Glycoproteomics analysis to examine the role of chlamydial protease-like activity factor

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ABSTRACT

Purpose: Examination of the role the chlamydial protease-like activity factor targets has in altering protein expression and relative abundance of post translational modification by monitoring global glycopeptide changes.

Methods: Hela 229 cells were infected with *Chlamydia trachomatis* L2 RST5 CPAF-sufficient strain and RST17 CPAF-deficient strain, enriched for glycopeptides and analyzed on a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer. Thermo Scientific™ Proteome Discoverer™ software with Byonic node was used for data analysis.

Results: Overall we identified over 5000 unique intact glycopeptides translating into over 600 unique glycoproteins. Number of unique glycopeptides/glycoforms with levels greater than two-fold change were observed upon infection with *Chlamydia trachomatis* L2 RST5 CPAF-sufficient and -deficient strains.

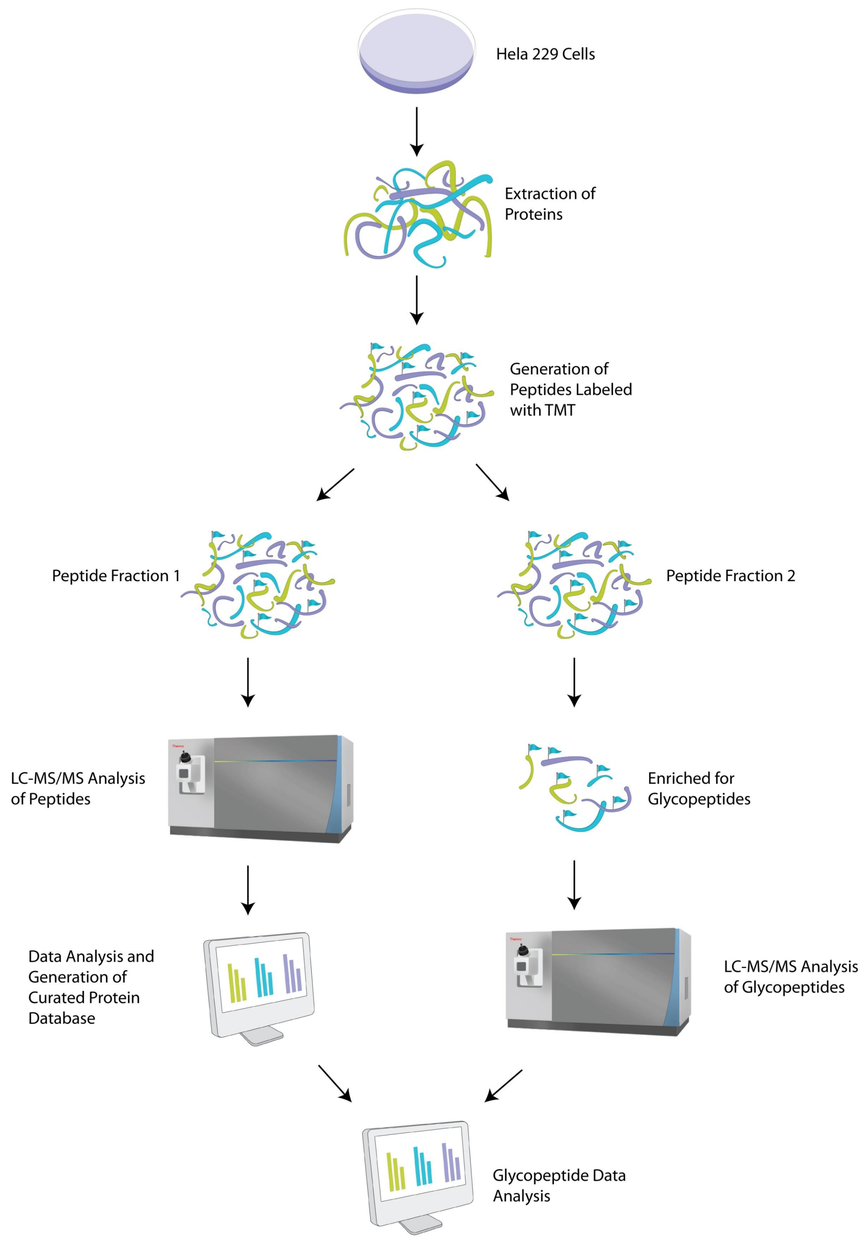
INTRODUCTION

Recently proteomics studies have been performed to examine the role of chlamydial protease-like activity factor (CPAF), a secreted virulence factor and its role in immune evasion.[1] However, experiments thus far have ignored the presence of glycopeptides that are prevalent in the sample, as large scale intact glycopeptide analysis remains challenging by mass spectrometry due to the complexities associated with the glycopeptide structure. Not only must one sequence the peptide backbone, but also site localization and glycan composition are required for intact glycopeptide analysis. The challenge is further compounded owing to the fact that traditional fragmentation techniques are not ideal for glycopeptide sequencing. Here, we undertake qualitative and quantitative intact glycopeptide analysis to examine the role of CPAF targets and derive additional insights from glycoproteomics experiments.

