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Synchronized human osteoblasts exhibit circadian rhythmicity in expression of genes involved in collagen turnover

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The human circadian system modulates cell activities along the 24-h time scale to assure predictive adaptation of individuals to the 24-h day/night alternations; it operates through interlaced molecular loops involving the recurrent expression of clock genes within each cell, and a central synchronization system in the suprachiasmatic nuclei of the hypothalamus. In cell cultures, exposure to serum treatment allows to obtain *in vitro* models of synchronized cells to investigate circadian periodicity in cell morphology and activities. Osteoblasts play a key role in regulating bone matrix collagen turnover; since biochemical markers of bone formation/resorption exhibit circadian variations in healthy humans, we investigated the circadian pattern of collagen turnover-related genes in primary cultures of synchronized osteoblasts.

Cells obtained from the mandibular bone of a healthy 20-year-old woman were grown with 199 medium plus 10% fetal bovine serum (FBS) and antibiotics. At the 4th passage, fully confluent cultures were treated with 50% FBS for 2 hours and then maintained in 199 medium containing 10% FBS. From the end of the serum shock, at 4 hour intervals for 48 hours, cells were harvested and the extracted total RNA was analysed for the expression of clock genes (Bmal1, Cry1) and collagen turnover-related genes (COL-I, MMP-1, MMP-2, TIMP-1, LH2b, SPARC) by real-time PCR. Rhythmometric analysis of the data was performed by the cosinor method.

In osteoblasts exposed to serum shock, the expression of clock genes exhibited circadian rhythmicity ($p < 0.05$) starting from the end of the serum treatment for 28 hours. Also the expression of genes related to collagen synthesis and maturation (COL-I and LH2b), and degradation (MMP-2, TIMP-1) showed circadian rhythmicity ($p < 0.05$), every gene being rhythmically expressed for 24 hours, starting 4, 8, or 16 hours from the end of the synchronizing stimulus. The results suggest a role of clock genes in controlling the circadian expression of downstream genes related to the collagen turnover in bone and rise the hypothesis that responses to substances affecting bone remodelling might display variations depending upon time of treatment, leading to different outcomes.

Key words

Circadian rhythms, human osteoblasts, gene expression, collagen turnover