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Photoactivation of TAZ via Akt/GSK3β signaling pathway promotes osteogenic differentiation

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ABSTRACT

Osteogenesis disorder is involved in osteoporosis and other related bone diseases, in which osteogenic differentiation is essential. Osteogenic differentiation is a complicated process regulated by intricate signal transduction networks. It has been reported that low-power laser irradiation (LPLI) has an osteogenic potential by promoting osteoblast differentiation. However, the molecular mechanisms remain to be understood. In this study, we reveal a novel mechanism that Akt/GSK3β/TAZ (transcriptional co-activator with PDZ-binding motif) signaling pathway plays a crucial role in LPLI-enhanced osteoblast differentiation. Photomodulation by LPLI activated Akt/GSK3β pathway which inhibited TAZ phosphorylation, leading to the increase of TAZ protein level and nuclear aggregation. Meanwhile, knockdown of TAZ suppressed osteogenic differentiation promoted by LPLI. Further study showed that LPLI promoted the interaction between TAZ and core-binding factor 1 (Cbfa1), up-regulating the transcription of osteopontin (OPN) and osteocalcin (OCN) and the activity of alkaline phosphatase (ALP). However, inhibition of Akt/GSK3β pathway reversed the effects of TAZ on osteogenic differentiation induced by LPLI. Taken together, for the first time, we report that LPLI promotes osteoblast differentiation via TAZ activation dependent on Akt/GSK3β signaling pathway.

1. Introduction

Osteoporosis is one of the most widespread degenerative diseases in association with the negative remodeling balance between bone resorption and formation, resulting in serious consequences, such as the promotion of bone fracture and disability (Schett and Bozec, 2014). Currently only symptomatic treatments are available in the form of bone resorption inhibitors, and the effect of these treatments on increasing or recovering bone mass is relatively small. Therefore, a new therapeutic strategy is urgently needed to stimulate new bone formation and correct the imbalance of trabecular microarchitecture characteristic of established osteoporosis. Osteogenic differentiation is a complex procedure involving numerous signaling cascades, which synergizes to direct the higher-order functions of bone formation, repair, and maintenance (Chubinskaya and Kuettner, 2003). This may have important therapeutic consequences, for example, affecting osteogenic differentiation by modulating transcription factors, which leads to an increase in bone formation.

Transcriptional co-activator with PDZ-binding motif (TAZ) is involved in the development of multiple organs by modulating the activity of transcription factors, such as paired box homeotic gene 3 (PAX3), paired box homeotic gene 8 (PAX8), myogenic differentiation antigen (MyoD), nuclear factor of activated T-cells (NFAT5), peroxisome proliferator-activated receptor γ (PPARγ) and so on (Chan et al., 2009; Jang et al., 2012; Jeong et al., 2010; Jung et al., 2009; Murakami et al., 2005; Ota and Sasaki, 2008; Varelas et al., 2008; Wrighton et al., 2008; Zhang et al., 2009). The critical regulation for TAZ activity is subcellular localization and protein level controlled by phosphorylation modification which leads to TAZ ubiquitylation and degradation. TAZ phosphorylation is regulated by multiple kinases, including casein kinase 1 (CK1), glycogen synthase kinase 3β (GSK3β) and large tumor suppressor (Lats) (de Cristofaro et al., 2011; Hergovich and Hemmings, 2010; Huang et al., 2012; Kanai et al., 2000; Lei et al., 2008; Liu et al., 2010, 2011; Mahoney et al., 2005). Core-binding factor 1 (Cbfa1)/Runt-related

Abbreviations: LPLI, low-power laser irradiation; ALP, alkaline phosphatase; BMP2, bone morphogenetic protein 2; Cbfa1, core-binding factor 1; CK1, casein kinase 1; DEX, dexamethasone; GSK3β, glycogen synthase kinase 3β; LATS, large tumor suppressor; MyoD, myogenic differentiation antigen; NFAT5, nuclear factor of activated T-cells 5; OPN, osteopontin; OCN, osteocalcin; PAX3, paired box homeotic gene 3; PAX8, paired box homeotic gene 8; PDK1/2, phosphoinositide dependent kinase 1/2; PI3K, phosphatidyl Inositol 3-kinase; PPARγ, peroxisome proliferator-activated receptor γ; RTK, receptor tyrosine kinases; TAZ, transcriptional co-activator with PDZ-binding motif.

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gene 2 (Runx2) is identified as an osteoblast-specific transcription factor. Cbfa1 controls osteoblast differentiation through regulating the activity of alkaline phosphatase (ALP), and the expression of osteocalcin (OCN), a1 (I) collagen, bone sialoprotein and osteopontin (OPN) (Ducy et al., 1997; Fujita et al., 2004; Komori et al., 1997; Kwun et al., 2010; Zhao et al., 2005). Byun and Hong reported that TAZ could promote osteoblast differentiation by enhancing Cbfa1-dependent transcriptional activation (Byun et al., 2012a,b; Hong et al., 2005). Cui et al. also found that TAZ as a transcriptional co-activator might be critical in the regulation of bone-specific genes expression (Cui et al., 2003). All of these studies demonstrate that TAZ plays an important role in osteoblast differentiation and has been used as a vital marker for indicating osteogenic potential (Cho et al., 2010; Jung et al., 2009; Zhao et al., 2009).

