

Dear Editors and Reviewers,

We are submitting our revised manuscript entitled "**O-GlcNAcylation may be a key regulatory factor in promoting osteogenic differentiation of BMSCs**" (Manuscript NO: 90591, Editorial) to the *World Journal of Stem Cells* for your reconsideration of its suitability for publication. All authors have read and approved the revised manuscript. We deeply appreciate the time and effort you have spent in reviewing our manuscript. We have learned much from the reviewers' comments, which are valuable and constructive. After studying the comments and your advice carefully, we have made a corresponding revision. Words in yellow are the changes we have made in the new manuscript. Our responses to the comments are listed below:

**1. *Mention the derivation of the UDP-GlcNAc molecule***

**Reply:** Thanks for your advice. We have added the derivation of the UDP-GlcNAc molecule in the first paragraph of the main text.

**2. *Other important functions may be highlighted in a single statement including cancer metastasis***

**Reply:** Thanks for your suggestion. We strongly agree that the topic of O-GlcNAc and cancer metastasis is indeed a very fascinating area of research that has been extensively documented (e.g. PMID: 37279852; PMID: 37838167; PMID: 37392316). However, this Editorial focuses on the narrow scope of O-GlcNAc and the osteogenic differentiation of BMSCs. Therefore, it is less appropriate to add a paragraph describing O-GlcNAc and cancer metastasis. Nonetheless, considering that this is an paper on O-GlcNAc and bone metabolism, we specifically searched for articles on O-GlcNAc and osteosarcoma that you might be interested in. We have provided a brief review of O-GlcNAc and osteosarcoma, which is at the end of the "Point by Point responses" section. Thanks again for your comments.

**3. *In the osteogenesis process O-GlcNAc is consistently increases or is it a dynamic process***

**Reply:** Thank you for your question. Existing evidence suggests that O-GlcNAcylation was positively associated with osteoblast differentiation. The expression of O-GlcNAcylation (as detected by the expression of RL2 protein) is increased during osteogenic differentiation rather than dynamically changing.

**4. *What happened to O-GlcNAc in inflammatory arthritis***

**Reply:** Thanks for your suggestion. Similarly, since the topic of our paper is not closely related to inflammatory arthritis, we thought it would be inappropriate to add this section to our manuscript. However, we also specifically searched for articles on O-GlcNAc and inflammatory arthritis that you might be interested in. We have provided a brief review of O-GlcNAc and inflammatory arthritis, which is at the end of the "Point by Point responses" section. Thanks again for your comments.

**5. *Language polishing***



**Reply:** The revised manuscript has been modified by the "Elsevier Language Editing" service. The certification of language polishing has been resubmitted.

**6. ABBREVIATIONS**

**Reply:** We have modified the abbreviations throughout the main text according to the rules for abbreviations.

*7. Please add the Core tip section. The number of words should be controlled between 50-100 words.*

**Reply:** We have added Core Tip on the first page of the revised manuscript.

*8. Please provide the PMID and DOI citation numbers to the reference list and list all authors of the references. If there is no PMID or DOI, please provide the website address.*

**Reply:** We have added the names of all authors and added DOIs or the website address in the References.

*9. The main text of the manuscript must include two parts: "Introduction" and "Conclusion".*

**Reply:** We have added two subtitles, "Introduction" and "Conclusion", to the corresponding places in the main text.

*10. Abbreviations other than special types of words such as COVID-19 and SARS-CoV-2 are not allowed in the article title, and no more than 18 words are allowed.*

**Reply:** We have replaced BMSCs in the title with bone marrow mesenchymal stromal cells according to the abbreviation rules.

We hope the Reviewers will be satisfied with the revisions for our manuscript. If you have any questions about our review, please do not hesitate to contact us.

Thanks and Best regards!

