Biochemical and Biophysical Research Communications 433 (2013) 226–231

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

IGF1 promotes osteogenic differentiation of mesenchymal stem cells derived from rat bone marrow by increasing TAZ expression

Peng Xue, Xuelun Wu, Lixin Zhou, Huan Ma, Yan Wang, Yan Liu, Jianxia Ma, Yukun Li *

Department of Endocrinology, The Third Hospital of Hebei Medical University, 139 Ziqiang Road, Shijiazhuang, Hebei Province 050000, PR China

ARTICLE INFO

Article history:
Received 18 February 2013
Available online 5 March 2013

Keywords:
Mesenchymal stem cell
Osteogenic differentiation
IGF1
TAZ

ABSTRACT

Whether insulin-like growth factor 1 (IGF1) inhibits or promotes the osteogenic differentiation in vitro remains controversial. Moreover, the biological mechanisms and signaling pathways by which IGF1 affects osteogenic differentiation remain obscure. Transcriptional coactivator with PDZ-binding motif (TAZ) plays a vital role in the osteogenic differentiation of mesenchymal stem cells (MSCs), and strongly activates runt related transcription factor 2 (RUNX2)-driven genes during the terminal osteogenic differentiation. In the present study, we found that IGF1 increased the ALP activities and calcium depositions of MSCs derived from rat bone marrow dose-dependently, with a peak at 100–200 ng/ml. IGF1 increased TAZ and RUNX2 expression mainly at the early stage of osteogenic differentiation, but increased OCN expression at the late stage. Our data further demonstrated that down-regulation of TAZ expression by siRNA inhibited the IGF1 induced increase in osteogenic differentiation. Moreover, UO126 (the MEK–ERK inhibitor), not LY294002 (the PI3K-Akt inhibitor), inhibited the IGF1 induced increase in TAZ expression. Taken together, we provide evidence to demonstrate that IGF1 promotes the osteogenic differentiation of rat MSCs by increasing TAZ expression, and that the increased TAZ expression induced by IGF1 is mostly mediated by the MEK–ERK pathway.

1. Introduction

Mesenchymal stem cells (MSCs) constitute a small population of pluripotent cells within the bone marrow, which differentiate into adipocytes, osteoblasts, chondrocytes or myocytes under the influence of particular signaling pathways [1]. The mechanisms that fine-tune the balance between the osteoblast and adipocyte differentiation of MSCs are likely to be of medical importance. For instance, decreased bone formation accompanied by an increase in bone marrow adipogenesis occurs in many elderly people [2]. Transcriptional coactivator with PDZ-binding motif (TAZ), a β-catenin-like molecule, was originally identified as a 14-3-3-interacting cellular protein. It plays an important role in regulating the balance between the osteoblast and adipocyte differentiation of MSCs. TAZ promotes the differentiation of MSCs into osteoblastic lineages by increasing runt related transcription factor 2 (RUNX2) expression and blocks the differentiation of MSCs from adipocyte lineages by decreasing peroxisome proliferator activated receptor gamma (PPARγ) expression [3–5].

Insulin-like growth factor 1 (IGF1) is a multifunctional peptide that regulates the cell growth, differentiation, and the expression of extracellular matrix proteins [6]. Generally, IGF1 mediates its effects by binding to IGF1 receptor on the cell surface, activating the inherent tyrosine kinase activity of the receptor, and enabling internalization of the receptor ligand complex to instigate signaling cascades, and to ultimately affect gene expression and protein synthesis [7]. In this process, a number of downstream mediators are activated, such as two classic IGF1 signaling pathways: the MEK–ERK pathway and the PI3K-Akt pathway [8–13]. In particular, IGF1 is a key protein in bone formation and remodeling [14,15]. IGF1 has been revealed to inhibit the osteogenic differentiation of mouse MSCs through the repression of osteocalcin (OCN) release and alkaline phosphatase (ALP) activity [16]. However, most studies report that IGF1 enhances the osteogenic differentiation in vitro [17–22]. Thus, whether IGF1 inhibits or promotes osteogenic differentiation remains controversial. Moreover, biological mechanisms and signaling pathways by which IGF1 affects osteogenic differentiation remain obscure.

In the present study, we investigated the effects of IGF1 on the osteogenic differentiation of MSCs derived from rat bone marrow and the role of TAZ expression on IGF1 activity. Our data indicated that IGF1 promoted the osteogenic differentiation of rat MSCs by increasing TAZ expression, and that the increased TAZ expression induced by IGF1 was mostly mediated by the MEK–ERK pathway.

* Corresponding author.
E-mail address: liyukun1225@163.com (Y. Li).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.088

2. Materials and methods

2.1. Cell isolation and culture

All animal experiments in this study were approved by the Local Committee of Animal Use and Protection of Hebei Medical University. A total of fifteen 4-week-old male Sprague–Dawley rats were obtained from the Centre of Laboratory Animal Science at Hebei Medical University. The rats were euthanized by CO_2 and sterilized in 75% ethanol for 5 min before surgery. While maintaining sterile technique, the bilateral femora and tibias were dissected. Using a 5-ml syringe, the bone marrow cells were flushed out of the marrow cavity with Dulbecco's modified Eagle's medium (DMEM, Hyclone, USA). The cells were washed three times with DMEM, and then seeded at a concentration of $5 \times 10^5/cm^2$ in 60-mm plastic dishes (Costar) containing DMEM supplemented with 10% fetal bovine serum (FBS, Hyclone, USA). Cells were allowed to expand in 37 °C incubator with 5% CO_2 until confluence was reached to 75–80%, with medium changed every 3 days to remove the nonadherent cells. The cells were lifted with 0.25% trypsin–EDTA (Gibco, Canada) and seeded in 60-mm plastic dishes at $5 \times 10^4/cm^2$ as passage 1. Cells at passage 3 were used in subsequent experiments.

