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MicroRNA in the molecular mechanism of the circadian clock in mammals

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1. ABSTRACT

The biochemical activity of mammals is controlled by an internal timekeeping mechanism driving a clock to run in approximate 24-hour (circadian) cycles. In mammals, this circadian clock is located both in the suprachiasmatic nuclei (SCN) and peripheral oscillators. Recently, microRNAs have emerged as significant players in circadian clock timing. The biological implications of miRNAs are extended further by recent studies that microRNAs are expressed in the SCN and peripheral circadian oscillators. In this study, we review recent work revealing the role of microRNAs in the molecular mechanism of circadian clock in mammals.

2. INTRODUCTION

Every aspect of human physiology is controlled by an internal timekeeping mechanism, which drives a clock to run in approximate 24-hour cycles. In mammals, this circadian clock is located in the suprachiasmatic nuclei (SCN) (1, 2), a bilateral structure within the hypothalamus that contains approximately 20,000 neurons with independent, self-sustained oscillations that are light responsive. The inherent timing capacity of the SCN is derived from autonomous neuronal oscillators (3, 4). In mammals, besides the SCN, the master circadian pacemaker also resides in synchronized rhythms of peripheral oscillators. The brain as well as in peripheral

organs, are under the influence of the SCN clock. The circadian pacemaker is likely to respond to the daily changes in the light-dark cycle by resetting its phase. Thus, the inherent clock timing process is tightly regulated by changes in the daily lighting cycle.

At a molecular level, the clockwork is comprised of a series of interlocked positive and negative transcription/translation feedback loops that drive rhythmic expression of critical clock components (5-8). The loops include positive elements and negative elements. On the one hand, positive elements include transcription factors and activators of transcription of negative element genes. Clock and Bmal are positive elements in mammals, which possess basic helix-loop-helix (bHLH) and Per-Arnt-Sim (PAS) domains. These 2 proteins form heterodimers (Clock: Bmal) and activate transcription via Ebox elements in the promoter regions of clock genes. On the other hand, the negative elements include three Period genes (Perl-3) and two Cryptochrome genes (Cryl, 2) in mammals, which suppress transcriptional activities of the positive elements and thus decrease the synthesis of the corresponding gene products. Though the negative elements do not share homologous domain structures, there are common characteristics in their dynamics that their transcripts and proteins exhibit robust circadian oscillations in their expressions. In the differences between the positive elements and negative elements, the positive elements show relatively weak fluctuation or no rhythm in their expressions of transcripts and gene products while the negative elements do not. Since the phosphorylation of the negative elements could relate to their turnover, it is associated with the enhancement of changes observed in the negative elements. Kinases responsible for the phosphorylation of the negative elements include CKIc and CKIS in mammals (9, 10). Dbt is a homologue of mammalian CKIe and Shaggy is a homologue of mammalian glycogen synthase kinase-3beta (GSK3beta).

MicroRNAs are short non-coding RNAs that can regulate their target gene expression post-transcriptionally and are implicated in the regulation of a wide array of biological processes (11). Mature microRNAs are small RNA molecules, typically 19-25 nucleotides long, derived from sequential RNase III-dependent cleavages of longer transcripts (12, 13). In humans, there are more than 1000 unique microRNAs (14), and 20-30% of the transcriptome is subject to miRNA-targeted regulation (15, 16). MicroRNAs are predicted to target over 50% of all human protein coding genes. By binding to partially complementary target sites within the 3' untranslated regions (3' UTRs) of select messenger RNAs, microRNAs act as potent negative regulators of protein translation either by directing the mRNA for degradation or inhibiting its translation into the encoded protein (17). Each microRNA is capable of regulating multiple target genes, leading to complex changes in genetic networks by the action of a single miRNA. Although the biological roles of only a small fraction of identified miRNAs have been elucidated, in mammals, these miRNAs regulate processes essential to cell growth, embryogenesis, stem cell maintenance, hematopoietic cell differentiation, and brain development (18-21). Thus, many of the biological processes seem to be controlled by microRNAs to some degree (23). Moreover, microRNAs have emerged as another avenue by which the circadian clock is regulated. About 10% of all mammalian transcripts are under circadian regulation, (24) suggesting that the transcripts may be regulated at a transcriptional/post-transcriptional level. Thus, this paper reviews the role of microRNA in the molecular mechanism of circadian clock in mammals and aims to explore the contributions of microRNA to circadian clock function or vice versa.

