Ann Med Res 2021;28(7):1417-22

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.07.712

Original Article

Expression level of UCMA as a candidate molecular target in osteoarthritis

Hamza Malik Okuyan[1,3], Ahmet Arslan[2,3], Yusuf Ziya Igci[3], Bulent Gogebakan[4], Nilufer Bilgic[4,5], Kubra Gunduz[4,5], Dilara Sonmez[4,6]

[1]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Sakarya University of Applied Sciences, Sakarya, Turkey
[2]Department of Medical Genetics, Faculty of Medicine, Tekirdag Namık Kemal University, Gaziantep, Turkey
[3]Department of Medical Biology, Graduate School of Health Sciences, Gaziantep University, Gaziantep, Turkey
[4]Department of Molecular Biochemistry and Genetics, Graduate School of Health Sciences, Hatay Mustafa Kemal University, Hatay, Turkey
[5]Department of Medical Biology, Hamidiye Institute of Health Sciences, University of Health Sciences, Istanbul, Turkey
[6]Department of Molecular Medicine, Institute of Aziz Sancar Experimental Medicine Research, Istanbul University, Istanbul, Turkey

Copyright@Author(s) - Available online at www.annalsmedres.org
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

Abstract

Aim: Osteoarthritis (OA) is a degenerative joint disorder that damages cartilage, synovium and subchondral bone, and there is yet no effective treatment for OA. The identification of novel therapeutic methods is crucially needed for better treatment of OA. Upper zone of growth plate and cartilage matrix associated (UCMA) was discovered as a chondrocyte specific protein in 2008, but its expression is solely not specific to cartilage tissue. Although UCMA is implicated in cartilage and bone metabolic processes, the molecular function of UCMA in OA is not elucidated yet. We aimed to examine the potential effect of UCMA in osteoblast metabolism associated with OA.

Materials and Methods: We created an *in vitro* OA model by inducing osteoblast cell line with IL-1β. The expression levels of 12 related genes were determined using the qRT-PCR method. The MMP1 and OPG expression levels in the supernatants of cells were detected with ELISA.

Results: We found that there was a dramatic increase in the levels of UCMA expression and other OA-related markers. We showed that IL-1β triggered the expression of main transcription factors playing a role during bone formation. MMP1 and OPG synthesis and secretions were increased in IL-1β induced-hFOB1.19 cell line significantly.

Conclusion: Our study, as the first one using the human osteoblast cell line, provides good evidence about the potential value of UCMA in the pathophysiology of OA and shows that UCMA can be a promising molecular target to develop therapeutic approaches for OA.

Keywords: hFOB1.19; gene expression; inflammation; osteoarthritis; UCMA

INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disorder that damages cartilage, synovium and subchondral bone. OA, which decreases life quality and increases health expenses, is the most important reason for the physical disability in developed countries, and its incidence is predicted to double up till 2020 (1). Although OA is accepted as a prevalent and severe disorder, very few studies focus on this health issue, OA pathophysiology is still fully unknown, and it causes structural defects and cellular changes in all articular regions such as bone, cartilage and synovium (2,3). The current therapies against OA have limited and diverse side effects (4). Thus, it is crucially required to detect novel therapeutic approaches and molecular targets for the OA treatment (3). Although cartilage destruction leading to chondrocyte apoptosis and extracellular degradation is a prominent feature of OA, subchondral bone sclerosis and osteophyte formation are also connected with OA pathogenesis. Therefore, some studies suggest that targeting bone tissue should be useful for the treatment of OA (5). In response to the changed biochemical surroundings of the diseased joint, the cellular physiologies of the osteoblasts, synoviocytes and chondrocytes change during OA (2,6). Especially, osteoblasts show different cellular behaviours and gene expression patterns throughout the disease course. The deregulated gene expression in osteoblasts negatively affects various mechanisms such as bone mineralization and remodelling, cytokine synthesis, and thereby,

