

学位論文の要旨

氏名 高野 恵

学 位 论 文 名 Investigating the Regulatory Mechanism of Glucose Metabolism
by Ubiquitin-like Protein MNSFβ

発 表 雑 誌 名 Molecular Biology Reports
(巻, 初頁～終頁, 年) (51, 1053, 2024)

著 者 名 Megumi Kono, Kyoko Yamasaki, Morihiko Nakamura

論文内容の要旨

INTRODUCTION

Monoclonal nonspecific suppressor factor β (MNSF β), a member of the ubiquitin-like protein family, is involved in various biological functions, such as cell proliferation, immunological responses, and apoptosis. The C-terminal glycine doublet of ubiquitin, which participates in the isopeptide bond formation during conjugation, is a conserved sequence even in MNSF β . MNSF β forms covalent bonds with certain lysines of specific target proteins, including endophilin II and Bcl-G. Unlike ubiquitin, MNSF β may not be involved in the degradation of targeted proteins. While MNSF β regulates glycolysis, its effects are related to GLUT1. Hypoxia-inducible factor-1 α (HIF-1 α) controls GLUT1 expression in multiple cell lines, but whether MNSF β affects HIF-1 α remains unclear.

The heterodimeric transcription factor HIF-1 increases the expression of enzymes implicated in glucose metabolism. HIF-1 comprises two subunits: an oxygen-labile HIF-1 α subunit and a constitutively stable HIF-1 β subunit. HIF-1 α has an oxygen-dependent degradation domain (ODDD) that binds the E3 ubiquitin ligase von Hippel Lindau protein (pVHL). Prolyl hydroxylases (PHDs) hydroxylate the two proline residues within the ODDD, which recruits pVHL, and HIF-1 α is ubiquitinated and degraded by the 26S proteasome. In hypoxia, PHD inhibition impedes pVHL binding. This is followed by HIF-1 α stabilization, dimerization with HIF-1 β , coactivator recruitment, and binding to the promoter region of target genes. The asparaginyl hydroxylase factor-inhibiting HIF-1 α (FIH) suppresses HIF-1 α transcriptional activity. FIH causes hydroxylation of HIF-1 α in the carboxy-terminal transactivation domain

(C-TAD), which prevents HIF-1 α binding to transcriptional coactivators, such as CREB-binding protein (CBP)/p300. The histone acetyltransferase CBP/p300 are relevant to HIF-1 α / β heterodimer formation. Acetylated CBP/p300 interacts with the C-TAD of HIF-1 α to enhance gene expression. Hypoxia, alongside many oncogenic and inflammatory stimuli, promotes HIF-1 α activation and accumulation.

In this study, MNSF β alters both glycolysis and mitochondrial metabolism, markedly affecting glucose metabolism and cytokine production, and HIF-1 α may be involved in this regulatory mechanism.

MATERIALS AND METHODS

We used two types of cells, the macrophage-like cell line Raw264.7 and peritoneal macrophages harvested from female BALB/c mice. Peritoneal macrophages were harvested 4 days after intraperitoneal injection of 1.5 ml of sterile 4% Brewer's thioglycollate medium. The cells were collected by centrifugation at 400 \times g for 5 min, washed and seeded. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

Cells were transfected with siRNAs for 48 h or cDNAs for 18 h and were used in various experiments. HIF-1 α mRNA expression was analyzed by RT-PCR. The DNA binding activity of HIF-1 α was evaluated by EMSA. Western blotting was performed to detect HIF-1 α , acetyl-p300/CBP, FIH and PHD2 protein expression. Intracellular ROS was evaluated with a ROS Assay Kit -Highly sensitive DCFH-DA- (Dojindo). Intracellular ATP was detected using an ATP Assay Kit-Luminescence (Dojindo). The mitochondrial oxygen consumption rate (OCR) was evaluated using an Extracellular OCR Plate Assay Kit (Dojindo). Glucose and lactate in the supernatant were detected using a glucose assay kit-WST (Dojindo) and glycolysis cell-based assay kit (Cayman). The cytokine expressions in cell culture supernatants were detected using a Proteome Profiler Mouse Cytokine Array Kit, Panel A (R&D Systems).

Statistical significance was analyzed by Student's t-test and expressed as *P* values. *p* < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

MNSF β knockdown increased OCR and ROS production in Raw264.7 cells, which showed a markedly decreased ATP level upon oligomycin treatment. In addition, previous studies show that MNSF β siRNA reduces lactate in the culture supernatant and glucose consumption in Raw264.7 cells, suggesting that MNSF β knockdown shifts the primary ATP production pathway from glycolysis to OXPHOS.

HIF-1 α and autoacetylation activate CBP/p300, which is involved in various gene

expression. MNSF β siRNA decreased HIF-1 α expression and transcriptional activity and acetyl-CBP/p300 expression. The decreased HIF-1 α transcriptional activity probably resulted from decreased HIF-1 α and acetyl-CBP/p300 expression. It is unlikely that MNSF β is involved in protein degradation, as previously reported. CBP/p300, which binds to HIF-1 α , is a histone acetylase that acetylates DNA, thereby reducing the binding between histones and DNA and recruiting transcription factors to the binding site. Therefore, the decrease in acetyl-CBP/p300 may inhibit the binding of HIF-1 α to DNA. Further experiments are needed to determine how MNSF β affects HIF-1 α and acetyl-CBP/p300 expression.

Our evaluation of the effects of MNSF β on the regulation of glucose metabolism in murine peritoneal macrophages, which are not cancer cells, revealed no changes in lactate secretion, glucose consumption, or ROS production. Notably, HIF-1 α expression in peritoneal macrophages remained at a low level in the unstimulated state. These results suggest HIF-1 α mediation of these MNSF β -induced metabolic changes at least in Raw264.7 cells. The MNSF β knockdown-triggered changes may have been difficult to observe in unstimulated peritoneal macrophages because these cells primarily depend on OXPHOS for ATP production. In macrophages, LPS promotes the glycolytic pathway. The MNSF β knockdown-triggered reduction in LPS-stimulated HIF-1 α protein expression in peritoneal macrophages suggests that MNSF β can regulate the glycolytic pathway in LPS-stimulated peritoneal macrophages. The present study implicates MNSF β in glucose metabolism and inflammatory responses. As the effect of MNSF β on glycolysis and mitochondrial metabolism is a new finding in the regulation of glucose metabolism, MNSF β can be a potential target for the treatment of diseases, such as cancer, that involve metabolic changes due to HIF-1 α activation. MNSF β also alters cytokine production pattern, suggesting its involvement in inflammatory processes through HIF-1 α signaling. This may help in the elucidation of new mechanisms in inflammatory diseases. Detailed molecular mechanisms of how MNSF β specifically regulates HIF-1 α and how this regulation affects metabolic pathways and cytokine productions might require further elucidation. Overall, MNSF β may be an important ubiquitin-like protein that regulates multiple functions of macrophages.

CONCLUSION

MNSF β affects both glycolysis and mitochondrial metabolism, suggesting HIF-1 α involvement in the MNSF β -regulated glucose metabolism in Raw264.7 cells.

The study primarily utilizes the Raw264.7 cell line, which may not fully represent the behavior of human macrophages. Thus, caution should be exercised when extrapolating these results to humans.