N-glycoproteome from a cancer cell line and its non-tumorigenic cell line combining Fbs1-GYR N-glycopeptide enrichment and trapped-ion-mobility-quadrupole-time-of-flight

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Introduction

N-glycosylation is implicated in the development and progression of many cancer types and efficient methodologies are required for this type of study. Our group described in 2017[a] that Fbs1-GYR enrichment outperformed the established lectin enrichment method and offered a deeper analysis and greater coverage of the human serum N-glycoproteome. Fbs1 recognizes the core motif Man3GlcNAc2 preferring high mannose N-glycans. On the other hand, analysis of N-glycopeptides is still an analytical challenge for LC-MS/MS with respect to electrospray ionization, chromatographic separation and structural elucidation by collision induced dissociation experiments requiring improvements in the capabilities of mass spectrometry. In this work, we have combined Fbs1-GYR N-glycopeptide enrichment technology with parallel accumulation serial fragmentation (PASEF) on a trapped ion mobility spectrometry - quadrupole time-of-flight mass spectrometer to study the comprehensive glycopeptide profiles in HCT116 cancer cells and their non-tumorigenic DNMT1/3b double knockout cells (DKO1) ([a] Chenet.al.,2017,NatureCommunications - 10.1038/ncomms15487).

Methods

Cell lysate from serum-starved HCT116 and DKO1 cells were digested with trypsin and N-glycopeptides were enriched by Fbs1-GYR. The enriched samples were submitted to MSMS analysis using timsTOF Pro 2 coupled to nanoELUTE (Bruker Daltonics) using a C18 IonOpticks column (25 cm x 75 µm, 1.6 µm). Data were searched on PMI-Byos v3.11-1 (Protein Metrics Inc.) against Uniprot Human Database (56,000 protein sequences) and a human glycan database containing 132 N-glycan structures (FDR = 1%). The number of peptide-spectrum matches (PSMs) were used for glycosylation quantification. All samples were analyzed on a 45-minute reverse-phase gradient loading 400 ng of sample on column. Instrument parameters were systematically tuned towards optimal energy stepping collision induced dissociation (CID) and combining lower- and higher-energy for glycopeptide fragmentation allowing the online acquisition of glycan and peptide moieties within a single tandem MS experiment.

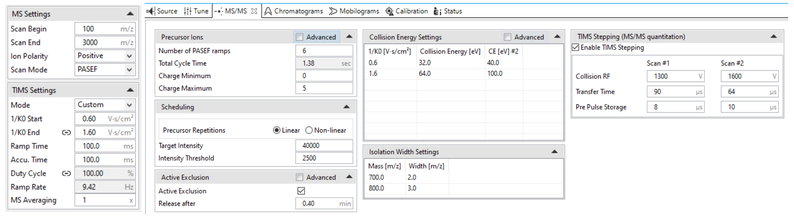


Figure 1. MS parameters setup for glycopeptides method.

Results

Global proteome (not enriched) identified around 29,000 and 31,000 PSMs for HCT116 and DKO1 cells, respectively. However, only 0.28% (HCT116) and 0.42% (DKO1) of the PSMs came from N-glycopeptides. By applying Fbs1-GYR enrichment technology and using the 45-minute gradient, the number of PSMs increased to 60,000 PSMs for both cell lines with PSM values for N-glycopeptides corresponding to 42.6% (HCT116) and 48.3% (DKO1). Thus, Fbs1-GYR enabled more than 100-fold enrichment of N-glycopeptides (fig. 2). In addition to these numbers, over 1,000 glycoproteins and 2,300 N-glycosites were identified. Interestingly, glycopeptides show a greater spread of mobility values than do native peptides (fig. 3). Hundred's glycopeptides found in the sample have the same m/z and retention time but distinct ion mobility values what suggest different glycoforms (data not shown).

Label free quantitative analysis was performed and compared by T-test, showing several glycoproteins as potential cancer biomarkers for this specific cell line (orange dots in fig. 4A). In parallel, western blots using HRP labeled M6P receptor was performed as an orthogonal methodology in the presence and absence of PNGase F as shown in the figure 3B where AMPN (Aminopeptidase N) and EPCAM (epithelial cell adhesion molecule) glycoproteins are shown (fig. 4B).

