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Chapter 2

Circadian Clock Gene Regulation in Aging and Drug Discovery

Yufeng Li, Yanqi Dang, Shuang Ling and Jin-Wen Xu

Additional information is available at the end of the chapter

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Abstract

The circadian clock is an endogenous timer in prokaryotes and mammals. Resting and adjusting the internal clock can assist in pacing the daily routine. Growing evidence indicates that the circadian clock and aging process are closely associated. The disruption of the circadian clock leads to accelerated aging and increased incidence of various diseases. In particular, elderly people are more vulnerable and have a higher risk of diseases than do young people. In this study, we reviewed studies on aging and circadian rhythms over the last decade, with a focus on circadian clock gene regulation in aging and drug discovery for targeting the circadian clock in diseases.

Keywords: Bmal1, clock, circadian clock genes, aging, drug discovery

1. Introduction

Circadian rhythms affect almost all daily activity behavioral patterns, physiology, and gene expression. Circadian rhythms indicate the appropriate time for various activities, such as consuming food and mating, and gradually form the circadian clock [1]. The circadian clock is an internal timekeeping system, which facilitates adaptation to the external world in anticipation of daily environmental changes. A 24-h circadian rhythm pattern has been observed in almost all cells [2]. Circadian clocks coordinate external day and night cycles with diverse environmental and metabolic stimuli. However, a disrupted circadian clock causes various diseases, such as insomnia, diabetes, cancer, and metabolic syndrome [3]. The ability of the brain timing system and function of circadian rhythm-regulating genes decline with age. The

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disruption of circadian rhythms disrupts the coordination among body systems. Aging damages body homeostasis and results in a subhealth status [4]. Consequently, the systems of elderly people are more vulnerable than those of young people [5]. The disruption of circadian rhythms adversely affects the aging process and increases the risk of diseases. A set of circadian clock genes form negative feedback loops that can regulate transcription, with a 24-h circadian oscillation [4, 6]. The circadian function significantly affects the aging process. Chronic destructions of the circadian function are associated with the occurrence of various age-related diseases, such as cancer and premature aging. Therefore, studies are warranted to determine how the circadian clock genes orchestrate interactions between the internal physiology and the aging progress.

In this study, we reviewed studies within the last decade on aging and circadian rhythms and the mechanism underlying the maintenance of biological processes by the circadian clock, with a focus on circadian clock gene regulation in aging and antiaging drug discovery.

2. Circadian clock

2.1. Structure of the circadian system

Living systems possess an exquisite internal biological clock, and the major function of which is to regulate the daily sleep–wake cycle [7]. The circadian clock follows a rhythm of approximately 24 h and ensures accurate adaptation to external daily rhythms through a powerful endogenous timing system [8]. The circadian clock also drives numerous molecular and cellular processes by generating oscillations. Virtually, all body cells have an autonomous circadian clock [9, 10], which is composed of a central clock existing in the suprachiasmatic nucleus (SCN) neuron and peripheral clocks. Clock, Bmal1 (brain and muscle ARNT-like protein-1), Pers, and Crys constitute a set of circadian oscillation genes in mammals [11].

2.1.1. Suprachiasmatic nucleus

The central circadian clock, located in the SCN of the anterior hypothalamus, is the primary circadian pacemaker [12]. The SCN comprises a network of approximately 20,000 neurons. Each neuron is considered to have an oscillator of the autonomous circadian clock. As the neurons are joined and oscillated in a consistent manner [13], the SCN neuron can generate an autonomous circadian clock similar to other cells [14, 15]. The SCN as the primary circadian pacemaker regulates independent gene expressions through neuronal firing [16].

The retina captures optical signals and transmits signals to the SCN (**Figure 1**) [17]. SCN neurons organize coupling mechanisms that ensure their synchronization even in darkness [18]. SCN neurons change the gene expression levels by converting electrical information into chemical information [16]. Neuronal firing frequency can synchronize the other cells of the body with rhythmic changes [19]. The central clock is controlled by external signals; food and light are the strongest signals affecting the clock (**Figure 1**). Once synchronized, the central clock consequently mediates the synchronization of the peripheral clocks through signaling [20].

