Comprehensive Quantitative Analysis of Ovarian and Breast Cancer Tumor Peptidomes

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Overview

- Top-down analysis of the peptidome can provide insights to which conventional bottom-up proteomics are blind.
- Developed an effective and robust analytical platform for comprehensive analyses of the tissue peptidome, which is suitable for high throughput quantitative studies.
- Peptidomic profiles reflect tumor-associated protease activity and are expected to provide unique and complementary results to those obtainable from conventional bottom-up proteomics approaches.

Introduction

- Aberrant degradation of proteins is associated with many pathological states including cancers.[1,2]
- Mass spectrometric analyses of the tumor peptidome, the intracellular and intercellular products of protein degradation, have the potential to provide biological insights on proteolytic processing in cancer.[3]
- Effective approaches for robust peptidomic identification and quantification are lacking, and suffer confounding factors and biases due to sample handling and processing.
- We have previously shown the potential of peptidomics using top-down approaches.[4]
- In this study we demonstrate the effectiveness and utility of a new peptidomics platform for comprehensive characterization of ovarian and breast tumor peptidomes.

Methods

Samples

Table 1. Summary of the ovarian cancer (OvCa) tumor samples and patient-derived xenograft (PDX) breast tumor samples

A. Summary of the OvCa patient tumor samples

Patient name	Cancer type	Room Temperature delay
A	OvCa	0, 5, 30, 60 min
B	OvCa	0, 5, 30, 60 min
C	OvCa	0, 5, 30, 60 min

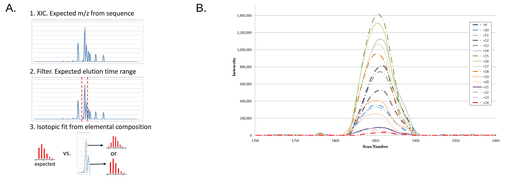
B. Summary of the PDX breast tumor samples

Sample name	Cancer subtype	WHIM line	Room Temperature delay
WHIM6-0-a	Basal BrCa	WHIM6	0 min
WHIM6-60-a	Basal BrCa	WHIM6	60 min
WHIM6-0-b	Basal BrCa	WHIM6	0 min
WHIM6-60-b	Basal BrCa	WHIM6	60 min
WHIM2	Basal BrCa	WHIM2	N/A
WHIM16	Luminal BrCa	WHIM16	N/A

Workflow applied

- **Protein extraction**
  - Add extraction buffer (0.25% acetic acid with the protease inhibitors)
  - Homogenize/sonicate on ice bed
  - Centrifuge @ 14,000 × g, 4 °C for 30 min
- **Ultrafiltration**
  - Filter with 30 kDa MWCO filters @ 8,000 × g, 4 °C
  - Concentrated the sample in Speed-Vac
  - Final peptide/protein yield: 0.3%
- **LC-MS/MS analysis**
  - LC: 70 cm × 75 μm i.d. column; 3-μm Jupiter C18; 240-min gradient; Waters UPLC
  - MS: Orbitrap Velos; 60K for MS and 15K for MS/MS (CID); top6 MS/MS
- **Data analysis**
  - MS/MS data: Sequest + MS-Align ⇒ create one peptide database
  - LC-MS data: accurate mass and time (AMT) tag or Informed Quantitation (IQ) analysis

New Informed Quantitation (IQ) approach applied



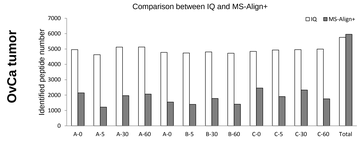
- Significantly improved sensitivity
- Provides more accurate quantification
- Better distinguishes overlapping features
- Improves FDR and reduces "missing data"

Solid lines : charge states chosen for MS/MS fragmentation
Dashed lines: charge states present but not chosen for fragmentation

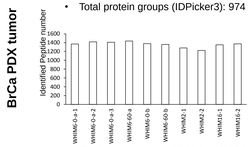
Results

Good coverage and reproducibility provided by the tumor peptidomics platform

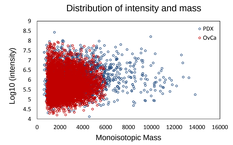
Comprehensive coverage provided by IQ approach