Received: 08.07.2020 **Accepted:** 12.08.2020 **Available online:** 12.07.2021
Corresponding Author: Hamza Malik Okuyan, Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Sakarya University of Applied Sciences, Sakarya, Turkey **E-mail:** hmokuyan@subu.edu.tr

contributes to the development of OA (7). Moreover, the subchondral pathogenesis in OA is closely associated with the deregulated expressions of Receptor activator of NF-kB ligand (RANKL) / Osteoprotegerin (OPG), and these molecules are prominently expressed in osteoblasts (8,9). Interleukine-1 Beta (IL-1β), as a prominent factor, has been reported to stimulate catabolic activity in osteoblasts by the synthesis of RANKL and IL-6 which contribute to pathological subchondral changes (10,11). Vitamin K is an intriguing candidate in the prevention and therapy of pathologies, such as calcification due to its role in skeletal metabolism (12-14). It was shown in the related studies that, subclinical levels of vitamin K elevated the progression risk of the knee OA (15). Upper zone of growth plate and cartilage matrix associated (UCMA, also termed as Gla rich protein, GRP) is one of the most recent members of the vitamin K-dependent proteins (VKDP). It is highlighted that UCMA is likely to be a molecular target for the treatment of some diseases like kidney diseases and OA (16-18). UCMA is a protein that has a high affinity tendency to calcium (Ca) mineral and possesses 16 gla residues (19). The molecular mechanism of UCMA is not fully known. It is asserted that UCMA can function as a modulator in the utilization of the Ca deposits in the extracellular matrix (ECM) (16). Recent studies have shown that OA was directly related to the accumulation of basic calcium phosphate crystals (20-24). Also, UCMA was implicated in the connection between cartilage and bone tissues during OA (25), it can function as a mediator regarding inflammation and calcification processes (26), and it is considered an inhibitor of vascular calcification (27). Conversely, no remarkable phenotypic alterations were found in the cartilage and bone of UCMA knocked down mice (28). However, UCMA is needed for skeletal development and calcification in knock-down zebrafish. (14) It is known that UCMA expression is present in early chondrocytes, but it disappears along with the maturation and hypertrophy of chondrocytes. In a study examining the effect of BMP2 on the regulation of UCMA expression, it was observed that BMP2 suppressed UCMA expression in chondrogenic and primary chondrocytes in a dose-dependent manner (29). BMP2 is responsible for the induction of osterix (Osx) transcription factor expression during osteogenesis. BMP2 regulates the gene expressions of bone metabolism-related proteins such as runt-related transcription factor 2 (Runx2), Osx, collagen type-1, and osteopontin (OPN) (30,31). Runx and Osx transcription factors playing a role in the differentiation of osteoblast and nodule formation also regulate UCMA up-regulation (32).

In the current study, to determine the possible role of UCMA in OA pathophysiology, osteoblastic cell line hFOB1.19 was used as an *in-vitro* experimental model. We aimed to examine the potential effect of UCMA in osteoblast metabolism associated with OA. We created an *in vitro* OA model by inducing osteoblast cell line with IL-1β.

MATERIALS and METHODS

Cell Culture and IL-1β Treatment

The human osteoblast cell line was used in the *in vitro* experiments, and this hFOB1.19 cell line was bought from American Type Culture Collection. (Manassas, VA 20108, USA). hFOB1.19 Cells were propagated in culture with Dulbecco's modified Eagle medium:F12 (DMEM), which supports the growth of osteoblast. G418 (0.3 mg/mL, Sigma, Missouri, USA) and 10% fetal bovine serum as growth support was added to the medium. hFBO1.19 cells were kept in a humidified atmosphere, including 5% CO_2 at 34°C. The hFOB1.19 cells culture medium was replaced twice a week, and the hFOB1.19 cells were seeded into the 6 well plates at the confluence of 80%. Cells were incubated with 5 and 10 ng/ml IL-1β for 48 (48 h) and 72 hours (72 h).

