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**Glycoproteomic Characterization of the Cancer-Specific Marker Carcino Embryonic Antigen (CEA)**

CEA is a tumor marker for the clinical management of colorectal cancer which has the specific utility of monitoring post-operative disease recurrence. After surgery to remove cancerous tissue, the level of CEA in blood can be periodically monitored using an immunoassay. If the levels begin to rise above 6.0 ng/mL there is a high correlation with recurrence of the cancer (Fakih *et al*, Oncology 2006).

CEA, like many tumor markers is a glycoprotein and there is a significant body of work showing protein glycosylation is greatly affected by diseases such as cancer. Here we demonstrate the qualitative and quantitative analysis of CEA glycoforms using an LC-MS/MS based workflow.

**Methods**

1µg of purified human CEA protein (AbCam P/N 81699) was separated on a 10% Bis-Tris NuPage gel (Invitrogen) in the MES buffer system. The window corresponding to the modified protein was excised and processed by in-gel digestion using a robot (ProGest, DigiLab) and Promega sequencing grade trypsin.

The digested sample was analyzed by nano LC/MS/MS with a Waters NanoAcquity HPLC system interfaced to a ThermoFisher LTQ Orbitrap Velos. Peptides were loaded on a trapping column and eluted over a 75µm analytical column at 350nL/min; both columns were packed with Jupiter Proteo resin (Phenomenex). The mass spectrometer was operated in data-dependent mode, with MS performed in the Orbitrap at 60,000 FWHM resolution and MS/MS performed in the LTQ. The fifteen most abundant ions were selected for MS/MS.

**Results**

Glycopeptides and the individual glycoforms of each glycopeptide were identified and characterized by manual analysis of the LC-MS/MS data. A snap shot of the identified glycoforms of a single glycosite are captured in the table below.

| Glycan Compostion | Experimental *m/z* (z) | Composition of the Glycans for Peptide LQLSNGNR | Theoretical m/z (z) | Error (ppm) |
|---|---|---|---|---|
| 1 | 978.4319 (+2) | $(GlcNAc)_2(Man)_3(Hex)$ | 978.4308 (+2) | 1.09 |
| 2 | 998.9454 (+2) | $(GlcNAc)_2(Man)_3(HexNAc)$ | 998.9441 (+2) | 1.27 |
| 3 | 1059.4591 (+2) | $(GlcNAc)_2(Man)_3(Hex)_2$ | 1059.4572 (+2) | 1.76 |
| 4 | 1078.4303 (+2) | $(GlcNAc)_2(Man)_3(Hex)(HexNAc)$ | 1079.9705 (+2) | 0.71 |
| 5 | 1160.9979 (+2) | $(GlcNAc)_2(Man)_3(Hex)_2(HexNAc)$ | 1160.9969 (+2) | 0.83 |

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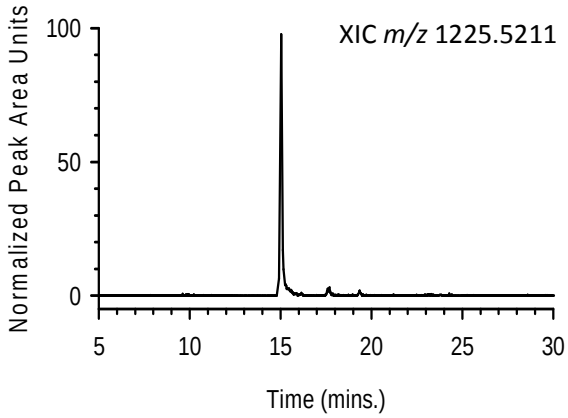
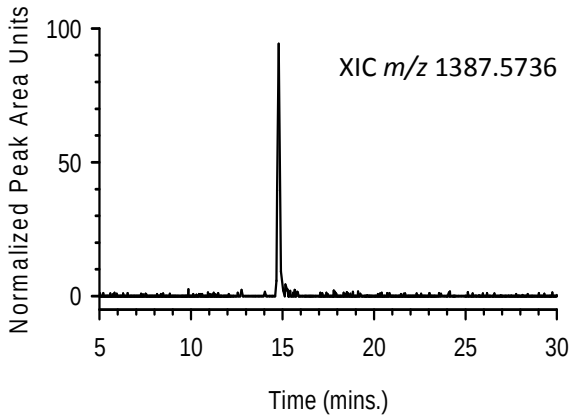
Identified glycoforms were quantified using High Resolution/ Accurate Mass (HR/AM) approach. Typical HR/AM chromatograms are shown below.

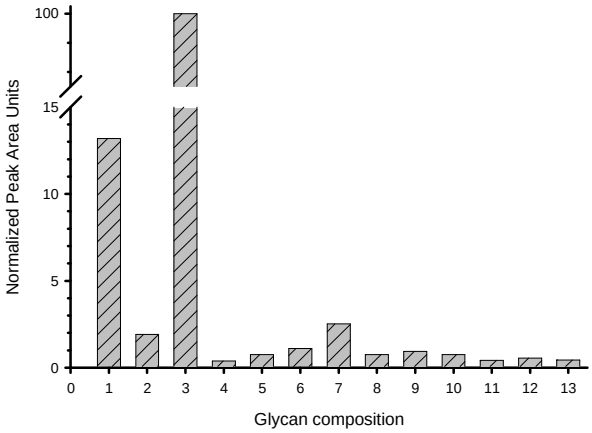



The extracted ion chromatogram (XIC) for the glycopeptide corresponding to Glycan composition 7 in the chart opposite. Data were generated using a 10ppm extraction window.

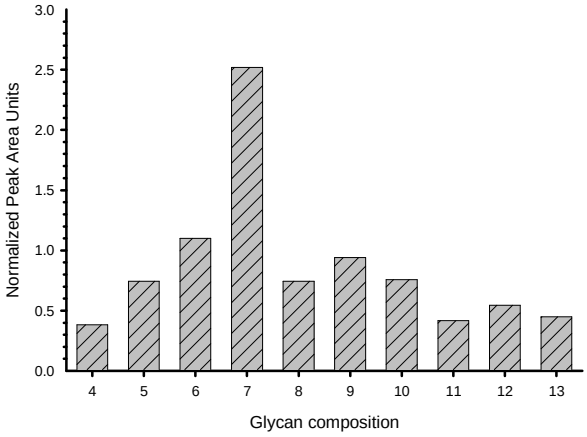



The extracted ion chromatogram (XIC) for the glycopeptide corresponding to Glycan composition 13 in the chart opposite. Data were generated using a 10ppm extraction window.

Chromatographic peak area data is normalized to the most abundant glycoform and displayed as a bar chart.




In the chart above Glycan composition 1 and 3 are clearly the most abundant. These peptides represent the $(GlcNAc)_2(Man)_3(Hex)$ and $(GlcNAc)_2(Man)_3(Hex)_2$ forms of the LQLSNGNR peptide. The position of the glycan is indicated by the underscore.




In the chart above Glycopeptides 1 and 3 have been removed to enable a detailed view of the remaining less abundant glycoforms. Of these Glycopeptide 7 is the most abundant. Glycopeptide 7 is the (GlcNAc)2(Man)3(Hex)(HexNAc)(NeuNAc) form of LQLSNGNR.

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