Microchip CE-MS Analysis of Sialylated N-glycan linkage Isomers

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Overview

- Linkage isomers of sialylated N-glycans are of great significance for diagnosing and monitoring diseases.
- Qualitative and quantitative analysis of **sialylated N-glycan linkage isomers** remains challenging due to their low abundance and limited isomeric separation techniques.
- A novel strategy based on microchip capillary electrophoresis–mass spectrometry (Microchip CE-MS) was developed.
- Fast and specific analysis of **α-2,3- and α-2,6-linked sialylated N-glycan linkage isomers** can be achieved.

Methods

- A novel strategy based on CE-MS workflow was utilized for this work combining fast CE separation with high-resolution mass spec identification.
- Front-end CE and NanoESI was performed by ZipChip™ interface from 908Devices Inc.
- Only positively charged components in the sample can be analyzed based unique microchip CE principle.
- CE separation was implemented in a liquid BGE solution composed of the mixture of methanol and water (ZipChip™ Metabolite assay kit) through a 22 cm length CE separation channel (ZipChip™ HR chip).
- MS analyses were performed by interfacing the device with a QExactive HF MS from Thermo Fisher.

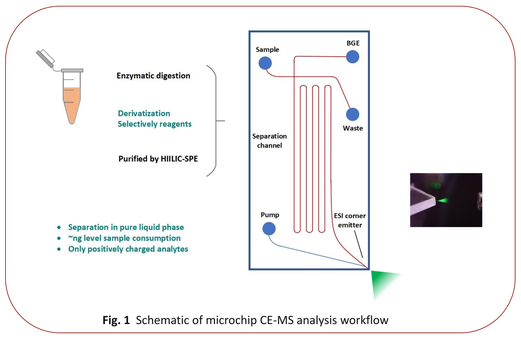

Fig. 1 Schematic of microchip CE-MS analysis workflow

- N-glycans from glycopeptides or glycoproteins were prepared with standard enzymatic digestion procedure (PNGase F in ABC buffer).
- N-glycans from serum samples were prepared through quantification (Protein Assay Kit), reduction (DTT), alkylation (IAA), followed by enzymatic digestion with PNGase F.
- Released Glycan were further derivatized on the sialic acid site, the different reagents were used depend on the application. The solution was then purified by cotton-wool-based HILIC-SPE.

Results

◆ By **two steps** linkage-specific derivatization, α-2,3- and α-2,6-Sialic acid linkage isomers were differentiated.

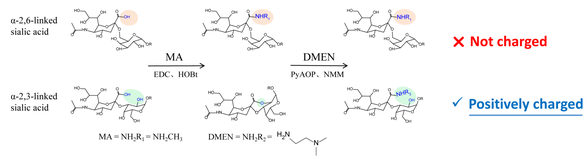

Fig. 2 Schematic of two steps Linkage-Specific Derivatization workflow of Sialylated N-Glycans

- α-2,3-sialylated N-glycans **positively charged** while α-2,6-sialylated N-glycans are **not** charged.

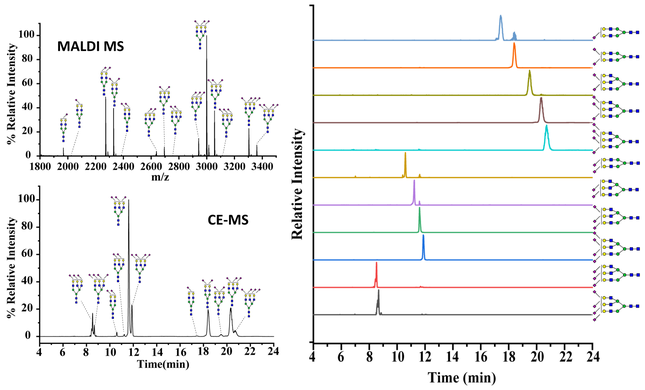

Fig. 3 MS analysis of sialylated N-glycans from fetuin and microchip CE separation spectrum

- All α-2,3-sialylated N-glycans were migrated in CE separation according to charge, size and shape of glycans, and the CE-MS data is in accordance with the MALDI MS data.
- A total of 33 α-2,3-sialylated glycoforms can be detected via microchip CE-MS.
- Method was further validated cross 10 serum samples from healthy individuals.

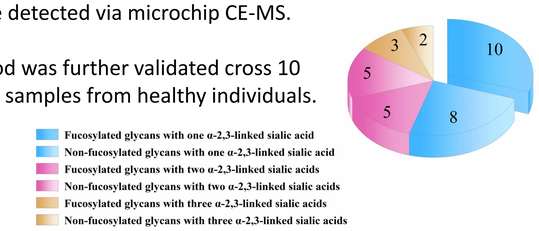

Fig. 4 Composition analysis of 33 detected α-2,3-sialylated glycoforms

◆ The serum samples from 11 healthy individuals (**HC**) and 11 **Ovarian Cancer** (**OC**) patients were profiled and compared.

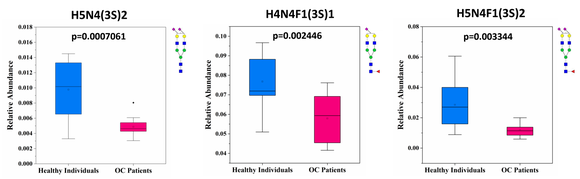

Fig. 5 Significant difference analysis between healthy and OC group

- OPLS-DA score plot showed clear different distribution of between **HC** and **OC** samples, from screened seven α-2,3-sialylated N-glycans, **six** of them was found as new candidate biomarkers by this work (more details in Ref. 1).