3. MOLECULAR CONTROL THROUGH MICRORNA

Since the circadian timekeeping in mammals is organized in a hierarchical fashion, as mentioned above, that includes the 'master' circadian clock SCN and the 'Peripheral' circadian clocks, we review the regulation of microRNA in SCN clock and peripheral circadian oscillators respectively as follows.

3.1 MicroRNAs and SCN clock

In the mammalian SCN, microRNAs are involved in clock timing and entrainment. Cheng *et al* (25) have found that microRNA (miR)-219 played a role in modulating the circadian clock located in the SCN. Since miR-219 affected per1 expression, it can influence the core circadian transcriptional loop. They also found that miR-219 was a target of the CLOCK and BMAL1 complex, and exhibited robust expression during circadian rhythms. In an *in vivo* antisense silencing study, they demonstrated that miR219 shortens the circadian period. However, miR-219 was not found to directly target per1 (25).

Furthermore, Cheng *et al* (25) demonstrated the role of miR-132 in modulating the SCN clock. It is similar to miR-219 in that miR-132 showed oscillatory expression in wild-type mice, but not in circadian mutant mice. MiR-132 also influenced the core circadian transcriptional loop by indirectly affecting per1 expression. They found that miR-132 is induced by photic entrainment cues via a MAPK/CREB-dependent mechanism, modulates clock-gene expression, and attenuates the entraining effects of light, which is different from miR-219 (25).

MiR-132 is one of the Ca(2+)/cAMP response element-binding protein-regulated microRNAs, which attenuates its capacity to reset, or entrain, the clock. Genes involved in chromatin remodeling (Mecp2, Ep300, Jarid1a) and translational control (Btg2, Paip2a) are direct targets of miR-132 in the mouse SCN. Coordinated regulation of these targets underlies miR-132-dependent modulation of Period gene expression and clock entrainment: the mPer1 and mPer2 promoters are bound to and transcriptionally activated by MeCP2, whereas PAIP2A and BTG2 suppress the translation of the PERIOD proteins by enhancing mRNA decay. These findings suggest that miR-132 can be selectively enriched for chromatin- and translation-associated target genes and be an organizer of chromatin remodeling and protein translation within the SCN clock, thereby fine-tuning clock entrainment (26).

A study about direct effects of miR-132 on sleep gives further understanding of miR-132 and SCN in response to light. Davis *et al* (27) reported that intracerebroventricular application of a miRNA-132 mimetic (preMIR-132) decreased duration of non-rapid-eye-movement sleep (NREMS) while simultaneously increasing the duration of rapid eye movement sleep (REMS) during the light phase. Further, preMIR-132 decreased electroencephalographic (EEG) slow-wave activity (SWA) during NREMS. In addition, after ventricular or supracortical injections of preMIR-132, the mimetic-induced effects specifically occurred only during NREMS. The spontaneous cortical levels of miRNA-132 were lower at the end of the sleep-dominant light period compared with at the end of the dark period in rats. These results suggest that miRNAs play a regulatory role in sleep (27). Study on miRNAs and sleep homeostasis found the expression of 10 miRNAs changed with sleep deprivation in sham-lesioned mice, 5 of which increased (miR-410, −212, −29c, −29b-2, and −708) and 5 decreased (let-7e, miR-137, −22, −219-2, and −99a) (28). These results about miRNAs and sleep contribute to further study on the relationship between miRNAs and photic control in SCN.

3.2 MicroRNAs and peripheral circadian oscillators

Besides regulating the circadian clock in SCN, miRNAs also play a role in regulating peripheral circadian oscillators. In a study on circadian rhythm regulation of the retina (29), miRNAs, which were involved in circadian rhythm regulation of the retina, were identified. The miRNA expression profiling with retinal RNA harvested was performed at noon (Zeitgeber time 5) and midnight (Zeitgeber time 17), and then 12 miRNAs were identified, including members of the miR-183/96/182 cluster with diurnal variation in expression pattern. MiR-96, miR-182, and miR-183 are on mouse chromosome 6qA3 with conservation of synteny to human chromosome 7q32.2, which are members of a polycistronic, sensory organ-specific paralogous miRNA cluster. The target of miR-96 and miR-182 is MITF, a transcription factor required for the establishment and maintenance of retinal pigmented epithelium. These findings suggest that miR-96 and miR-182 are involved in circadian rhythm regulation, perhaps by modulating the expression of adenylyl cyclase VI (ADCY6) (29). Another finding from the study on miR-182 and circadian rhythms was abnormal processing of pre-miR-182 in patients carrying the T allele of the rs76481776 polymorphism (30). This polymorphism may contribute to the dysregulation of circadian rhythms in major depressive patients with insomnia, which could influence expression levels of the mature form of miR-182 and might increase downregulation in some of its target genes (30). These findings suggest that miR-182 is an important miRNA in mediating peripheral circadian oscillators, and is involved in pathogenesis of some diseases.

