Marrying SILAC and iTRAQ: relative protein quantification by isobaric stable isotope labelling with immonium ion splitting (ISIS)

Mara Colzani, Alexandra Potts, Patrice Wardel, Frédéric Schütz, Manfredo Quadroni

University of Lausanne, Epalinges, Switzerland; Swiss Institute of Bioinformatics, Lausanne, Switzerland

Overview

We present a novel strategy for protein relative quantification based on a new metabolic stable isotope labelling method followed by LC-MS/MS analysis.

• Mass spectrometry and stable isotope labelling can be used for quantification of complex mixtures of proteins:
  • chemical derivatization is used to perform in vitro peptide labelling
    ➔ MS based quantification (e.g. ICAT)
  • MS/MS based quantification (e.g. TMT)
  • metabolic labelling incorporates in vivo stable isotope-labelled amino acids during protein synthesis in cultured cells
    ➔ MS based quantification (e.g. SILAC)

• Here we present a novel approach that combines metabolic isotope labelling and MS/MS based quantification.

Introduction

We present a novel strategy for protein relative quantification based on a new metabolic stable isotope labelling method followed by LC-MS/MS analysis.

Materials and methods

LC-MS system: Ultimate (LC Packing) nano HPLC on line with ESI-QTOF (API QSTAR Pulsar) / Applied Biosystems/SCIEX). Peptides loaded on a reverse phase column (PepMAP C18; 3 mm, 100 Å) were separated by a 2-45% acetonitrile gradient.

• Protein identification: Mascot.dll script from Analyst software was used to extract non-identified proteins. Proteins were identified using Mascot (Matrix Science), restricted to human taxonomy.

• Data analysis: An in-house built script extracted from Mascot.dat file the intensity values at the following m/z values at the following:

1. 15 N light/13 C heavy
2. 15 N labelled
3. 13 C labelled

Results

1. Peak intensities in un-labelled sample

Analysis of intensity and occurrence of signals at:

- 72.081 and 86.097 m/z (natural Val and Ile/Leu immonium ions)
- 73.078 and 87.094 m/z (expected values for heavier immonium ions)

Results:

• peaks at 72.081 and 86.097 m/z were specific for Val and Ile/Leu
• peaks at 73.078 and 87.094 m/z were weak and related to the presence of peaks at 72.081 and 86.097 m/z, respectively
• intensity of heavy and light peaks were consistent with the predicted natural isotope distribution
• natural Val, Ile and Leu immonium ions, in combination with the 13 C and 15 N labelled analogues, resulted suitable for our quantification strategy

2. 15 N and 13 C labelling

Co-elution, MS and MS/MS spectra of digested protein mixtures obtained from cells fully labelled with 13 C and 15 N were analyzed:

- the labelling was complete
- 15 N and 13 C peptides were isobaric, co-eluted and presented nearly identical MS/MS spectra, but different reporting immonium ions in MS/MS spectra
- at our working resolution, the immonium ion peak of asparagine (at 87.056 m/z) was distinguishable from those of heavy Ile/Leu

Method validation

3. Quantification of a normalized mixture

Two identical cultures of SKM282 cells were differentially labelled. The lysates were mixed in 1:3 ratio and one fraction was analyzed by ISIS.

• identification of 127 proteins (2035 peptides)
• 97% of the identified peptides contained at least one label
• ISIS method computed 80 protein ratios (63%)
• ratio was close to the expected value of 0.333 (mean ratio = 0.340, mean std. err. = 0.023)

4. High-confidence ratios identification

We set threshold statistical values to identify high-confidence ratios (minimum t-value = 9 and maximum p-value = 0.001).

• 65 ratios out of 80 passed the filter and were considered “high confidence”

Method application

5. Differential analysis of melanoma cells

SBCL2 and SKM28 are melanoma cells at different stages of tumor progression. The two cell cultures were differentially labelled; the lysates were mixed in 1:1 ratio and analyzed by ISIS.

• identification of 582 proteins (13504 peptides)
• quantification of 527 proteins: 502 high-confidence ratios (86%)
• identification and quantification of proteins changing > 2-fold

Conclusions

• The method allows the identification and the quantification of proteins differentially expressed in two different cell lines
• The ISIS strategy combines the advantages of metabolic labelling and MS/MS-based quantification