Circadian clock control of hepatic lipid metabolism: role of small heterodimer partner (Shp)

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ABSTRACT

Hepatic steatosis, the accumulation of triglyceride droplets in the hepatocytes, is a common hepatic pathology seen in subjects with obesity/metabolic syndrome and those with excessive alcohol use. The pathogenesis underlying hepatic steatosis is complex. Recent studies have shown the specific role played by the molecular clock mechanism in the control of lipid metabolism and that the disruption of these tissue clocks may lead to the disturbances in lipid homeostasis. This review reports a novel role of small heterodimer partner in maintaining triglyceride and lipoprotein homeostasis through neuronal PAS domain protein 2.

INTRODUCTION

Circadian regulation and its regulation in cellular metabolism

The cellular metabolism is under the tight control of a cell-autonomous circadian clock. The clock controls and drives gene and protein expression in a rhythmic fashion, which in turn affects the time-of-day regulation of glucose, bile acid and lipid metabolism.[1–3] The molecular clock acts as a self-sustainable pacemaker generating the rhythmicity over the 24-hour period. It consists of an input pathway by environmental cues and the output mechanisms that control cellular physiological and biochemical processes.[4] The master circadian oscillator is located in the hypothalamic suprachiasmatic nucleus; however, self-sustaining clocks are also found in the peripheral tissues.[5 6] The circadian clock is consisted of a series of autoregulatory transcriptional translational feedback loops (TTFLs): a positive loop comprising the heterodimerization of neuronal PAS domain protein 2 (NPAS2), bHLH-PAS proteins brain and muscle ARNT-like protein1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK) and a negative loop consisting of *cryptochrome* (*cry*) and *period* (*per*) genes.[5] The TTFLs act through E-box regulatory elements in their target genes and an interconnecting loop that consists of REV-ERB*α*/*β* *and* retinoic acid-related orphan nuclear receptor (ROR)*α*/*β*/*γ*. REV-ERB*α*/*β* *and* ROR*α*/*β*/*γ* *control the transcription processes by acting on the ROR elements in clock/Npas2/clock and Bmal1 gene promoters*.[5] Clock output, a critical aspect of the circadian system, subsequently generates the rhythmic regulation of enzymes and hormones over the 24-hour period.[5] A common hepatic pathology seen in patients with excessive alcohol use and those with obesity/metabolic syndrome is hepatic steatosis.[7–9] The accumulation of triglyceride droplets in the hepatocytes is a complex process resulting from the imbalance between fatty acid synthesis and oxidation. Alcohol can inhibit mitochondrial fatty acid β-oxidation through the changes in the redox state.[9] It, directly or indirectly, regulates transcription factors that are involved in fatty acid oxidation (peroxisome proliferator-activated receptor α) and fatty acid synthesis (sterol regulatory element-binding protein 1c, SREBP-1c), leading to the inhibition of fatty acid oxidation and increasing in lipogenesis.[9] In non-alcoholic fatty liver disease, the increase in intrahepatic lipogenesis through the activation of SREBP-1c has been found to be related to the induction of endoplasmic reticulum (ER) stress response and high levels of circulating tumor necrotic factor α.[9–11] As a result, genes regulating lipid syntheses which are under the control of these transcription factors, such as *fatty acid synthase* (*Fas*), *acetyl coA carboxylase* (*ACC*) and *3-hydroxy-3-methylglutaryl-coA reductase* (*hmgcr*), in cholesterol synthesis are disturbed.[9 12] In addition to alteration in lipid metabolism, bile acid synthesis is also impaired in patients with alcoholic and non-alcoholic fatty liver diseases.[4 13–16]

During chronic alcohol feeding, the levels of *clock* and *Bmal1 did not differ in mice fed with ethanol compared to pair-fed controls a*cross the 24-hour period.[4] However, the expression of hepatic *Npas2*, another component of the positive limb of the TTFL, was decreased by approximately fourfold in alcohol-fed group, particularly at Zeitgeber time 0 (ZT0) and ZT4, and elevated at ZT12.[4] For the interlocking TTFL, expression of *Rev-erbβ* and *Rev-erbα* was elevated at ZT0, and *Rev-erbα* additionally at ZT20. The alterations in clock-controlled genes associated with fatty acid oxidation (*acyl-coenzyme A thioesterase* (*Acot1*), *pparα*), lipoprotein (*lipoprotein lipase* (*Lpl*)), fatty acid synthesis (*ACC* and *Fas*) and cholesterol metabolism (*hmgcr*) were observed.[4] Furthermore, several of these rhythmic genes had changes in their temporal profiles. Hepatic bile acid synthesis is also under the control of clock. Its process involves coordinated expression of *Rev-erbα*/*β*, albumin site D-binding protein (*DBP*) and E4 promoter-binding protein 4 (*E4BP4*), which regulate the temporal