Gene Expression Analysis

Total RNA isolation from cell cultures was performed by using a miRNeasy mini kit (Qiagen, Hilden, Germany). Then, RNA concentration was measured spectrophotometrically. cDNA was synthesized by using RT^2 HT First Strand Kit (Qiagen, Maryland, USA). Quantitative Real-Time Polymerase Chain Reactions were conducted by using SYBR Green qPCR Mastermix Kit (Qiagen, Maryland, USA) with Rotor-Gene Q (Qiagen, Hilden, Germany). Specific Primers for interested genes (UCMA, MGP, OC/BGLAP, OPN/SPP1, NF-κB1, RANKL/ TNFSF11, OPG/TNFSF11B, MMP1, RUNX2, Osx/Sp7, BMP2, BMP7, GAPDH [housekeeping gene] were purchased from Qiagen (Table 1). The following PCR conditions were used: initiation step at 95 °C for 10 min, then second step at 95 °C for 15 sec and third step at 60 °C for 1 min (40 cycles of amplification).

Table 1. Gene list

Gene	NCBI RefSeq	Catalog No
UCMA	NM_145314	PPH11256A
MGP	NM_000900	PPH17932F
OC/BGLAP	NM_199173	PPH01898A
OPN/SPP1	NM_000582	PPH00582E
NF-κB1	NM_001165412	PPH00204F
RANKL/ TNFSF11	NM_003701	PPH01048F
OPG/ TNFSF11B	NM_002546	PPH01049B
MMP1	NM_001145938.1	PPH00120B
RUNX2	NM_001015051	PPH01897C
Osx/Sp7	NM_152860	PPH00705A
BMP2	NM_001200	PPH00549C
BMP7	NM_001719	PPH00527A
GAPDH	NM_001256799	PPH00150F

ELISA

Concentrations of MMP1 and OPG in supernatants of cultured hFOB1.19 cells were analyzed using an enzyme-linked immunosorbent assay (ELISA, R&D Systems). Samples were measured spectrophotometrically at 450 nm using a microplate reader (Multiskan Go, Thermoscientific, Vantaa, Finland).

Statistical Analysis

As previously stated, we utilized the Gene Globe Data Analysis Center (Qiagen) to assess qPCR data (33). Relative quantification RT-PCR in the present study was performed in triplicate. We also used the Delta Delta Ct (ΔΔCt) method for data analysis and normalized the raw gene expression data with housekeeping gene GAPDH as an internal control. We stated all qPCR results as the "fold-change" ($2^{\wedge(-\Delta\Delta Ct)}$) and considered that fold-change > 2 was upregulated and <1 is as down-regulated.. Using the values in the control group and treatment groups were calculated p values according to a student's t-test. We plotted all graphics in the present study using GraphPad Prism. ELISA data were indicated as mean±SEM. The comparison of the groups was conducted by using a one-way ANOVA or t-test. P<0.05 values were accepted as significant. Data analysis was conducted with IBM SPSS (V23).

RESULTS

We investigated the expression levels of UCMA and OA related genes with the qRT-PCR. Firstly, we found that UCMA was expressed in the hFOB1.19 osteoblast cell line. Then, we analyzed the association of UCMA with OA in human osteoblastic cells, which are the main cell type involved in OA pathogenesis. Our results indicated that the levels of UCMA expression and the genes related to mineralization, i.e. MGP, BGLAP and SPP1, were also upregulated in our cell culture OA model. The increased levels of the prominent OA-related markers were confirmed in our *in-vitro* OA model. Among these genes, the highest expression levels belonged to UCMA (P<0.05) (Figure 1a). The most remarkable difference regarding the UCMA levels was observed at 48 h (P<0.05) (Figure 1a), but we did not detect any significant difference at 72 h. The results revealed that IL-1β stimulation triggers a rapid response in the expression of UCMA in osteoblast cells. In parallel, most of the mineralization markers at 48 h were found to be expressed in a similar way (Figure 1b, c and d). We also detected that the expression levels of MGP were upregulated at a high dosage and 72 h, while the expression levels of BGLAP were downregulated at a low dosage and 72 h (P<0.05). In contrast, we did not observe any difference in the expression levels of BGLAP and SPP1 at a high dosage of IL-1b and 72 h. Also, we found that there are no significant differences in the expression levels of MGP and SPP1 at a low dosage of IL-1b and 72h.