Moreover, lability and plasticity of the phase in the intrinsic period are two critical functions of the central clock [21]. As the phase is labile, the length of the intrinsic period leads to different phases [22]. The waveform of the SCN amplitude is mainly related to the light cycle. The waveform is narrow with a high amplitude in short photoperiods, whereas it is broader with a low amplitude in long photoperiods. The circadian waveform in SCN oscillation is strong correlated with the SCN and behavioral rhythms [23].

2.1.2. Peripheral clocks

Peripheral and central clocks have been discovered in various tissues. One study reported the ubiquity of peripheral clock and its mechanism in both SCN and other cells [24]. Another study reported that cultured SCN cells maintained a firm rhythmic pattern through photoreception, also expressed in many organs, such as the liver, lungs, and kidneys [25]. In addition, numerous mammalian peripheral tissues exhibit circadian oscillation; consequently, oscillations are suppressed when the SCN is absent [9]. Thus, a delayed feedback loop originally associating the same components is considered to be composed of the rhythm-generating molecular circuitry, which is constructed by both SCN and peripheral cells [16]. Several pivotal physiological functions are influenced by light–dark oscillations in peripheral organs (**Figure 1**), including the heart, liver, lung, kidney, and skeletal muscles [26].

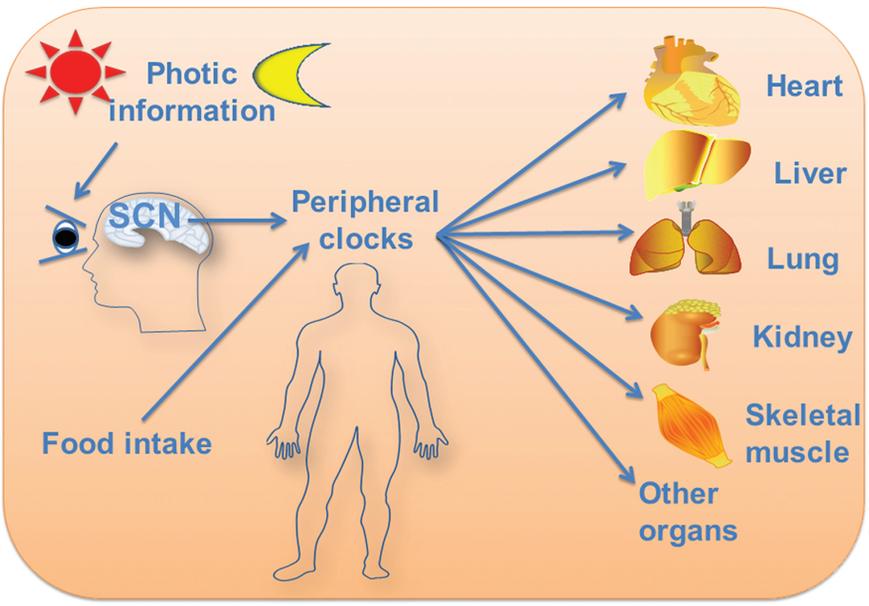


Figure 1. Structure of the circadian system. The retina captures photic information and transmits signals to the SCN. Food and light transmit signals to the peripheral clock.

Local peripheral oscillators can be synchronized by neuronal signals and stimulating hormones. The SCN sends signals to all body systems, coordinating the feeding–fasting cycles [27, 28]. Although the SCN functions as the master synchronizer of the entire system, food intake can disrupt the control in peripheral clocks. A change in the feeding schedule alters the phase in the central and peripheral clocks in the liver [29]. Moreover, light information is transmitted

to the adrenal gland, liver, and pancreas by the SCN, which distributes a rhythmic signal to all tissues of the peripheral organs [30]. The central neural and peripheral tissues maintain the normal neurological and metabolic homeostasis in the sleep–wake cycle [31]. The endogenous mechanism of oscillation in peripheral cells is a gene regulatory network to generate sustained oscillations. A group of genes forms the core network of the mammalian circadian clock, which can function even in the absence of external inputs in individual cells [32]. Numerous signaling pathways participate in the phase entrainment of peripheral clocks and warrant additional studies.

2.2. Circadian clock and diseases

The circadian clock regulates the sleep–wake cycle, metabolism, hormone secretion, and other physiological processes. A recent study suggested that chronic circadian disruption is deleterious to health and causes myocardial infarction, sudden cardiac death, and aortic aneurysm rupture risk [33]. Circadian dysregulation has extensive consequences not only on glucose control but also on inflammation. REV–ERBα and retinoid-related orphan receptors (RORs), which are downstream proteins of the signaling pathway, can affect adipogenesis and thrombosis through circadian clock regulation. This study indicates that metabolic and circadian pathways, which involve the nuclear receptor superfamily, are associated to the central node [34].

