Quantitative analysis of endogenous peptides with LC-MS/MS directly from complex secretome samples

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Complete Software for Proteomics

Overview

Purpose: To develop a software tool for endogenous peptide analysis direct from secretome samples.

Methods: An automated multiple-round search was proposed by combining database search and de novo sequencing to facilitate analysis of the secretome beyond the dominant tryptic fragments.

Results: A workflow for direct analysis of endogenous peptides in complex secretomes.

Introduction

Endogenous peptides released into body fluids contain highly relevant information such as regulatory biomolecules with both diagnostic and therapeutic potential. A major analytical challenge in secretory peptide research is the low abundance of the analyte proportional to the large volume of extracellular matrix proteins (Figure 1). The common analytical solution to this problem is to enrich the peptide fraction of a complex sample by physical removal of the most abundant proteins. As sample depletion of abundant matrix proteins (such as albumin in plasma or cell culture media) is known to remove low abundant signalling peptides, biologists interested in decoding the (poly)peptide language of cells prefer not to do so. It has been reported that high resolution LC-MS/MS allows for the discovery of signalling peptides directly from their typical cell biological culture media [1]. Here we report a data processing workflow for direct quantitative analysis of endogenous peptides in complex secretomes, without the removal of highly abundant background proteins.

Figure 1. Extracellular proteome analytical challenges

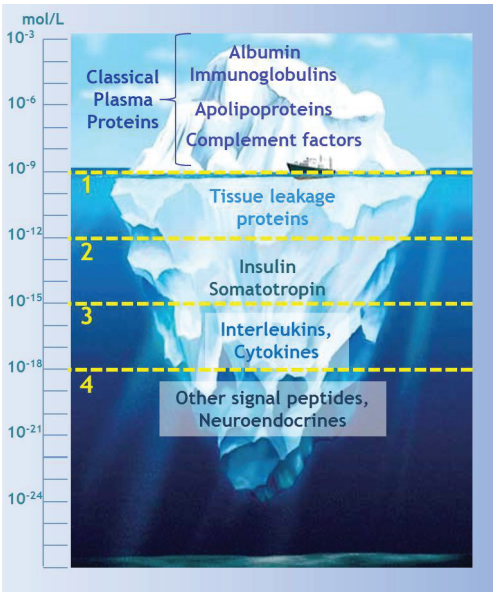
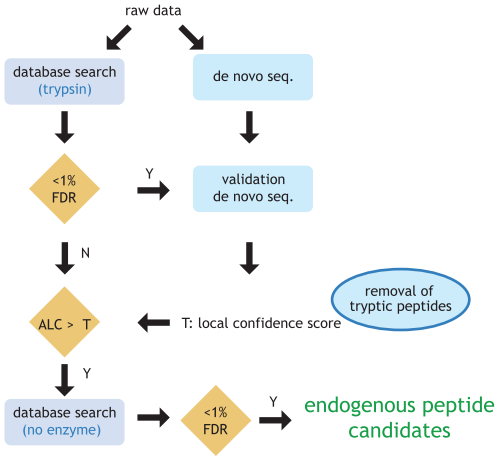


Figure 2. Workflow for the identification of endogenous peptides



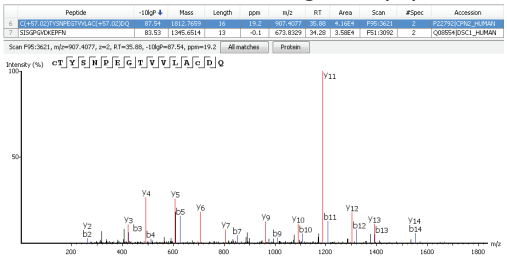
Methods

A high-resolution mass spectrometric workflow combining database search with de novo sequencing was proposed to facilitate analysis of the secretome beyond the dominant tryptic fragments. After a first round of database searching, with the tryptic enzyme specificity, all confident matches were filtered out. The remaining unidentified MS/MS spectra with good de novo sequencing tags underwent a second round database search without enzyme specificity (Figure 2). This allows the identification of endogenous peptides with high sensitivity and accuracy. Peptides were quantified with intensity-based label-free quantification.

Experimental

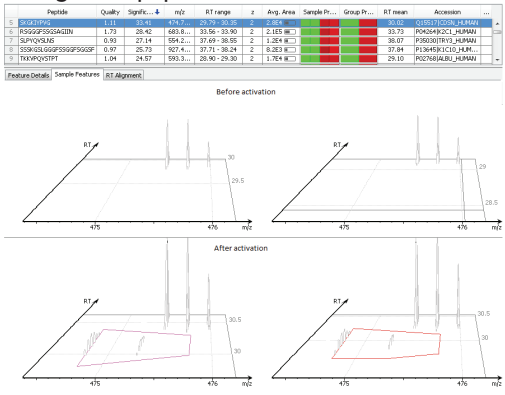
Human naive T-cells (CD25-, CD4+) were put in primary culture in typical DMEM medium supplemented with 7.5% FCS (fetal calf serum) and activated in vitro at t=0. Extracellular medium sampling was done at t=6, 24 and 48 hrs post-activation. Samples were conditioned FCS-containing medium of a selected population of human T-cells, which were reduced, alkylated, fractionated over C4 RP-HPLC, digested with trypsin and run by LC-MS with an LTQ Orbitrap Velos. Given a complex two-dimensional LC-MS/MS dataset (>130 GB) of conditioned medium of two differently treated primary human cell cultures sampled at three time points, PEAKS was supplemented with the described novel data analysis workflow. This allows the identification of small endogenous peptides (examples in Table 1) in the presence of a very busy background of predominant tryptic peptides, which made the former invisible in standard analyses. Label-free quantitation in PEAKS Q shows that these (poly)peptide profiles contain biomarkers for specific physiological or pathological conditions (Figure 3).

Table 1. Candidates of endogenous peptides



- Data are currently being validated
- GO analysis confirms relevance (majority is annotated in database as “secreted”)

Figure 3. Label-free quantitative analysis of endogenous peptides



Conclusions

Direct analysis of endogenous peptides in complex secretomes without phyical removal of abundant proteins is possible using PEAKS software.

References

1. Pinkse, M., et al. Modern MS based unlabeled secretome analysis of conditioned culture media reveals novel aspects of cell biology, from microorganisms to human, 61st ASMS poster 2013.

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