Moreover, we investigated the mRNA expression levels of NF-κB1, RANKL, OPG and MMP1, which closely related to OA pathogenesis in the present study. The increased level of RANKL, which is part of the RANKL/RANK/OPG axis, implies its role in the OA progression. We found that the expression levels of NF-κB1, RANKL, OPG and MMP1 gene expression levels were significantly increased at 48 h (P<0.05) (Figure 2). The mRNA expression level of RANKL was conspicuously increased following UCMA after IL-1β treatment (P<0.05) (Figure 2b). We also detected that the expression levels of NF-κB1 and OPG were elevated at 72 h, similar to 48 h (P<0.05) (Figure 2a and 2c). We did not detect any difference in the expression levels of RANKL at 72 h after a low dosage of IL-1 treatment (P>0.05) (Figure 2b).

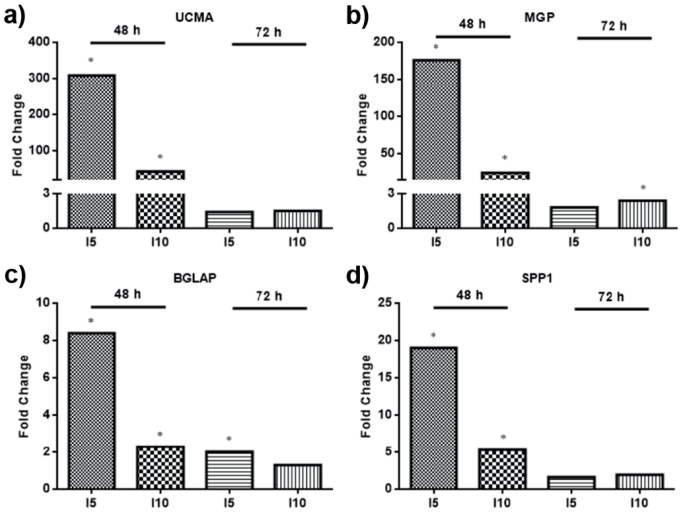

Figure 1. Expression levels of UCMA and mineralization markers in OA. The expression patterns of UCMA (a), and mineralization markers; MGP (b), BGLAP (c), and SPP1 (d) after 48 h and 72 h treatment with IL-1β in hFOB1.19 human osteoblast cell line. All data were expressed as fold change. I5: IL-1β, 5 ng/ml; I10: IL-1β,10 ng/ml (*p < 0.05)

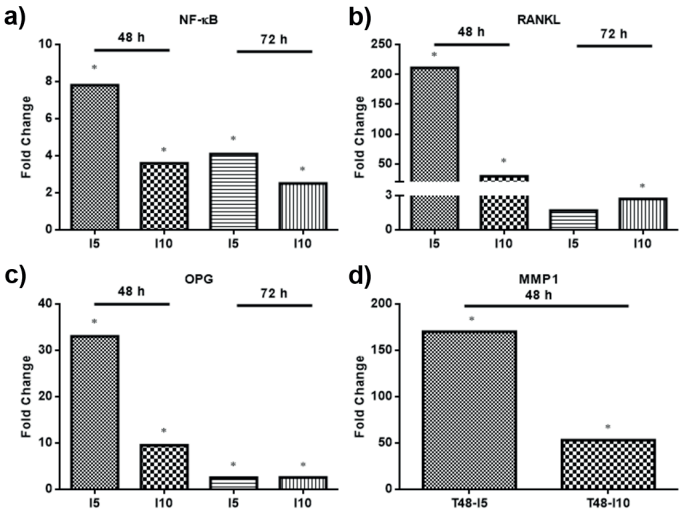

Figure 2. Association of RANKL/OPG system with inflammatory situations in OA. Gene expression levels of NF-κB (a), RANKL (b), and OPG (c), after 48 h and 72 h, and MMP1 (d) after 48 h treatment with IL-1β in hFOB1.19 human osteoblast cell line. All data were expressed as fold change. I5: IL-1β,5 ng/ml; I10: IL-1β,10 ng/ml (*p < 0.05)

We also analyzed the gene expression pattern with respect to cellular differentiation in human osteoblast cells. After IL-1β treatment, the expression levels of Runx2, Osx, BMP2, and BMP7 genes significantly increased (P<0.05) (Figure 3). In particular, we found that these markers increased markedly at low IL-1B dosage. The results of our study demonstrated that IL-1β promoted cell differentiation markers in OA (P<0.05) (Figure 3).