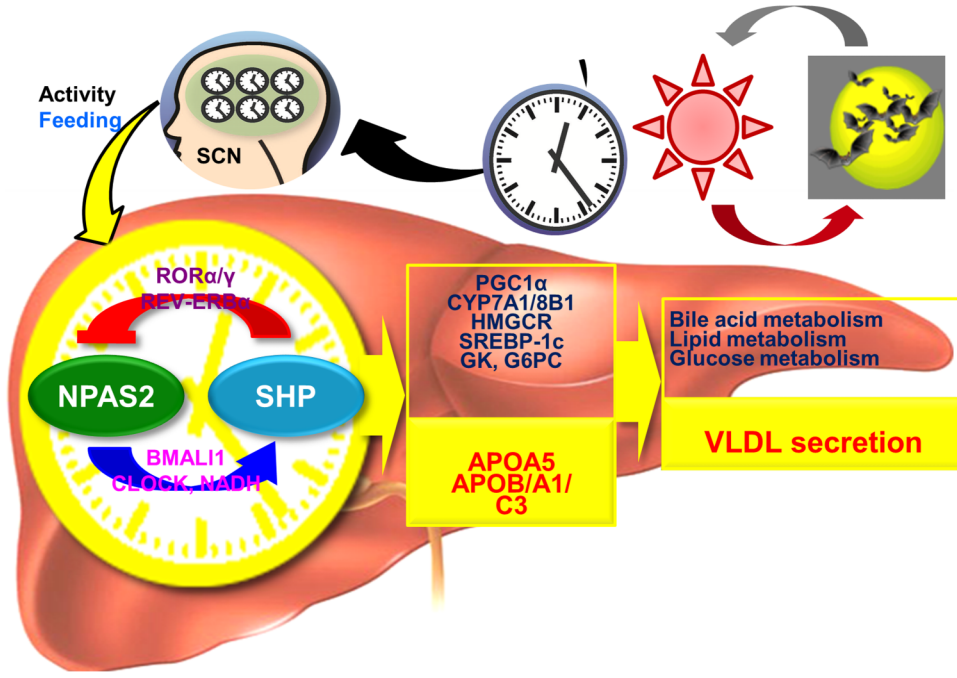

Figure 1 SHP in circadian clock-mediated control of hepatic metabolism. SHP is an important component in the hepatic circadian clock network. In the hepatocytes, there is a feedback regulatory loop between Npas2 and Shp. SHP inhibits Npas2 transcription by repressing Rorα/γ transactivation of the Npas2 promoter or by enhancing Rev-erbα inhibition. NPAS2 then activates *Shp* gene expression through CLOCK or by binding rhythmically to the *Shp* promoter. The interplay between NPAS2 and SHP maintains bile acid, lipid, glucose and lipoprotein homeostasis through the regulation of numerous genes involved in the process. SHP, small heterodimer partner; VLDL, very-low-density lipoprotein.

expression of *Cyp7a1*. In alcohol-fed mice, hepatic *Dbp* and *Cyp7a1* were upregulated at ZT4, and *E4bp4* was downregulated.[4] We observed the shifts in the phases of *Rev-erbα*, *Rev-erbβ* and *Dbp*. The *Cyp7a1 diurnal* waveform was significantly altered with the expression occurring at different phases of cycle in a biphasic pattern, with a major peak at ZT4, and the CG of expression was antiphasic, dramatically delayed by ~11 hours.[4] Our study clearly showed the disturbance of the circadian system in hepatic steatosis,[4] though the exact mechanism is still elusive.

