Pathway analysis in proteomics

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Motivation

Recent developments of high throughput techniques in proteomics and mass spectrometry allow the identification and quantitation of hundreds to thousands proteins in a single experiment. The analysis of such data, although still partial if thinking at the entire picture of the complete proteome, might contain enough information to describe the complex mechanisms regulating biological processes. We will present here a novel approach called Pathway Analysis of quantitative proteomics data and use it for the identification of modulated pathways in different mammalian cell lines.

Methods

Total protein extracts were analyzed in a typical expression-proteomics label free experiment. Briefly, whole cell lysates were treated for in solution trypsin digestion and injected into an LTQ-Orbitrap mass spectrometer. Proteins were identified via Mascot Server. Using a Label Free quantitation Approach, the identified proteins are then quantified based on their MS ion count, retention time and chromatographic peak intensity. The data obtained have been analyzed by Pathway Search Engine (Zubarev R. et al. J. Proteomics 2008) that uses known signaling mechanisms to identify and quantify activated pathways. The analysis passes through the Key Node filtering step. A key node is a molecule found on pathway intersections in the upstream vicinity of the genes from the input list. Each found key-node receives a score reflecting its connectivity. Key-nodes with the highest score are then selected, and downstream genes are chosen as a subset for subsequent mapping onto the pathways.

Results

Analyzing the changes in the expression of the 300-600 proteins identified with High Accuracy Mass Spectrometry and quantified with a label free quantitation approach, it was possible to identify and quantify up and down-regulated pathways that can facilitate the understanding of a molecular mechanism.

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