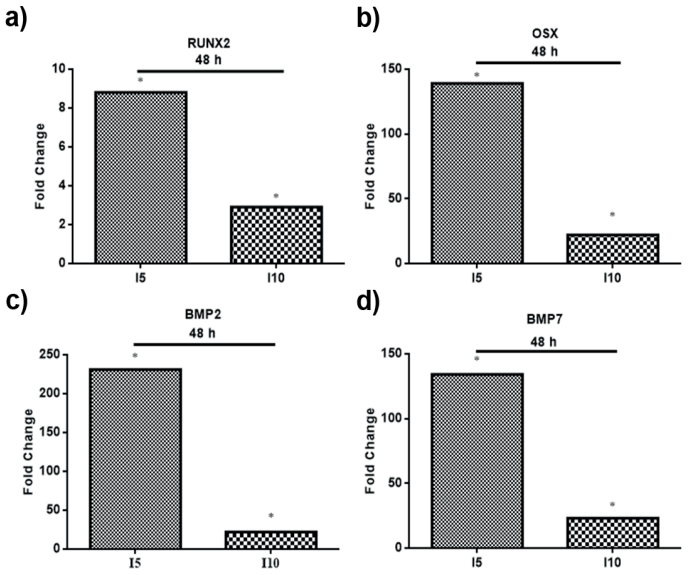

Figure 3. Gene expression patterns of cell differentiation markers; Runx2 (a), Osx (b), BMP2 (c), and BMP7 (d) after 48 h treatment with IL-1β in hFOB1.19 human osteoblast cell line. All data were expressed as fold change. I5: IL-1β, 5 ng/ml; I10: IL-1β,10 ng/ml (*p < 0.05)

In order to detect the effect of pro-inflammatory cytokine IL-1β on the protein levels of OPG and MMP1, which contribute to abnormal bone metabolisms related to OA in the Osteoblast cell line, we analyzed the protein levels with ELISA. As depicted in Figure 4a and 4b, the synthesis and secretions of OPG and MMP1 in hFOB1.19 Osteoblasts were significantly elevated depending on the dosage and treatment duration of IL-1β (P<0.05).

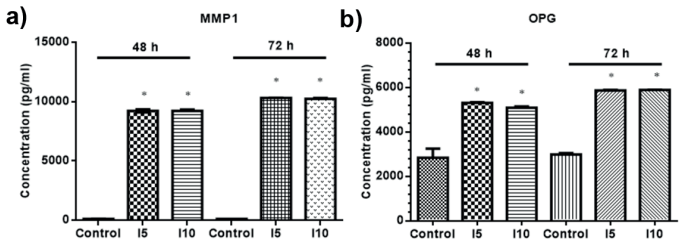

Figure 4. Protein levels (pg/ml) of MMP1 (a) and OPG (b) in the supernatant of the hFOB1.19 cells after IL-1β stimulation for 48 h and 72 h measured with ELISA. All data were stated as mean ± SEM. I5: IL-1β, 5 ng/ml; I10: IL-1β,10 ng/ml (*p < 0.05)

DISCUSSION

In the current study, we showed the role of UCMA in inflammatory processes during OA pathogenesis in hFOB1.19 human osteoblast cell line for the first time. In human osteoblast cells stimulated with proinflammatory IL-1β cytokine, we obtained that gene expression levels of UCMA upregulated, which is associated with the high levels of OA and osteoblast related genes. Our results in this study are in line with the findings of another study conducted with other cell types (synoviocytes and chondrocytes) affected by OA (26). Cavaco et al. (2016) induced synoviocytes and chondrocytes with IL-1β to evaluate the inflammatory aspect of UCMA. Then, when they checked the expression levels of UCMA and some OA-related genes (COX2, MMP13), they noticed that the expression levels of these genes peaked 3 h after IL-1β stimulation and decreased gradually between 6-72 h (26). Partially similar to this study we found that while the expression levels of UCMA and other OA-related genes (RANKL, MMP1, OPG, MGP, BGLAP, and SPP1) peaked at 48 h after stimulation of osteoblasts with IL-1β, the expression levels of these genes decreased at 72 h after IL-1β treatment. Together with the findings of the previous studies, it can be ascertained that IL-1β stimulation yields similar outputs in different OA cell line models (osteoblast, chondrocyte, synoviocyte). We demonstrated in our study that there was a similar gene expression pattern between UCMA and inflammation-induced markers mentioned above. This particular outcome suggests that UCMA can play a role in OA after stimulated with inflammation.

