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EXPRESSION LEVELS OF UCMA IN TUMOR NECROSIS FACTOR-A-INDUCED HUMAN OSTEOBLAST CELLS

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ABSTRACT: Osteoarthritis (OA) is the most common degenerative joint disease affecting millions of people in the world. OA is characterized with several anomalies which can be observed in all joints such as degradation in articular cartilage, osteophyte formation, sclerosis within subchondral bone, and inflammation. Although some causative events leading to OA, e.g. genetics, aging, and trauma cannot be prevented, it is possible to improve the course of the disease on the cellular level by means of better understanding the molecular pathway regarding cytokines. Tumor necrosis factor alpha (TNF-α) is one of the catabolic cytokines playing role in OA pathogenesis. Upper zone of growth plate and cartilage matrix associated (UCMA) is a new member of vitamin K-dependent protein family. UCMA is also a promising target for treatment and prevention of some diseases. However, the exact physiological function of UCMA in human diseases is still unknown. The aim of our study is to investigate the expression levels of UCMA in TNF-α induced human osteoblast cells to understand whether UCMA is involved in the cytokine-related regulation of OA pathogenesis. We used TNF-α-induced human osteoblast (hFOB1.19) cell culture model for better understanding the role of UCMA in OA pathogenesis. Our results showed that UCMA can be involved in inflammatory pathways as in line with previous studies.

KEYWORDS: UCMA, TNF-α, Osteoarthritis, hFOB1.19, Osteoblast

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1.INTRODUCTION

Osteoarthritis is a degenerative joint disease affecting millions of people in the world [1, 2]. It is reported among the people over 60 years of age worldwide that 18% of women and 10% of men have symptomatic OA [1, 2]. OA is characterized with several anomalies which can be observed in all joints such as degradation in articular cartilage, osteophyte formation, sclerosis within subchondral bone, and inflammation [3, 4]. There is still no effective treatment against OA which leads to reduction in life quality and increase in health expenditures and the current treatment of OA has severe side effects [5, 6]. Although some causative events leading to OA, e.g. genetics, aging, and trauma cannot be prevented, it is possible to improve the course of the disease on the cellular level by means of better understanding the molecular pathway regarding cytokines [7]. Osteoblasts are responsible for formation, mineralization, and reconstitution of bone tissue [8]. Bone formation and reconstitution processes are deregulated in some common bone disorders such as OA and osteoporosis and osteoblasts play a central role in the pathogeneses of these disorders [8]. During OA progression period, the cytokines that are synthesized in articular region have negative effects on anabolic and catabolic processes [9]. Tumor necrosis factor alpha (TNF-α) is one of the catabolic cytokines playing role in OA pathogenesis [9, 10]. Particularly, TNF-α triggers the secretion of osteoclastogenic activity-related proteins; receptor activator of NF-κB ligand (RANKL) and interleukin-6 (IL-6) [11]. Other than this, osteocalcin or so called bone gamma-carboxyglutamate (BGLAP) is a vitamin-K-dependent osteoblast specific protein. In a previous study it was reported that BGLAP was involved in mineralization processes as well as in the activation and differentiation of osteoblasts [8]. Upper zone of growth plate and cartilage matrix associated (UCMA) is a new member of vitamin K-dependent protein family [12]. In addition, UCMA is a protein with a high affinity tendency towards calcium mineral (Ca) and bearing 16 gla residues [13]. Besides it was previously emphasized that UCMA is also a promising target for treatment and prevention of some diseases [12]. It was highlighted in recent studies that UCMA provides a key connection between cartilage and bone tissues, can be a mediator related to calcification and mineralization, and can also function as an inhibitor in calcification of cardiovascular system [14-16]. However, the exact therapeutic function of UCMA in human diseases is still unknown. The aim of our study is to investigate the expression levels of UCMA in TNF-α induced human osteoblast cells to understand whether UCMA is involved in the cytokine-related regulation of OA pathogenesis. We used TNF-α-induced human osteoblast (hFOB1.19) cell culture model.

2. MATERIALS AND METHODS

Cell Culture and TNF-α Treatment

We purchased the human osteoblast cell line hFOB1.19 from American type culture collection (Manassas, VA 20108, USA). Cell culture media to grow cells consisted of Dulbecco's modified Eagle medium: F12, 10% fetal bovine serum, and G418 (0.3 mg/mL, Sigma, Missouri, USA). We

incubated the cells in a humidified atmosphere with 5% CO2 at 34°C. We refreshed the cell media twice per week and passaged the cells when the confluence reached to 80%. We treated the cells with TNF-α (5 and 10 ng/ml, R&D Systems Minneapolis, MN, USA) for 24 and 48 hours (24 h and 48 h).