Small heterodimer partner

The small heterodimer partner (SHP, NR0B2) serves as an important regulator of lipid[17 18] and bile acid metabolism[19 20] and of circadian rhythms in the liver.[21 22] SHP, an orphan member of the nuclear receptor superfamily, has a distinct structure due to the lack of DNA-binding domain.[21] SHP binds to the AF-2 domain (the C-terminal transcription activation domain located within the ligand binding protein of ligand-regulated and constitutive active NRs) through two functional LXXLL-related motifs (also called NR-boxes), which are located in the putative N-terminal helix 1 of the ligand-binding domain and in the C-terminal region of helix 5.[23] In general, SHP is a negative regulator and it inhibits the transcription activities after its binding to a number of nuclear receptors or transcription factors.[24 25] Numerous studies suggest that SHP has pleiotropic roles in the pathology of chronic liver diseases.[26 27] SHP, as a transcriptional repressor of nuclear receptors[28] (and review by Zhang *et al*[21]), involves in the pathogenesis of hepatic steatosis[29 30] by regulating the transcriptional activity of lipogenic transcription factors.[31] The time-of-day changes in the regulation of triglyceride metabolism under the control of *Clock* gene is also mediated by *Shp*.[22] However, it is unclear on how Shp controls liver clock machinery and the rhythmicity of intrahepatic metabolites.

SHP/neuronal PAS domain protein 2 axis regulates the oscillation of liver lipid metabolism

Using the transcriptomic approach, we found a significant disruption in the rhythmicity over 24-hour period of several important hepatic genes involving in the metabolism of lipid, cholesterol, fatty acid and bile acid in *Shp* null *($Shp^{-/-}$)* mice when compared to wild-type counterparts.[32] For genes regulating lipid metabolism, *Pparγ1* was significantly decreased, whereas *Acc* was moderately downregulated in *$Shp^{-/-}$* mice.[32] However, the expression of peroxisome proliferator-activated receptor (*Ppar*)*α* and very-low-density lipoprotein (VLDL) receptor (*Vldlr*; cholesterol uptake) was markedly increased in *$Shp^{-/-}$* mice.[32] To further explore the mechanism, we found that the core clock gene, especially hepatic *Npas2* mRNA, was strongly upregulated in *$Shp^{-/-}$* mice, suggesting a direct inhibition by SHP. In the core clock machinery pathway, RORα and RORγ *can activate Npas2, while* REV-ERBα represses its activity.[5 33] We thus hypothesized that the inhibitory effect of SHP on *Npas2* transcription is through its binding with retinoic acid-related orphan receptor (ROR)α, RORγ or REV-ERBα. We found that SHP can interact with RORγ and REV-ERBα, but not with RORα protein. It inhibits the activation of the *Npas2* promoter by RORγ.[32]

Coexpression of SHP with REV-ERBα further inhibited RORα activity, suggesting that SHP acts as a corepressor of REV-ERBα.[32] Taken together, we found that SHP is a unique transcriptional repressor of *Npas2* through crosstalk with RORα,γ and REV-ERBα.[32]

The next important question is whether there is the mechanistic link between the changes in the core clock component, *Npas2*, and hepatic lipid metabolism or steatosis under *Shp*-deficient condition. Using the loss-of-function approach by knocking down *Npas2* with *siNpas2*, we found that *siNpas2* triggered severe steatosis in $Shp^{-/-}$ liver. Interestingly, VLDL secretion was markedly inhibited by siNpas2 in $Shp^{-/-}$ mice.[32] Under this condition, the expression of apolipoprotein (*Apo*) *B*, an activator of VLDL secretion, was significantly reduced.[32] Our data suggested that knockdown of Npas2 in $Shp^{-/-}$ liver induced hepatic steatosis and accumulation of intrahepatic triglyceride by inhibiting VLDL secretion.[32] These data support the notion that SHP is an important intracellular switch coordinating circadian metabolic functions. SHP is also involved in homocysteine metabolism as the rhythmic gene expression regulating its metabolism is significantly altered in $Shp^{-/-}$ mice.[34] The schematic diagram on the role of *Shp* in controlling hepatic metabolism is shown in figure 1.

Conclusion

Circadian clocks control multiple physiological and metabolic pathways[2 22 32 35–38] This review reports a novel interplay between SHP and NPAS2 and the circadian controls of lipoprotein and lipid metabolism by NPAS2. Dysregulation of NPAS2 is associated with alcoholic and non-alcoholic fatty liver disease. Because of the feedback regulatory loop between Npas2 and Shp, further investigation is needed to explore the role of SHP as a molecular switch in regulating important metabolic function and whether modulating SHP may serve as a new therapeutic potential for fatty liver disease and other metabolic disorders.[39]

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