2.2. Differentiation induction and IGF1 addition

Osteogenic differentiation was induced by culturing cells in the osteogenic medium (DMEM supplemented with 10% FBS, 10^{-8} M dexamethasone [Sigma, USA], 10 mM β-glycerophosphate [Sigma, USA], and 50 μg/ml ascorbic acid [Sigma, USA]). Recombinant rat IGF1 powder (R&D systems, USA) was dissolved in sterile PBS as the manual describes. MSCs cultured in the osteogenic medium were regarded as control (or IGF1 untreatment) group; and cultured in the osteogenic medium containing IGF1 were regarded as IGF1 treatment group.

2.3. Small interfering RNA transfection

Small interfering RNA (siRNA) duplex oligo targeting TAZ mRNA (SiTAZ) and non-targeting duplex oligo as a negative control (SiCON) were synthesized by Shanghai Genepharma Corporation, China. After seeding for 24 h, cells were transfected with 2 μg/ml of siRNAs, using RNAi-Mate (Shanghai Genepharma Corporation, China), as recommended by the manufacturer's instructions, and cultured in the osteogenic medium with or without IGF1. Since the calcium depositions of MSCs lasted for 21 days, cells were subjected to three rounds of transfection, prior performing experiments [23]. The sequences of SiTAZ and SiCON were listed as follows:

SiTAZ: 5′-GGCCAGAGAUAUUUCCUUATT-3′, 5′-UAAGGAAAUAUCUCUGGCCTT-3′;

SiCON: 5′-UUCUCCGAACGUGUCACGUTT-3′, 5′-ACGUGACACGUUCGGAGAATT-3′.

2.4. Inhibitor study

Ten micrometer MEK–ERK inhibitor UO126 (Beyotime Institute of Biotechnology, China) and 10 μM PI3K-Akt inhibitor LY294002 (Beyotime Institute of Biotechnology, China) were added into medium 1.5 h before the addition of osteogenic supplements or IGF1.

2.5. ALP activity assay

MSCs were seeded in 24-well plates (Costar) for ALP activity assay. Cells were harvested and resuspended in 250 μl culture supernatants, followed by cell breaking with an ultrasound breaker. After centrifugation, the ALP activities in the cell supernatants were quantified by an ALP Detection Kit (Nanjing Jiancheng Biotech Institute, China) and a spectrophotometer at a wavelength of 520 nm. Each value was normalized to the protein concentration.

2.6. Alizarin red staining (AR-S)

MSCs were seeded in 35-mm plastic dishes (Costar) for AR-S. Cells were washed twice with PBS and fixed with 4% paraformaldehyde at room temperature for 10 min. Then the dishes were washed three times with distilled water and incubated with 0.1% AR (Sigma, USA) at 37 °C for 30 min. Cells were then washed thoroughly with distilled water and the images were acquired using a scanner. Then, AR was destained with 10% cetylpyridinium chloride (Sigma, USA) in 10^{-3} M sodium phosphate for 30 min at room temperature. And the calcium concentrations were determined according to the absorbance at 562 nm using a standard calcium curve prepared in the same solution. All data were normalized to total protein content [24].

2.7. Real-time reverse transcription-polymerase chain reaction (Real-time RT-PCR)

MSCs were seeded in 24-well plates (Costar) for total RNA isolation. Cells were harvested using TRIzol (Invitrogen, USA) and total RNA was isolated according to the manufacturer's protocol. The

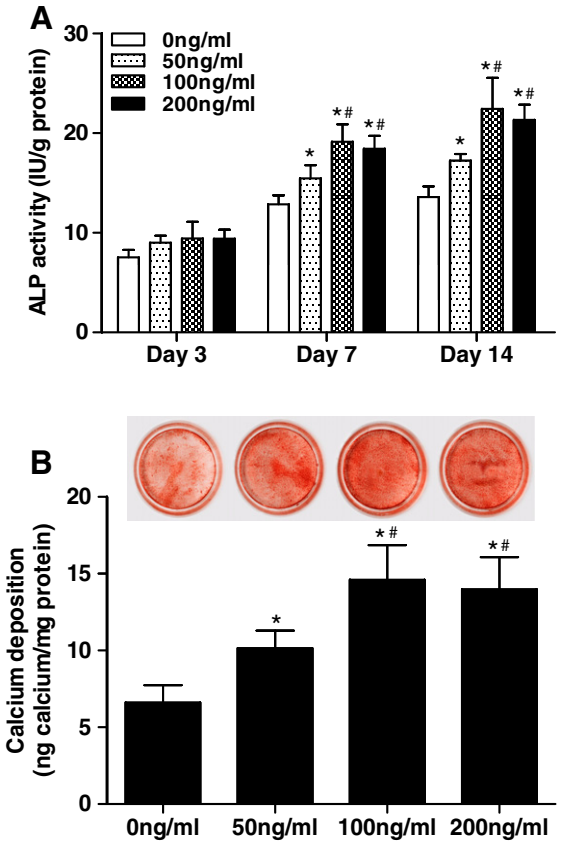

Fig. 1. IGF1 promoted the osteogenic differentiation of MSCs. (A) ALP activities of MSCs after the stimulation by IGF1 at 0–200 ng/ml for 3–14 days. $^*P < 0.05$ vs. 0 ng/ml control; $^{\#}P < 0.05$ vs. 50 ng/ml. (B) AR-S of MSCs after the stimulation by IGF1 at 0–200 ng/ml for 21 days. $^*P < 0.05$ vs. 0 ng/ml control; $^{\#}P < 0.05$ vs. 50 ng/ml. Bar graphs showed the means ± SD from three independent experiments ($n = 3$).