RNA Isolation and Quantitative Real-Time RT-PCR

We used miRNeasy mini kit (Qiagen, Hilden, Germany) to isolate total RNA from cells. We measured RNA concentration MultiskanTM GO spectrophotometrically (ThermoFisher, Finland). Then, cDNA reaction was performed with single step RT-PCR by using RT^2 HT First Strand Kit (Qiagen, Maryland, USA). We used gene-specific primers (Qiagen, Table 1) to measure mRNA levels of UCMA, TNFSF11, BGLAP, IL-6, and GAPDH as the housekeeping gene for internal normalization.

Table 1: List of primers for qRT-PCR

Name	NCBI Reference Sequence	Catalog No:
UCMA	NM_145314.2	PPH11256A
BGLAP	NM_199173.5	PPH01898A
TNFSF11	NM_003701.3	PPH01048F
IL-6	NM_000600	PPH00560C
GAPDH	NM_002046.5	PPH00150F

Statistical Analysis

To analyze gene expression studies obtained from qRT-PCR, we utilized Gene Globe Data Analysis Center (Qiagen, online service). The analysis method is based on Delta Delta Ct (ΔΔCt). In short, the raw data (Ct values) of each gene was normalized with housekeeping gene (GAPDH) as the internal control. Then the treatment and control groups were compared and the ratio was calculated and expressed as a "fold change" by using the formula $2^{-\Delta\Delta Ct}$. We performed all experiments in duplicates. Student's t test was used to compare control and treatment groups by using replicate $2^{-\Delta\Delta Ct}$ values. Only if the p value is less than 0.05, we considered the difference as significant. We presented all data as mean±SEM. We used GraphPad Prism version 6.0 (GraphPad Software) to produce graphics and Adobe Photoshop C5.1 software to design and present art work. SPSS v.22 was used for statistical tests.

3. RESULTS AND DISCUSSION

Effect of TNF-α on gene expression levels of UCMA, BGLAP, TNFSF11, and IL-6 in hFOB1.19 cells

In order to determine the effect of inflammatory cytokine TNF-α on UCMA in human osteoblast cells, we measured UCMA expression levels with qRT-PCR method. As shown in Fig. 1A, UCMA

mRNA levels vary in a dose and time dependent manner. Especially at 48 hours (h), 5 ng/ml TNF-α treatment resulted in a significant increase in UCMA expression levels ($p<0.05$). At the same time point, 10 ng/ml TNF-α treatment did not lead to any significant difference in UCMA mRNA levels although the fold change was greater than 2 ($p>0.05$). We also analyzed the gene expression levels of BGLAP, another vitamin K-dependent protein like UCMA, to observe TNF-α treatment effect (Fig. 1B). At 48 h with 5 ng/ml TNF-α treatment, BGLAP mRNA levels were downregulated as its fold change value was less than 1 ($p>0.05$). Similarly, at the same time point with 10 ng/ml TNF-α treatment, there was no significant difference in BGLAP expression levels since fold change value was less than 2 ($p>0.05$).

