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6404/28: “LiP-Quant, an Automated Chemoproteomic Approach to Identify Drug Targets in Complex Proteomes”

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Background

Target identification is a critical step in elucidating the mechanism of action (MoA) for bioactive compounds. For phenotypic drug discovery pipelines, current unbiased, label-free chemoproteomics-based methods rely predominantly on the modulation of target thermal stability upon drug binding. We developed an automated drug target deconvolution workflow combining limited proteolysis with mass spectrometry (LiP-Quant) that exploits protein structural alterations, as well as steric effects driven by drug binding. A major advantage of LiP-Quant is its unique focus on the detection of signature peptides that discern ligand binding, peptides that are generated by a limited digestion and identified by proteomic analysis. Here we demonstrate the performance of LiP-Quant using two well-characterized kinase inhibitors (KIs), Selumetinib (SE) and Staurosporine (ST), as well as two natural product-derived phosphatase inhibitors (PIs) Calyculin A and Fostriecin in human cell lysate. Furthermore, LiP-Quant can be deployed to estimate in-lysate EC50 value of compound binding.

Methods

Mechanically sheared HeLa cell lysate was incubated with compound at multiple concentrations. Next, a limited digest was performed using proteinase K. Finally, the limited digests were processed to peptides with trypsin for mass spectrometry analysis. A project-specific spectral library was generated using data-dependent acquisition (DDA) mass spectrometry and for quantitative analysis data-independent acquisition (DIA) data were recorded and analyzed using Spectronaut.

Results

Herein, we demonstrate the ability of our LiP-Quant approach to identify unique peptides generated by the binding of either a highly specific (selumetinib) or broad specificity (staurosporine) KI in human cell line lysate. While > 20 kinases met the qualifying LiP-score cutoff for staurosporine, the direct targets MEK1 and MEK2 were clearly identified as main hits in the unbiased ranking by LiP scores. Both cases represent a highly specific enrichment given

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that we quantified > 100,000 peptides in each of the experiments. These findings confirm our approach's ability to identify genuine drug targets regardless of drug specificity in a complex biological matrix. To characterize the specificity of LiP-Quant, we treated lysate with two separate protein PIs. According to literature calyculin A targets protein phosphatase 1 and 2 (PP1A and PP2A) and fostriecin also targets PP2A, in addition to protein phosphatase 4 (PP4C) but does not bind PP1A. Robust phosphatase identification was achieved for both calyculin A and fostriecin treatment. Importantly, with LiP-Quant, we could recapitulate the known relative affinities of the PIs towards their respective targets.

Conclusions

Collectively, this data demonstrate that LiP-Quant can be used to effectively identify protein drug targets and characterize the binding properties in complex proteomes, without compound modification or labeling, and regardless of the specificity of the compound. These capabilities make LiP-Quant a powerful target deconvolution and identification strategy.

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