Low-power laser irradiation (LPLI), as a relatively noninvasive technique, with light spectrum from the visible to near-infrared range can modulate various biological processes (Karu, 1989). Recently, a large number of studies have suggested that LPLI is a well-accepted therapeutic tool in the treatment of regeneration limitation and wound healing through regulating cell survival, proliferation and differentiation (Alfredo et al., 2012; Antunes et al., 2007; Feng et al., 2012; Hawkins et al., 2005; Huang et al., 2013; Kazem Shakouri et al., 2010; Karu, 1987; Liang et al., 2012; Liu et al., 2007; Wu et al., 2011b; Zhang et al., 2009, 2012). Increasingly, reports demonstrate LPLI has a positive effect on muscle-skeletal conditions and osteogenic differentiation (Bouvet-Gerbettaz et al., 2009; Fávaro-Pípi et al., 2011; Hou et al., 2008; Kiyosaki et al., 2010; Pires-Oliveira et al., 2008; Renno et al., 2007; Stein et al., 2005; Stein et al., 2008). Therefore, LPLI could be an effective therapeutic strategy in treating bone disease, such as osteoporosis. However, the relevant intracellular molecular signaling pathways underlying LPLI treatment are not fully elucidated.

In this study, a novel cellular signaling mechanism is proposed that Akt/GSK3β/TAZ signaling pathway activated by LPLI enhances osteoblast differentiation. We demonstrate for the first time that LPLI increases the protein level and nuclear aggregation of TAZ through inhibition of its serine phosphorylation dependent on Akt/GSK3β signaling. Moreover, LPLI promotes the interaction between TAZ and Cbfa1, which leads to transactivation of OPN and OCN. These findings highlight the essential role of TAZ in LPLI-enhanced osteoblast differentiation, providing deeper insight into the therapeutic potential for osteoporosis and other related bone diseases by targeting TAZ.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (Grand Island, NY, USA). LiCl was purchased from BioMol Research Laboratories, Inc. (Plymouth Meeting, PA, USA). Lipofectamine™ 2000 reagent was purchased from Invitrogen (Carlsbad, CA). API-2, ascorbic acid and β-glycerophosphate were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Wortmannin and FTS were purchased from Cayman Chemical (Ann Arbor, MI). The concentrations of LiCl, wortmannin, API-2, FTS, ascorbic acid and β-glycerophosphate used in our experiments were 10 mM, 100 nM, 1 μM, 10 μM, 50 μg/ml, and 10 mM, respectively. The antibodies used for western blot included antibodies against TAZ, β-actin and Histone H3 (Santa Cruz, CA); phospho-Akt (Ser473), Akt, phospho-GSK3β (Ser9), GSK3β; phospho-β-catenin (Ser675), β-catenin, phospho-Lats1 (Thr1079), Lats1 (Cell Signaling Technology, Danvers, MA); Cbfa1, OPN (Bioworld Technology, Inc.). TAZ-shRNA and negative control shRNA (NC shRNA) were synthesized by GenePharma Co., Ltd. (Shanghai, China).

2.2. Cell culture and transfection

The mouse pluripotent mesenchymal precursor cell line C2C12 was obtained from Department of Life Science, Southern Medical University. MC3T3 and C3H10T1/2 cells were purchased from the Chinese academy of sciences in Shanghai. The cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 in air. When the cells grew to 70–80% confluence, transfections were performed using Lipofectamine™ 2000 reagent according to the manufacturer's instructions in serum-free medium. The serum-free medium was replaced with fresh culture medium after 5 h and then cells were incubated for an additional 24–48 h for expression.

2.3. Low-power laser irradiation

All groups of cells were starved for 18 h and subsequently irradiated by He—Ne laser (632.8 nm, 12 mW, 13.33 mW/cm^2, HN-1000, Guangzhou, China) at a fluence of 2 J/cm^2. Cell treated with LPLI once a day for a week and then every other day. The chemicals were added into the culture medium 30 min before LPLI treatment. The entire procedure was carried out at room temperature. Throughout each experiment, the cells were kept either in a complete or very dark environment, except when subjected to light irradiation, to minimize the ambient light interference (Feng et al., 2012; Liang et al., 2012; Zhang et al., 2012).

2.4. Alizarin red staining

The C2C12 cells were washed twice with PBS (pH 7.4) and then fixed in 10% formalin for 15 min. Subsequently the cells were stained with 1% alizarin red solution for 5 min (Ichida et al., 2004). For quantification, alizarin red stain was recovered in 10% acetic acid, heated to 85 °C for 10 min and cooled by ice, and read in duplicate using an auto microplate reader at 450 nm.

2.5. Alkaline phosphatase activity assay

The C2C12 cells were washed twice with PBS (pH 7.4), lysed in 1% Triton X-100 on ice, and then centrifuged followed by cell lysing with a sonicator. After centrifugation, the ALP activities in the cell supernatants were quantified by an ALP Detection Kit (Nanjing Jiancheng Biotechnology Institute, Nanjing, China) and auto microplate reader at 520 nm (Hong et al., 2009). Each value was normalized to total protein concentration.

2.6. Immunofluorescence (IF)

The C2C12 cells were fixed in 4% paraformaldehyde for 15 min at room temperature and then were permeabilized in 0.1% Triton-X 100 for 30 min and were washed five times with PBS after every step (pH 7.4). Samples were incubated in blocking buffer (1% bovine serum albumin in PBS) for 1 h at room temperature, followed by incubation with anti-TAZ antibody at 4 °C overnight. Cells were washed five times for 5 min in PBS, and then FITC-conjugated secondary antibodies (Proteintech Group, Chicago) were added for 2 h at a room temperature. Nuclei were stained with PI (10 μg/ml). After five additional washes with PBS, the slides were mounted and analyzed by confocal laser scanning microscopy.

2.7. Cellular fractionation

To prepare cytoplasmic and nuclear fractions, cells were washed in ice-cold PBS, scraped and homogenized in ice-cold hypotonic buffer (10 mM HEPES pH 7.4; 10 mM KCl; 1.5 mM $MgCl_2$; 1 mM