MATERIALS AND METHODS

Hela 229 cells were infected with *Chlamydia trachomatis* L2 RST5 CPAF-sufficient strain and RST17 CPAF-deficient strain. 150 ug of proteins were extracted from the cell lysates in triplicates from each infection, digested with trypsin and labeled with Thermo Scientific™ Tandem Mass Tag™ 6-plex Reagents (TMT) per manufacturer's protocol. Proteins from Hela 229 cells infected with the sufficient strain was labeled with 127 and 130 label, deficient strain with 128 and 131 label and the control Hela 229 were labeled with 126 and 129 label. Upon labeling samples were enriched for glycopeptides with strong anion exchange (SAX) column.[2] The enriched glycopeptides were and analyzed using a Thermo Scientific™ EASY-nLC™ 1000 with a Thermo Scientific™ EASY-Spray™ C18 PepMap column (2um, 100A, 75umx50 cm) on an Orbitrap Fusion Lumos mass spectrometer. [2] Glycopeptide MS2 data was collected by DDA using the following filters: the monoisotopic precursor selection, intensity threshold of 2e5, dynamic exclusion of 30 sec, and ions selected were fragmented both with HCD and ETD. Data analysis were performed using Proteome Discoverer 2.1 software using the Byonic node (Protein Metrics). Proteomics experiment were also performed for the sample. Figure 1 shows the schematic representation of the workflow undertaken.

Figure 1. Schematic representation of the workflow.



The identification of LC-MS/MS glycopeptide data can be challenging. To minimize false positives, we performed both proteomics and glycoproteomics experiment (Figure 1). Total lysate samples were initially searched from the standard DDA proteomics LC-MS/MS run to identify the proteins present in the sample using conventional database search approach. The initial protein identifications allow for creation of a custom database that we can use to search in a more targeted glycoproteomics identification search. We use unmodified peptides from these survey runs to created a curated database of proteins that can also be glycoproteins will be present. The smaller curated protein database is used to search the intact glycopeptide data. This strategy not only minimizes the false positives but also speeds up the search time.

Figure 2. LC-MS/MS of tryptically digested Hela 229 cells infected with *Chlamydia trachomatis* L2 RST5 CPAF-sufficient strain and RST17 CPAF-deficient strain. Top chromatogram shows the XIC of 204.087 (indicative of HexNAc) for the full run. Subsequent XICs show 10 and 1 minute windows respectively. Bottom spectra is a representative HCD glycopeptide spectrum.

