Comprehensive, Quantitative, Intact Proteoform Measurements of Patient-Derived Breast Tumor Xenografts Using an Improved Top-Down Proteomics Pipeline

Tao Liu1*, Paul Piechowski1, Samuel Payne1, Sangtae Kim2, Jungkap Park1, Christopher Wilkins1, Carrie Nicora1, Yufeng Shen1, Rui Zhao1, Anil Shukla1, Ronald Moore1, Sheri Davies1, Shunqiang Li2, Reid Townsend3, Matthew Ellis4, Emily Boja4, Henry Rodriguez4, Karin Rodland1, and Richard D. Smith1

1Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 2Department of Medicine, Washington University, St. Louis, MO 3Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 4National Cancer Institute, National Institutes of Health, Bethesda, MD

Overview

Many biologically active proteoforms cannot be identified or distinguished by conventional bottom-up approaches since the information is lost by tryptic digestion.

• Introducing deep proteomics measurements with available genomic data enables characterization of protein variants, including coding polymorphisms, processing products, and post-translational modifications.

There is an urgent need to be able to make comprehensive and reproducible measurements of proteoforms in biological or clinical samples.

The available patient-derived xenografts (PDX) for two breast cancer subtypes (luminal B and basal) provide an ideal system for this evaluation.

Introduction

The widely used bottom-up proteomics approaches in many cases cannot identify or distinguish many different processed and/or modified versions of the gene products whose expression is critical for understanding the development and treatment of cancers.

Bottom-up proteomics approaches are inherently limited by the "peptide-to-protein" inference problem, affecting both accuracy and precision of the quantification.

We have recently developed a significantly improved top-down proteomics pipeline that allows for comprehensive quantitative intact proteoform measurements.

Application of this pipeline to the comparative analysis of PDX models of basal and luminal B breast cancer subtypes provided new insights into cancer biology.

Comprehensive and reproducible measurements of proteoforms can be achieved using the improved top-down proteomics approach.

Methods

Sample preparation

Washington human-mouse (WHIM) PDX samples representing luminal B (P31 and P33) and basal (P15 and P16) breast cancer subtypes.

The top-down measurements provided unique information on up-regulation of many proteoforms, variants, additional protein modifications, and phosphorylation sites.

Conclusions

Our top-down proteomics pipeline provided significant improvement in proteome coverage, precision of the quantification, and the ability to identify statistically significant changes on proteoform abundances, in comparison to another recent report that analyzed proteoforms in the same PDX samples.

Although still relatively limited in coverage compared to the conventional bottom-up approach, the top-down analysis provides a complementary view of the proteoforms (e.g., truncated and/or post-translationally modified proteoforms, variants, additional protein modifications).

The top-down, bottom-up, and peptidomics analysis of the same PDX samples showed the same degree of changes in proteoform/peptide abundances between the two subtypes.

The top-down measurements provided unique information on up-regulation of many proteoforms of histones and 60S ribosomal proteins as well as key pathway level changes (e.g., phosphorylation of the luminal subtype). This holds great promise in providing new insights into cancer biology.

Fractionation and new technologies (e.g., SLIM IMS) will greatly improve proteome coverage.

References

1. Li et al., Cell Rep. 2013 Sep 26;4(6):1116-30

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