Circadian rhythm and sleep disorders lead to an increased incidence of metabolic syndrome [35]. Chronic sleep curtailment may affect the increase in the prevalence of diabetes and obesity. The effect may change glucose metabolism by reducing energy consumption [36]. Hypothalamic–pituitary–thyroid axis rhythms are endocrine rhythms and are accurately governed by the circadian system [37]. Evidence suggests that a seasonal change in thyroid hormone availability in the hypothalamus, and pituitary gland is a crucial element [38]. Sex-differentiated circadian timing systems exist in the hypothalamic–pituitary–gonadal (HPG) axis, the hypothalamic–adrenal–pituitary (HPA) axis, and sleep-arousal systems [39]. When various stressors appear, the HPA axis adjusts the circadian rhythms of the peripheral clocks through the glucocorticoid receptor [40]. In addition, the female HPG axis is regulated by a molecular clock in gonadotropin secretion because it relates to the timing of gonadotropin secretion in ovulation and parturition [41]. The circadian timing system and estradiol sensitive neural circuits driving the HPG axis functioning include gonadotropin-releasing hormone secretion and preovulatory luteinizing hormone [42].

Moreover, a strong association exists between the circadian clock and hormone secretion. The human placenta synthesizes the melatonin-regulating circadian system of many tissues, and the unusual secretion adversely affects fetal and maternal health [43]. The circadian system, which is composed of a family of clock genes, also regulates hormonal production and activity [44]. Maternal melatonin is a circadian signal for oscillating the fetal SCN clock. However, maternal melatonin probably cannot control the adrenal gland [45]; therefore, the circadian clock system affects the fetus during embryonic development.

Adequate sleep is crucial for maintaining the function of circadian rhythms. The lack of sleep disrupts circadian rhythms. Impaired endocrine and physiological circadian rhythms affect

the quantity of immune cells [46]. Circadian oscillations can mediate cognitive performance through sleep. However, it cannot work correctly in case of the environmental disturbance of the clock, including shift work and schedules [47]. Circadian systems are disrupted through inadequate melatonin secretion, and the altered clock gene expression can cause human metabolic syndrome and cardiovascular diseases [35].

3. Circadian clock gene regulation in aging

Circadian oscillations regulate transcription by using a set of genes, thus establishing an autoregulatory feedback loop. A study reported that circadian gene expression is widespread through the body [19]. Animal studies have revealed that a disrupted circadian clock function accelerated the development of aging phenotypes. The temporal precision of circadian system is lost with advancing age. Growing evidence indicates that aging is affected by circadian clocks. Circadian clocks can change gene expression, physiological functions, and daily cycles. The circadian system leads to various age-related pathologies and exhibits a weak precision with advancing age. The aged SCN shows changes in the expression of vasoactive intestinal polypeptide [48]. Circadian rhythms of the electrical activity are decreased [49]. When a young SCN is transplanted into aged animals, the behavioral rhythms functioning in locomotion is improved [50]. These studies indicate that the SCN is crucial to improve the age-related circadian system, which may be deteriorated in aged individuals.