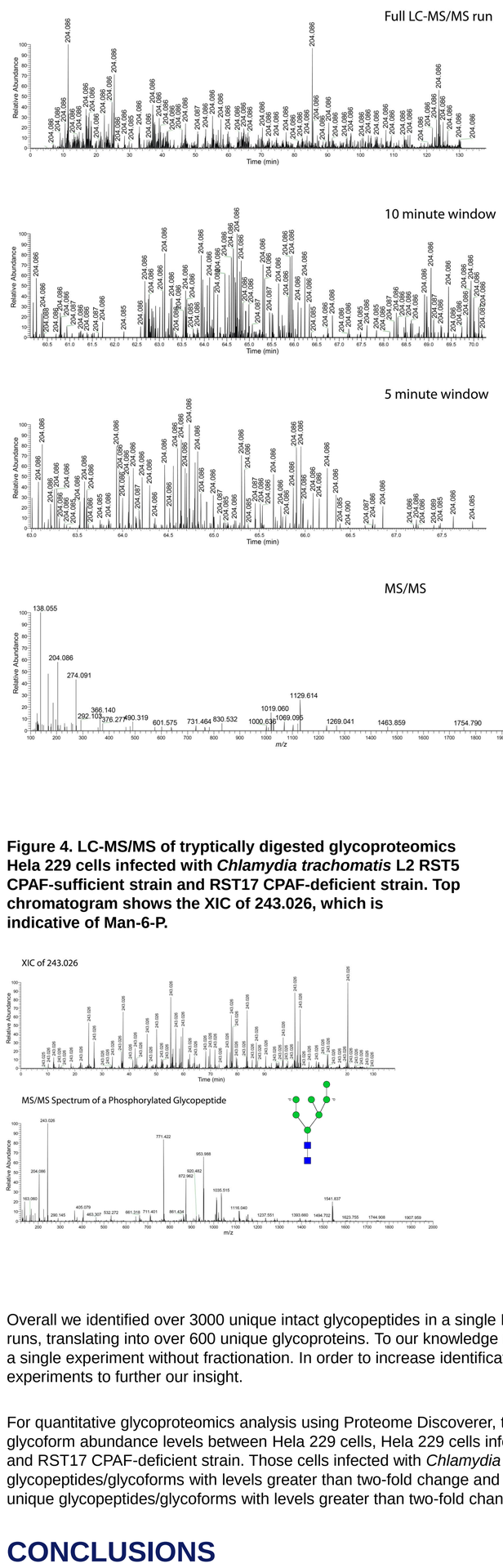


Figure 3. LC-MS/MS of tryptically digested proteomics and glycoproteomics Hela 229 cells infected with *Chlamydia trachomatis* L2 RST5 CPAF-sufficient strain and RST17 CPAF-deficient strain . Top chromatogram shows the XIC of 204.087 for the proteomics run and the bottom for the glycoproteomics run. 10 and 1 minute windows shown respectively.

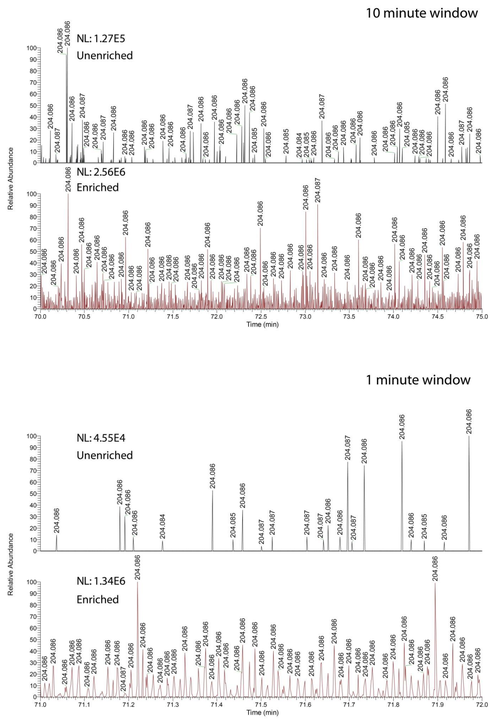
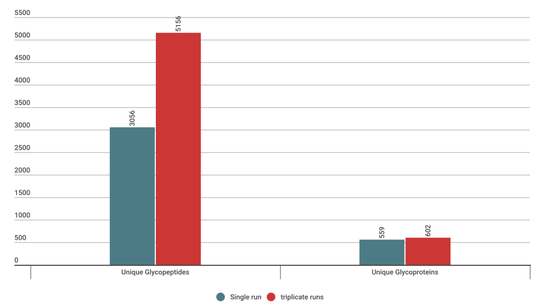


Figure 4. LC-MS/MS of tryptically digested glycoproteomics Hela 229 cells infected with *Chlamydia trachomatis* L2 RST5 CPAF-sufficient strain and RST17 CPAF-deficient strain. Top chromatogram shows the XIC of 243.026, which is indicative of Man-6-P.

Figure 5. Unique glycopeptides and glycoproteins identified in single and triplicate runs.



Overall we identified over 3000 unique intact glycopeptides in a single LC-MS/MS run and over 5000 unique intact glycopeptides in three runs, translating into over 600 unique glycoproteins. To our knowledge this is by far the largest number of intact glycopeptides reported in a single experiment without fractionation. In order to increase identification we have fractionated the sample and are currently doing experiments to further our insight.

For quantitative glycoproteomics analysis using Proteome Discoverer, there were differences in glycoprotein and/or glycopeptide glycoform abundance levels between Hela 229 cells, Hela 229 cells infected with *Chlamydia trachomatis* L2 RST5 CPAF-sufficient strain and RST17 CPAF-deficient strain. Those cells infected with *Chlamydia trachomatis* L2 RST5 CPAF-sufficient strain showed 23 unique glycopeptides/glycoforms with levels greater than two-fold change and cells infected with RST17 CPAF-deficient strain showed 81 unique glycopeptides/glycoforms with levels greater than two-fold change.

CONCLUSIONS

- This study represents the first qualitative and quantitative glycoproteomics analysis to examine the role of chlamydial protease-like activity factor targets
- Over 5000 unique glycopeptides and 600 glyoproteins identified representing the largest number reported in a single study. Number of unique glycopeptides/glycoforms were observed to show change in expression upon infection
- Further functional and cellular pathways analysis with additional glycoproteomics experiments are planned for the future

REFERENCES

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2. Totten SM, Feasley, CL, Bermundez A, Pitteri SJ. J Proteome Res 2017 Mar 3; 16 (3)1249-1260.

TRADEMARKS/LICENSING