Surmann-Schmitt et al. reported in their studies that UCMA was expressed in the differentiation of osteoblast (29). However, Viegas et al. observed that UCMA was expressed in chondrocytes, osteocytes and osteoblasts (19). Also, in the previous studies, UCMA expression and regulation were detected in osteoblasts (19,29,32). Intriguingly, in a study conducted by Lee et al. (2015), they indicated that UCMA, which is a direct target of Runx2 and Osx at the transcriptional level, is a stimulatory factor in the differentiation of osteoblast bone formation (32). It was demonstrated in the same study that UCMA gene expression significantly decreased in Runx2 and Osx knock-out mice (32). Additionally, in order to analyze how UCMA gene expression is regulated by Runx2 and Osx, they transfected a mouse pre-osteoblast cell line (MC3T3-E1) with vectors carrying Runx2, Osx and both Runx2-Osx encoding regions (32). Their findings revealed that UCMA gene expression in osteoblasts increased in all over-expression conditions (Runx2, Osx and both Runx2-Osx) (32). These results suggested that Runx2, together with Osx regulates the gene expression of UCMA in MC3T3-E1 cell line (32). Apart from these results, we obtained in our study that the gene expression of UCMA, Runx2 and Osx can be triggered by IL-1β induction in hFOB1.19 cell line. Moreover, thus evidently, UCMA gene expression can be promoted by both IL-1β cytokine and Runx2 and Osx transcription factors. Nevertheless, in another study conducted with chondrogenic cells, it was asserted that UCMA was suppressed by BMP2, which is responsible for Osx upregulation (37). These contradictory findings reveal the fact that further researches are needed to understand the molecular mechanism of UCMA.

MGP and OC are other members of VKDP family like UCMA (26). In our *in vitro* OA model, we found a considerable increase in MGP and OC gene expression levels compared to the control group. We also demonstrated a similar gene expression pattern between UCMA, MGP and OC in the current study. Besides, the highest rise in the gene expression levels was observed in UCMA among these three genes. It was shown that, along with UCMA, the levels of MGP and OC in articular chondrocytes and synoviocytes increased depending on the calcification

that contributes to OA progression (26). In another study utilized UCMA overexpressing osteoblasts, it was detected that OC levels were upregulated and showed a similar gene expression pattern with UCMA (32). On the other hand, it has not been clarified yet whether UCMA directly upregulated OC expression. Based on our results, we can suggest that MGP, just like UCMA, can be a strong candidate as a molecular target for OA.

It was reported in previous studies that SPP1 involves in several cellular mechanisms such as mineralization, bone morphogenesis and inflammation (34). In a study with fibroblast-like synoviocytes, it was shown that induction of mineralization increased the gene expression levels of SPP1. In the same study, expression levels of UCMA, MGP and BGLAP showed a similar pattern with the increasing levels of SPP1 (26). Moreover, in another study investigating osteoblast differentiation processes, it was shown that SPP1 gene expression levels increased in UCMA over-expressing cells (32). In our study, we demonstrated that SPP1, an important gene for osteoblasts, can be induced with IL-1β in hFOB1.19 cell line.

The osteoblasts are characterized by deregulated gene expressions in OA, and the secretions of osteoblasts affect the physiology of osteoclasts, which involved in subchondral pathogenesis (11,35). In the previous studies, a close connection has been highlighted between changes in subchondral bone with OA and OPG – RANK – RANKL expressions (9). Articular inner balance is regulated with OPG/RANK/RANKL signalling pathway, and during OA pathogenesis, the pathway is deregulated, and a reduction in OPG/RANKL ratio is observed (9). Hereby, in subchondral bone, an increase in osteoporosis and subchondral intensity exacerbates OA progression by means of osteoclast accumulation (36-39). We observed in our study that the highest gene expression levels belonged to RANKL after UCMA, and thereby OPG/RANKL ratio was decreased in osteoblasts

Together with this, there are some discrepancies in the literature about UCMA. In a study performed by Eitzinger et al. (2012), which was about the function of UCMA, especially in mouse skeletal tissues, they did not observe any noticeable phenotypic alteration in bones and cartilages of UCMA knock-out mice (28). However, Neacsu et al. (2011) showed in a study performed with zebra fish that UCMA is necessary for skeletal development and calcification (14).