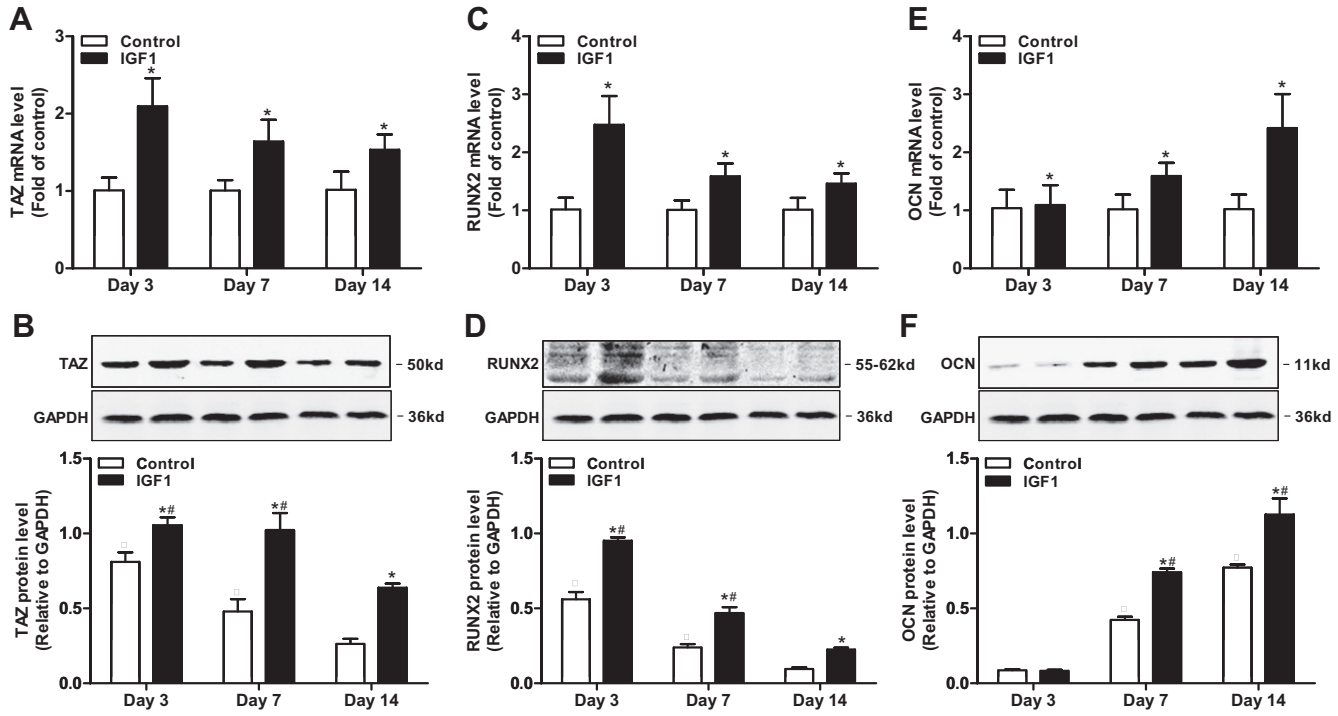

Fig. 2. IGF1 increased TAZ, Runx2 and OCN expression at the different stages of osteogenic differentiation. (A) Real-time RT-PCR for the relative TAZ mRNA levels. $^{*}P < 0.05$ vs. control. (B) Western blot analysis for the relative TAZ protein levels. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. day 14 for IGF1 treatment group; $^{\triangle}P < 0.05$ vs. day 14 for control group. (C) Real-time RT-PCR for the relative RUNX2 mRNA levels. $^{*}P < 0.05$ vs. control. (D) Western blot analysis for the relative RUNX2 protein levels. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. day 14 for IGF1 treatment group; $^{\triangle}P < 0.05$ vs. day 14 for control group. (E) Real-time RT-PCR for the relative OCN mRNA levels. $^{*}P < 0.05$ vs. control. (F) Western blot analysis for the relative OCN protein levels. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. day 3 for IGF1 treatment group; $^{\triangle}P < 0.05$ vs. day 3 for control group. Bar graphs show the means ± SD from three independent experiments ($n = 3$).

first-strand cDNA was synthesized using M-MLV Reverse Transcriptase (GeneCopoeia, USA). Real-time RT-PCR was performed using All-in-One™ qPCR Mix (GeneCopoeia, USA) in a quantitative PCR System (Corbett). All primers were synthesized by the same manufacturer (Sangon Biotech, China). Real-time RT-PCR reaction conditions were: 95 °C for 1 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 20 s and 72 °C for 20 s. The relative expression was calculated according to the ratio of the copy numbers of the target genes (TAZ, RUNX2 and OCN) to the housekeeping gene GAPDH in each sample. The relative gene expression values were evaluated by the $2^{-\Delta\Delta Ct}$ method [25,26]. Sense and antisense primers were listed as follows:

GAPDH: 5′-AGTTCAACGGCACAGTCAAGG-3′, 5′-AGCACCAGCATCA CCCCAT-3′;

TAZ: 5′-ATGTTGACCTCGGGACTTTGG-3′, 5′-GAGGAAGGGCTCGC TTTTGT-3′;

RUNX2: 5′-GCACCGACAGCCCCAACTT-3′, 5′-CCACGGGCAGGGTC TTGTT-3′;

OCN: 5′-CAGGAGGGCAGTAAGGTGG-3′, 5′-CAGGGGATCTGGGTA GGG-3′.

