical role for CREBH in the human TG metabolism. The identification of CREBH as a stress-induced metabolic regulator has important implications in the understanding and treatment of metabolic diseases.

4. CREBH and hepatic glucose metabolism

Phosphoenolpyruvate carboxykinase (PEPCK-C) and glucose-6-phosphatase (G6Pase) are important in the regulation of gluconeogenesis (71,72). CREBH(N) has been demonstrated to increase PEPCK-C and G6Pase transcription by binding CRE in the promoters of these genes via a CREB/CREB-regulated transcriptional coactivator 2 (CRTC2)-dependent manner (30). Notably, knockdown of hepatic CREBH by small hairpin RNA results in reduced hepatic glucose production by suppressing PEPCK-C and G6Pase expression (30). Chanda *et al* (31) observed that CB1R signaling mediated by CREBH induced hepatic gluconeogenesis in primary hepatocytes; and the knockdown of CREBH attenuated the CB1R signaling-mediated upregulation of hepatic gluconeogenesis.

In addition, CREBH serves a potential role in the regulation of insulin resistance (INR). Notably, iron excess has been

associated with reduced insulin sensitivity and with disease progression, whereas iron removal has been demonstrated to be beneficial (73-75). Iron metabolism is controlled by hepcidin, which is a downstream target gene of CREBH (27). Thus, CREBH has a critical association with insulin sensitivity. Hepatic CB1R and CREBH gene expression levels are higher in various models of insulin resistance (30,76). Chanda *et al* (32) demonstrated that CB1R-mediated activation of CREBH increased DAG production and phosphorylation of protein kinase C ε type, which induces INR effects via disrupting the insulin receptor signaling pathway. CB1R activation under CREBH-deficient conditions failed to induce DAG production, ultimately leading to the recovery of insulin receptor signaling component activity. Therefore, the suppression of CB1R/CREBH signaling may reduce INR. However, the study by Chanda *et al* (32) did not directly assess insulin resistance in mice, such as with an insulin tolerance test. As mentioned above, fenofibrate, a PPARα agonist, has been demonstrated to reduce plasma glucose and insulin levels and to increase insulin sensitivity mediated by CREBH (77). By contrast, Zhang *et al* (33) observed that CREBH knockout mice are less responsive to insulin than WT mice, which does not support the hypothesis that targeted inhibition of CREBH would be useful for improving insulin resistance. Therefore, the function of CREBH in controlling insulin sensitivity remains to be determined, however, these findings in rodent and human primary hepatocytes indicate that CREBH is important in the hepatic glucose metabolism.

5. CREBH and inflammation

CREBH is crucial in the activation of an acute inflammatory response. The acute phase response (APR) is systemic inflammatory component of innate immunity, which is an ancient metazoan adaptation mechanism initiated by chemical structures presented by invading microorganisms or exposed by damage to the host (78-80). Zhang *et al* (22) demonstrated that the expression levels of C-reactive protein (CRP) and serum amyloid P-component, whose synthesis occurs predominantly in APR, were significantly reduced in the fetal livers of CREBH knockdown mice compared with the RNAi control mice. It was also observed that CREBH and ATF6 interact and bind to the same conserved element in the promoter of CRP to synergistically activate expression upon ER stress, suggesting that CREBH was required to activate the APR. In addition, in line with other studies by Zhang *et al* (22) and Misra *et al* (35) it was demonstrated that ERRγ increased CRP promoter activation via regulating CREBH. CREBH expression was also observed to be induced by ER stress and proinflammatory cytokines, including interleukin (IL)6, IL1β or tumor necrosis factor α (TNFα), in hepatoma cells and in the liver. Notably, in ER stress, cleavage of CREBH, and APR induced by proinflammatory cytokines, occurred in the liver, but not in hepatoma cell lines; suggesting this is impaired and not comparable to that in the liver *in vivo* (22). Furthermore, hepcidin, another APR protein, is important in the anemia of inflammation (81) and is also transcriptionally regulated by CREBH (27,29). In addition, mRNA expression levels of other major APR proteins, including serum amyloid A1, A2 and A3, fibrinogen and α1-acid glycoprotein in CREBH knockdown fetal livers, were similar to those in the control fetal livers, suggesting that CREBH is not required for induction of all APR genes (22).

6. Roles of CREBH on NAFLD

NAFLD is defined as a pathological accumulation of fat in the form of TG in the liver, not as a result of alcohol consumption (82). It is an inclusive term that includes a spectrum of liver pathologies from simple steatosis to non-alcoholic steatohepatitis (NASH). The liver is important in the lipid metabolism, including importing and manufacturing FFAs, storing TGs in LDs and exporting lipids as VLDL to the serum. Alterations in any of these processes may result in the development of NAFLD (83). Donnelly *et al* (84) observed that under pathophysiological conditions, ~60% of hepatic TGs derive from FFAs from adipose tissues, 26% from *de novo* lipogenesis and 15% from the diet. FFAs derived from adipose tissues and *de novo* lipogenesis are termed non-esterified fatty acids. The excess of FFAs stimulates TG synthesis. The TGs are stored as LDs within hepatocytes, or lipidated by apolipoprotein B100 within the lumen of ER and subsequently secreted into the blood as VLDLs (85) via the Golgi apparatus (86). Therefore, an excess of FFAs and TG, or impaired VLDL assembly or secretion, may result in excessive lipid accumulation in the liver. Accumulation of lipids in the liver further stimulates existing hepatic INR by generation of lipid-derived secondary messengers, such as DAG and ceramides (87). Furthermore, lipid accumulation in the liver is also linked with the progression of ER stress, mitochondria stress and impaired autophagy, resulting in the condition termed lipotoxicity (88). This latter event may result in an immune response by the Kupffer cells and hepatic stellate cells, which leads to the progression of NASH, hepatic cirrhosis, and in certain severe cases, hepatocellular carcinoma (89).

