Göteborg, 2020

Mass spectrometric analysis of proteoglycans –

Novel tools for studying prohormones in insulin-producing cells

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Wallenberg Conference Center, Europe, Medicinaregatan 20, fredagen den 18 December 2020, klockan 13:00

av **Mahnaz Nikpour**

Fakultetsopponent:

Professor Jeremy E Turnbull

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Avhandlingen baseras på följande delarbeten

I. A glycoproteomic approach to identify novel proteoglycans. Noborn F, **Nikpour M**, Persson A, Sihlbom C, Nilsson J, and Larson G. Accepted for publication in Methods in Molecular Biology, Special volume on Glycosaminoglycans: Chemistry & Biology, 2020.

II. Proteoglycan profiling of human, rat and mouse insulin-secreting cells. **Nikpour M**, Nilsson J, Persson A, Noborn F, Vorontsov E, and Larson G. Manuscript

III. Structural domain mapping of proteoglycan-derived glycosaminoglycans from rat insulinoma cells. Persson A, **Nikpour M**, Vorontsov E, Nilsson J, and Larson G. Manuscript

IV. Establishing B4galt7 knock-down clones of the rat INS-1 832/13 insulinoma cell line for studying biological effects of downregulation of GAG biosynthesis. **Nikpour M**, Madsen TD, Satir DM, Gomez Toledo A, Nilsson A, Persson A, Noborn F, Schjoldager KT, and Larson G. Manuscript

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Sweden, 2020

Abstract

Proteoglycans (PGs) are proteins that carry one or more negatively charged glycosaminoglycan (GAG) chains. Proteoglycans have been identified in essentially all multicellular organisms, and are implicated in a wide range of biological and pathological processes. To further decipher the influence of GAG glycosylation on biological and pathological events in humans, detailed structural characterization of PGs/GAGs is needed. However, studying the PGs/GAGs is very challenging and has been hampered by the limited number of analytical tools available. In this thesis work, we have developed and applied glycoproteomics and glycomics methods to characterize PGs and their GAG structures and investigated their possible influence on the cellular characteristics of insulin-producing cells. The workflow included isolation, enrichment, and enzymatic depolymerization of PG/GAG structures followed by structural analysis using liquid chromatography tandem mass spectrometry. We identified several different chondroitin/dermatan sulfate (CS/DS) and heparan sulfate (HS) PGs, some of which are novel PGs. Several of the identified PGs, such as chromogranin-A (CgA) belong to the granin family of secretory granules which are typically co-stored, co-processed and co-released with the insulin hormone. Considering that several of the PGs identified belong to the granin family, and that the granins are important for the biogenesis of dense-core secretory granules, we started to explore the cellular effects of blocking the GAG glycosylation in rat INS-1 832/13 cells by using the CRISPR/Cas9 technique. Our data showed that B4galt7-KO clones had a major, but not always complete, block of the GAG glycosylation of the CgA protein. Furthermore, the cellular localization of CgA as well as its proteolytic processing was different in KO cells compared to WT cells. Further studies of the effects of downregulation of the GAG biosynthesis in these clones are ongoing.

In summary, our structural findings may assist in elucidating the influence of GAG modifications on the storage, processing, and secretion of peptide hormones of endocrine cells, with particular relevance to insulin-secreting beta cells. Given the paramount importance of insulin on glucose homeostasis, these novel aspects of GAG glycosylation presented herein may provide new insights into diabetes research and future treatment strategies.

Keywords: proteoglycan, glycosaminoglycan, mass spectrometry, secretory granule, prohormones, CRISPR/Cas9

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