Global In-Depth Quantitative Phosphoproteomic Analysis of HIV Infected Cells using the Orbitrap Fusion Tribrid Mass Spectrometer

Eliuk S[1], Johnson J[2], Zabrouskov V[1], Krogan N[2]
[1]Thermo Fisher Scientific, San Jose, CA, [2]University of California San Francisco, CA

Overview

Purpose: To determine the quantitative changes in the phosphoproteome of human cell lines following infection by the human immunodeficiency virus (HIV) in order to characterize the HIV-human interface.

Methods: Global identification and quantification of changes in human phosphoproteome in response to HIV was performed on SILAC-labeled Jurkat cells after HILIC/TiO_2 fractionation /enrichments using the new Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer and Thermo Scientific EASY-ETD™ source.

Results: More phosphopeptides were identified using a combination of ETD and CID fragmentation in a single run (via novel data dependent decision tree with dynamic scan management) than when using CID fragmentation alone. This permitted precise phosphosite localization on more phosphopeptides.

Introduction

A complete understanding of the molecular events following infection by the human immunodeficiency virus (HIV) is key for developing therapeutic strategies. We present an in-depth, quantitative proteomic analysis of HIV-infected cells by monitoring the changes in the global phosphorylation status of host proteins.

This unbiased systems approach has enabled the identification of exciting new contributors involved in the pathogenesis of HIV infection, which could ultimately serve as therapeutic targets.

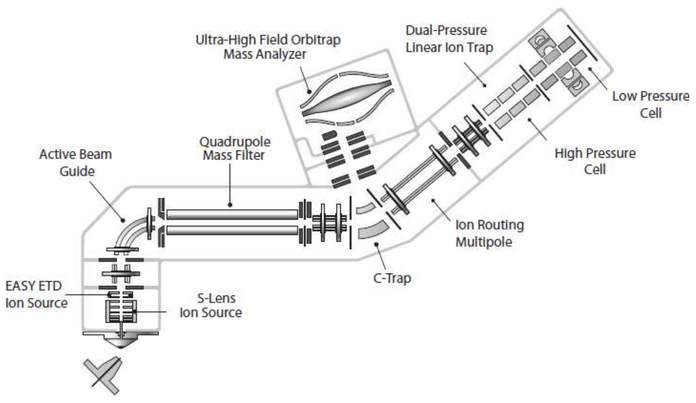


FIGURE 1. The Orbitrap Fusion Tribrid MS with novel architecture based on mass resolving quadrupole, ultra-high field Orbitrap mass analyzer, ion routing multipole, and dual-pressure linear ion trap (Q-OT-qIT).

Methods

Sample Preparation

Jurkat T cells were labeled (and reverse labeled) in light/heavy SILAC medium and either infected with HIV (strain NL4-3) or mock-infected. Harvested cells were lysed, digested and fractionated using HILIC/TiO_2 for enrichment of phosphorylated peptides.

Liquid Chromatography

A Thermo Scientific EASY-nLC™ 1000 coupled with an EASY-Spray™ source (25 cm x 0.075 mm ID, 2µm particle size) column was used for online separation of peptides over a 90 minute gradient. The mobile phases were 0.1% formic acid/water and 0.1% formic acid/acetonitrile. A 90 min linear gradient (5-22% B over 70 min) was used with a flow rate of 300 nL/min.

Mass Spectrometry

Data was acquired on the new Thermo Scientific Orbitrap Fusion Tribrid MS (Figure 1) using either solely collisional induced dissociation (CID method) or a combination of ETD/CID fragmentations (DDDT method)[1]. The former consisted of 1 full scan followed by data-dependent CID MS2. The latter consisted of 1 full scan followed by data-dependent CID or ETD scans triggered based on pre-defined parameters (precursor charge density; Figure 2).

For both methods, full scan resolution of 120,000 (at *m/z* 400) was used and fragment ions were detected in the linear ion trap.