2.8. Western blot analysis

MSCs were seeded in 60-mm plastic dishes (Costar) for total protein isolation. Proteins were separated by 12% SDS–PAGE and transferred to a PVDF membrane using a semidry transfer apparatus (Hoefer) for 1.5 h at room temperature. Membranes were blocked with 5% milk in TBST for 2 h at 37 °C, and incubated with primary antibodies against TAZ (1:1000, Cell Signaling, USA), RUNX2 (1:200, Boster, China), OCN (1:200, Boster, China) or GAPDH (1:3000, Bioworld, USA) at 4 °C overnight. Membranes incubated with IRDye800® conjugated secondary antibody (1:20,000, Rockland, USA) for 1 h at 37 °C, following scanning with the Odyssey Infrared Imaging System (Li-COR Biosciences). Then the integrated intensity for each detected band was determined with Image J, v.1.46.

2.9. Statistics

Quantitative results were expressed as mean ± standard deviation (SD). All experiments were replicated three times. Independent samples t test, and one way analysis of variance (ANOVA) followed by Student Newman Keuls (S–N–K) post hoc analysis were performed with SPSS, v.13.0. Values were considered statistically significant at $P < 0.05$.

3. Results

3.1. IGF1 promoted the osteogenic differentiation of MSCs

We first tested whether IGF1 affected the osteogenic differentiation of rat MSCs. ALP activity assay results revealed that 50–200 ng/ml IGF1 increased the ALP activities of MSCs at day 7 and day 14, respectively. In addition, the ALP activities in 100–200 ng/ml groups were higher than 50 ng/ml group (Fig. 1A). Similarly, AR-S results revealed that 50–200 ng/ml IGF1 increased the calcium depositions of MSCs at day 21. In addition, the calcium depositions in 100–200 ng/ml groups were higher than 50 ng/ml group (Fig. 1B). Thus, IGF1 treatment could promote the osteogenic differentiation of MSCs dose-dependently, with a peak at 100–200 ng/ml. In the subsequent experiments, we used IGF1 at the concentration of 100 ng/ml.

