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Review Article

Implications of Circadian Rhythms on Metabolic Disorders

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Abstract

A vital role of circadian rhythms is to enable an organism to predict or adapt to environmental oscillations, including internal and external cues. These physiological and behavioral circadian rhythmicities are exhibited by all mammals and are generated by intracellular levels of circadian oscillators, which are composed of transcriptional/translational feedback loops involving a set of circadian-clock genes. These circadian-clock genes play important roles in regulating not only circadian rhythms but also energy homeostasis and metabolism. Increasing evidence shows that mutations or knockouts of circadian-clock genes or disruptions of the circadian rhythm initiate metabolic disorders. Similarly, high-nutrient diets influence the expression levels of circadian-clock genes in the liver. Changes in the cellular redox potential affect the activity of circadian-clock gene transcription factors and the expressions and functions of circadian-clock genes, which regulate energy metabolism. The characterizations of circadian-clock genes have potential therapeutic relevance with respect to the pathogenesis and treatment of obesity-related metabolic diseases including type 2 diabetes and metabolic syndrome. (*Tzu Chi Med J* 2009;21(4):285–288)

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1. Introduction

Rhythmicity in organisms is exhibited in several forms. The longest rhythm cycles about once per year and exerts annual changes. In contrast, the shortest rhythm cycles about once per second, such as the heart beating, and maintains the life of vertebrates. Additionally, seasonal and monthly rhythms are also common, such as mating and maternal behavior in animals, and the menstrual cycle in primates. However, the most common rhythm in nature is the circadian rhythm. The word "circa" means "about" and "diem" or "dies" means "day", so the circadian rhythm is a cycle of approximately 24 hours. This phenomenon is conserved in all eukaryotes and even some prokaryotes. A primary role of the circadian rhythm is to entrain the organism to environmental cues, so that an animal is able to anticipate, predict or adapt to environmental fluctuations, such as food availability and predator risk. The circadian rhythm is also critical to the synchronization and relative phasing of diverse internal physiological processes and molecular pathways (1). In mammals, the pacemaker of circadian rhythm is located in the hypothalamic

suprachiasmatic nucleus (SCN). Several studies have shown a series of circadian-clock genes in the SCN that regulate circadian rhythms [1].

2. Molecular mechanisms of circadian-clock systems

The circadian-clock genes, including the circadian locomotor output cycles kaput (*Clock*), brain and muscle-Arnt-like 1 (*Bmal1*), Period 1 *(Per1*), *Per2*, *Per3*, cryptochrome 1 (*Cry1*) and *Cry2* as well as their translational products, proteins, consist of a complex circuitry of transcriptional/translational regulatory feedback loops, which drive the circadian rhythmicity. In brief, *Clock* and *Bmal1* encode proteins that are members of the basic helix-loop-helix (b-HLH)-period-Arnt-Single-minded (PAS) transcription factor family. CLOCK and BMAL1 proteins heterodimerize in the cytoplasm to form a complex and activate the transcription of target genes containing E-box (5′-CAGCTG-3′) as well as E-box-like promoter sequences [1], and then they mediate other core circadian-clock genes. These other core circadian-clock genes include the *Per* genes (*Per1*, *Per2*, and *Per3*), paralogous members of the PAS protein family, and *Cry* genes (*Cry1* and *Cry2*). PER and CRY proteins form a heteromultimeric complex that translocates to the nucleus and directly inhibits transcriptional activity of the CLOCK-BMAL1 complex, thereby lowering *Per* and *Cry* mRNA levels. Thus, this circuitry involves a primary loop with CLOCK and BMAL1 as transcriptional activators, and PER and CRY proteins as transcriptional repressors [1–4]. Moreover, *Per*s and *Bmal1* have robust oscillation in opposite phases correlating with their opposing functions [5–7]. All these circadian-clock genes exhibit a 24-hour rhythm in SCN cells and peripheral tissues, except for *Clock*, which has been shown not to oscillate in the SCN [8] and other tissues [7]. Several other genes also appear to be important in sustaining biological clock function. Casein kinase I epsilon (CKIε) can phosphorylate the PER proteins to enhance their instability and degradation [8–11]. *Bmal1* expression is negatively regulated by the transcription factor reverse erythroblastosis virus α (REV-ERBα) [12], and positively regulated by retinoic acid receptor-related orphan receptor α (RORα) through the ROR response element (RORE) [13]. Thus, *Bmal1* oscillation is driven by a rhythmic change in RORE occupancy by RORs and REV-ERBα.

3. Effects of circadian-clock gene mutations and metabolic disorders

Recent studies have suggested that disruption of circadian rhythms may lead to manifestations of metabolic syndrome [14–16]. Evidence also suggests that loss of the circadian rhythmicity of glucose metabolism may contribute to the development of metabolic disorders, such as type 2 diabetes, in both animals [17–19] and humans [17,18,20]. The direct linkage between circadian mechanism dysfunction and metabolic abnormalities is demonstrated by phenotypes of circadian-clock gene mutants, knockouts or disruptions.