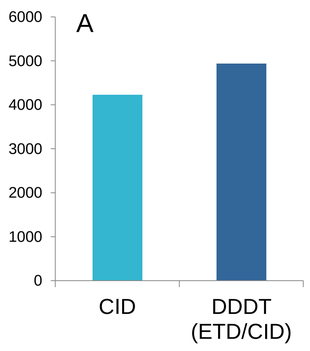
Data Analysis

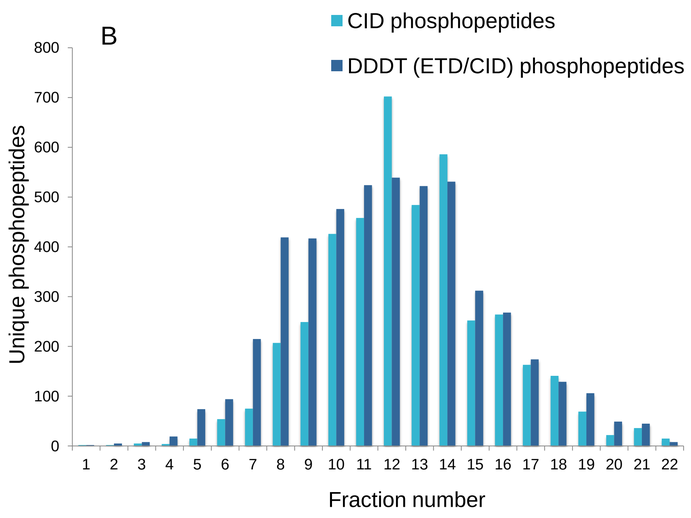
Data were analyzed using Thermo Scientific Proteome Discoverer™ software v1.4. PhosphoRS[2] module was used to specify exact localization of phosphate within candidate peptide sequences.

FIGURE 2. New method editor software permits easy development of advanced acquisition methods using an intuitive drag-and-drop interface.

Results

FIGURE 3. Analyses of tryptic phosphopeptides using either CID or DDDT methods. A) DDDT identified more total unique phosphopeptides than CID. B) Phosphopeptides identified per fraction are shown.





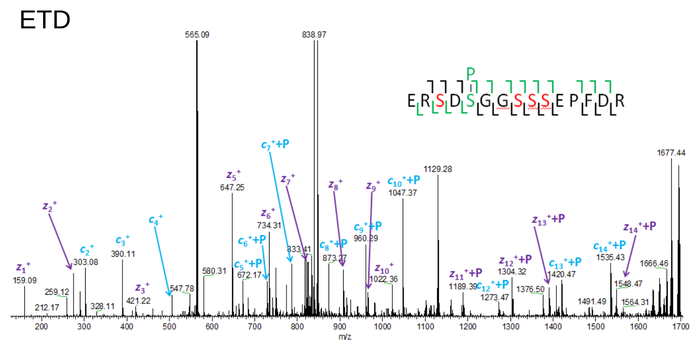
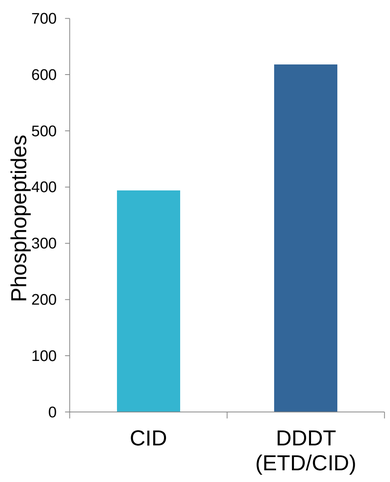


FIGURE 4. ETD fragmentation permits more complete fragment ion coverage across the sequence of the peptide enabling precise localization of the sites of phosphory-lation, particularly when multiple sites of potential phosphorylation are present.

FIGURE 5. Phospho-peptides with confident site localization. DDDT acquisition enables a precise phosphosite localization in significantly more cases. Data for single phosphopeptide fraction are shown after processing with PhosphoRS module.



