Novel molecular regulators of bone turnover

**O-GlcNAcylation on NUP153 regulates the early stages of osteoclastogenesis through MYC nuclear translocation**

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**Background:** Bone homeostasis is maintained by the balance between bone formation and resorption. In inflammatory arthritis, such as rheumatoid arthritis (RA), the pro-inflammatory environment promotes osteoclast differentiation and skews this balance towards bone resorption, leading to destructive bone erosion and bone loss. O-GlcNAcylation is one of the most common post-translational modifications, which attaches a single N-acetylglucosamine (GlcNAc) molecule to the serine or threonine of the target protein. O-GlcNAcylation is controlled by the activities of a single pair of enzymes: OGT, which facilitates the transfer of GlcNAc onto proteins; OGA, which removes GlcNAc from proteins. The activity of O-GlcNAcylation has been reported to be involved in several cellular events, such as transcription, translation, intracellular trafficking, and differentiation. We previously showed that the dynamics of O-GlcNAcylation are essential for osteoclast differentiation. TNF-α, a key pro-inflammatory factor in RA, intensifies the O-GlcNAcylation dynamics. Inhibition of OGT arrests osteoclast precursors at early stages, whereas OGA inhibition blocks osteoclast maturation. However, the molecular mechanism of these regulations remains unclear.

**Objectives:** We aimed to identify the O-GlcNAcylation targets in osteoclast precursors in a pro-inflammatory milieu and to decipher the molecular mechanism of O-GlcNAcylation-mediated regulation of osteoclastogenesis.

**Methods:** We first identify the O-GlcNAc-dependent molecular pathways in osteoclast precursors with pharmacological OGT and OGA inhibition by RNA sequencing. Then, we identified the O-GlcNAcylated proteins by mass spectrometry-based proteomics analysis and confirmed by immunoprecipitation. We found the potential molecular mechanism by combining the data from transcriptomics and proteomics. The proposed mechanism was further validated through siRNA-mediated knockdown and high-content screening analysis.

**Results:** Our transcriptomics data showed that OGT inhibition arrested osteoclast differentiation at early stages through interfering the cytokine signaling and metabolic adaption. The upstream analysis proposed MYC as the most potent regulator for the transcriptomic profile under OGT inhibition. Recent studies proposed MYC as a master regulator for metabolic reprogramming during osteoclast differentiation. However, O-GlcNAcylation of MYC was not detected by mass spectrometry, suggesting indirect effects of O-GlcNAcylation on MYC signaling in osteoclast precursors. We detected upregulated levels of O-GlcNAc on NUP153, MTDH, RBM27, IFI207 upon RANKL+TNFα stimulation. An integrated analysis of transcriptomic and proteomic data by Ingenuity Pathway Analysis indicated that NUP153 might regulate the most DEGs among all the identified targets and indicated potential of NUP153 to regulate nuclear trafficking of MYC. Subcellular fractionation and confocal microscopy showed enhanced MYC nuclear translocation upon RANKL+TNFα stimulation, which could be blocked by NUP153 knockdown or OGT inhibition. Functionally, knockdown of NUP153 arrested cells at similar stages to OGT inhibition and reduced bone resorption ability. Together, these results suggest a model, in which O-GlcNAcylation regulates the shuttling activity of the nuclear pore complex NUP153 to control the access of MYC to the nucleus during osteoclast differentiation.

**Conclusion:** Our results indicated that OGT inhibition arrests osteoclastogenesis at early stages through hampering MYC-dependent metabolic adaption. NUP153 was proposed as the most potent O-GlcNAcylation target by multi-omics data integration. NUP153-mediated MYC nuclear trafficking is required for osteoclast differentiation. These findings reveal the molecular mechanism of O-GlcNAcylation-dependent osteoclastogenesis and provide therapeutic insights on targeting O-GlcNAcylation in pathologic bone resorption.

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**Assessment of the effect of tofacitinib on bone marrow adipocytes and bone-forming osteoblasts under non-inflammatory and inflammatory conditions**

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**Background:** Systemic inflammation is the main factor underlying secondary osteoporosis in patients with rheumatoid arthritis (RA). The JAK inhibitors, such as Tofacitinib (Tofa), can control systemic inflammation and have beneficial effects on bone in various models. This might be due to direct effects on the bone microenviroment and not exclusively based on their anti-inflammatory function. Bone marrow adipocytes (BMAds) are abundant in the bone microenvironment. The effect of JAK inhibitors on BMAds is unknown, but evidence suggests that there is competition between human bone marrow-derived stromal cell (hBMSCs) differentiation routes toward BMAds and osteoblasts (Ob) in osteoporosis.

**Objectives:** To determine in various models whether Tofa influences directly BMAd cell commitment toward adipogenesis and osteoblastogenesis. Then, in a prospective pilot study, to investigate the potential effects of Tofa on bone marrow adiposity in patients with RA.

**Methods:** To study the effect of Tofa on cellular commitment, hBMSCs were cultured for 3 days in appropriate Ob- and BMAd-differentiation media (Ob-3d and BMAd-3d), together with Tofa at 200, 400 (equivalent to a therapeutic dose of 5mg twice a day in RA patients) or 800nM. To mimic inflammatory conditions, TNFα was added to the media at a dose of 1ng/ml. This study was also conducted on mature BMAds and a similar treatment was applied for 6 days to mature BMAds at 14 days of differentiation (BMAd-20d). The impact of Tofa was determined by gene expression profile analysis, western-blots analysis and cell density monitoring. In parallel, in a pilot study of 9 RA patients treated with Tofa 5mg twice a day (TOFAT study: NCT04175886), proton density fat fraction (PDFF) was measured by (Dixon technique) at the lumbar spine at the start of treatment and at 6 months.

**Results:** In non-inflammatory conditions, the gene expression of Runx2 decreased in Ob-3d treated with Tofa 400 and 800nM(p<0.05). Conversely, BMAd-3d treated with Tofa (at 200, 400 and 800nM) exhibited a substantial increase in the gene expression of PPARγ2/CEBPα and Perilipin 1 (a marker associated with lipid droplet formation) compared to controls (p<0.05). The increase in the expression of Perilipin 1 was also confirmed at the protein level. In inflammatory conditions, BMAd-3d and Ob-3d markers decreased considerably (PPARγ2 and RUNX2, respectively, p<0.05), but the addition of Tofa did not change the expression profiles of Ob-3d compared to TNFα controls. On the contrary, the analysis of PPARγ2 gene expression showed that Tofa limited the negative effect of TNFα on BMAd differentiation (p<0.05). The positive effect of Tofa on mature adipocyte (BMAd-20d) under inflammatory conditions was also supported by an increase in the density of differentiated BMAdss (p<0.001). These findings were consolidated by an increase in PDFF at 6 months of treatment with Tofa in RA patients (+6.9%, p<0.01).

**Conclusion:** Overall, in vitro and clinical results suggest a stimulatory effect of Tofa on BMAds commitment and differentiation, which does not support a positive effect of Tofa on bone.

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