CONCLUSION

In conclusion, the present study provides sound evidence about the potential value of UCMA in the pathophysiology of OA. So far as we know, this is the first study investigating UCMA in a human osteoblast cell line. Besides, further in vivo and in vitro researches are needed to improve understanding of the molecular function of UCMA in OA pathogenesis.

Competing Interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical Approval: No ethical certification was needed for the present in vitro study.

REFERENCES

1. Hosnijeh FS, Runhaar J, van Meurs JB, et al. Biomarkers for osteoarthritis: Can they be used for risk assessment? A systematic review. Maturitas 2015;82:36-49.
2. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone 2012;51:249-57.
3. Kirwan JR, Elson CJ. Is the progression of osteoarthritis phasic? Evidence and implications. J Rheumatol 2000;27:834-6.
4. Kraus VB, Blanco FJ, Englund M, et al. Call for standardized definitions of osteoarthritis and risk stratification for clinical trials and clinical use. Osteoarthritis Cartilage 2015;23:1233-41.
5. Kwan Tat S, Lajeunesse D, Pelletier JP, et al. Targeting subchondral bone for treating osteoarthritis: what is the evidence? Best Pract Res Clin Rheumatol 2010;24:51-70.
6. Findlay DM, Atkins GJ. Osteoblast-chondrocyte interactions in osteoarthritis. Curr Osteoporos Rep 2014;12:127-34.
7. Maruotti N, Corrado A, Cantatore FP. Osteoblast role in osteoarthritis pathogenesis. J Cell Physiol 2017;232:2957-63.
8. Romas E, Gillespie MT, Martin TJ. Involvement of receptor activator of NFkappaB ligand and tumor necrosis factor-alpha in bone destruction in rheumatoid arthritis. Bone 2002;30:340-6.
9. Kwan Tat S, Pelletier JP, Lajeunesse D, et al. The differential expression of osteoprotegerin (OPG) and receptor activator of nuclear factor kappaB ligand (RANKL) in human osteoarthritic subchondral bone osteoblasts is an indicator of the metabolic state of these disease cells. Clin Exp Rheumatol 2008;26:295-304.
10. Rigoglou S, Papavassiliou AG. The NF-kappaB signalling pathway in osteoarthritis. Int J Biochem Cell Biol 2013;45:2580-4.
11. Kwan Tat S, Padrines M, Theoleyre S, et al. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev 2004;15:49-60.
12. Price PA, Williamson MK, Haba T, et al. Excessive mineralization with growth plate closure in rats on chronic warfarin treatment. Proc Natl Acad Sci U S A 1982;79:7734-8.
13. Luo G, Ducy P, McKee MD, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997;386:78-81.
14. Neacsu CD, Grosch M, Tejada M, et al. Ucmaa (Grp-2) is required for zebrafish skeletal development. Evidence for a functional role of its glutamate gamma-carboxylation. Matrix Biol 2011;30:369-78.

