Modification (O-GlcNAcylation) of proteins by O-linked N-acetylglucosamine occurs in the nucleus and in the cytoplasm. O-GlcNAcylated proteins are particularly relevant to chronic human diseases including diabetes, cancer, neurodegenerative disorders, and cardiovascular disease. Increased flux through the hexosamine biosynthesis pathway promotes the O-GlcNAcylation, and has been implicated in the development of insulin resistance and diabetes complications. In our previous immunohistochemical study, we demonstrated that the O-GlcNAcylation level increased in various tissues including kidney from diabetic GK rats, which is an animal model of type 2 diabetes.

To identify marker proteins that change in their extent of O-GlcNAcylation in the diabetic kidney from GK rats, we separated total kidney proteins 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 analysis. The localization and the quantity of these O-GlcNAcylated-proteins were analyzed by in situ Proximity ligation assay (PLA). O-GlcNAcylated proteins that changed significantly in the degree of O-GlcNAcylation were identified as cytoskeletal proteins (α-actin, α-tubulin, α-actinin 4, myosin) and mitochondrial proteins (ATP synthase, pyruvate carboxylase). Results of immunoprecipitation and immunoblot studies, as well as in situ PLA demonstrated that the extent of O-GlcNAcylation of the above proteins increased in the diabetic kidney. Immunoelectron microscopy revealed that α-actinin 4 increased in the foot process of podocytes and the proximal tubules. To further examine the changes of the O-GlcNAcylation of glomerular proteins accompanied with diabetic nephropathy, we isolated glomerulus from kidney and performed proteomic analysis.

It was revealed that some glomerulus-specific proteins including synaptopodin were O-GlcNAcylated. To elucidate the role of O-GlcNAcylation of glomerular proteins in the diabetic nephropathy the morphological changes of the glomerular epithelial cells were examined under the various conditions in vitro.

References

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