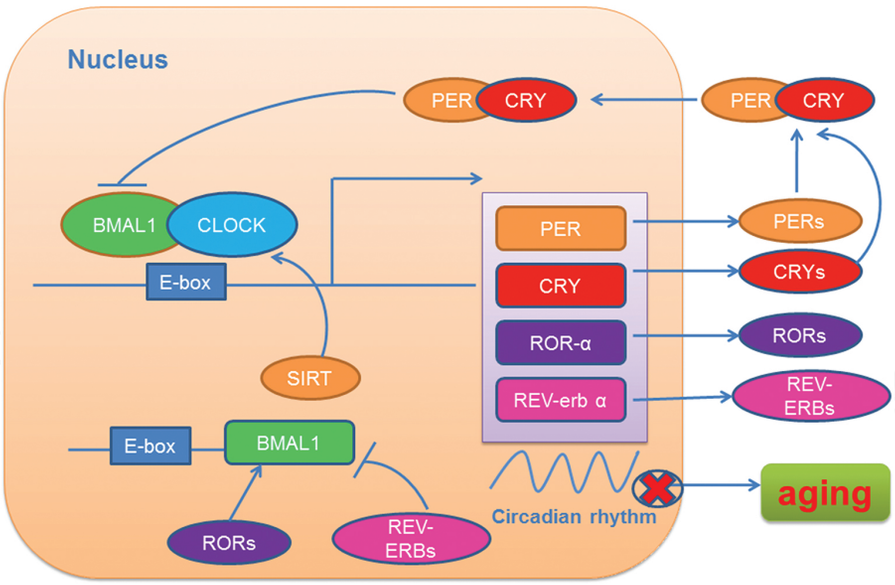


Figure 2. Circadian clock gene regulation in aging.

Bmal1 and clock activate the expression of period (Per), cryptochrome (Cry), RORα, and REV–ERBα. The negative elements of the clock PER and CRY proteins, which are associated with Clock–Bmal1 at E-box sites, enter the nucleus. REV–ERBα competes with each other for their binding DNA in the Bmal1 promoter, and the expression of Bmal1 is repressed.

3.1. Effects of Bmal1 genes on the aging process

Bmal1 belongs to the family of the basic helix–loop–helix (bHLH)–PAS domain containing transcription factors [51]. Bmal1 is a transcriptional factor and major component of the circadian clock; it plays a critical role in accelerating aging and the development of age-related pathologies. Bmal1 as the core clock gene regulates the expression of other circadian clock genes, which affects the physiological circadian rhythm [52]. Bmal1 deficiency has been shown to significantly shorten lifespan and accelerate senescence. *Bmal1*$^{-/-}$ mice have a shorter lifespan and exhibit cataracts, organ shrinkage, and other symptoms of premature aging [53].

Bmal1 may be directly associated with premature aging and reduced lifespan. Recent studies have reported a significantly weakened function of circadian rhythms in aged animals. The disturbances in circadian rhythms may cause thrombosis, a critical result of age-related cardiovascular diseases. Genetic ablation of the gene Bmal1 gene in mice significantly elevates the circulating von Willebrand factor, fibrinogen, and PAI-1 [54]. Bmal1 regulates BDH1 and PIK3R1; thus, Bmal1 affects metabolism, cell signaling, and the contractile function of the heart. Bmal1 predicts impairments in ketone body metabolism and depressing glucose utilization in the hearts of cardiomyocyte-specific Bmal1-knockout mice [55].

Bmal1 is crucial for regulating oxidative stress. Oxidative stress maintains reactive oxygen species (ROS) homeostasis and senescence through the hypoxia-inducible factor-mediated pathway. *Bmal1*$^{-/-}$ cells do not induce replicative senescence but rather stress-induced senescence. Stress-induced senescence causes cell and organ aging [56]. A recent study revealed a significantly decreased rhythmic function in aged animals. The study reported that the activity of the mammalian target of rapamycin complex 1 pathway is controlled by the circadian clock through Bmal1-dependent mechanisms. This regulation evidenced an association between aging and metabolism [57]. Bmal1 in the heart also determines the pathological consequences of the chronic disruption of the circadian clock. *Bmal1*$^{-/-}$ mice develop dilated cardiomyopathy with aging and decreased cardiac performance with changes in titin [58]. In addition, Bmal1 controls the blood coagulation pathway by changing platelet numbers and altering the vascular function. This control results from arterial and venous thrombogenicity by attenuating nitric oxide and anticoagulant factor synthesis [59].

3.2. Effects of Clock on the aging process

Clock is a circadian clock protein similar to Bmal1 and is a transcription factor of the circadian system. Bmal1 and Clock have different functions in modulating the biological function. The deficiency of Clock does not induce any significant age-related changes in organs and tissues; however, they are affected in *Bmal1*$^{-/-}$ mice [60]. The results of Clock and Bmal1 deficiency in physiology are different. Clock encodes a novel bHLH–PAS domain protein of transcription factors [61, 62]. Clock is a unique gene with various features, including DNA binding, protein dimerization, and domain activation [62]. Clock is widely recognized to have circadian functions. The deficiency of the Clock protein significantly affects longevity. The aging of Clock-deficient mice results in a higher rate of pathologies, cataracts, and dermatitis than that of wild-type mice [60].

