Abstract of Doctoral Dissertation

Degree requested Doctor of Life Science Applicant's name YOGESH K V

Title of Doctoral Dissertation

Synthesis of Complex Glycopeptides Toward Novel Cancer Marker Discovery
(複雑な糖ペプチドの合成による新しい癌マーカーの探索)

AGP, alpha-1-acid glycoprotein is one of the major glycoproteins in plasma synthesized in the liver and secreted into the blood where its concentration is 1g/L in healthy normal individuals. This concentration level of AGP in plasma alters significantly since glycosylation pattern extensively varies during inflammation, metastasis and other diseases, mostly with respect to the degree of branching, sialylation and fucosylation results in multi-antennary hyper-branched *N*-glycans reveals its importance as potential biomarker for diagnosis. There are five glycosylation sites in a peptide backbone of AGP gives a platform to share over 40% of its molecular mass by carbohydrate content in which the majority (85-90%) being either tri- or tetra-antennary. Hence qualitative and quantitative study of specific glycopeptide rather than protein candidate is more precise for biomarker discovery mainly because of heterogenicity of glycan. However, Prior studies on largescale glycomics of over 3500 human serum samples revealed that serum glycoproteins of cancer patients often bear dominantly specific glycoforms, namely branched tri- and tetra-antennary *N*-glycans, beyond cancer species when compared with normal control groups. Hence an absolute quantification of tryptic glycopeptide fragments of AGP carrying such *N*-glycans can be realize as a disease biomarker required for diagnosis.

Recently, MRM (Multiple Reaction Monitoring) analysis is explored as a potential method for quantitative proteomics and glycoproteomic study. However, specific synthetic glycopeptide as an internal standard are essential to establish a new method for characterization and quantification of glycopeptides using MRM analysis and therefore it is possible to realize absolute quantification by its combination with target synthetic glycopeptide as an internal standard.

In this study, we have synthesized tryptic glycopeptide fragments of AGP chemo-enzymatically, carrying complex type tri-antennary *N*-glycans. One of the synthetic glycopeptide fragments was used as an internal standard, characterized and prepared SRM assay using SRM based LC-MS MS analysis. Finally, for the first-time, an absolute quantification in a human serum is established. We demonstrate that synthetic glycopeptides facilitate selected reaction monitoring (SRM)-based targeted glycoproteomics toward the discovery of peptides having such cancer-relevant *N*-glycoforms directly from tryptic digests of whole human serum glycoproteins. SRM assay using synthetic glycopeptide **1**, ^{40}Ser-Val-Gln-Glu-Ile-Gln-Ala-Thr-Phe-Phe-Tyr-Phe-Thr-Pro-Asn-Lys-Thr-Glu-Asp-Thr-Lle-Phe-Leu-Arg^{63} having an asialo tri-antennary *N*-glycan at Asn54 residue, as a calibration standard allowed for rapid and absolute quantitation of the tryptic fragment derived from serum α1- acid glycoprotein carrying a focused *N*-glycoform of cancer patients and healthy controls at a range between 40~1600 fmole/μL without any enrichment process of the target glycoprotein.

We demonstrated for the first-time high potentials of quantitative glycoproteomics targeting serum tryptic glycopeptides as new class biomarkers that can be monitored directly without any enrichment process of the parent glycoproteins. Remarkably, use of structure-defined synthetic glycopeptides as a calibration standard in SRM assay allowed for the absolute quantitation of the focused glycopeptides

elaborated during tryptic digestion of the whole serum glycoproteins. a disaccharide azidoalcohol **4** derived from abundant locust bean gum galactomannan would enable the synthesis of a variety of branched *N*-glycoforms both triantennary and tetra-antennary *N*-glycans when combined with a series of enzyme-assisted modifications. Particularly, *trans*-glycosylation activities of engineered *endo*-glycosidases to the GlcNAc-peptides as acceptors using preformed oligosaccharide oxazolines as donor substrates might be a key to expand the feasibility of this approach. Our extensive efforts to construct a library of such structure-defined glycopeptides will provide nice tools not only to discover novel disease biomarkers but to understand the significance and molecular mechanism in the dynamic and site-specific protein glycosylations.