Results

◆ By **one step** linkage-specific derivatization, α-2,3- and α-2,6- Sialic acid linkage isomers were distinguishably identified by CE-MS.

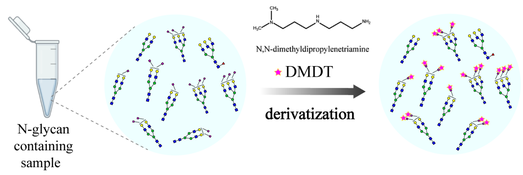

Fig. 6 Schematic of one step Linkage-Specific Derivatization workflow of Sialylated N-Glycans

- Several amino reagents were optimized and **DMDT** was selected. Both α-2,3- and α-2,6- sialic acid linkage isomers can be positively charged to 3-A2-DMDT and 6-A2-DMDT after derivatized by DMDT and separated by CE-MS in a much shorter time.

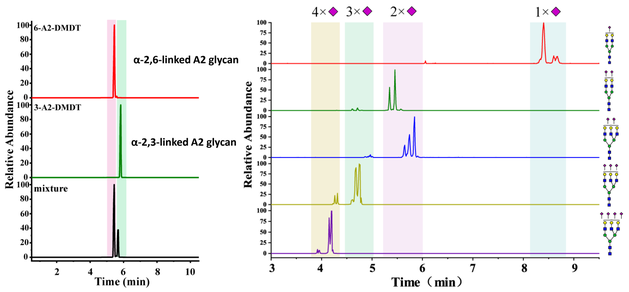

Fig. 7 Microchip CE separation of sialylated N-glycans linkage isomers derivatized with amino reagent DMDT

- In less than 10 mins, sialylated N-glycans with different number of sialic acids were well separated in CE separation in an orderly manner according to the number of sialic acids.
- More sialic acids carried more charge and migrates faster in CE separation with shorter migration time.
- MD simulation utilized to explore the conformation difference of linkage isomers and depicted 3-A2-DMDT with a much looser structure than 6-A2-DMDT, lead to a larger steric hindrance result in longer migration time. (more details in Ref. 2).

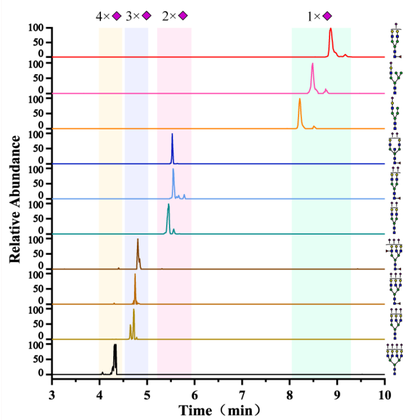

Fig. 8 The overlaid EIEs of sialylated N-glycans from serum after one-step sialic acid derivatization by CE-MS.

- The serum samples from **cervical cancer** patients were analyzed
- Separation time within 10 mins
- Higher number of sialylated N-glycans detected

Results

- A total of 52 sialylated N-glycans were detected within 10 minutes from serum sample

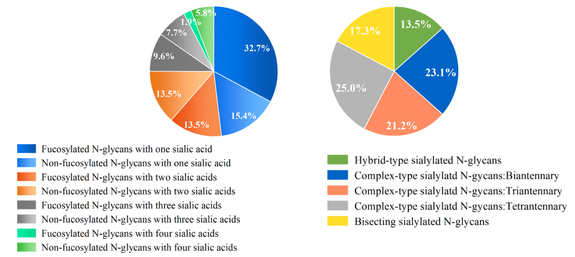

Fig. 9 Distribution of sialylated N-glycans categorized by the degree of sialylation and fucosylation

◆ The serum samples from 35 healthy individuals (**HC**) and 35 **Cervical Cancer** (**CC**) patients were profiled and compared.

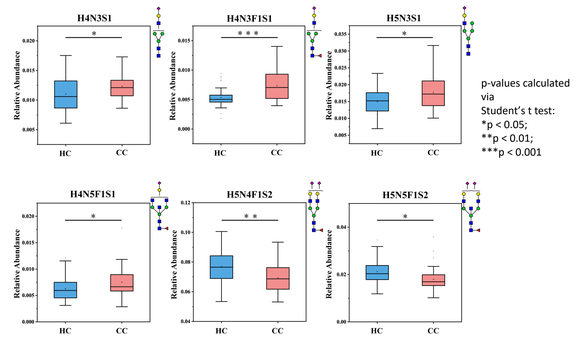

Fig. 10 Box plots for the relative abundance of several sialylated N-glycans between serum from 35 HC and 35 CC

- The significant difference analysis was based on p-value calculated via Student's t test between the two groups.
- **Six** sialylated N-glycans could be potential serum biomarkers of **Cervical Cancer** , and sample-sample correlation and quantitative stability by OPLS-DA score plot were also investigated and confirmed.

Summary

- A novel strategy for high-sensitivity profiling of sialylated N-glycans was developed by microchip CE-MS and linkage-specific derivatization.
- And successfully qualitative and quantitative analysis of sialylated N-glycans from disease including ovarian and cervical cancer patients, demonstrated a high-throughput and low-cost workflow for biomarker screening and discovery for different diseases.

References

1. Anal. Chem. 2021, 93, 13, 5537–5546
2. Anal. Chem. 2022, 94, 11, 4666–4676