When Clock is a mutant gene, the pattern of circadian gene expression loses circadian behavior and is disrupted [61, 62]. While Clock in mice is knocked out, the robust circadian rhythms in spontaneous activity are still expressed [63]. Bmal1 forms a heterodimer as the transcriptionally active complex to drive circadian rhythmicity in Clock-deficient animals [64]. In addition, neuronal PAS domain protein 2 functionally replaces the Clock activity in Clock-deficient mice [65]. Age-related circadian changes are caused by both wild-type and heterozygous Clock mutants [66]. Clock–Bmal1 complex activate the transcription of other genes with E-box elements in their promoters [67]. The dimers translocate to the nucleus and obstruct the Clock–Bmal1 complex, thereby hindering the additional transcription of the other genes [68] and ultimately leading Clock and Bmal1 to increase Per transcription and restart the cycle [69]. Clock, as the core component of circadian transcription, operates in complex with another protein. Clock deficiency was shown to cause age-related cataracts [60]. Clock-deficient mice lose body weight, relative organ weight, or ectopic calcification during aging. These mice maintain behavioral rhythms, indicating that peripheral circadian oscillators require Clock [70].

3.3. Effects of the Clock–Bmal1 complex on the aging process

In animals, a transcription–translation loop that revolves around the transcription factors Clock and Bmal1 forms circadian oscillators. The core positive element of the clock is Clock–Bmal1, which is the heterodimeric transcription factor output of circadian locomotor cycles [71]. The negative elements of the clock PER and CRY proteins, associated with Clock–Bmal1 at E-box sites, enter the nucleus [64, 72] (**Figure 2**). The transcriptional activity of Clock–Bmal1 is suppressed by recruiting the SIN3–histone deacetylase (HDAC) complex and preventing transcriptional termination [72, 73]. Histone acetyltransferases (HATs) and HDACs generate rhythms in histone acetylation and the circadian rhythms [74]. Mixed-lineage leukemia protein-1, a H3K4 methyltransferase, has a nonredundant role in the circadian oscillator [75]. The Jumonji C domain-containing H3K4me3 demethylase family and AT-rich interaction domain-containing histone lysine demethylase 1a form a Clock–Bmal1 complex; the complex is then recruited to the Per2 promotor, simultaneously enhancing transcription by Clock–Bmal1 [76]. The transcription factors Clock and Bmal1, which form heterodimers and bind to an E-box enhancer element, regulate gene transcription [77, 78]. Bmal1 and Clock activate the genes Per and Cry. Once a certain concentration of PER and CRY proteins accumulate, they form BMAL1–CLOCK complex, consequently inhibiting gene transcription (**Figure 2**) [79, 80].

Clock–Bmal1 is not simply a passive consequence of negative feedback protein but rather induces proactive regulation [81]. As the core component of molecular clock, the Clock–Bmal1 complex controls numerous clock genes [82]. This complex may activate as well as repress transcription, and the switch depends on the interaction of Clock–Bmal1 with Cry [83]. The Clock–Bmal1-dependent recruitment of HATs promotes the periodic disruption of Clock–Bmal1 [84]. Clock–Bmal1 as a signaling molecule resets the clock through the Ca^{2+}-dependent protein kinase C pathway [85, 86]. The cAMP response element-binding protein activates the Clock–Bmal1 complex that rapidly resets phase and mediates the acute transactivation of Clock–Bmal1, consequently inducing immediate-early Per1 transcription [87]. Clock–Bmal1

DNA binding promotes rhythmic chromatin opening and remodeling. It mediates the rhythmic transcription factors binding to Clock–Bmal1 and the transcriptional output, suggesting that Clock–Bmal1 drives rhythmic gene expression and biological functions [88].

3.4. Effects of PER and CRY on the aging process

The Per and Cry genes can combine with the E-box domain promoters using Clock and Bmal1, thus driving the transcription of messenger RNA [79, 80]. Per and Cry genes play a negative transcriptional feedback loop in mouse [89]. When PER and CRY proteins reach adequately high levels, they form dimer feedback to the nucleus. The Clock–Bmal1 complex is then binded to turn off transcription [4]. Regulatory kinases Case in Kinase I epsilon (CKIε) of rodents phosphorylates PER and degrades it to feed back to the cell nucleus [90, 91]. CKIε masks the mPER1 nuclear localization signal, and mPer2 causes mPer1–mPer2 heterodimer formation in the cytoplasm. Phosphorylation-dependent cytoplasmic retention may be the reason for CKIε regulating the mammalian circadian rhythm [91]. In aged animals, Per1 transcription is induced by light and reduced with a significantly longer delay to resynchronization [92], whereas in young animals, the disruption of the Per genes results in insensitivity to light [89, 93].