Yours Sincerely,

Prof. Guoxin Ni

02/02/2024



### **O-GlcNAcylation and osteosarcoma (unpublished)**

Osteosarcoma is one of the most common primary malignant bone tumors in adolescents with a 5-year survival rate of less than 20%. Currently, the main treatment strategy for osteosarcoma is surgery combined with chemotherapy(1). Some studies have revealed the presence of high expression of OGT and O-GlcNAcylation in different solid tumor tissues. O-GlcNAcylation is able to affect tumor glucose metabolism, metastasis, proliferation, vascular invasion and drug resistance by modifying tumor-associated proteins(2,3). Recently, a clinical study examined O-GlcNAcylation, OGT, and OGA expression in bone specimens derived from 109 patients diagnosed with osteosarcoma. The results showed that high OGA expression was significantly associated with longer overall survival and metastasis-free period in patients with stage IIB, and positively correlated with good response to chemotherapeutic agents (i.e., tumor tissue necrosis percentage  $\geq 90$ ). Further multivariate analysis indicated that OGA expression was an independent predictor of good prognosis for osteosarcoma(4). In addition, cancer cells are able to promote signaling pathways associated with tumorigenesis by increasing uptake of glucose and glutamine, which can upregulate the flux of the HBP pathway and increase O-GlcNAcylation levels(5). It has been shown that HBP pathway dysregulation is significantly associated with the prognosis of osteosarcoma patients. Five HBP-related genes (OGT, UAP1, GPI, MGEA5, and PGM3) can be used to guide immunologic and targeted therapies for osteosarcoma, with significant predictive value for osteosarcoma prognosis(6).

ROCK2 can modulate the level of target protein phosphorylation/activity to regulate downstream gene expression. ROCK2 has been shown to promote cancer cell proliferation, which is closely associated with cancer progression and poor prognosis(7). Deng et al. (8) showed that ROCK2 could promote the proliferation of osteosarcoma cells. Overexpression of ROCK2 promotes OGT protein stability by inhibiting OGT degradation and ubiquitination through the ubiquitin-proteasome system, which ultimately promotes osteosarcoma cell proliferation and tumor growth and increases resistance to TRAIL(8). In addition, Sun et al. (9) showed that lncEBLN3P was able to enhance OGT expression by targeting miR-200a-3p, which ultimately promotes metastasis and drug resistance in osteosarcoma. Unfortunately, the studies related to O-GlcNAcylation and osteosarcoma have rarely been validated by animal experiments.

In summary, the expression level of O-GlcNAcylation was significantly elevated in patients



with osteosarcoma. O-GlcNAcylation accelerates tumor progression by regulating tumor cell proliferation, differentiation, and migration and is strongly associated with adverse clinical outcomes. Therefore, the development of O-GlcNAcylation-related specific inhibitors provides a new therapeutic direction for osteosarcoma treatment. Although O-GlcNAcylation has shown strong therapeutic potential in the treatment of osteosarcoma, the potential regulatory mechanisms of O-GlcNAcylation in osteosarcoma remain unclear. More studies are still needed to further investigate the role of O-GlcNAcylation in osteosarcoma. In addition, the efficacy and safety of O-GlcNAcylation inhibitors in vivo need to be validated by extensive preclinical and clinical trials.

## References

1. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: Current Treatment and a Collaborative Pathway to Success. *J clin oncol*. 2015;33(27):3029-35. PubMed PMID: 26304877.
2. Cheng YU, Li H, Li J, Li J, Gao Y, Liu B. O-GlcNAcylation enhances anaplastic thyroid carcinoma malignancy. *Oncol lett*. 2016;12(1):572-8. PubMed PMID: 27347182.
3. He XF, Hu X, Wen GJ, Wang Z, Lin WJ. O-GlcNAcylation in cancer development and immunotherapy. *Cancer Lett*. 2023;566:216258. Epub 20230604. doi: 10.1016/j.canlet.2023.216258. PubMed PMID: 37279852.
4. Sombuthaweeri T, Wu S, Chamusri N, Settakorn J, Pruksakorn D, Chaiyawat P, et al. Relationship Between O-GlcNAcase Expression and Prognosis of Patients With Osteosarcoma. *Appl immunohisto m m*. 2022;30(1):e1-e10. PubMed PMID: 34469899.
5. Lee JB, Pyo KH, Kim HR. Role and Function of O-GlcNAcylation in Cancer. *Cancers (Basel)*. 2021;13(21). Epub 20211026. doi: 10.3390/cancers13215365. PubMed PMID: 34771527; PubMed Central PMCID: PMC8582477.
6. Su Z, Wang C, Pan R, Li H, Chen J, Tan J, et al. The hexosamine biosynthesis pathway-related gene signature correlates with immune infiltration and predicts prognosis of patients with osteosarcoma. *Front Immunol*. 2022;13:1028263. Epub 20221006. doi: 10.3389/fimmu.2022.1028263. PubMed PMID: 36275679; PubMed Central PMCID: PMC9582954.
7. Calò LA, Ravarotto V, Pagnin E, Simioni F, Nalesso F. Rho kinase activity and atrial fibrillation: insights from chronic kidney disease and dialysis patients. *J hypertens*. 2019;37(5):1096-7. PubMed PMID: 30920496.
8. Deng X, Yi X, Huang D, Liu P, Chen L, Du Y, et al. ROCK2 mediates osteosarcoma progression and TRAIL resistance by modulating O-GlcNAc transferase degradation. *Am J Cancer Res*. 2020;10(3):781-98. Epub 20200301. PubMed PMID: 32266091; PubMed Central PMCID: PMC7136927.
9. Sun MX, An HY, Sun YB, Sun YB, Bai B. LncRNA EBLN3P attributes methotrexate resistance in osteosarcoma cells through miR-200a-3p/O-GlcNAc transferase pathway. *J Orthop Surg Res*. 2022;17(1):557. PubMed PMID: 36544170.