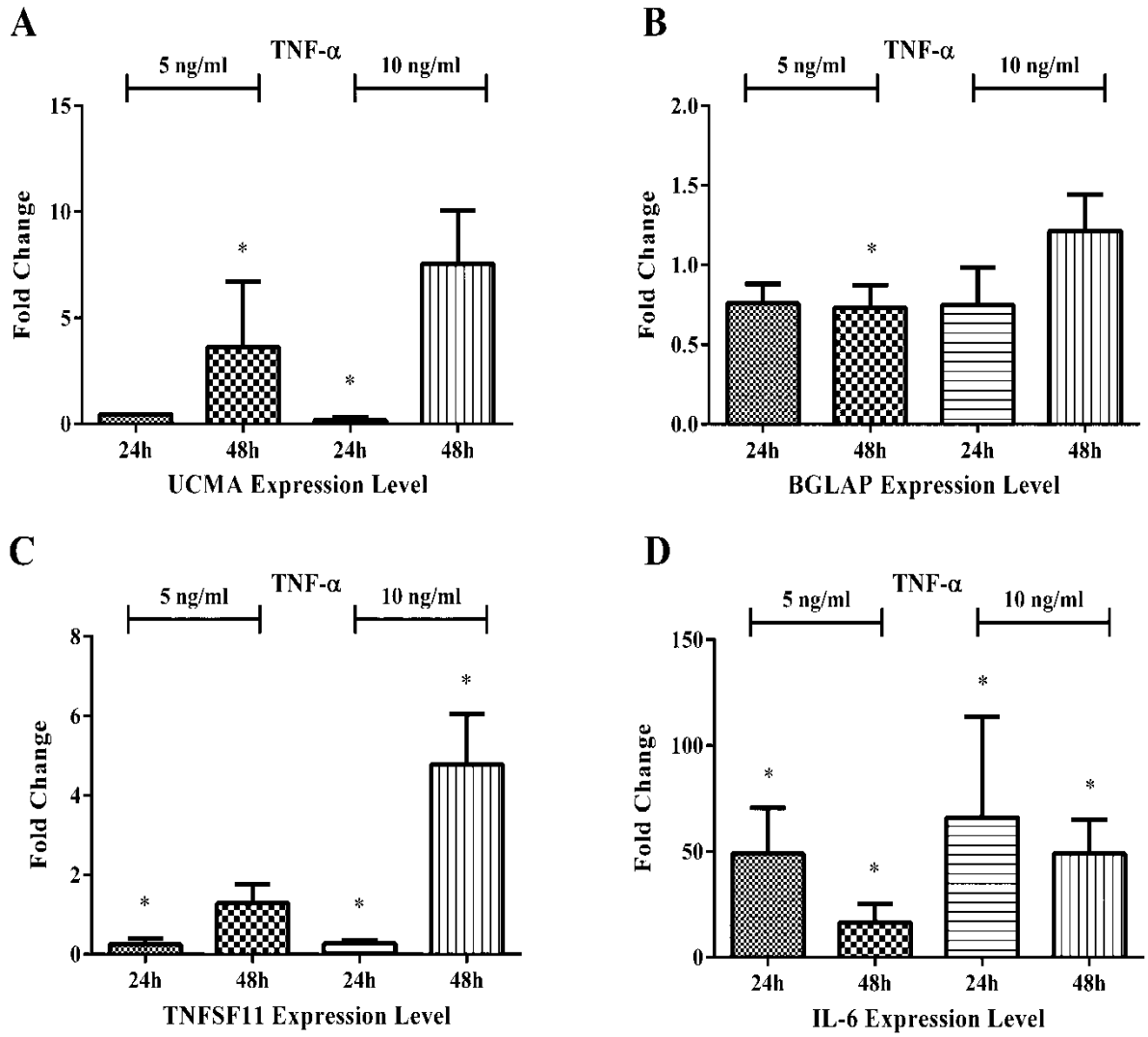

Figure 1: Gene expression levels of UCMA (A), BGLAP (B), TNFSF11 (C), and IL-6 (D) after TNF-α stimulation in hFOB1.19 cell line.

Two different concentrations of TNF-α (5 and 10 ng/ml) and two different treatment durations (24h and 48h) were depicted in different motifs on graph bars. The gene expression levels were shown as "fold change" compared to the control. Data are representative of two independent experiments and were presented as mean±SEM. *$p<0.05$.