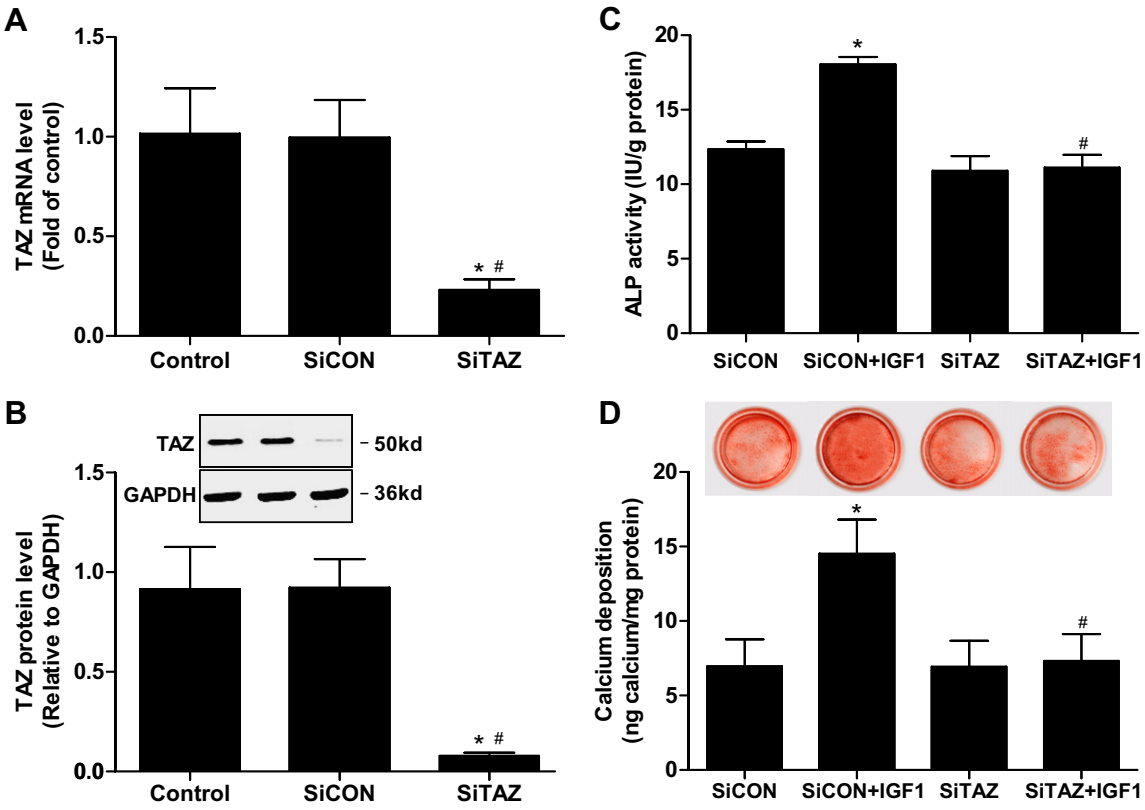

Fig. 3. SiTAZ transfection offset the effects of IGF1 on osteogenic differentiation. (A) Real-time RT-PCR for the relative TAZ mRNA levels at day 3. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. SiCON group. (B) Western blot analysis for the relative TAZ protein levels at day 3. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. SiCON group. (C) ALP activities of MSCs transfected with SiTAZ or SiCON, and cultured in the osteogenic medium with or without IGF1 after 7 days. $^{*}P < 0.05$ vs. SiCON group; $^{\#}P < 0.05$ vs. SiCON + IGF1 group. (D) AR-S of MSCs transfected with SiTAZ or SiCON, and cultured in the osteogenic medium with or without IGF1 after 21 days. $^{*}P < 0.05$ vs. SiCON group; $^{\#}P < 0.05$ vs. SiCON + IGF1 group. Bar graphs show the means ± SD from three independent experiments ($n = 3$).

3.2. Effects of IGF1 on TAZ, Runx2 and OCN expression

We investigated the effects of IGF1 at 100 ng/ml on TAZ, Runx2 and OCN expression at day 3, 7 and 14, respectively. Real-time RT-PCR results revealed that IGF1 upraised TAZ and RUNX2 mRNA levels at day 3, 7 and 14, respectively (Fig. 2A and C), and upraised OCN mRNA levels at day 7 and 14, respectively (Fig. 2E). Western blot analysis further verified these results. Moreover, either in IGF1 treatment or untreatment group, the TAZ and RUNX2 protein levels at days 3–7 were higher than day 14, but the OCN protein levels at days 7–14 were higher than day 3 (Fig. 2B, D, and F).

3.3. SiTAZ transfection offset the effects of IGF1 on osteogenic differentiation

Both real-time RT-PCR and Western blot analysis results suggested that SiTAZ transfection significantly decreased TAZ expression compared with control or SiCON group (Fig. 3A and B). Furthermore, SiTAZ transfection blocked the IGF1 induced increases in the osteogenic differentiation of MSCs, which expressed not only in the ALP activity assay, but also in the AR-S. Firstly, the ALP activities were significantly reduced in IGF1 + SiTAZ treatment group compared with IGF1 + SiCON treatment group, and there was no significant difference of the ALP activities among SiCON, SiTAZ and IGF1 + SiTAZ treatment group (Fig. 3C). Secondly, the AR-S results were consistent with the ALP activity assay results (Fig. 3D).

3.4. IGF1 increased TAZ expression mediated by the MEK–ERK pathway rather than the PI3K-Akt pathway

UO126 and LY294002 are the inhibitors for the MEK–ERK pathway and the PI3K-Akt pathway, respectively. Real-time RT-PCR results revealed that the TAZ mRNA levels were significantly reduced in IGF1 + UO126 treatment group compared with IGF1 treatment group. However, there was no significant difference of the TAZ mRNA levels between IGF1 + LY294002 and IGF1 treatment group. Moreover, there was no significant difference of the TAZ mRNA levels among control, UO126, IGF1 + UO126 and LY294002 treatment group (Fig. 4A). Furthermore, Western blot analysis results were consistent with the real-time RT-PCR results (Fig. 4B).

4. Discussion

Many research groups are currently attempting to identify molecules that stimulate osteoblast differentiation for the development of drugs for osteoporosis. Whether IGF1 inhibits or promotes osteogenic differentiation in vitro remains controversial in the previous studies. In our study, we determined the effects of IGF1 on the osteogenic differentiation of rat MSCs. We found that IGF1 could increase the ALP activities and mineralization capacities of rat MSCs dose-dependently, with a peak at 100–200 ng/ml.

We also found that IGF1 promoted the osteogenic differentiation of rat MSCs by increasing TAZ expression based on the two ways as follows: firstly, IGF1 treatment substantially up-regulated TAZ expression at the initial stage of osteogenic differentiation;

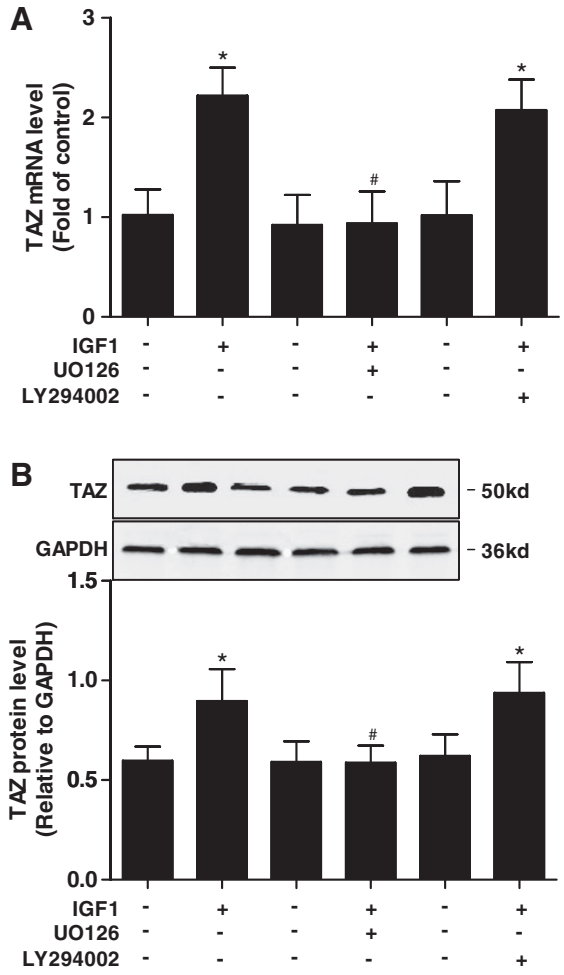

Fig. 4. IGF1 increased TAZ expression mediated by the MEK–ERK pathway. (A) Real-time RT-PCR for the relative TAZ mRNA levels at day 3. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. IGF1 treatment group. (B) Western blot analysis for the relative TAZ protein levels at day 3. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. IGF1 treatment group. Bar graphs show the means ± SD from three independent experiments ($n = 3$).

