Targeting the human glycoproteome

New enrichment protocols and mass spectrometric analyses reveal unique and novel glycosylation sites

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i lokal Hjärtat, Sahlgrenska Universitetssjukhuset, Göteborg, fredagen den 2 mars 2012 kl. 13:00.

av

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I. Nilsson, J., Ruetschi, U., Halim, A., Hesse, C., Carlsohn, E., Brinkmalm, G., and Larson, G. (2009) Enrichment of glycopeptides for glycan structure and attachment site identification, *Nat Methods 6*, 809-811.

II. Halim, A*., Brinkmalm, G*., Ruetschi, U., Westman-Brinkmalm, A., Portelius, E., Zetterberg, H., Blennow, K., Larson, G., and Nilsson, J. (2011) Site-specific characterization of threonine, serine, and tyrosine glycosylations of amyloid precursor protein/amyloid beta-peptides in human cerebrospinal fluid, *Proc Natl Acad Sci U S A 108*, 11848-11853.

III. Halim, A., Nilsson, J., Ruetschi, U., Hesse, C., and Larson, G. (2011) Human urinary glycoproteomics; attachment site specific analysis of N-and O-linked glycosylations by CID and ECD, *Mol Cell Proteomics*. doi:10.1074/mcp.M111.013649

IV. Halim, A., Ruetschi, U., Larson, G., and Nilsson, J. Glycosylation motifs in the GalNAc O-glycosylation of cerebrospinal fluid proteins, *Manuscript*.

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Glycosylation is one of the most common and structurally diverse post-translational modifications of proteins. Given that protein glycosylation is involved in various cellular processes, the characterization of site-specific N- and O-linked glycosylations is becoming increasingly important. However, current mass spectrometry-based technologies, i.e. proteomics and glycomics, are unable to resolve the site-specific glycosylation pattern of glycoproteins.

The primary aim of this thesis was to develop glycoproteomic techniques for mass spectrometric analysis of glycoproteins. A sialic acid capture-and-release method, based on hydrazide chemistry, for selective enrichment of N- and O-linked glycopeptides from complex biological samples was developed. Enriched glycopeptides were separated by reversed phase liquid chromatography and analyzed by Fourier transform ion cyclotron mass spectrometry (FTICR MS) utilizing collision induced dissociation (CID) and electron capture dissociation (ECD) fragmentation techniques.

Initially, both N- and O-glycopeptides from sialylated glycoproteins of human cerebrospinal fluid (CSF) were enriched and characterized. Subsequently, a targeted O-glycoproteomics approach was developed, allowing for sequence analysis of preferred O-glycosylation sites of glycoproteins. The applicability of the sialic acid capture-and-release strategy was further demonstrated for human urine, a technically more challenging biological fluid. The LC-MS/MS analyses revealed unique N- and O-glycosylations, many of which were previously unknown, both for CSF and urinary glycoproteins. In e.g. CSF, a series of O-glycopeptides with Thr linked O-glycans in the vicinity of the β-secretase cleavage site of the amyloid precursor protein (APP) were identified. Additionally, amyloid beta (Aβ) peptides, originating from APP, were immunoprecipitated from CSF samples for a targeted glycoproteomic analysis. These analyses revealed that a series of Aβ peptides were uniquely modified with sialylated O-glycans at a specific Tyr residue. A relative increase of such Tyr O-glycosylated Aβ peptides was observed in CSF samples from Alzheimer's disease (AD) patients compared to non-AD patients, suggesting that these Aβ glycopeptides may potentially be used as biomarkers of AD.

Keywords: Glycobiology, Glycoproteomics, Mass spectrometry

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