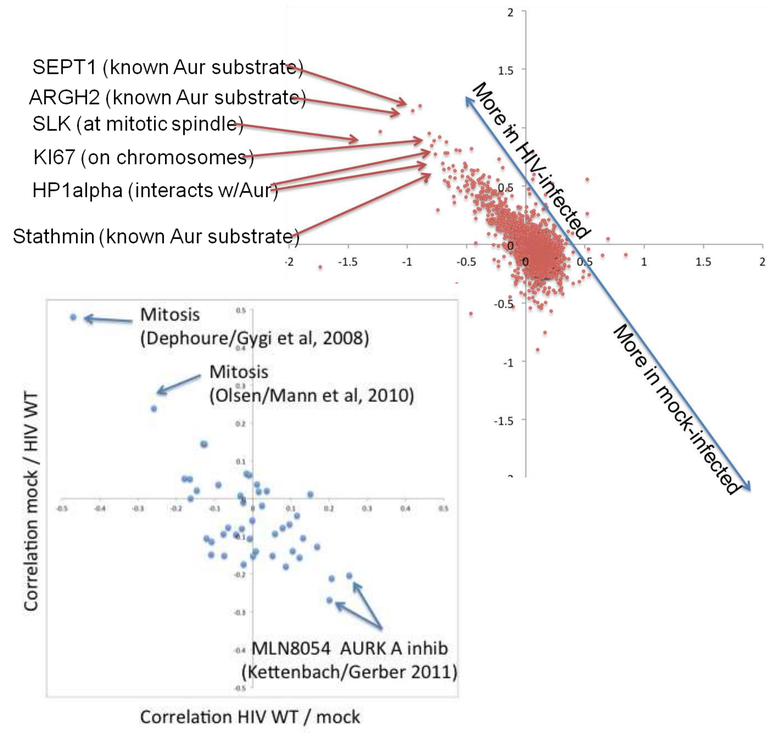


FIGURE 6. Aurora kinases are activated by HIV infection. SILAC phosphorylation profiles comparing HIV-infected to mock-infected Jurkat cells were checked against PTMfunc database (http://ptmfunc.com) of post-translational modifications[3]. PTMfunc identified a correlation with two independent mitosis phosphoproteomics data sets, and an anti-correlation with an aurora kinase inhibitor data set. Manual inspection of the most heavily phosphorylated peptides confirmed strong activation of aurora kinases in HIV-infected cells.

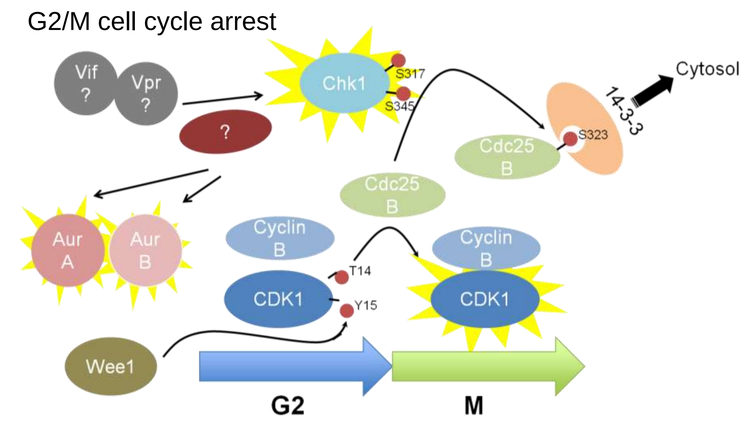


FIGURE 7. HIV arrests cells by bypassing the full DNA damage response. Our phosphoproteomics analysis indicates that Chk1-dependent cell cycle arrest is achieved while maintaining aurora kinase activity. The HIV proteins VIF and VPR are both required for cell cycle arrest[4]. The mechanisms by which this arrest occurs is unknown, but our data suggests it acts through an intermediate in the DNA damage pathway.

Conclusion

- Jurkat T cells infected with HIV (strain NL4-3) or mock-infected were used for proteomic experiments to establish changes in phosphorylation abundance.
- Quantitative analysis of the samples was performed on an Orbitrap Fusion Tribrid mass spectrometer. This novel mass spectrometer, with its unique architecture, was engineered to optimize both speed and efficiency enabling sensitive MS/MS analysis at a high rate.
- Due to the quadrupole isolation, parallelization of Orbitrap and ion trap detection, and pipelining of ion injection and mass analysis, the extremely high resolution full scan data necessary for accurate SILAC quantitation was acquired with simultaneous acquisition of sensitive MS/MS spectra using both ETD and CID fragmentation.
- This comprehensive data set enabled the determination of proteins with altered abundance of phosphorylation which may play a role in the signaling cascade leading to the severe and life threatening pathogenesis of HIV infection.

References

1. Swaney DL, McAlister GC, Coon JJ. Nat. Methods 2008, 5, 959.
2. Taus T, Kocher T, Pichler P, *et al.* J. Proteome Res. 2011, 10, 5354.
3. Beltrao P, Albanèse V, Kenner LR, *et al.* Cell. 2012, 150, 413.
4. Sakai K, Dimas J, Lenardo MJ. PNAS U S A. 2006, 103, 3369.

Thermo SCIENTIFIC