secondly, down-regulation of TAZ expression by siRNA inhibited the IGF1 induced increase in osteogenic differentiation. TAZ plays a vital role in the osteogenic differentiation of MSCs. It has a single WW domain, and the WW domain of TAZ binds strongly to the sequence motif Pro-Pro-X-Tyr. This motif can be found within the regulatory region of RUNX2, and TAZ could strongly activate RUNX2-driven genes during the terminal osteogenic differentiation [3]. Previous studies reported that TAZ expression changes substantially as MSCs initiate differentiation, and several cytokines could up-regulate TAZ expression. For instance, it had been revealed that TAZ increased several fold when MSCs were stimulated to undergo osteogenic differentiation by the treatment with bone morphogenic protein 2 (BMP-2) [4]. Moreover, tumor necrosis factor alpha (TNF-α), dexamethasone and phorbaketal-A could also stimulate osteoblast differentiation through the TAZ mediated Runx2 activation [27–29].

Except for TAZ, the up-regulation of RUNX2 and OCN in IGF1 treatment group also suggested that IGF1 could enhance the osteogenic differentiation of MSCs. In addition, the protein levels of RUNX2 at days 3–7 were higher than day 14, but the protein levels of OCN at days 7–14 were higher than day 3. Therefore, we inferred that IGF1 could increase RUNX2 expression at the early stage of osteogenic differentiation, but increase OCN expression at the late stage. In fact, RUNX2 and OCN are two key osteoblast markers in the process of osteogenic differentiation: (1) RUNX2 is necessary for MSCs osteogenic differentiation [30,31] and serves as an early transcriptional regulator [32–34]. The interaction of TAZ and RUNX2 promotes the differentiation of MSCs into osteoblastic lineage. After promoting MSCs differentiation into pre-osteoblasts, RUNX2 drives them into immature osteoblasts and to produce bone matrix [35–37]. (2) OCN, a small γ-carboxyglutamate protein preferentially expressed by osteoblasts [38], is secreted in the late stage of osteoblasts differentiation under the control of Runx2 [39]. For instance, vitamin D has been revealed to modulate the expression of OCN gene [40], and is able to increase OCN gene transcription by several fold [41]. Runx2 factors, by interacting with their cognate sites that flank the vitamin D response element, enhance the vitamin D responsiveness [42]; on the contrary mutations of Runx2 sites inhibit vitamin D enhanced OCN promoter activity [43].

Our data demonstrated that UO126 (the MEK–ERK inhibitor), not LY294002 (the PI3K-Akt inhibitor), inhibited the IGF1 induced increase in TAZ expression. Thus, we inferred that IGF1 increased TAZ expression mostly mediated by the MEK–ERK pathway rather than the PI3K-Akt pathway. MEK1/2 and ERK1/2 belong to a family of serine/threonine kinases that play essential roles in the signal transduction by modulating gene transcription in response to changes in the cellular environment [44]. Celil et al. [45] reported that the activities of RUNX2 and osterix were regulated via the MEK–ERK pathway. Wu et al. [46] reported that stretch promoted the osteoblastic differentiation of MSCs by the MEK–ERK activating RUNX2 pathway. These two studies both determined the close relationship between the MEK–ERK pathway and the RUNX2 expression. Furthermore, TAZ is the upstream enhancer for RUNX2 in the process of osteoblastic differentiation.

In summary, our data demonstrate that IGF1 can promote the osteoblastic differentiation of rat MSCs. This stimulative effect is due to the increase of TAZ expression and the subsequent up-regulation of RUNX2 and OCN. When the MEK–ERK pathway is inhibited, the IGF1 induced increase in TAZ expression is offset.

Acknowledgments

This work was supported by National Natural Science foundation of Hebei Province (No: C2009001179) and Grant from Hebei Provincial Health Bureau (No: 20090316).

References

[1] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[2] M.E. Nuttall, J.M. Gimble, Is there a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis?, Bone 27 (2000) 177–184
[3] J.H. Hong, M.B. Yaffe, TAZ: a beta-catenin-like molecule that regulates mesenchymal stem cell differentiation, Cell Cycle 5 (2006) 176–179.
[4] J.H. Hong, E.S. Hwang, M.T. McManus, A. Amsterdam, Y. Tian, R. Kalmukova, E. Mueller, T. Benjamin, B.M. Spiegelman, P.A. Sharp, N. Hopkins, M.B. Yaffe, TAZ, a transcriptional modulator of mesenchymal stem cell differentiation, Science 309 (2005) 1074–1078.
[5] H. Jeong, S. Bae, S.Y. An, M.R. Byun, J.H. Hwang, M.B. Yaffe, J.H. Hong, E.S. Hwang, TAZ as a novel enhancer of MyoD-mediated myogenic differentiation, FASEB J. 24 (2010) 3310–3320.
[6] S. Blom, P. Holmstrup, E. Dabelsteen, The effect of insulin-like growth factor-I and human growth hormone on periodontal ligament fibroblast morphology, growth pattern DNA synthesis, and receptor binding, J. Periodontol. 63 (1992) 960–968.
[7] R.J. Romanelli, A.P. LeBeau, C.G. Fulmer, D.A. Lazzarino, A. Hochberg, T.L. Wood, Insulin-like growth factor type-I receptor internalization and recycling mediate the sustained phosphorylation of Akt, J. Biol. Chem. 282 (2007) 22513–22524.
[8] Y. Li, Y.H. Song, J. Mohler, P. Delafontaine, ANG II induces apoptosis of human vascular smooth muscle via extrinsic pathway involving inhibition of Akt phosphorylation and increased FasL expression, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) H2116–H2123.