In the next step, TNFSF11 expression levels were measured after TNF-α induction of hFOB1.19 cells (Fig. 1C). After 24 h induction with both 5 and 10 ng/ml TNF-α, TNFSF11 expression levels decreased significantly (fold change < 1, $p<0.05$). On the other hand, after 48 h treatment with TNF-α resulted in a significant increase in TNFSF11 mRNA levels as the fold change was greater than 2 ($p<0.05$). As to our knowledge, IL-6 mRNA levels are regulated by TNF-α in various cell types. Therefore, in the last part of our study, we analyzed how IL-6 expression was affected from TNF-α treatment in a time and dose dependent manner in hFOB1.19 human osteoblast cells (Fig. 1D). After 24 h- and 48 h-long TNF-α treatment, there was a significant increase in IL-6 mRNA levels ($p<0.05$). Furthermore, the fold change values at 24 h time point were greater than the ones at 48 h for both dosages. In previous studies it was reported that, cytokines in general provide pivotal contribution to the OA progression and TNF-α, a proinflammatory cytokine, has a strong catabolic effect during OA pathogenesis [9, 10]. In the present study, for the first time as to our knowledge, we analyzed the potential role of TNF-α stimulation on the regulation of UCMA expression in hFOB1.19 human osteoblast cell line. Viegas et al. detected UCMA expression in osteoblast and osteocytes, as well as in chondrocytes [13]. Lee et al. also analyzed and confirmed the expression of UCMA in MC3T3-E1 osteoblast cell line [17]. Besides in the same study they suggested that, UCMA is regulated by runt-related transcription factor 2 (RUNX2) and osterix (Osx) proteins during bone formation process and osteoblast differentiation [17]. In line with these results, we confirmed in our study the presence of UCMA expression in hFOB1.19 human osteoblast cell line. Moreover, UCMA expression levels can vary during osteoblast differentiation. Although RUNX2 and Osx are upregulated during early stages of osteoblast differentiation, UCMA expression levels are quite low [17]. Despite UCMA expression is observed during osteoblast differentiation, the action mechanism of UCMA in OA pathogenesis through TNF-α pathway is not fully known. The data obtained in our study revealed that UCMA mRNA levels vary depending on dosage and duration of TNF-α which can have a promoting effect on UCMA expression levels. Particularly, although UCMA expression was downregulated at 24 h, it is upregulated at 48 h. Recent studies about the role of UCMA in inflammatory processes are taking attention of many researchers [14, 18]. Cavaco et al. investigated the role of UCMA in inflammatory pathways regarding OA pathogenesis. They found that in OA-derived chondrocytes and synoviocytes induced with TNF-α like proinflammatory cytokine IL-1β, the expression levels of inflammatory markers (COX2 and MMP13) as well as UCMA were higher compared to the control [14]. In the same study, COX2 and MMP13 levels decreased after treating IL-1β-induced cells with UCMA [14]. These results indicate that UCMA can be a promising anti-inflammatory agent for the treatment of OA [18]. Viegas et al. also tested anti-inflammatory effect of UCMA in human leukocyte and THP-1 monocyte cell culture model. Their results demonstrated that gene expression levels of UCMA were upregulated in the cells induced with inflammatory stimulators like lipopolysaccharide (LPS) [18]. In addition they also showed that, the levels of

inflammatory markers such as TNF-α and prostaglandin E2 (PGE2) decreased in UCMA-treated cells [18]. Likewise UCMA, another vitamin K-dependent protein BGLAP plays role in OA pathogenesis. Previously it was shown that, TNF-α can promote a decrease in BGLAP synthesis [8, 19]. However, in our study, there was no significant change in BGLAP mRNA levels after TNF-α induction. Another prominent pro-inflammatory cytokine involved in OA pathogenesis is IL-6. Previous studies stated that TNF-α activates IL-6 production and initiates inflammatory reaction cascade in osteoblasts [9, 20, 21]. In the present study, we analyzed alterations in IL-6 mRNA levels by inducing hFBOB1.19 cells with TNF-α. Depending on treatment dosage and duration, we observed that the highest increase in gene expression levels belonged to IL-6. Our results showed that TNF-α promotes IL-6 expression in short time intervals. Another target molecule that we analyzed is TNFSF11, an osteoclastogenic cytokine, playing an active role in osteoporosis and its secretion is increased by TNF-α [11, 22, 23]. We observed in the present study that, TNFSF11 mRNA levels were upregulated after TNF-α treatment at higher dose and exposure time.

4. CONCLUSION

Our results suggest that UCMA expression can be regulated with TNF-α and it can be a potential candidate as a therapeutic agent targeting inflammatory pathways for OA treatment. Nonetheless, further studies about physiological importance of UCMA will provide a better understanding regarding inflammatory pathogenesis of OA.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

REFERENCES

1. Osteoarthritis: A Serious Disease. Osteoarthritis Research Society International. 2016:1e103.
2. Nelson AE. Osteoarthritis year in review 2017: clinical. Osteoarthritis Cartilage. 2018;26(3):319-25.
3. Castaneda S, Roman-Blas JA, Largo R, Herrero-Beaumont G. Subchondral bone as a key target for osteoarthritis treatment. Biochem Pharmacol. 2012;83(3):315-23.
4. Sharma AR, Jagga S, Lee SS, Nam JS. Interplay between cartilage and subchondral bone contributing to pathogenesis of osteoarthritis. Int J Mol Sci. 2013;14(10):19805-30.
5. Birimoglu Okuyan C, Okuyan HM, Terzi MY, Kalacı A. Tai Chi as a Physiotherapeutic Approach against Osteoarthritis and Oxidative Stress. Saudi J. Med. Pharm. Sci. 2018;4(1A):49-53.
6. Cutolo M, Berenbaum F, Hochberg M, Punzi L, Reginster JY. Commentary on recent therapeutic guidelines for osteoarthritis. Semin Arthritis Rheum. 2015;44(6):611-7.

