Studies on *O*-GlcNAc Modification of NF-κB p65 Subunit

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NF-κB is a transcription factor and is important for expressing variable proteins related to inflammatory and immune responses. It is composed of a heterodimer of p65 and p50 subunits and is located in the cytoplasm by I-κB. When the nuclear localization signal is transduced, I-κB is degraded by ubiquitin-dependent manner and NF-κB is transported to nucleus. Functions of NF-κB are regulated by various posttranslational modifications like phosphorylation and acetylation. Also, NF-κB p65 is modified by *O*-GlcNAc (*O*-linked *N*-acetylglucosamine), but the exact function of *O*-GlcNAc on NF-κB p65 has not been studied well. *O*-GlcNAc is dynamically modified to serine or threonine residues of nuclear and cytoplasmic proteins by two enzymes, *O*-GlcNAc transferase (OGT) and O-GlcNAcase. In this study, roles and sites of *O*-GlcNAc modification on NF-κB p65 using human lung carcinoma cell, A549 and STZ-induced diabetic mice have been investigated. We show that the treatment of STZ (streptozotocin), an *O*-GlcNAcase inhibitor, increases *O*-GlcNAc modification and the activity of NF-κB p65 without the degradation of I-κB and decreases the binding of NF-κB p65 with I-κB. Also, we identified Thr 352 as a target for *O*-GlcNAc modification and transcriptional activation in response to STZ by

mutational analysis and ESI-MS/MS analysis. Our finding suggests that *O*-GlcNAc modification on Thr 352 of NF-κB p65 subunit increases the transcriptional activity and the increment of *O*-GlcNAc on NF-κB p65 subunit may be a reason for diabetic-associated NF-κB p65 subunit activation in diabetic mice. Therefore, our data may contribute to understanding of diabetes and its complications-associated NF-κB activation.