[9] Y.H. Song, Y. Li, J. Du, W.E. Mitch, N. Rosenthal, P. Delafontaine, Muscle-specific expression of IGF-1 blocks angiotensin II-induced skeletal muscle wasting, J. Clin. Invest. 115 (2005) 451–458.
[10] P. Delafontaine, Y.H. Song, Y. Li, Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 435–444.
[11] T. Kofidis, J.L. de Bruin, T. Yamane, L.B. Balsam, D.R. Lebl, R.J. Swijnenburg, M. Tanaka, I.L. Weissman, R.C. Robbins, Insulin-like growth factor promotes engraftment, differentiation, and functional improvement after transfer of embryonic stem cells for myocardial restoration, Stem Cells 22 (2004) 1239–1245.
[12] Y. Li, Y. Higashi, H. Itabe, Y.H. Song, J. Du, P. Delafontaine, Insulin-like growth factor-1 receptor activation inhibits oxidized LDL-induced cytochrome C release and apoptosis via the phosphatidylinositol 3 kinase/Akt signaling pathway, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 2178–2184.
[13] M. Goto, A. Iwase, T. Harata, S. Takigawa, K. Suzuki, S. Manabe, F. Kikkawa, IGF1-induced AKT phosphorylation and cell proliferation are suppressed with the increase in PTEN during luteinization in human granulosa cells, Reproduction 137 (2009) 835–842.
[14] F. Minuto, C. Palermo, M. Arvigo, A.M. Barreca, The IGF system and bone, J. Endocrinol. Invest. 28 (2005) 8–10.
[15] C.J. Rosen, Insulin-like growth factor I and bone mineral density: experience from animal models and human observational studies, Best Pract. Res. Clin. Endocrinol. Metab. 18 (2004) 423–435.
[16] Z. Huang, P.G. Ren, T. Ma, R.L. Smith, S.B. Goodman, Modulating osteogenesis of mesenchymal stem cells by modifying growth factor availability, Cytokine 51 (2010) 305–310.
[17] T. Ueland, GH/IGF-I and bone resorption in vivo and in vitro, Eur. J. Endocrinol. 152 (2005) 327–332.
[18] S. Srouji, I. Blumenfeld, A. Rachmiel, E. Livne, Bone defect repair in rat tibia by TGF-beta1 and IGF-1 released from hydrogel scaffold, Cell Tissue Bank. 5 (2004) 223–230.
[19] I. Blumenfeld, S. Srouji, Y. Lanir, D. Laufer, E. Livne, Enhancement of bone defect healing in old rats by TGF-beta and IGF-1, Exp. Gerontol. 37 (2002) 553–565.
[20] S. Wang, J. Mu, Z. Fan, Y. Yu, M. Yan, G. Lei, C. Tang, Z. Wang, Y. Zheng, J. Yu, G. Zhang, Insulin-like growth factor 1 can promote the osteogenic differentiation and osteogenesis of stem cells from apical papilla, Stem Cell Res. 8 (2012) 346–356.
[21] Y. Yu, J. Mu, Z. Fan, G. Lei, M. Yan, S. Wang, C. Tang, Z. Wang, J. Yu, G. Zhang, Insulin-like growth factor 1 enhances the proliferation and osteogenic differentiation of human periodontal ligament stem cells via ERK and JNK MAPK pathways, Histochem. Cell Biol. 137 (2012) 513–525.
[22] A.M. Osyczka, P.S. Leboy, Bone morphogenetic protein regulation of early osteoblast genes in human marrow stromal cells is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase signaling, Endocrinology 146 (2005) 3428–3437.
[23] R. Rubin, A. Arzumanyan, A.R. Soliera, B. Ross, F. Peruzzi, M. Prisco, Insulin receptor substrate (IRS)-1 regulates murine embryonic stem (mES) cells self-renewal, J. Cell. Physiol. 213 (2007) 445–453.
[24] K. Johnson, K. Pritzker, J. Goding, R. Terkeltaub, The nucleoside triphosphate pyrophosphohydrolase isozyme PC-1 directly promotes cartilage calcification through chondrocyte apoptosis and increased calcium precipitation by mineralizing vesicles, J. Rheumatol. 28 (2001) 2681–2691.
[25] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[26] S.A. Bustin, Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays, J. Mol. Endocrinol. 25 (2000) 169–193.
[27] H.H. Cho, K.K. Shin, Y.J. Kim, J.S. Song, J.M. Kim, Y.C. Bae, C.D. Kim, J.S. Jung, NF-kappaB activation stimulates osteogenic differentiation of mesenchymal stem cells derived from human adipose tissue by increasing TAZ expression, J. Cell. Physiol. 223 (2010) 168–177.
[28] D. Hong, H.X. Chen, Y. Xue, D.M. Li, X.C. Wan, R. Ge, J.C. Li, Osteoblastogenic effects of dexamethasone through upregulation of TAZ expression in rat mesenchymal stem cells, J. Steroid Biochem. Mol. Biol. 116 (2009) 86–92.
