Peptide Mapping for Quantitative Profiling of Peptide Variants using PEAKS

PEAKS® Complete Software for Proteomics

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Overview

Purpose: To develop a software tool able to analyze and quantitate single amino variations on a large-scale.
Methods: A unique peptide mapping tool was proposed which is able to map all LC-MS signals eluted from the mass spectrometer for each identified protein. With PEAKS' de novo capabilities, the signals display not only identified peptide LC-MS data, but as well as de novo only LC-MS data.
Results: A new method for large-scale quantitative analysis of single amino acid variations

Introduction

Global quantification of the single amino acid variations (SAVs) is essential to investigate their roles in disease progression. Most proteomic software tools are only able to interpret peptide fragments through database searches and map database hits to the protein sequences. As a result, mutated peptides are neither identified nor mapped to the protein sequences, thus failing to provide direct quantitative analysis. This study demonstrates the benefits of using PEAKS, a de novo assisted search engine, to maximize the performance of peptide mapping. Subsequent quantification of these peptide variants also provides large-scale quantitative analysis of SAVs.

Methods

A thorough analysis of the LC-MS/MS data using a complete software package PEAKS, was proposed to ensure a feasible method for the analysis of single amino acid variants on a large scale data set. An initial database search was performed to identify all proteins in the sample. These proteins then became the candidates for searching SAVs. For the remaining unidentified spectra, de novo sequencing of these scans were conducted. Resulting high confident de novo sequence tags were then mapped to the corresponding protein candidates (figure 1). To further analyze these tags, homology search of the spectra were performed within the peptide maps to find single amino acid variants (figure 2 and 3). Finally, relative quantification of mutant and normal forms of the peptide could ultimately be determined by feature intensities of their extracted ion chromatograms (figures 3, 4 and 5).

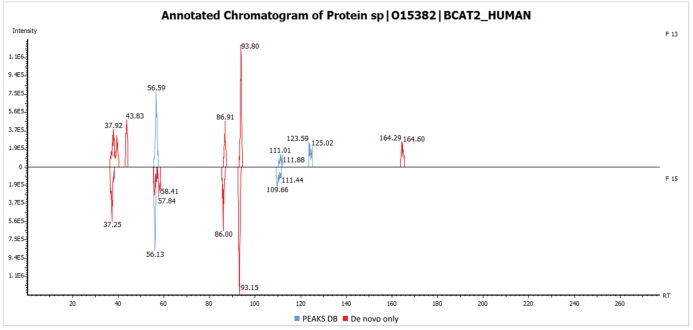

Figure 1. Peptide mapping of PEAKS DB and de novo only results

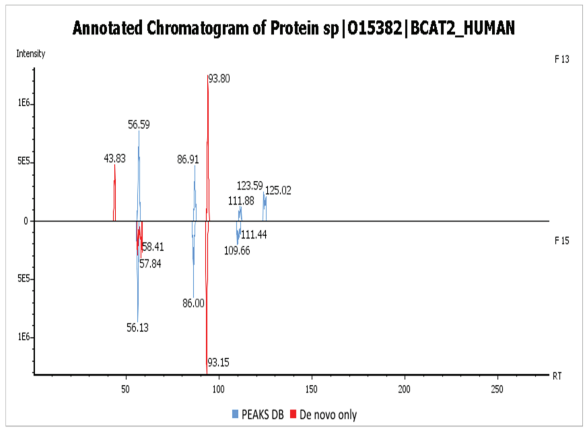

Figure 2. Peptide mapping of PEAKS SPIDER results

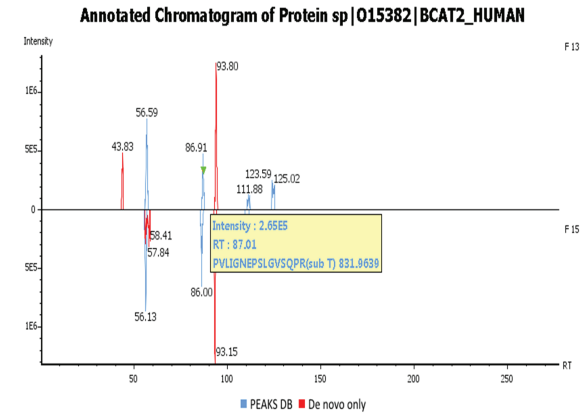

Figure 3. Identified single amino acid variant from SPIDER search

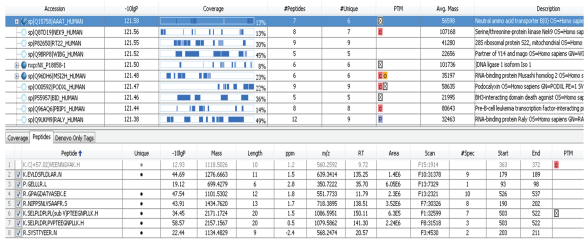

Figure 4. PEAKS displays corresponding peptide intensities for quantitative analysis

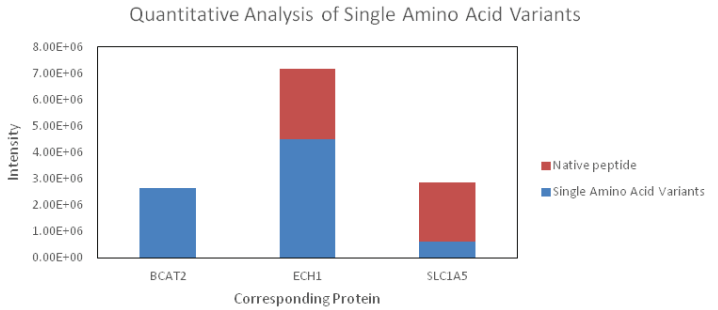

Figure 5. Quantitative analysis for confirmed Chromosome 19 SAVs

Experimental

Glioma stem cell (GSC) lines, which were isolated and prepared by The University of Texas M.D. Anderson Cancer Center, were reduced, alkylated and analyzed by LC-MS/MS on a Thermo Orbitrap Elite mass spectrometer (Lichti, et. al., 2015). The LC-MS/MS data was analyzed using PEAKS with 10 ppm parent mass error tolerance and 0.025 Da fragment mass tolerance. A maximum of 2 missed cleavages were allowed, in addition to one non-specific cleavage. Carbamidomethyl cysteine was set as a fixed modification, whereas variable modifications included methionine oxidation as well as phosphorylation on STY. Database search was performed using the custom database with FDR estimation enabled. Highly confident de novo tags were then further analyzed using PEAKS SPIDER. Peptides with a -10logP score >30, which contained SAVs or post-translational modifications (PTMs), were selected for further validation.
As many as 312 unique variation sites were quantified. 19 SAV-containing peptides have been verified. Those peptides represent 19 SAVs in 17 chromosome 19 proteins. The peptide maps generated by PEAKS indicate that this approach could not only serve to maximize peptide maps of any protein, but to also provide quantifiable information regarding these variants presented as peptide profiles (figure 3).

Conclusions

Large-scale quantitative analysis of single amino acid variants is made feasible using PEAKS' automated peptide mapping tool.

References

1. Lichti, C.F., Mostovenko, E., Wadsworth, P.A., Lynch, G.C., Pettitt, B.M., Sulman, E.P., Wang, Q., Lang, F.F., Rezeli, M., Marko-Varga, G., Vegvari, A., Nilsson, C.L.: Systematic identification of single amino acid variants in glioma stem-cell derived chromosome 19 proteins. J. Proteome Res. 14, 778-786 (2015).