In addition, the miR-192/194 cluster was identified as a potent inhibitor of the entire Period gene family. In accordance, the exogenous expression of miR-192/194 leads to an altered circadian rhythm. This finding has uncovered a new mechanism for the control of the circadian clock at the post-transcriptional level, which is different from the biological clock mechanism involved in core transcriptional unit Bmal/CLOCK (31).

In liver, most metabolic pathways are under circadian control, and hundreds of protein-encoding genes are thus transcribed in a cyclic fashion. MiRNAs in peripheral circadian oscillators of liver could be related to some diseases. Microarray-based study of mouse liver for 48 h at 4-hour intervals reported miRNA-mRNA pairs in the regulation of circadian rhythm (32). Circadian miRNA-mRNA target pairs are defined as the pair of both elements of which show circadian expression patterns and the sequence-based target relationship of which can be predicted. There is an inverse correlation between expression of circadian initiators Clock and Bmal and their corresponding miRNAs miR-181d and miR-191, while a positive correlation was exhibited between circadian suppressors including Per, Cry, CKIe and Rev-erba and their corresponding miRNAs. In this study, genomic location analysis revealed that the intronic region showed higher abundance of cyclic than non-cyclic miRNAs targeting circadian genes while other (i.e., 3-UTR, exon and intergenic) regions showed no difference. This suggested that miRNAs are involved in the regulation of peripheral circadian rhythm in mouse liver by modulating Clock:Bmal1 complex (32). The peroxisome proliferator-activated receptor (PPAR)beta/delta and PPARalpha coactivator Smarcd1/Baf60a was identified as a novel miR-122 target. PPARs belong to the nuclear hormone receptor super-family and are well-known metabolic regulators. PPARalpha and PPARbeta/delta serve predominantly catabolic functions. These PPARs show circadian expression in liver. The rhythmic transcription extended to the locus specifying miR-122, a highly abundant, hepatocyte-specific microRNA suggesting an involvement of the circadian metabolic regulators of the PPAR family in miR-122-mediated metabolic control (33). In mouse liver, a liver specific miRNA miR-122 regulated Nocturnin expression (34). Nocturnin is a circadian clock-regulated deadenylase thought to control mRNA expression post-transcriptionally through poly(A) tail removal. The expression of Nocturnin is robustly rhythmic in liver at both the mRNA and protein levels, and mice lacking Nocturnin are resistant to diet-induced obesity and hepatic steatosis. That study found that the normal rhythmic profile of Nocturnin expression in liver was shaped in part by miR-122, which suggests that the role of miR-122 in regulating Nocturnin expression may be an important intersection between hepatic metabolism and circadian control (34). Identifying specific miRNAs and their targets that are critically involved in circadian rhythm will provide a better understanding of the regulation of circadian-clock system in liver.

The circadian oscillator also governs intestine physiology. The peripheral circadian oscillator in jejunum was studied in rat model. Balakrishnan *et al* (35) first reported the circadian rhythmicity of specific microRNAs in rat jejunum. They reported a link between anti-proliferative miR-16 and the intestinal proliferation rhythm and point to miR-16 as an important regulator of proliferation in jejunal crypts.