[29] M.R. Byun, A.R. Kim, J.H. Hwang, M.K. Sung, Y.K. Lee, B.S. Hwang, J.R. Rho, E.S. Hwang, J.H. Hong, Phorbaketal A stimulates osteoblast differentiation through TAZ mediated Runx2 activation, FEBS Lett. 586 (2012) 1086–1092.
[30] J. Li, Q. Zhao, E. Wang, C. Zhang, G. Wang, Q. Yuan, Transplantation of Cbfa1-overexpressing adipose stem cells together with vascularized periosteal flaps repair segmental bone defects, J. Surg. Res. 176 (2012) e13–20.
[31] T. Komori, H. Yagi, S. Nomura, A. Yamaguchi, K. Sasaki, K. Deguchi, Y. Shimizu, R.T. Bronson, Y.H. Gao, M. Inada, M. Sato, R. Okamoto, Y. Kitamura, S. Yoshiki, T. Kishimoto, Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts, Cell 89 (1997) 755–764.
[32] S. Takeda, J.P. Bonnamy, M.J. Owen, P. Ducy, G. Karsenty, Continuous expression of Cbfa1 in nonhypertrophic chondrocytes uncovers its ability to induce hypertrophic chondrocyte differentiation and partially rescues Cbfa1-deficient mice, Genes Dev. 15 (2001) 467–481.
[33] G. Karsenty, E.F. Wagner, Reaching a genetic and molecular understanding of skeletal development, Dev. Cell 2 (2002) 389–406.
[34] A. Yamaguchi, T. Komori, T. Suda, Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1, Endocr. Rev. 21 (2000) 393–411.
[35] T. Komori, Regulation of osteoblast differentiation by transcription factors, J. Cell. Biochem. 99 (2006) 1233–1239.
[36] I. Kobayashi, T. Kiyoshima, H. Wada, K. Matsuo, K. Nonaka, J.Y. Honda, K. Koyano, H. Sakai, Type II/III Runx2/Cbfa1 is required for tooth germ development, Bone 38 (2006) 836–844.
[37] T.M. Liu, E.H. Lee, Transcriptional regulatory cascades in Runx2-dependent bone development, Tissue Eng. Part B (2012) [Epub ahead of print].
[38] A. Neve, A. Corrado, F.P. Cantatore, Osteocalcin: skeletal and extra-skeletal effects, J. Cell. Physiol. 228 (2012) 1149–1153.
[39] L. Carvallo, B. Henriquez, R. Paredes, J. Olate, S. Onate, A.J. van Wijnen, J.B. Lian, G.S. Stein, J.L. Stein, M. Montecino, 1alpha,25-dihydroxy vitamin D3-enhanced expression of the osteocalcin gene involves increased promoter occupancy of basal transcription regulators and gradual recruitment of the 1alpha,25-dihydroxy vitamin D3 receptor-SRC-1 coactivator complex, J. Cell. Physiol. 214 (2008) 740–749.
[40] M.E. Martinez, M.T. del Campo, S. Medina, M. Sanchez, M.J. Sanchez-Cabezudo, P. Esbrit, P. Martinez, I. Moreno, A. Rodrigo, M.V. Garces, L. Munuera, Influence of skeletal site of origin and donor age on osteoblastic cell growth and differentiation, Calcif. Tissue Int. 64 (1999) 280–286.
[41] K. Ozono, J. Liao, S.A. Kerner, R.A. Scott, J.W. Pike, The vitamin D-responsive element in the human osteocalcin gene Association with a nuclear proto-oncogene enhancer, J. Biol. Chem. 265 (1990) 21881–21888.
[42] R. Paredes, G. Arriagada, F. Cruzat, A. Villagra, J. Olate, K. Zaidi, A. van Wijnen, J.B. Lian, G.S. Stein, J.L. Stein, M. Montecino, Bone-specific transcription factor Runx2 interacts with the 1alpha,25-dihydroxyvitamin D3 receptor to up-regulate rat osteocalcin gene expression in osteoblastic cells, Mol. Cell. Biol. 24 (2004) 8847–8861.
[43] A. Javed, S. Gutierrez, M. Montecino, A.J. van Wijnen, J.L. Stein, G.S. Stein, J.B. Lian, Multiple Cbfa/AML sites in the rat osteocalcin promoter are required for basal and vitamin D-responsive transcription and contribute to chromatin organization, Mol. Cell. Biol. 19 (1999) 7491–7500.
[44] A.G. Turjanski, J.P. Vaque, J.S. Gutkind, MAP kinases and the control of nuclear events, Oncogene 26 (2007) 3240–3253.
[45] A.B. Celil, P.G. Campbell, BMP-2 and insulin-like growth factor-I mediate Osterix (Osx) expression in human mesenchymal stem cells via the MAPK and protein kinase D signaling pathways, J. Biol. Chem. 280 (2005) 31353–31359.
[46] Y. Wu, X. Zhang, P. Zhang, B. Fang, L. Jiang, Intermittent traction stretch promotes the osteoblastic differentiation of bone mesenchymal stem cells by the ERK1/2-activated Cbfa1 pathway, Connect. Tissue Res. 53 (2012) 451–459.