## **O-GlcNAcylation and inflammatory arthritis (unpublished)**

Previous studies have proposed that dysregulation of O-GlcNAcylation affects inflammatory signaling pathways such as NF- $\kappa$ B, MAPK, and PI3K/AKT, which can lead to a variety of inflammatory diseases(1). Arthritis is a disease primarily associated with autoimmune reactions, infections, metabolic disorders and trauma, such as osteoarthritis (OA) and rheumatoid arthritis (RA). It has been shown that O-GlcNAcylation is closely related to the pathogenesis of OA and RA mainly. OA is a chronic degenerative joint disease characterized by cartilage degeneration, synovial inflammation and subchondral bone remodeling. Pathologic changes in articular cartilage are recognized as one of the key drivers of OA. Impairment of chondrocyte function disrupts the metabolic balance between extracellular matrix synthesis and catabolism, leading to articular cartilage degeneration(2, 3). It was shown that increased O-GlcNAcylation induced a significant expansion of the height of the growth plate and the height of the hypertrophic zone in neonatal mice and promoted hypertrophic differentiation of chondrocytes(4). A significant increase in O-GlcNAcylation was also found in degenerated articular cartilage in clinical samples of OA and in animal models of OA(5, 6). In addition, IL-1 $\beta$ , a key catabolic cytokine in OA, was able to induce further accumulation of O-GlcNAc in chondrocytes from OA patients, suggesting that the inflammatory environment may contribute to the increased O-GlcNAcylation of cartilage in OA, which may exacerbate cartilage damage(5). In addition to cartilage degeneration, the role of synovial inflammation in the pathologic changes of OA is equally noteworthy, as it is closely associated with painful symptoms and has value in determining prognosis(7, 8). Although glucosamine (GlcN) inhibits inflammatory factor production in synoviocytes and thus exhibits a protective effect against OA(9, 10), the underlying anti-inflammatory mechanism of GlcN remains unclear. Since GlcN can act as an inducer of HBP, it has been speculated that GlcN has the potential to exert an anti-inflammatory effect by regulating cellular function through O-GlcNAcylation of target proteins(11, 12). Using DNA microarray analysis, a team of researchers found that more than half of the GlcN-regulated genes were mediated by O-GlcNAcylation in the IL-1 $\beta$ -stimulated human synovial cell line MH7A. It has also been found that GlcN can inhibit pro-inflammatory cytokine expression, while OGT inhibitors can partially restore GlcN-regulated pro-inflammatory cytokine expression(13). Thus, O-GlcNAcylation is closely related to the process of anti-inflammatory regulation of OA synoviocytes by GlcN.