In vivo data indicates that in metabolic syndrome disorders, such as NAFLD, levels of ER stress markers in the liver and other tissues are increased, and liver damage occurs (80-93). CREBH is an ER-bound transcription factor activated by ER stress and it is a key metabolic regulator required to activate expression of the genes involved in *de novo* lipogenesis, TG and cholesterol biosynthesis, FA elongation and oxidation, lipolysis and lipid transport in response to ER stress (33). Previous studies have demonstrated that fasting- and high fat diet (HFD)-induced fatty liver was more pronounced in CREBH$^{-/-}$ mice compared with the WT mice (33,70), while adenoviral delivery of CREBH inhibited HFD-induced steatosis in WT mice (77). In addition, NAFLD is associated with inflammation and fibrosis, increased hepatocyte ballooning, lobular and portal inflammation, Mallory bodies and collagen deposition were observed in CREBH null mice following the AHF diet (33). A histological scoring system for NAFLD was used (94,95), demonstrating that the CREBH null mice developed profound NASH following the AHF diet. Increased levels of the key indicators of hepatotoxicity were also observed, including alanine aminotransferase and aspartate aminotransferase, and NASH-associated pro-inflammatory cytokines (96), such as TNFα and IL6 (33). Notably, fasting induced hepatic steatosis and an increase in

CREBH expression levels (36), however, increased expression levels of CREBH maintains lipid homeostasis by regulating expression of the genes involved in the lipid metabolism (33). CREBH also inhibited HFD-induced steatosis in WT mice (77), suggesting there is a negative feedback control mechanism in the involvement of CREBH in the development of NAFLD.

CREBH is suggested to be involved in the development of NAFLD by regulating the lipid metabolism and maintaining insulin sensitivity. Accumulation of lipids in the liver stimulates existing hepatic insulin resistance, which further stimulates hepatic SREBP-1c production, resulting in increased *de novo* synthesis of fatty acids (97). As mentioned above, CREBH regulates insulin sensitivity by regulating hepcidin to control iron levels and mediating CB1R to disrupt hepatic insulin receptor signaling. Fenofibrate has been demonstrated to reduce plasma glucose and insulin levels and increase the insulin sensitivity mediated by CREBH (77). In addition, CREBH prevents insulin-induced SREBP-1c expression by markedly increasing the promoter activity of insulin induced gene 2, whose downregulation mediates insulin-stimulated transcriptional activity of SREBP-1c (77). The previous studies demonstrated that CREBH is key in the involvement of NAFLD by regulating insulin sensitivity.

As mentioned above, CREBH is also associated with inflammation in the liver (22,35). The development of NAFLD involves insulin resistance and increased inflammation. Furthermore, inflammation in the development of NASH further impedes insulin signaling (98). Cytokine production of IL-6 and TNF-α is increased in NASH and may be involved in its pathogenesis (99). CREBH expression and cleavage may be induced by IL-6 and TNF-α to activate the APR (22), thus, the present review hypothesized that CREBH is a protective factor in response to inflammation by maintaining the balance of glucose and lipid metabolism by mediating gene expression in the liver for the two processes. In addition, TLR4 is activated by LPS or gut microbiota, which is also associated with NAFLD. The activated TLR4 increases hepatic expression levels of TNF-α and increases hepatic steatosis and inflammation during the development of NAFLD (52). CREBH is regulated by TLR4 (48), suggesting that CREBH may be activated by TLR4 and is, thus, involved in the development of NAFLD. The biological relevance of this regulation remains to be elucidated but CREBH may provide a potential therapeutic target in NAFLD.

7. Conclusions

Various studies have elucidated the roles of CREBH. Via the regulation by ER stress, CB1R signaling, ERRγ, SMILE, nutrition, PPARα and HNF4α, CREBH promotes the genes encoding lipogenic regulators, TG synthesis enzymes, enzymes or regulators in lipolysis and lipid transport, FA elongation enzymes and FA oxidation or cholesterol biosynthesis enzymes to regulate hepatic lipid metabolism. By binding CRE in the promoter of PEPCK-C and G6Pase, it is involved in hepatic glucose metabolism in a CRTC2-dependent manner. In addition, CREBH regulates iron metabolism and mediates CB1R signaling, thereby regulating insulin sensitivity. Furthermore, CREBH is crucial in the activation of an acute inflammatory response. As inflammation and disorders of glucose and lipid metabolism are the predominant factors in NAFLD, targeting CREBH appears to be a promising effective therapeutic strategy.

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