指導教授氏名 Instructing Professor Commissioned Professor	指導役割 Role
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学位論文要旨
Dissertation Abstract

岡山大学大学院医歯薬学総合研究科

専攻分野　機能再生･再建科学 Department 口腔形態学分野	身分　大学院生	氏名 Name	オウ　ヨウ 翁　瑶 WENG　Yao

論文題名 Title of Doctoral Dissertation	O-GlcNAcylation drives calcium signaling towards osteoblast differentiation: a bioinformatics-oriented study O-GlcNAcylation 型糖鎖修飾は，細胞内カルシウムシグナリングと連動して骨芽細胞の分化を制御する

論文内容の要旨（2000字程度）Dissertation Abstract (approx. 800 words)

Objective: The O-linked β-*N*-acetylglucosaminylation (O-GlcNAcylation) is a protein post-translational modification. O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) are responsible for addition and removal of O-linked β-N-acetylglucosamine (O-GlcNAc) moieties of proteins, respectively. I investigated the correlation of the change in the O-GlcNAcylation pattern with the expression of bone markers as well as the relationship between O-GlcNAcylation and intracellular calcium levels.

Methods: OSMI-1 (OGT inhibitor) and Thiamet-G (OGA inhibitor) were used for suppressing and promoting O-GlcNAcylation, respectively. To examine the role of O-GlcNAcylation levels on osteoblast differentiation, MC3T3-E1 osteoblastic cells treated with OSMI-1 or Thiamet-G were subjected to ALP activity assay and ALP and Alizarin red staining. Correlations between the levels of O-GlcNAcylation and the expression of osteogenic markers as well as OGT were evaluated by qPCR and western blotting. The O-GlcNAcylated proteins, detected by a pan-specific anti-O-GlcNAc antibody, assumed to correlate with Runx2 expression were determined using Pearson's and Spearman's correlation test and retrieved from several databases retrieved then used for further bioinformatics analysis. Intracellular calcium ($[Ca^{2+}]_i$) was monitored in MC3T3-E1 cells treated with OGT and OGA inhibitors using a confocal laser-scanning microscope (CLS). To investigate the influence of $[Ca^{2+}]_i$ changes and calcium signal on the OSMI-1/Thiamet-G-mediated changes in the expression of Runx2, Bsp, OCN, and OGT via an orthogonal experimental design. In this study, a wide variety of reagents related to intracellular calcium movement were used. These were: EGTA, a membrane-impermeable calcium chelator; BAPTA-AM, a membrane-permeable $[Ca^{2+}]_i$ chelator; A23187, a calcium ionophore; and W7, a calmodulin antagonist. The interaction effect between O-GlcNAcylation and $[Ca^{2+}]_i$ on osteogenic marker expression was determined using stable OGT knockdown MC3T3-E1 cells.

Results: OSMI-1 (10 μM) and Thiamet-G (100 μM) inhibited and enhanced the osteoblast differentiation of MC3T3-E1 cells, respectively. OSMI-1 treatment decreased

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ALP activity, the intensity of ALP staining, and calcium deposition in MC3T3-E1 cells, whereas Thiamet-G treatment increased them. OGT expression was negatively controlled by the feedback regulation on changes in O-GlcNAc levels. OSMI-1 clearly decreased the mean relative density of the total O-GlcNAcylation but increased both mRNA and protein expression of OGT; while Thiamet-G increased the O-GlcNAc level but decreased both mRNA and protein expression of OGT. The O-GlcNAcylated proteins with different molecular weights were changed heterogeneously along with the osteoblast differentiation. Seven bands (218, 152, 117, 96, 75, 59, and 30 kDa) were clearly detected by a pan-specific anti-O-GlcNAc antibody during osteoblast differentiation. The time-course profile of global O-GlcNAcylated proteins showed a distinctive pattern with different molecular weights during osteoblast differentiation. O-GlcNAc distribution contributes to the switching from the early to late stages of osteoblast differentiation. The protein expression of Runx2 was significantly correlated with 152, 117, 75, and 30-kDa O-GlcNAcylated proteins in the DMSO, OSMI-1, and Thiamet-G groups using Pearson's and Spearman's correlation test. Based on a literature mining, 21 Runx2-related O-GlcNAcylated proteins were identified as being related to osteoblast differentiation. Bioinformatic analysis revealed that some calcium-related annotations were significantly enriched in the retrieved protein data. "Calcium" was also a high-frequency word from all related GO terms and UniProt Keywords. In the functional enrichment analysis, "GO:0019722_calcium-mediated signaling" and "GO:0071277_cellular response to calcium ion" were related to $[Ca^{2+}]_i$ with a considerable "List Hits" percentage. Treatment with OSMI-1 and Thiamet-G rapidly decreased and increased the $[Ca^{2+}]_i$ in MC3T3-E1 cells, respectively. From the orthogonal designed experiments, OSMI-1 and Thiamet-G significantly influenced the mRNA expression of osteogenic markers (Runx2, Bsp, and OCN). Changes of $[Ca^{2+}]_i$ also significantly influenced the mRNA expression of osteogenic markers. Significant interaction effect between the changes of $[Ca^{2+}]_i$ and O-GlcNAcylation was detected on Runx2, Bsp, and OCN. The interaction effect between the changes in O-GlcNAcylation and $[Ca^{2+}]_i$ was further examined using the stable OGT knockdown cells. $[Ca^{2+}]_i$ changes induced by EGTA, BAPTA-AM, or A23187 significantly changed the mRNA expression of Runx2, Bsp, OCN in the control group; however, these $[Ca^{2+}]_i$-induced changes were interrupted by OGT knockdown for the expression of Runx2, Bsp, and OCN.

Conclusion: These findings suggested that O-GlcNAcylation interacts with $[Ca^{2+}]_i$ and elicits osteoblast differentiation by regulating the expression of osteogenic markers. The literature-mining results also provided a lot of informative hints for future work. Testing its deduction will improve our understanding of the exact mechanism underlying osteoblast differentiation.