RA is a common chronic and systemic autoimmune disease characterized by aggressive inflammation, overgrowth of synovial tissue, and progressive joint erosion, ultimately leading to loss of joint function and deformity(14). It has been shown that O-GlcNAcylation can affect macrophage function to regulate the immune response process in the organism(15, 16). Accumulating evidence shows that O-GlcNAcylation is highly expressed in fibroblast-like cells and synovial tissues of RA patients compared to normal tissues. The inflammatory milieu in RA promotes O-GlcNAcylation levels(1, 17). Inhibition of O-GlcNAcylation of TAB1 in fibroblast-like cells from RA patients reduces TAK1 autophosphorylation and suppresses TAK1 activation(17). Phosphorylation of TAK1 is an important determinant of downstream signaling in human RASFs. Induction of TAK1 activation leads to activation of the downstream pathways MAPK and IKK thereby promoting the release of inflammatory mediators and tissue destruction in RA(18). Kim et al. (19) found that increased O-GlcNAcylation promoted TNF- $\alpha$ -stimulated proliferation of fibroblast-like synoviocytes and expression of RA-associated proinflammatory cytokines, and exacerbated synovial proliferation and bone destruction in RA. O-GlcNAcylation of the NF- $\kappa$ B subunit p65 can increase the expression levels of downstream genes by promoting nuclear translocation of p65, DNA binding of proteins, and enhanced transcriptional activity, thereby enhancing the TNF- $\alpha$ -induced inflammatory process. In addition, Th1 and Th17 cells play a key role in the pathogenesis of RA. O-GlcNAcylation of p65 may exacerbate the severity of arthritis by contributing to the transformation of T cell populations into Th1 and Th17 cells(19, 20).

Thus, O-GlcNAcylation is involved in different pathological processes in arthritis, such as chondrocyte hypertrophy, synovial proliferation and inflammation. The modulation of O-GlcNAcylation may be a potential therapeutic strategy for arthritis. However, the potential regulatory mechanism of O-GlcNAcylation in arthritis needs to be further investigated.

## References

1. Li Y, Xie M, Men L, Du J. O-GlcNAcylation in immunity and inflammation: An intricate system (Review). *Int j mol med*. 2019;44(2):363-74. PubMed PMID: 31198979.
2. Lubbers R, van Schaarenburg RA, Kwekkeboom JC, Levarht EWN, Bakker AM, Mahdad R, et al. Complement component C1q is produced by isolated articular chondrocytes. *Osteoarthritis cartilage*. 2020;28(5):675-84. PubMed PMID: 31634584.



