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# Native N-Glycome of Single Mammalian Cells and ng-Level Blood Isolates Deciphered Using Label-Free Capillary Electrophoresis-Mass Spectrometry

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**Summary**

Aberrant glycosylation represents an attractive source of potential biomarkers for the diagnostic, prognostic, and treatment monitoring of various human diseases, including autoimmune, genetic, oncological, and neurodegenerative pathologies. Contrary to the rapidly growing field of single-cell proteomics, single-cell glycomics (SCG) demonstrated lagging progress. Deciphering cell-to-cell glycome variations and developing glycomic techniques for sub-microliter volumes of minimally invasive liquid biopsies (e.g., blood, circulating rare cells, extracellular microvesicle isolates) can be crucial in designing advanced diagnostic and therapeutic approaches. In this work, we developed an integrated platform coupling online in-capillary sample processing with high-sensitivity label-free capillary electrophoresis-mass spectrometry (CE-MS) for N-glycan profiling of single mammalian cells and ng-level amounts of blood-derived protein, extracellular vesicle, and total plasma isolates. Native N-glycans were enzymatically released from the cell surface prior to their inline CE-MS analysis in the described proof-of-concept experiments. Direct and unbiased characterization and quantification of single-cell surface N-glycomes were demonstrated for HeLa and U87 cells, with the detection of up to 100 N-glycans per single cell. Specific N-glycosylation patterns were demonstrated for HeLa and U87 single cells, based on a thorough differential analysis of qualitative and quantitative SCG profiles. Significant differences in fractional distributions and abundances of the N-glycans detected in HeLa and U87 single cells were observed, reflecting unique molecular features for each cell type. Interestingly, N-glycome alterations were observed at the single cell level when HeLa and U87 cells were stimulated with lipopolysaccharide (LPS), which manifested the change in the phenotypic cell state reflected on the cell surface. Overall, we demonstrated in the presented here proof-of-concept study that our developed SCG workflow could effectively and accurately characterize the single-cell N-glycome of different mammalian cell lines and detect N-glycome alterations at the single-cell level.

The developed workflow was also applied to the profiling of ng-level amounts of blood-derived isolates of protein (e.g., total IgG, total IgM), extracellular vesicles, and total plasma, resulting in over 170, 220, and 370 quantitated N-glycans, respectively. These numbers of identified N-glycans corresponding to sub-0.5 ng-levels of serum proteins and nL/pL-levels of plasma isolates, largely exceed the profiling results reported in other N-glycan profiling studies of similar complexity blood-derived isolates.