A New Strategy for Quantitative Proteomics Using Isotope-Coded Protein Labels

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Why Proteomics?

Principle of ICPL

Analysis of apoptotic human cells

Data Analysis
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The Facts

One Genome

Different Proteomes

(Orgyia antiqua, L.)
Why Proteomics?

DNA → mRNA → Preprotein → Processed Protein → Complex / Interaction

Amount?  Localization?  Associated Proteins?
Dynamic?  Active Form?  Proteins?
The Challenge

30,000 genes

- splicing
- modifications
- degradation

> 300,000 protein species / organism

~ 50,000 protein species / cell

- highly dynamic
- large variability in amount
- no amplification possible
- heterogeneous molecules
State of the Art

Number of proteins/cell

Copies/cell

Sequence coverage

Covered

Uncovered

- 50 000

- 40 000

- 30 000

- 20 000

- 10 000

- 5 000

- 1 000

- 100%

- 80%

- 60%

- 40%

- 20%

- 100%

- 60%

- 40%

- 20%
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ICPL™-Isotope Coded Protein Label

Mouse with specific tumor

One organ

Lyse cells – solubilize proteins

Tumor Tissue

Healthy Tissue

Tumor Proteome

Healthy Proteome

Lyse cells – solubilize proteins
Differential Labeling

Labeling of all proteins with ICPL

Freeze status on the protein level

Tumor Proteome

Healthy Proteome

Combine
Reduction of Complexity

Reduction of complexity on protein level

Cleave proteins into peptides

Reduction of complexity on peptide level
Quantification and Identification

- MS-scan - Detect labeled peptide pairs
- Select differentially expressed peptide pairs
- Analyze sequence by MS/MS
- Protein identification by database search
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Analysis of Human Cell Extracts

Healthy (125µg) → Label with light tags → Combine & digest (Glu-C) → SCX-LC → 20 Fractions → RP-LC → 20 x 192 = 3840 MALDI-SPOTS

Apoptosis (125µg) → Label with heavy tags → Combine & digest (Glu-C) → SCX-LC → 20 Fractions → RP-LC → 20 x 192 = 3840 MALDI-SPOTS
SCX-LC Separation

20 Fractions a 2 min

Gradient: In 25 min from 2-50% B, then in 10 min to 100% B
A: 25% ACN, 10mM KH$_2$PO$_4$, pH = 3
B: 25% ACN, 10mM KH$_2$PO$_4$, pH = 3, 500mM KCl
RP-LC Separation
Spotting MALDI-Targets

Tray for six 192 MALDI-targets, in total 1152 Spots
Protein Quantification and Identification

MALDI-TOF/TOF
MS- and Data Analysis

Mass Spectrometer

Spotfire Server

MASCOT Server

MS-Data

Precursor

MSMS-Data

T2 System - TOF TOF

T2 ORACLE

DB ORACLE
Data Overview I
Data Overview II
Data Analysis

**Spotfire DecisionSite**

1) Elimination of false positive identified peptides
2) Deletion of incorrect quantified peptides
3) Detection of post-translational modified peptides
4) Data visualisation
5) Data exchange
Data Analysis

Spotfire DecisionSite

1) Elimination of false positive identified peptides
2) Deletion of incorrect quantified peptides
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Elimination of False Positive Identifications

2522 peptides with identification probability >80%
Elimination of False Positive Identifications

1037 peptides with identification probability >98%
Data Analysis

**Spotfire DecisionSite**

1) Elimination of false positive identified peptides

2) **Deletion of incorrect quantified peptides**

3) Detection of post-translational modified peptides

4) Data visualisation

5) Data exchange
Deletion of Incorrect Quantified Peptides

1037 peptides with identification probability >98%
Deletion of Incorrect Quantified Peptides

950 peptides correctly quantified and identified
Data Analysis

**Spotfire DecisionSite**

1) Elimination of false positive identified peptides
2) Deletion of incorrect quantified peptides
3) Detection of post-translational modified peptides
4) Data visualisation
5) Data exchange
Analysis of PTMs

The dataset was searched for the following post-translational modifications (PTMs):

- Acetylation (K and protein n-terminus)
- Formylation (protein n-terminus)
- Methylation (K and R)
- Phosphorylation (S, T, Y)
Detection of Post-translational Modifications

63 peptides are post-translational modified
Spotfire Decision Site

1) Elimination of false positive identified peptides
2) Deletion of incorrect quantified peptides
3) Detection of post-translational modified peptides
4) Data visualisation
5) Data exchange
Scatter-Plot of all Peptides
Zoomed Scatter-Plot
Generation of Average Ratios

Protein is not regulated
Generation of Average of all Ratios

Important for normalisation of the protein regulation
Generation of Protein Summary Table
Generation of Protein Summary Table

Number of unique proteins: 413
Generation of Protein Summary Table
Protein Summary Table

Unfortunately only available as web page and has to added manually into DecisionSite
Importing Protein Summary Table Data
Importing Protein Summary Table Data
Normalisation of Mean Ratio

Expression: \( \frac{\text{Mean Ratio}}{0.716} \)
Final Visualisation of Protein Regulation
Final Visualisation of Protein Regulation
Up-regulated Proteins

2 Ratios
List of all Up-regulated Proteins
Data Analysis

Spotfire DecisionSite

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Data Exchange with Spotfire Posters
Results

• In total 3840 MS-spectra (13.3h) and 13462 pairs for MS/MS-analysis (37.4h) were acquired

• This corresponds to 413 unique proteins (average = 2.3 peptides/protein)

• 31 proteins were found to be up- and 33 down-regulated during apoptosis

• 25 acetylation, 2 methylation and 2 phosphorylation sites could be identified
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