3. Liu Z, Zhang H, Wang H, Wei L, Niu L. Magnolol Alleviates IL-1 $\beta$ -Induced Dysfunction of Chondrocytes Through Repression of SIRT1/AMPK/PGC-1 $\alpha$  Signaling Pathway. *J interf cytok res.* 2020;40(3):145-51. PubMed PMID: 31916911.
4. Andrés-Bergós J, Tardío L, Larranaga-Vera A, Gómez R, Herrero-Beaumont G, Largo R. The increase in O-linked N-acetylglucosamine protein modification stimulates chondrogenic differentiation both in vitro and in vivo. *J biol chem.* 2012;287(40):33615-28. PubMed PMID: 22859309.
5. Tardío L, Andrés-Bergós J, Zachara NE, Larrañaga-Vera A, Rodríguez-Villar C, Herrero-Beaumont G, et al. O-linked N-acetylglucosamine (O-GlcNAc) protein modification is increased in the cartilage of patients with knee osteoarthritis. *Osteoarthr cartilage.* 2014;22(2):259-63. PubMed PMID: 24333294.
6. Largo R, Tardío L, Andrés-Bergós J, Herrero-Beaumont G. Study of the O-linked-N-acetyl-glucosaminylation of proteins induced by high doses of glucosamine and its correlation with osteoarthritis progression. *Osteoarthritis and Cartilage.* 2012;20:S126. doi: <https://doi.org/10.1016/j.joca.2012.02.160>.
7. Mathiessen A, Conaghan PG. Synovitis in osteoarthritis: current understanding with therapeutic implications. *Arthritis Res Ther.* 2017;19(1):18. Epub 20170202. doi: 10.1186/s13075-017-1229-9. PubMed PMID: 28148295; PubMed Central PMCID: PMC5289060.
8. Wang X, Blizzard L, Halliday A, Han W, Jin X, Cicuttini F, et al. Association between MRI-detected knee joint regional effusion-synovitis and structural changes in older adults: a cohort study. *Ann rheum dis.* 2016;75(3):519-25. PubMed PMID: 25550336.
9. Kapoor M, Mineau F, Fahmi H, Pelletier JP, Martel-Pelletier J. Glucosamine sulfate reduces prostaglandin E(2) production in osteoarthritic chondrocytes through inhibition of microsomal PGE synthase-1. *J rheumatol.* 2012;39(3):635-44. PubMed PMID: 22089456.
10. Hua J, Sakamoto K, Kikukawa T, Abe C, Kurosawa H, Nagaoka I. Evaluation of the suppressive actions of glucosamine on the interleukin-1 $\beta$ -mediated activation of synoviocytes. *Inflamm Res.* 2007;56(10):432-8. doi: 10.1007/s00011-007-7020-7. PubMed PMID: 18026701.
11. Suh HN, Lee YJ, Kim MO, Ryu JM, Han HJ. Glucosamine-induced Sp1 O-GlcNAcylation ameliorates hypoxia-induced SGLT dysfunction in primary cultured renal proximal tubule cells. *J Cell Physiol.* 2014;229(10):1557-68. doi: 10.1002/jcp.24599. PubMed PMID: 24591095.
12. Hwang SY, Hwang JS, Kim SY, Han IO. O-GlcNAcylation and p50/p105 binding of c-Rel are dynamically regulated by LPS and glucosamine in BV2 microglia cells. *Brit j pharmacol.* 2013;169(7):1551-60. PubMed PMID: 23646894.
13. Someya A, Ikegami T, Sakamoto K, Nagaoka I. Glucosamine Downregulates the IL-1 $\beta$ -Induced Expression of Proinflammatory Cytokine Genes in Human Synovial MH7A Cells by O-GlcNAc Modification-Dependent and -Independent Mechanisms. *PLoS One.* 2016;11(10):e0165158. PubMed PMID: 27776166.
14. Weyand CM, Goronzy JJ. The immunology of rheumatoid arthritis. *Nat Immunol.* 2021;22(1):10-8. Epub 20201130. doi: 10.1038/s41590-020-00816-x. PubMed PMID: 33257900; PubMed Central PMCID: PMC8557973.
15. Jitschin R, Bottcher M, Saul D, Lukassen S, Bruns H, Loschinski R, et al. Inflammation-induced glycolytic switch controls suppressivity of mesenchymal stem cells via STAT1 glycosylation. *Leukemia.* 2019;33(7):1783-96. Epub 20190124. doi: 10.1038/s41375-018-0376-6. PubMed PMID: 30679801.



16. Yang Y, Li X, Luan HH, Zhang B, Zhang K, Nam JH, et al. OGT suppresses S6K1-mediated macrophage inflammation and metabolic disturbance. *Proc Natl Acad Sci U S A*. 2020;117(28):16616-25. Epub 20200629. doi: 10.1073/pnas.1916121117. PubMed PMID: 32601203; PubMed Central PMCID: PMC7368321.
17. Umar S, Singh AK, Chourasia M, Rasmussen SM, Ruth JH, Ahmed S. Penta-o-galloyl-beta-d-Glucose (PGG) inhibits inflammation in human rheumatoid arthritis synovial fibroblasts and rat adjuvant-induced arthritis model. *Front Immunol*. 2022;13(null):928436. PubMed PMID: 36032089.
18. Ajibade AA, Wang HY, Wang RF. Cell type-specific function of TAK1 in innate immune signaling. *Trends immunol*. 2013;34(7):307-16. PubMed PMID: 23664135.
19. Kim HB, Lee SW, Mun CH, Yoon JY, Pai J, Shin I, et al. O-linked N-acetylglucosamine glycosylation of p65 aggravated the inflammation in both fibroblast-like synoviocytes stimulated by tumor necrosis factor- $\alpha$  and mice with collagen induced arthritis. *Arthritis res ther*. 2015;17(null):248. PubMed PMID: 26370562.
20. Lee SW, Park KH, Park S, Kim JH, Hong SY, Lee SK, et al. Soluble receptor for advanced glycation end products alleviates nephritis in (NZB/NZW)F1 mice. *Arthritis rheum-us*. 2013;65(7):1902-12. PubMed PMID: 23553192.