15. Misra D, Booth SL, Tolstykh I, et al. Vitamin K deficiency is associated with incident knee osteoarthritis. Am J Med 2013;126:243-8.
16. Cancela ML, Conceicao N, Laize V. Gla-rich protein, a new player in tissue calcification? Adv Nutr 2012;3:174-81.
17. Okuyan HM, Terzi MY, Ozcan O, et al. Association of UCMA levels in serum and synovial fluid with severity of knee osteoarthritis. Int J Rheum Dis 2019;22:1884-90.
18. Okuyan HM, Ozcan O, Dogan S, et al. Upper Zone of Growth Plate and Cartilage Matrix (UCMA) levels in patients with chronic kidney disease. Brazilian Archives of Biology and Technology 2020;63.
19. Viegas CS, Simes DC, Laize V, et al. Gla-rich protein (GRP), a new vitamin K-dependent protein identified from sturgeon cartilage and highly conserved in vertebrates. J Biol Chem 2008;283:36655-64.
20. Liu YZ, Jackson AP, Cosgrove SD. Contribution of calcium-containing crystals to cartilage degradation and synovial inflammation in osteoarthritis. Osteoarthritis Cartilage 2009;17:1333-40.
21. Fuerst M, Bertrand J, Lammers L, et al. Calcification of articular cartilage in human osteoarthritis. Arthritis Rheum 2009;60:2694-703.
22. O'Connell JX. Pathology of the synovium. Am J Clin Pathol 2000;114:773-84.
23. Hernandez-Santana A, Yavorskyy A, Loughran ST, et al. New approaches in the detection of calcium-containing microcrystals in synovial fluid. Bioanalysis 2011;3:1085-91.
24. Ea HK, Nguyen C, Bazin D, et al. Articular cartilage calcification in osteoarthritis: insights into crystal-induced stress. Arthritis Rheum 2011;63:10-8.
25. Stock M, Menges S, Eitzinger N, et al. A dual role for UCMA in osteoarthritis-Inhibition of aggrecanases and promotion of bone turnover. Arthritis Rheumatol 2017; 69:1233-45.
26. Cavaco S, Viegas CS, Rafael MS, et al. Gla-rich protein is involved in the cross-talk between calcification and inflammation in osteoarthritis. Cell Mol Life Sci 2016;73:1051-65.
27. Viegas CS, Rafael MS, Enriquez JL, et al. Gla-rich protein acts as a calcification inhibitor in the human cardiovascular system. Arterioscler Thromb Vasc Biol 2015;35:399-408.
28. Eitzinger N, Surmann-Schmitt C, Bosl M, et al. Ucma is not necessary for normal development of the mouse skeleton. Bone 2012;50:670-80.
29. Surmann-Schmitt C, Dietz U, Kireva T, et al. Ucma, a novel secreted cartilage-specific protein with implications in osteogenesis. J Biol Chem 2008;283:7082-93.
30. Sun J, Li J, Li C, et al. Role of bone morphogenetic protein-2 in osteogenic differentiation of mesenchymal stem cells. Mol Med Rep 2015;12:4230-7.
31. Lysdahl H, Baatrup A, Foldager CB, et al. Preconditioning Human Mesenchymal Stem Cells with a Low Concentration of BMP2 Stimulates Proliferation and Osteogenic Differentiation In Vitro. Biores Open Access 2014;3:278-85.
32. Lee YJ, Park SY, Lee SJ, et al. Ucma, a direct transcriptional target of Runx2 and Osterix, promotes osteoblast differentiation and nodule formation. Osteoarthritis Cartilage 2015;23:1421-31.
33. Garcia-Reyero N, Escalon L, Prats E, et al. Targeted Gene Expression in Zebrafish Exposed to Chlorpyrifos-Oxon Confirms Phenotype-Specific Mechanisms Leading to Adverse Outcomes. Bull Environ Contam Toxicol 2016;96:707-13.
34. Lund SA, Giachelli CM, Scatena M. The role of osteopontin in inflammatory processes. J Cell Commun Signal 2009;3:311-22.
35. Hofbauer LC, Schoppet M. Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. JAMA 2004;292:490-5.
36. Castaneda S, Roman-Blas JA, Largo R, et al. Subchondral bone as a key target for osteoarthritis treatment. Biochem Pharmacol 2012;83:315-23.
37. Kwan Tat S, Amiable N, Pelletier JP, et al. Modulation of OPG, RANK and RANKL by human chondrocytes and their implication during osteoarthritis. Rheumatology (Oxford) 2009;48:1482-90.
38. Trouvin AP, Goeb V. Receptor activator of nuclear factor-kappaB ligand and osteoprotegerin: maintaining the balance to prevent bone loss. Clin Interv Aging 2010;5:345-54.
39. Martin TJ. Historically significant events in the discovery of RANK/RANKL/OPG. World J Orthop 2013;4:186-97.