Towards single cell glycomics by CESI-MS

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As progressively more single-cell proteomics studies are performed, the analysis of post-translational modifications (PTMs) in a single-cell manner remains limited and requires high-resolution and highly sensitive platforms. In addition, protocols to profile 1 of the most abundant PTMs (glycosylation) with minute amounts of cells or low abundant proteins in a high-throughput manner remains scarce. This is unfortunate as there is a need for more in-depth studies to investigate the associations between glycosylation and various diseases, including cancer. In this study, to enhance the release efficiency and sensitivity, we further developed the release of *N*-glycans which would require only minute amounts with a 96-well polyvinylidene difluoride (PVDF) membrane-based approach. The optimized sample preparation was combined with a highly sensitive, sheathless capillary electrophoresis-mass spectrometry (CESI-MS) platform and its application was evaluated by characterizing the *N*-glycome from total plasma proteome and a pancreatic cancer cell line (PaTu-S) using various amounts of lysed cells (5 – 5000 cells). The ability to separate isomeric glycan species on the CESI-MS platform was investigated using *N*-glycans released from total plasma. An overall highly diverse *N*-glycome was observed, including the separation and identification of *N*-glycan isomers containing differently linked sialic acids. It is important to note that this workflow does not require a specific sialic acid derivatization step nor further purification after eluting the *N*-glycans from the PVDF membrane. A total of 58 *N*-glycans could be relatively quantified from 5000 PaTu-S cells using the optimized PVDF membrane-aided protocol. The four most abundant *N*-glycans could still be detected using ~50 cells after cell lysis (~0.2 cells were hydrodynamically injected using 90 nL). Future endeavors will focus on further optimizing the CESI-MS conditions to gain more insight into the *N*-glycome using minute amounts of samples and maintaining isomeric separation. Lastly, we envisioned the use of laser-capture microdissection to define the minimal amount of material that is required to obtain a representative *N*-glycome profile of a specific area of interest in tissues. This would allow us to investigate whether specific glycomic features are solely present in the invasive front of the tumor, cancerous region or neighboring healthy tissue. Eventually this research will contribute to gaining more in-depth knowledge about the role of glycosylation in tumor progression.

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