7. Mobasheri A. The future of osteoarthritis therapeutics: emerging biological therapy. Curr Rheumatol Rep. 2013;15(12):385.
8. Neve A, Corrado A, Cantatore FP. Osteoblast physiology in normal and pathological conditions. Cell Tissue Res. 2011;343(2):289-302.
9. Wojdasiewicz P, Poniatowski LA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm. 2014;2014:561459.
10. Lee AS, Ellman MB, Yan D, Kroin JS, Cole BJ, van Wijnen AJ, et al. A current review of molecular mechanisms regarding osteoarthritis and pain. Gene. 2013;527(2):440-7.
11. Rigoglou S, Papavassiliou AG. The NF-kappaB signalling pathway in osteoarthritis. Int J Biochem Cell Biol. 2013;45(11):2580-4.
12. Cancela ML, Conceicao N, Laize V. Gla-rich protein, a new player in tissue calcification? Adv Nutr. 2012;3(2):174-81.
13. Viegas CS, Simes DC, Laize V, Williamson MK, Price PA, Cancela ML. Gla-rich protein (GRP), a new vitamin K-dependent protein identified from sturgeon cartilage and highly conserved in vertebrates. J Biol Chem. 2008;283(52):36655-64.
14. Cavaco S, Viegas CS, Rafael MS, Ramos A, Magalhaes J, Blanco FJ, et al. Gla-rich protein is involved in the cross-talk between calcification and inflammation in osteoarthritis. Cell Mol Life Sci. 2016;73(5):1051-65.
15. Stock M, Menges S, Eitzinger N, Gesslein M, Botschner R, Wormser L, et al. A Dual Role of Upper Zone of Growth Plate and Cartilage Matrix-Associated Protein in Human and Mouse Osteoarthritic Cartilage: Inhibition of Aggrecanases and Promotion of Bone Turnover. Arthritis Rheumatol. 2017;69(6):1233-45.
16. Viegas CS, Rafael MS, Enriquez JL, Teixeira A, Vitorino R, Luis IM, et al. Gla-rich protein acts as a calcification inhibitor in the human cardiovascular system. Arterioscler Thromb Vasc Biol. 2015;35(2):399-408.
17. Lee YJ, Park SY, Lee SJ, Boo YC, Choi JY, Kim JE. Ucma, a direct transcriptional target of Runx2 and Osterix, promotes osteoblast differentiation and nodule formation. Osteoarthritis Cartilage. 2015;23(8):1421-31.
18. Viegas CSB, Costa RM, Santos L, Videira PA, Silva Z, Araujo N, et al. Gla-rich protein function as an anti-inflammatory agent in monocytes/macrophages: Implications for calcification-related chronic inflammatory diseases. PLoS One. 2017;12(5):e0177829.
19. Li YP, Stashenko P. Proinflammatory cytokines tumor necrosis factor-alpha and IL-6, but not IL-1, down-regulate the osteocalcin gene promoter. J Immunol. 1992;148(3):788-94.
20. Blanchard F, Duplomb L, Baud'huin M, Brounais B. The dual role of IL-6-type cytokines on bone remodeling and bone tumors. Cytokine Growth Factor Rev. 2009;20(1):19-28.

21. Fonseca JE, Santos MJ, Canhao H, Choy E. Interleukin-6 as a key player in systemic inflammation and joint destruction. Autoimmun Rev. 2009;8(7):538-42.
22. Palmqvist P, Persson E, Conaway HH, Lerner UH. IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-kappa B ligand, osteoprotegerin, and receptor activator of NF-kappa B in mouse calvariae. J Immunol. 2002;169(6):3353-62.
23. Xing L, Carlson L, Story B, Tai Z, Keng P, Siebenlist U, et al. Expression of either NF-kappaB p50 or p52 in osteoclast precursors is required for IL-1-induced bone resorption. J Bone Miner Res. 2003;18(2):260-9.