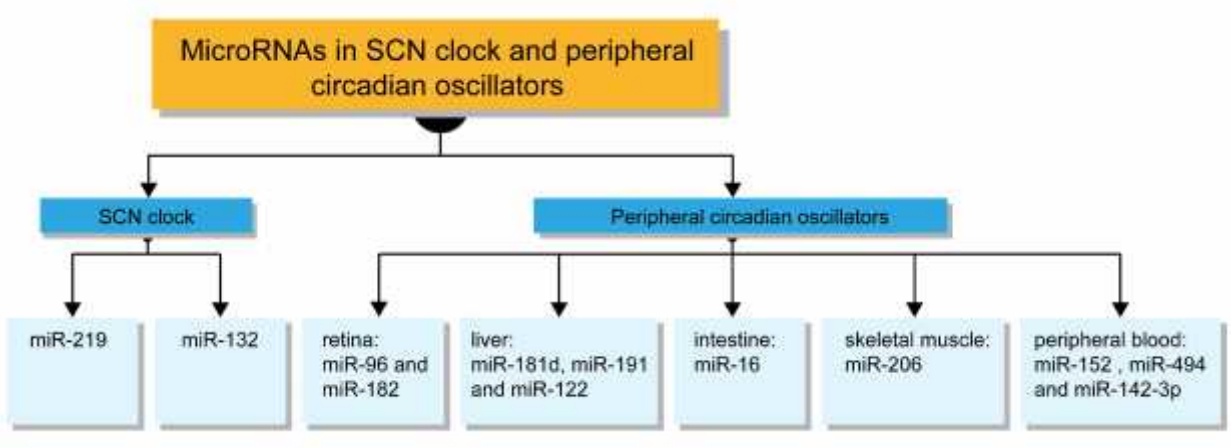

Figure 1. MicroRNAs in SCN clock and peripheral circadian oscillators. In SCN, miR-219 and miR-132 play a specific role in modulating the SCN clock. While in peripheral circadian oscillators, several microRNAs have been reported to involve in circadian rhythm regulation, such as miR-96 and miR-182 in retina, miR-181d, miR-191 and miR-122 in liver, miR-16 in intestine, miR-206 in skeletal muscle, and miR-152 , miR-494 and miR-142-3p in peripheral blood.

Furthermore, the circadian oscillator controls skeletal muscle. In skeletal muscle, miR-206 has a profound effect on the dynamic mechanism of the mammalian circadian clock. It is an important regulator of the circadian clock at the post-transcriptional level (36). MiR-206 has not only been identified as the only miRNA expressed in skeletal muscles, but also exhibited crucial roles in the regulation of the muscle development. The amplitude and frequency of the oscillation can be significantly altered through the miR-206-mediated control. MiR-206 can control the amplitude and control or alternate the frequency of the circadian clock, resulting in affecting the level of the gene expression and interfering with the temporal sequence of the gene production or delivery (36).

In peripheral blood, several miRNAs with Bmal as a predicted target were expressed in mice when exposed to a standard 12 h light:12 h dark photoperiod. Among these miRNAs, miR-152 and miR-494 were marked by diurnal oscillations with bimodal peaks in expression occurring near the middle of the day and 8 or 12 hr later during the night. Co-transfection of pre-miR over-expression constructs for miR-494 and miR-142-3p in HEK293 cells had significant effects in repressing luciferase-reported Bmal 3' UTR activity by 60%. These results suggest that these miRNAs may function as post-transcriptional modulators of Bmal, and circulating miRNAs may play a role in the regulation of the molecular clockworks in peripheral circadian oscillators (37).

In summary, in SCN, miR-219 and miR-132 play a specific role in modulating the SCN clock. While in peripheral circadian oscillators, several microRNAs have been reported to involve in circadian rhythm regulation, such as miR-96 and miR-182 in retina, miR-181d, miR-191 and miR-122 in liver, miR-16 in intestine, miR-206 in skeletal muscle, and miR-152 , miR-494 and miR-142-3p in peripheral blood (Figure 1).

4. CONCLUSIONS

Recent findings that microRNA affects key clock timing processes outlined here are a mere starting point for the examination of microRNAs and circadian clock timing. A deeper understanding about microRNAs affecting circadian clock timing processes could lead to the proposal of novel chronotherapeutic approaches to circadian-related diseases. There are numerous challenges that will need to be addressed for further studies on microRNAs in the molecular mechanism of the circadian clock in mammals.

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Abbreviations: SCN: suprachiasmatic nuclei; Bhlh: basic helix-loop-helix; PAS: Per-Arnt-Sim; 3' UTRs: 3' untranslated regions; miR: microRNA; NREMS: non-rapid-eye-movement sleep; EEG: electroencephalographic; ADCY6: adenylyl cyclase VI; PPAR: proliferator-activated receptor

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