

mRNA of Expression of Per 1 in Mice Bone Marrow Mesenchymal Stem Cells Irradiated by Red Laser

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Abstract

A core group of circadian genes regulate the circadian rhythms in mammalian cells. However, the mammalian cellular circadian rhythm in photobiomodulation remains unknown. A lot of evidence has shown that >20% of expressed mRNAs in bone tissues oscillate in a circadian manner. The aim of this paper is to investigate the mRNA expression of period 1 (per 1) in murine bone marrow-derived mesenchymal stem cells (BMSCs) which were irradiated by 635 nm red laser light. The cells were seeded in 35mm tissue-culture dishes at a density of 8×10^4 cells/dish and cultured in Dulbecco's modified Eagle's medium (DMEM). BMSCs were irradiated once by 635 nm red light with radiation energies 0 J/cm², 1 J/cm², 4 J/cm². mRNA expression of *per 1* via Semi-Quantitative Real-time RT-PCR at 0h, 6 h, 12 h, 18 h, 24 h. The genes displayed an oscillatory period of nearly 24 hours. And 635 m laser light changed the mRNA expression of *per 1*. We conclude that red light irradiation can affect the circadian rhythm of BMSCs.

Keywords

Photobiostimulation, Mesenchymal Stem Cells (MSCs), Period 1, Circadian Rhythm

1. Introduction

Low-level laser therapy (LLLT) was introduced by the work of Mester *et al.* [1] more than 30 years ago. It has been shown to modulate cellular proliferation [2]-[5]. Moreover, in a recent study by Abramovitch-Gottlieb *et al.* [6], a low-energy laser was found to stimulate the osteogenic phenotype of mesenchymal stem cells (MSCs) in a three-dimensional biomatrix.

Circadian rhythms are oscillations in the behavior and physiologic processes in organisms ranging from algae to man, and occur with a periodicity of approximately 24 h [7]. The core circadian rhythms genes include two cryptochrome genes (Cry1 and Cry2), three homologs of the period genes (Per1, Per2, and Per3), and the transcriptional activator genes Clock and Bmal1 (brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1) [8]. This oscillator is thought to be composed of interlocking auto regulatory feedback loops, the transcription/translation feedback loop. The PER1 and PER2 and CRY1 and CRY2 act as negative regulators of transcription driven by the BMAL1-CLOCK heterodimer.

Here we demonstrate that irradiation with a red laser (wavelength 635nm) alters the mRNA expression of primary mouse MSCs.

2. Materials and Methods

2.1. Cell Culture

MSCs were isolated from the bone shaft of femurs and tibias of 12-week-old male C57BL/6 mice. Briefly, both the ends of mice femurs and tibias were cut away from the epiphysis and the bone marrow was flushed out with a syringe with 0.5 mL of Dulbecco's modified Eagle minimal essential medium (DMEM)/low glucose supplemented with 10% fetal calf serum (FCS) (Hyclone, UT, USA), 100U/ml was placed into T-25 tissue (Greiner, Frickenhausen, Germany) culture flasks at 37°C in a 5% CO₂ atmosphere. The medium was changed every 3 d. When the proliferating cells became subconfluent, usually after 7 - 10 d, the cells were trypsinized with 0.25% trypsin (Gibco/BRL, CA, USA) in phosphate buffered saline (PBS). All cells used for the experiments were the third passage

2.2. Procedure of Irradiation

A laser with a continuous wavelength of 635 nm (a power output of 960mW) was used in this study. The diameter of light spot is 35 mm. Before the irradiation, BMSCs (passage 3) were synchronized by exposed to 1 μM dexamethasone for 2 hours. In this manner, 6 wells plate (Jet-Biofil, Guangzhou, China) were simultaneously irradiated on a clean bench. Total energy corresponding to 10 sec exposure was 1 J/cm², 40 sec exposure was 4 J/cm². As controls, 6-wells plate was placed on a clean bench without any irradiation.

2.3. Analysis by Semi-Quantitative Real-Time RT-PCR

Total RNA was extracted 24h after irradiation from rat BMSCs cultured in 35mm dishes by an acid guanidinium thiocyanate-phenol-chloroform extraction method. Cells were homogenized using TRIZOL reagent (Invitrogen, CA, USA), and total RNA was isolated. RNA was reverse transcribed with reverse transcriptase kit (Toyoba, Osaka, Jap). Aliquots of cDNA were amplified in a 25 μl polymerase chain reaction (PCR) reaction mixture which contained 10 nM 5'- and 3'-oligomers (period 1(5'-AGC AAG CCT TCC TCA ACC-3'; 5'-TTT AGA TCG GCA GTG GTG T-3'), β-actin (5'-AGC CAT GTA CGT AGC CAT CC-3'; 5'-CTC TCA GCT GTG GTG GTG AA-3').

