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Review

Q1 Circadian clock and bone biology

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ABSTRACT

Background: Many metabolic and physiological processes display circadian oscillations that are controlled by the internal circadian clock system. A molecular clock system is generated by the transcriptional-translational feedback loops of clock genes. Circadian oscillators reside in peripheral tissues where they receive timing cues from the central clock in the suprachiasmatic nucleus (SCN). Circadian rhythms have also been identified in bone and cartilage. Q2 Q3

Highlight: Recent findings demonstrated that bone metabolic functions of the major cell types namely osteoblasts, chondrocytes, and osteoclasts, are closely related to an intrinsic biological rhythm, named the circadian clock. Several studies have also shown that clock genes play significant roles in bone formation and resorption. Adrenergic receptor signaling in osteoblasts was previously reported to synchronize clock genes as well as genes that are important for osteoblast function such as Ptgs2 gene encoding prostaglandin G/H synthetase 2, which is a late-limiting enzyme for prostaglandin synthesis. Since the circadian clock regulates key molecules in cellular functions of osteoblasts and osteoclasts, this system may be closely involved in the cellular functions associated with bone remodeling.

Conclusion: This review focuses on the latest knowledge on the molecular mechanisms of biological clocks and their roles in bone biology, indicating the potential of this clock system in the treatment of bone disorders.

Contents

Abbreviations: AR, adrenergic receptor; BMAL1, brain and muscle arnt-like 1; BMP, bone morphogenetic protein; CCG, clock-controlled genes; CLOCK, circadian locomotor output cycles kaput; CRY, cryptochromes; E4BP4, E4 promoter-binding protein 4; FGF, fibroblast growth factor; Ihh, indian hedgehog; NFATc1, nuclear factor of activated T cells cytoplasmic 1; Nfil3, nuclear factor IL-3; OPG, osteoprotegerin; PER, period; PPR, PTH/PTHrP receptor; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; RANKL, receptor activator of NF-κB ligand; ROR, retinoid acid-related orphan receptor; SCN, suprachiasmatic nucleus; TRAP, tartrate-resistant acid phosphatase

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1. Introduction

Circadian rhythms are daily fluctuations over a period of approximately 24 h in physiology and behavior that have evolved in response to the Earth's rotation. The circadian clock, an evolutionarily conserved mechanism, is an essential time-keeping system that entrains the internal physiology to environmental cues. The circadian clock system comprises a hierarchal structure [1–3]. The main circadian rhythm is controlled by the central clock in the suprachiasmatic nucleus (SCN) located in the hypothalamus, and receives light input from the retina through the retino-hypothalamic tract. The SCN is regarded as the master circadian pacemaker that controls most of the physical circadian rhythms in mammals. Peripheral clocks are self-sustaining clock mechanisms existing in peripheral tissues where they receive timing cues from the SCN.

The circadian autoregulatory loop is generated by a transcriptional-translational mechanism that shares the same molecular components and is conserved between species. The circadian clocks in the SCN of the clock center, in peripheral tissues, and even in cultured cells essentially share the same mechanisms [4]. Circadian rhythms are controlled by a negative feedback mechanism involving clock genes. The transcription factor brain and muscle arnt-like 1 (BMAL1) is an essential positive regulator of the core molecular clock loop [5]. BMAL1 forms a heterodimer with circadian locomotor output cycles kaput (CLOCK), which recognizes a conserved E-box (CACGTG) to elicit target gene transcription. In the nucleus, BMAL1/CLOCK heterodimers directly activate negative regulators of the core clock circuit, the *Period* (*PER*) genes (*PER1*, *PER2*, and *PER3*) and *Cryptochromes* (*CRY1* and *CRY2*), through the E-Box, a transcription factor binding site located in the promoter region of *PER* genes [5–7]. These factors then inhibit the BMAL1/CLOCK-mediated gene transactivation, which, coupled with temporally controlled post-translational modifications (phosphorylation, ubiquitination, acetylation, and proteasome-mediated degradation), generates the daily oscillations of the molecular clock [8–11]. An additional transcriptional negative feedback loop involves the nuclear receptors Rev-erbα/β and retinoid acid-related orphan receptor (ROR)-α and forms a reinforcing mechanism that safeguards the robustness of the clock circuitry [12,13]. BMAL1/CLOCK activates Rev-erbα transcription, resulting in daily fluctuations in Rev-erbα expression, which in turn represses *Bmal1*. Together with positive transcriptional regulation by retinoid acid-related orphan receptors (RORα, β, γ), Rev-erbs/RORs generate rhythmic oscillations in *Bmal1* gene transcription, and occupancy of the *Bmal1* promoter by either Rev-erbα or RORα is crucial for proper timing of the core clock machinery [13].

