

Detection of O-GlcNAcylated Proteins by Glycoproteomics and in situ Proximity Ligation Assay (PLA)

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O-linked N-acetyl-D-glucosamine, termed O-GlcNAc, is a post-translational modification involved in modulation of signaling and transcription in response to cellular nutrients or stress by preventing O-phosphorylation. O-GlcNAc serves as a glucose sensor via hexosamine biosynthetic pathway. Elevated O-GlcNAc modification (O-GlcNAcylation) of proteins by increased flux through the hexosamine biosynthetic pathway has been implicated in the development of the insulin resistance and diabetic complications.

We previously demonstrated that the O-GlcNAcylation level increased in the kidney, cornea, sciatic nerve, liver and pancreas in the Goto-Kakizaki (GK) rat, which is an animal model of non-insulin dependent type (type 2) diabetes.

In this study to identify marker proteins of which O-GlcNAcylation level change in diabetic kidney, we carried out the glycoproteomic analysis. Total proteins of kidney were separated by two-dimensional gel electrophoresis. O-GlcNAcylated proteins were detected by the immunoblot using anti-O-GlcNAc antibody. Selected proteins that changed markedly in the O-GlcNAc level were identified by Mass Spectrometry (MS) analysis. The localization and the quantity of these O-GlcNAcylated-proteins were analyzed by in situ Proximity ligation assay (PLA) which was developed to examine protein-to-protein interaction and post-translational modification of proteins.