Deletion of *Bmal1* also induces arrhythmicity, the early onset of age-related pathologies such as myopathy and arthropathy, and altered hepatic carbohydrate metabolism [21]. *Bmal1* or *Clock* mutant mice exhibit suppressed diurnal variations in glucose and triglycerides as well as abolished gluconeogenesis, but the counter-regulatory responses of corticosterone and glucagon are retained [21]. *Clock* mutant mice have a greatly attenuated diurnal feeding rhythm and increased caloric intake, are hyperphagic and obese, and develop a metabolic syndrome of hyperleptinemia, hyperlipidemia, hepatic steatosis, and hyperglycemia [22]. The expression of hypothalamic peptides, ghrelin and orexin, which are related to energy balance as well as gluconeogenesis, are attenuated in these *Clock* mutant mice [22]. A combination of the *Clock* mutant with the leptin knockout (*ob/ob*) results in significantly heavier mice than the *ob/ob* phenotype [23]. Mutation in another core circadian-clock gene, *Per2* (*Per2* mutant mice), exhibits no diurnal feeding rhythm or glucocorticoid rhythm, but the corticosterone response to hypoglycemia is intact [24]. In addition, *Per2* mutant mice exhibit increased bone density [25]. Because bone and adipose tissue share a common ontogeny, it is possible that these findings may also have implications for adipogenesis [26]. Moreover, liver-specific deletion of *Bmal1* exhibited by hypoglycemia during fasting, exaggerates glucose clearance and loss of rhythmic expression of hepatic glucose regulatory genes, which shows a direct effect of the liver circadian-clock genes on glucose metabolism [27]. These findings suggest that disruption of circadian-clock genes causes impairment of glucose metabolism and leads to development of metabolic disorders.

4. Effects of high-fat diet on circadian-clock genes

In contrast to the comprehensible effects of circadian-clock gene mutations with metabolic disorders, the effects of a high-fat diet on the expression of circadian-clock genes are still controversial. Yanagihara et al reported that 8 weeks of a high-fat diet in female C57BL/6 mice had minor effects on the expression of circadian-clock genes such as *Clock*, *Bmal1*, *Per1*, *Per2*, *Cry1*, and *Cry2* in the liver [28]. Satoh et al

also reported similar results for levels of *Per1* and *Per2* in the liver of male ICR mice after high-fat diet treatment (29). Bray and Young also found that 4 weeks of high-fat-diet feeding had no significant effect on any circadian-clock gene oscillations in the rat liver (30). On the other hand, it has been reported that 6 weeks of a high-fat-diet decreases levels of *Clock* and *Bmal1* expression, but does not affect *Per1* in the livers of male C57BL/6 mice (31). Our recent studies found that chronic (11 months) treatment with a high-fat diet elevates the expression levels of *Bmal1*, *Per1*, *Per2*, *Per3*, *Cry1*, *Cry2* and *CK1ε*, while *Clock* shows no change in the livers of male C57BL/6 mice (7). It is possible that gender difference accounts for these controversial findings because we also found that female mice take longer to become obese from a high-fat diet (31,32). Moreover, a recent study also reported that a high-fat diet induces a phase-delay in circadian-clock gene expression and downstream genes, the circadian-clock-controlled genes (33). Therefore, a high-fat diet appears to affect circadian-clock gene expression.

5. Conclusion

Obesity and being overweight pose major risks for chronic diseases including type 2 diabetes, cardiovascular disease, hypertension, stroke, and certain forms of cancer (34). The pre-disease condition that is called metabolic syndrome has become the center of much attention; it is provoked by high-nutrient diets in both developed and developing countries (35). Although the detailed molecular mechanisms of circadian-clock genes involved in cellular energy status, energy metabolism, and metabolic disorders are not fully clear, growing evidence has demonstrated that changes in the cellular redox potential affect the activity of circadian-clock gene transcription factors (36,37), as well as the expression and function of circadian-clock genes, which regulate energy metabolism (21,22,38, 39). Because the liver is responsible for key elements of intermediary metabolism, such as the metabolism of carbohydrates, lipids and proteins (40), there are increasing studies on the relationships between the liver and circadian-clock genes. The interaction of circadian-clock genes within adipocytes is another important issue in comprehending the manifestation and development of metabolic disorders. Recently, expression levels of circadian-clock genes, *Per2*, *Bmal1* and *Cry1*, in adipose tissues were found to be related to human metabolic syndrome, and a close association between *Clock* polymorphisms, obesity and metabolic syndrome have also been reported (41,42). In conclusion, the characterization of circadian-clock genes has potential therapeutic relevance with respect to the pathogenesis and treatment of obesity-related metabolic diseases, such as type 2 diabetes and metabolic syndrome.

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