Circadian rhythms have also been identified in bone and cartilage [14–16]. A previous study reported circadian rhythms in the articular cartilage and growth plates in mouse *ex vivo* bone cultures [17]. Osteoblasts have been shown to express clock genes associated with the circadian signaling pathway. Bone mineralization in developing bone tissue has been demonstrated to be linked to circadian oscillator mechanisms using non-invasive Raman microscopy to track mineral accumulation in *ex vivo* neonatal murine calvarial organ cultures [18]. Osteoclast resorptive activity also exhibits circadian rhythmicity and is controlled by various endocrine hormones and cytokines [19–22]. The clock system has recently been identified as one of the major mechanisms controlling cellular functions. Circadian clock gene oscillations also participate actively in the functions of various cells. Previous studies demonstrated that clock genes play significant roles in bone formation and resorption, suggesting the potential of these genes as novel therapeutic targets for skeletal diseases. This review summarizes recent knowledge on clock and clock-controlled genes in the bone that have been shown to critically affect bone metabolism.

2. Circadian rhythms in endochondral bone formation

Recent studies identified an autonomous circadian clock in cartilage [17,23], and demonstrated that clock genes play significant roles in chondrocytes. Chondrocyte BMAL1 is required for the normal daily functions and integrity of adult articular cartilage [24]. Furthermore, a BMAL1 deficiency resulted in an accelerated aging phenotype, and has been suggested to play a role in the development of age-associated diseases such as osteoarthritis [24].

Endochondral ossification is an essential process of skeletal development in the fetal and neonatal periods [25]. During skeletal development, mesenchymal progenitor cells undergo a multistage differentiation process in which they proliferate and differentiate into bone- and cartilage-forming cells. Signaling molecules play important roles in the regulation of chondrocyte development [25]. Fibroblast growth factor (FGF) signaling [26,27], bone morphogenetic protein (BMP) signaling [28], and Indian hedgehog (Ihh) signaling [29–31] participate in regulating chondrocyte proliferation and differentiation. Parathyroid hormone (PTH)-related protein (PTHrP), regulated by Ihh and acting through the PTH/PTHrP receptor (PPR), is crucial for normal bone growth in the fetus and for subsequent bone growth in postnatal life [25,32]. PTHrP regulates the length of the columnar region by allowing continued proliferation of columnar chondrocytes and suppressing their terminal differentiation into post-mitotic hypertrophic chondrocytes [33]. On the other hand, previous studies using conditional knockout of BMAL1 showed that endochondral ossification is regulated by particular clock genes expressed in chondrocytes [34]. Takarada et al. demonstrated that the clock gene *Per1* negatively regulated chondrocyte differentiation by suppressing the functional E-box in the *Ihh* promoter [34]. Administration of PTH induced phase shifts in oscillations in the circadian clock in the growth plate and articular cartilage of a bone organ culture system. It also reset the circadian clock of chondrocytes generated during the process of bone fracture healing [35]. Hinoi et al. suggested that PTH induced the expression of *Per1* and *Per2* through the PPR in organ-cultured fetal metatarsals [36]. Therefore, the actions of PTHrP may mediate some of the circadian clock gene expression in chondrocytes. Collectively, these findings provide *in vivo* and *in vitro* evidence for the importance of the molecular clock in endochondral bone formation.

3. The circadian clock is involved in the regulation of bone metabolism

The central clock SCN is considered to orchestrate the circadian clock in peripheral tissues through neuronal and/or neuronal hormonal signals [37–40]. Although peripheral circadian oscillators have been implicated in the physiological functions of various tissues, the extent of their influence currently remains unknown [41,42]. Previous studies have characterized circadian clock gene oscillations in bone cells [22,43], and indicated that neuronal and/or hormonal signals stimulated oscillations in the circadian clock genes in osteoblasts and osteoclasts (Fig. 1). Clock genes, including *Per1*, *Per2*, and *Per3*, were found to be significantly upregulated by β-adrenergic receptor (AR) signaling in human osteoblasts [44]. The circadian expression of Fgf23, which is an important regulator of phosphate and vitamin D metabolism, in the skeleton was assessed by food intake and was found to be involved in the systemic activation of sympathetic tone [45]. In addition, AR signaling regulates the Ptgs2 gene encoding prostaglandin G/H synthetase 2, which is a late-limiting enzyme for prostaglandin synthesis, by driving clock genes in osteoblasts (Fig. 2) [46]. The BMAL/CLOCK complex transcribes a number of downstream targets, termed as clock-controlled genes (CCGs) that encode other transcription

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