- Total protein groups (IDPicker3): 974



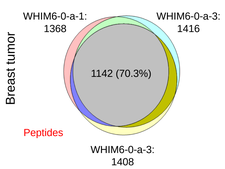
- Total protein groups (IDPicker3): 824



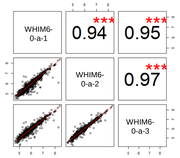
Mass and peptide MS intensity distribution of the peptidomes of the clinical ovarian tumor samples and Patient-derived xenograft breast tumors

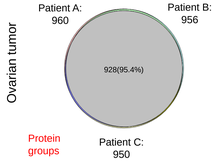
Good reproducibility for tumor peptidome analyses

Overlap of peptide identifications

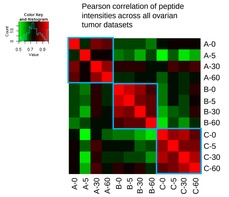


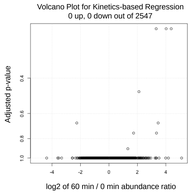
Correlation of peptide intensities

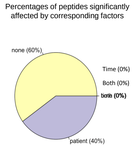




Post-excision delay of up to 60 min does not impact tumor peptidome characterization

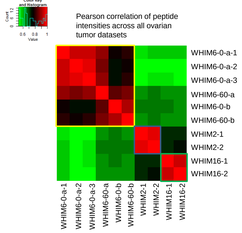


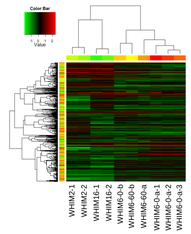


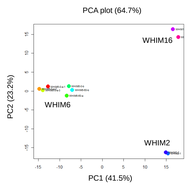


Peptidome profiles indicate tumor-specific proteolytic activities

Breast cancer subtypes separated by peptidome profiles

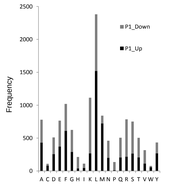




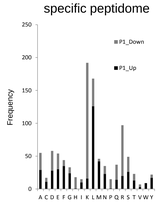


Proteolytic cleavage specificity for OvCa vs. BrCa

OvCa tumor peptidome

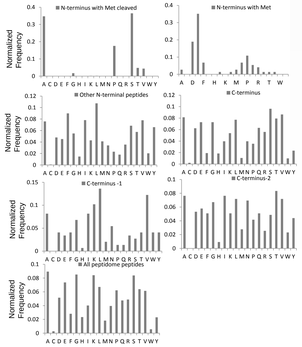


PDX tumor human-specific peptidome



Chymotrypsin and trypsin activities are the major contributor to both peptidomes, suggesting the involvement of proteasome-dependent pathway

Analyses of N- and C- terminal cleavage specificities revealed involvement of N-terminal Met Excision (NME) processing



Conclusions

- The reproducibility and coverage of a new tumor peptidomics pipeline have been demonstrated.
- Clinical tumor samples and patient-derived tumor xenograft samples, with post-excision delay of up to 60 min, have been determined as suitable for peptidomics analysis.
- Peptidomics profiles can be used to effectively separate different cancer types or subtypes, as well as individual patients.
- The peptidome, studied using top-down approaches, provides a practical and useful adjunct to bottom-up approaches.

Acknowledgements

Portions of this work were supported by the grant U24CA160019, from the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium (CPTAC), National Institutes of Health grant P41GM103493, and Department of Defense Interagency Agreement MIPR2DO89M2058. The experimental work described herein was performed in the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the DOE and located at Pacific Northwest National Laboratory, which is operated by Battelle Memorial Institute for the DOE under Contract DE-AC05-76RL0 1830.

References

1. Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 144, 646-674 (2011).
2. Lopezin-Otin C and Overall CM. Proteases degradomics: a new challenge for proteomics. *Nature Rev Mol Cell Biol*. 3, 509-519 (2002).
3. Villanueva et al. Differential exoprotease activities confter tumor-specific peptidome patterns. *J Clin Invest*. 116, 271–284 (2006).
4. Shen et al. Blood peptidome-degradome profile of breast cancer. *PLoS One*. 5, e13133 (2010).

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