2.4. Statistical Analysis

Results are presented as means ± S.D. of three independent experiments. Statistical significance was determined by analysis of variance (ANOVA), and P values of <0.05 were considered significant.

3. Result

Individual plates were harvested at 6 hour intervals for total RNA isolation and subsequent real time PCR analysis of mRNA expression (**Figure 1**). Non-irradiated BMSCs showed a robust circadian oscillating expression of per 1 with a period of nearly 24 hour. The peak acrophase was 6 h and 24 h, and the trough was 0.5 h and 18 h. Irradiated BMSCs showed different circadian rhythms with non-irradiated group. Both 1 J and 4 J groups had a trough at 6h when non-irradiated group had a peak. 4 J group also had a peak at 18 h and a trough at 24 h. 1 J had a similar wave shape with non-irradiated group since 12 h.

4. Discussion

As a novel reconstructive therapy, BMSCs are receiving attention because of their easy isolation, expansion, low immunogenicity and potential for long-term generation, making them ideal for repairing bone defects in bone bioengineering. BMSCs exhibit a fibroblast-like appearance, and can differentiate in vitro into various anchorage dependent cell types including but not limited to bone, cartilage and adipose tissue.

Circadian clock and cell cycle are global regulatory systems found in almost all organisms. The circadian clock shares a number of conceptual and molecular similarities with the cell cycle [9]. Both are periodic for 24

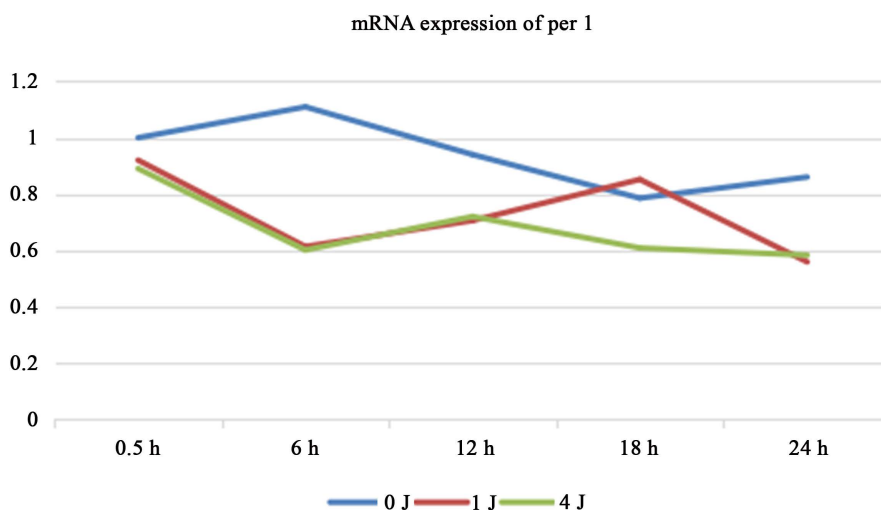


Figure 1. Real time PCR analysis was performed to examine expression of mRNAs Period 1 (per 1).

hours, and intrinsic to most cells. Similarly, both are based on the conceptual device of interlock auto-regulatory loops. Moreover, both rely on sequential phases of transcription, translation and protein modification and degradation. The circadian clock controls the expression of cell cycle-related genes; in contrast, circadian clockwork can oscillate accurately and independently of the cell cycle [10]. The circadian genes may regulate the cell proliferation via controlling cell cycle.

Some studies have shown that proliferation of MSCs can be promoted by laser irradiation [5] [11]. However, mechanism of cell proliferation induced by LLLI is still not fully understand. Kushibiki *et al.* in their study showed that laser irradiated MSCs altered the intracellular localization of the circadian rhythm protein CRY1 [12]. It is supposed that circadian rhythm maybe is one of the pathway to explain the photobiostimulation.

This study demonstrated the circadian expressions of per 1 in the mice BMSCs. The MSCs showed nearly 24 hours circadian rhythm, and irradiated MSCs showed different rhythms with non-irradiated group. 1 J group had a opposite wave shape to non-irradiated group's wav during the first 12 hours, then returned to the similar shape with the non-irradiated cells during the following 12 hours. 4J group had a totally opposite wave in the 24 hours period.

5. Conclusion

The study demonstrates that the abilities of multiple dose of laser at 635 nm to alter the circadian rhythm expression of per 1. We still not know the causal relationship between the circadian rhythm genes and photobiostimulation, however the findings provide more understanding about mechanism of cell proliferation induced by LLLI.

Acknowledgements

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