Advancing age reduces retinal sensitivity, which causes various age-related diseases. In elderly people, homeostatic sleep is association with the circadian clock gene Per3 in coding regions. The Per3 gene associates with a phase advance in the melatonin profile; therefore, elderly people experience more nocturnal wakefulness [5]. In situ hybridization for Per2 mRNA revealed that the age-related decrease in the diurnal rhythm amplitude in the hippocampus may aggravate cognitive deficits [94]. Pattern differences in clock gene expression can be associated with a depressive state. Under abnormal light–dark conditions, Per1 and Per2 genes may result in a depressive state [95]. The expression of rPer1, rPer2, or rCry1 mRNA is similar in both young and old SCN; however, when stimulated by light, aging reduces the gene expression [89]. A decreased Per gene expression suggests an impaired clock regulatory network and stress defense pathways may accelerate aging [96].

3.5. Effects of the SIRT1 gene on the aging process

Sirtuins belong to the silent information regulator (SIR)2 family of proteins [97, 98]. SIR2 and its orthologs regulate senescence in yeast, worms, and flies [99–101]. SIR2 retards senescence and extends the lifespan of diverse species through caloric restriction [101, 102]. Sirtuins are nicotinamide adenine dinucleotide (NAD^+)-dependent deacylases, which promote cell survival by suppressing apoptosis or senescence [103]. Sirtuins play key roles in expediting the resistance by increasing antioxidant pathways and facilitating DNA damage repair [104]. Seven mammalian sirtuins exist, and SIRT1 is one of the most crucial mammalian SIR2 orthologs [98]. SIRT1 is involved in cellular metabolism and circadian core clockwork machinery in biological systems [105]. The direct deacetylation activity and NAD^+ salvage pathway were found to correlate with SIRT1 in the circadian rhythm system [106]. In addition, SIRT1 regulates the Clock–Bmal1 complex, and deacetylates and degrades Per2 [79]. The SIRT1 activity contributes to disturbances in the acetylation of H3 and Bmal1 and transduces cellular

metabolic signals [105]. Moreover, SIRT1 has multiple downstream targets, including p53, Ku70, nuclear factor-kappaB, forkhead box O factors, peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-alpha (PCG-1α), PPARγ [98].

The central pacemaker is activated by SIRT1 to maintain robust circadian, and its decaying may accelerate aging [107]. SIRT1 can produce moderate changes in the intrinsic circadian period and is associated with age-related decline in the central clock. Some studies have reported a possible association between aging, sirtuins, and clock genes. SIRT1 activates the transcription of Bmal1 through PGC-1α to increase the amplitude of expression of BMAL1 and other proteins to control circadian rhythms in the SCN [108]. Recent studies have suggested that caloric restriction is a nongenetic manipulation that extends the lifespan; it is beneficial because SIRT1 has led to the search for sirtuin activators [109]. SIRT1 avoids mice getting diet-induced obesity by deacetylating and activating PGC-1α in the skeletal muscles [110]. In addition, the activity of the adipogenic nuclear receptor PPARγ is repressed by SIRT1 activation, thus reducing fat accumulation and adipocyte differentiation [98]. Moreover, SIRT1 mediates the lifespan extension through caloric restriction, affecting metabolism and lifespan in humans.

3.6. Effects of the ROR gene on the aging process

RORs are considered a core clock machinery because they regulate the cyclic expression of Bmal1 and Clock, thus providing an essential link between the positive and negative loops of the circadian clock [111]. RORs can be classified into three types: RORα, RORβ, and RORγ. RORα is the first member of the ROR subfamily and resembles the retinoic acid receptors and RXRs [112]. Both RORα and RORβ are required for the maturation of photoreceptors in the retina, and RORγ affects the development of several secondary lymphoid tissues [113].