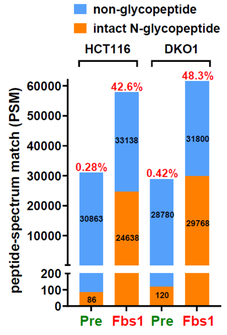


Figure 2. PSMs comparison before and after FBS1 enrichment.

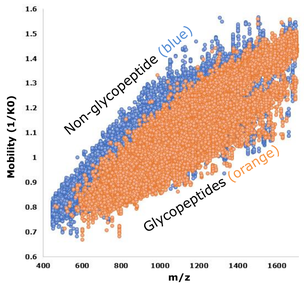
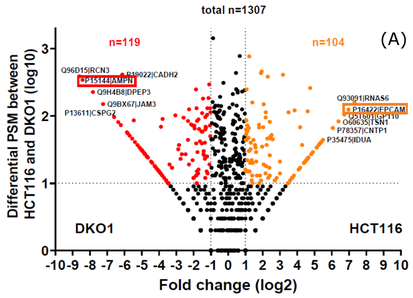


Figure 3. m/z versus mobility plot comparing native peptides and glycopeptides.



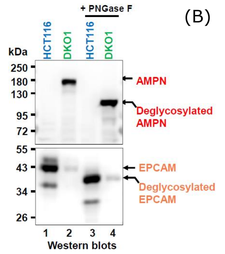


Figure 4. (A) Volcano Plot highlighting up-regulated proteins in HCT116 (scoring criteria ≥ 2 fold total PSM change and ≥ 10 PSM difference. (B) Western blots using HRP labeled M6P receptor .

Thus, western blots confirm mannose-6-phosphate (M6P) modification is significantly higher in HCT116 cells than that in DKO1 cells. Among several differentiated glycans compositions for these two different cell lines, M6P modification was up-regulated in the cancer cell line (fig. 5). Manose-6-phosphate is well known to be related to a sorting signal guiding lysosomal proteins to the lysosome.

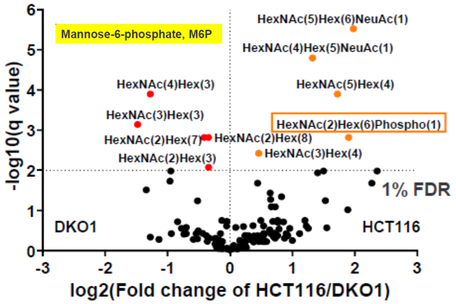


Figure 5. Volcano Plot comparing glycans composition (Scoring criteria ≤ 1% FDR).

M6P is a sequence tag that plays a pivotal role in transporting proteins from the Golgi complex and cell surface to the lysosome. Results for several individual proteins showed M6P modification in HCT116 cells is significantly higher (3.7-fold) than that in DKO1 cells (fig 6A). In total of 60 glycoproteins containing M6P, 52 glycoproteins are upregulated in HCT116 cells (fig 6B). Experiments are being performed to understand this overexpressed manose-6-phosphate in this cell line.

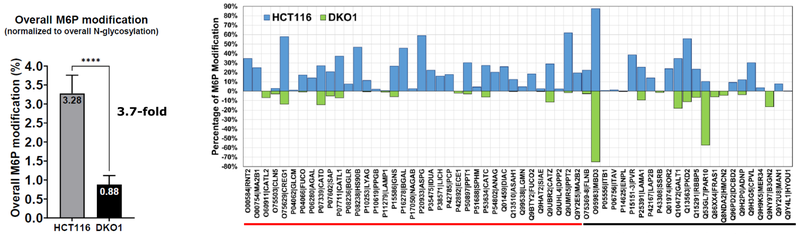


Figure 6. (A) M6P overall modification (%) for HCT116 and DKO1. (B) Percentage of M6P modification for individual proteins identified in HCT116 and DKO1 cells.

Conclusions

- Use of Fbs1-GYR enabled more than 100-fold enrichment of N-glycopeptides. This enables the simultaneous determination of N-glycan composition and N-glycosites with a deeper coverage and allows large-scale N-glycoproteomic studies.
- N-glycoproteomic data and western blots both showed manose-6-phosphate modification is significantly higher in HCT116 cancer cells that in DKO1 non-cancer cells.
- timsTOF Pro 2 combined to Fbs1-GYR N-glycopeptide enrichment delivered very high depth and quality proteomic data enabling a comparison of N-glycoproteomes of cell lines and it will help to understand epigenetic regulation of protein N-glycosylation.

timsTOF Pro 2