RORα, an orphan nuclear receptor, plays a crucial role in integrating the circadian clock and regulating the cardiovascular function. Four RORα isoforms exist in humans, namely RORα1–4, whereas only two isoforms α1 and α4 are present in mouse [114]. These isoforms participate in the different physiological processes and exhibit a distinct pattern of tissue-specific expression. For example, the expression of RORα1 and RORα4 was significantly higher in mouse cerebellum than in other tissues, whereas RORα4 is predominantly expressed in liver tissues [115]. RORα and REV–ERBα compete for the binding of their shared DNA-binding elements in the Bmal1 promoter (**Figure 2**) [116, 117]. RORα displays rhythmic expression patterns during the circadian cycle in some tissues. RORα expression shows a weak circadian oscillation in the liver, kidneys, retina, and lungs [114, 118, 119]. Moreover, RORα promotes Bmal1 transcription [116] through two RORα autonomous response elements [118]. RORα1 enhances the circadian amplitude of Bmal1 mRNA expression and regulates the downstream clock genes after serum shock [118]. In addition, the reduced expression of RORα is closely associated with aging in genetic hypertensive rats [120]. The enhancement of the RORα expression was shown to antiaging in human endothelial cells by facilitating Bmal1 transcription, [121]. The deficiency of RORα causes cerebellar hypoplasia and affects Purkinje cell survival and differentiation in aging [122]. RORα has been shown to restraint age-related degenerative, including muscular atrophy, immune deficiencies, osteoporosis, atherosclerosis, and inflammation [123,

124]. RORα plays a neuroprotective role during development and provides protection against this injury [124].

3.7. Effects of the REV–ERBα gene on the aging process

REV–ERB nuclear receptors are considered to be essential core clock components and serve as pivotal regulators of rhythmic metabolism [125]. REV–ERB has α and β isoforms, which coexpression in adipose tissues and the liver and brain [126, 127]. REV–ERBα, an orphan nuclear receptor, negatively regulates the activity of the Clock–Bmal1 complex. Its transcription is controlled through Per and Cry transcription [128]. REV–ERBα competes with activators of the ROR family of nuclear receptors for binding to specific ROR-responsive elements to ensure the rhythmic transcription of Bmal1 and Clock [118, 129]. The development of metabolic disorders indicates disrupted circadian rhythms [111]. REV–ERBA is associated with circadian rhythms and metabolism and is pivotal in regulating the core mammalian molecular clock (**Figure 2**). Furthermore, REV–ERBα controls the transcription of metabolic pathways while serving as a crucial output regulator.

Age is the essential risk factor for metabolic syndrome. REV–ERBα connects the core clock and numerous physiological processes. The deficiency of REV–ERBα results in asynchronous circadian rhythms, with a tendency for diet-induced obesity, impaired glucose, and lipid utilization leading to an increased risk of diabetes [130]. Evidence suggests that REV–ERB has opened new avenues for treating metabolic syndrome by affecting the circadian rhythm and metabolism [111]: for example, targeting REV–ERBα as a critical component of the peripheral clock to treat bipolar disorder by affecting glycogen synthase kinase 3 [131]. REV–ERB agonists can reduce dyslipidemia, hyperglycemia syndromes, and obesity [130, 132].

4. Drug discovery

New biological targets are being developed for discovering novel drugs. People can identify with aging, thus antiaging drugs constitute a lucrative market. Medical therapy contributes in delaying the aging process in humans. Researchers focus not only on the compounds having a validated target but also on herbs used as antiaging products in traditional medicine. Some drugs used to treat chronic diseases can be examined; these diseases are associated with senescence, such as cardiovascular diseases, type 2 diabetes mellitus, and cancer. Some studies on the compounds have been conducted, whereas studies on herbs are necessary. Many lead compounds and approved drugs are derived from herbs. In China, traditional Chinese medicine (TCM) has been investigated in large-scale clinical trials, with extensive research on aging concerns. With the development of longevity drugs, Chinese herbs have been demonstrated to retard aging, such as *Polygonum multiflorum*, Ginseng. Meanwhile, TCM is gradually attracting worldwide attention.

4.1. Compounds as antiaging drugs

4.1.1. Rapamycin

Rapamycin, used as an immunosuppressive drug, has been recently considered for antiaging treatments. Rapamycin retards multiple aspects of aging, including extending the lifespan and slowing aging in mice [133]. Rapamycin evidences an association between metabolism and longevity by controlling the target of rapamycin kinases to regulate cell growth and proliferation through mitogenic signals [134]. In addition, rapamycin extends mouse lifespan principally by inhibiting many aspects of cancer development [135]. Rapamycin appears to retard the effects of age on the liver, endometrium [133], and bone marrow [136]. Rapamycin was shown to reduce the effects of aging in mice [137]; it can retard clinically relevant β-amyloid and tau accumulation in aged CNS tissues [138].

4.1.2. Resveratrol

Resveratrol, a natural phenol, retards aging by selective SIRT1 activation. Resveratrol as an established antioxidant has multiple beneficial activities and delays the onset of age-associated diseases. Resveratrol can reduce the expression of SIRT1 by using an acetylated substrate and NAD^+ [109]. In yeast, resveratrol extends the lifespan by upregulating Sir2 or increasing DNA stability [99]. Resveratrol has antioxidant, antiaging, and antiangiogenic properties. Furthermore, resveratrol plays a dual role on the vasculature; it maintains vascular fitness through its anti-inflammatory and anticoagulant activities, whereas it inhibits angiogenesis to suppress tumor growth [139].

4.2. Herbs as antiaging drugs

4.2.1. Polygonum multiflorumygonum multiflorum

Polygonum multiflorum, a crucial TCM, is extensively used for health maintenance and disease treatment, particularly as an antiaging drug in China. An active component extracted from the roots of P. *multiflorum* Thunb is called 2,3,5,4'-tetrahydroxystilbene-2-O-β-d-glucoside (THSG). THSG, the major bioactive compound of *P. multiflorum*, has antioxidant [140], anti-inflammatory [141], and endothelial-protective activities [142]. Our previous study revealed that THSG prevents vascular senescence by increasing eNOS expression and SIRT1 activity and reducing the acetylation of p53 at the K373 site [143]. In another study, we indicated that THSG extends the lifespan by promoting the expression of the longevity gene Klotho [144]. The antioxidant capacity of THSG is similar to resveratrol or even stronger. Moreover, THSG possesses a potent antioxidant capacity and extends the lifetime of the nematode *Caenorhabditis elegans* [145]. THSG is a potent antiaging drug and has potential as a pharmaceutical antiaging drug.

4.2.2. Ginseng

Ginseng is a highly valued herb in Asia and is recorded in Chinese Pharmacopoeia. The major active components of ginseng are ginsenosides. The beneficial effects of ginseng have been

investigated; ginseng and its constituents have antianemic, antiaging, antioxidant, antineoplastic, and anti-stress activities. Rg1 and Rb1 improve the spatial learning ability by increasing the hippocampal synaptic density [109, 110]. Ginseng could play a crucial role in enhancing the defense system during the aging process. The mechanisms of ginsenosides warrant further investigation.

5. Conclusion

Considerable progress has been made to unravel the association between the circadian clock and aging. The role of circadian clocks in governing many other physiological systems has been reported; however, sufficient data are lacking. The circadian clock represents a nearly ubiquitous aspect of cellular regulation and molecular regulatory process, which exerts effects on organismal behavior. The researches on circadian clock genes regulation in aging have emerged recently, making the issue become attractive and outstanding.

The association between the circadian clock genes and aging might affect age-related diseases. The circadian clock is altered in normal aging, resulting in a decreased amplitude of rhythms in daily life, subsequently posing difficulties for humans to adjust to temporal changes. The study has major implications in determining why circadian rhythms diminish during aging and whether this decline could be reversed to tissue homeostasis and age-related diseases. However, additional studies are required to comprehensively understand the circadian clock effects on aging and obtain the target drug for extending the lifespan. Circadian genes must be extensively studied, including their different signaling pathways that are unknown, particularly the association between circadian genes and diseases. Focus on the effects of gene expression, translation, and signaling alterations on the biological clock would increase our knowledge regarding individualized treatment program and drug discovery.

Till date, studies have revealed several circadian clock genes that are crucial in regulating the lifespan. The manipulation of circadian clock genes would largely benefit the study of aging. This study not only explains circadian rhythm affects aging but also presents drugs for determining the therapeutic potential of targeting the circadian clock in diseases.

Author details

Yufeng Li, Yanqi Dang, Shuang Ling and Jin-Wen Xu*

*Address all correspondence to: jinwen.xu88@gmail.com

Murad Research Institute for Modernized Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China

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