Symposium on Protein Fractionation

Proteomic Analysis of Organ-Specific Breast Cancer Metastasis

Speaker: Emily I. Chen, Ph.D
The Scripps Research Institute
Department of Cell Biology
CANCER METASTASIS

BREAST CANCER METASTASIS

- Parent Breast Cancer Cell
- Brain Metastasis
- Lymph Nodes Metastasis
- Liver Metastasis
- Lung Metastasis
- Bone Metastasis
ULTIMATE GOAL OF THE STUDY

Protein Signature of Organ-Specific Metastasis

Parent Breast Cancer Cell

Brain Metastasis

Bone Metastasis
Characterization of Organ-Specific Breast Cancer Metastasis

Step 1: Generation of organ-targeted cancer cells

Heterogeneous Parent Breast Cancer Cells → Tumor/Host Interaction → Brain Metastasis

Heterogeneous Parent Breast Cancer Cells → Tumor/Host Interaction → Bone Metastasis

Step 2: Proteome analysis of organ-targeted cancer cells

Fractionation of complex cell lysate + MudPIT
(ProteomeLab PF 2D)
ProteomeLab PF 2D PROTEIN FRACTIONATION SYSTEM

1D Isoelectric focusing
2D reverse phase

By Beckman Coulter
MUDPIT
(Multidimensional Protein Identification Technology)

1. Sample preparation
2. Digestion
3. Purification of peptides
4. Preparation of column
5. MudPIT

SCX  RP

2D Chromatographic Separation of Peptides

Identify Proteins in Mixture

Mass spectrometer

Identify Proteins in Mixture

Digested Peptides

Mass spectrometer

Identify Proteins in Mixture

Digested Peptides

Mass spectrometer
PROTEOME ANALYSIS OF CELL LYSATES

1D Protein Fractionation (1mg)

↓

Protein Precipitation

↓

Rapigest + Trypsin Digest

↓

2D LC-MS/MS Analysis
Protein Fractionation by ProteomeLab PF 2D System

1 fraction → 10 fractions

pH Tracer

AU

Minutes
Protein Fractionation by ProteomeLab PF 2D System

Minutes

AU

BCM2 Bone

BCM2 Parent

BCM2 Brain
PEPTIDE FILTERING CRITERIA

+1 Xcorr 1.8
+2 Xcorr 2.5
+3 Xcorr 3.5
Minimum DeltCN: 0.08
Minimum Length: 7 a.a.
Maximum Length: 100 a.a.

BCM2 Parent  BCM2 Bone  BCM2 Brain

Peptide IDs  26,577  26,739  29,222
PROTEIN IDENTIFICATION IN THREE METASTATIC BREAST CANCER CELL LINES

Total Protein Identified: 3688
Parameter: minimum 2 peptides, half tryptic

BCM-2 Parent

BCM-2 Bone

BCM-2 Brain

798
207
599
324
274
588
SPECIFICITY OF PROTEIN SEPARATION

The graph shows the percentage of protein IDs across different fractions for BCM2 Parent, BCM2 Bone, BCM2 Brain. The x-axis represents the number of fractions, while the y-axis shows the percentage of protein IDs.

- BCM2 Parent: More specific
- BCM2 Bone: More abundant
- BCM2 Brain: Mixed characteristics
SPECIFICITY OF PROTEIN SEPARATION

BCM2 Parent

pH 7.82  7.52  7.22  6.92  6.62  6.32  6.02  5.71  5.45  5.30  3.98

Fraction Number

ALL
No Histones
Single Elution
Comparison Of Identified Proteins According To PI

![Graph showing comparison of identified proteins according to PI.]
Comparison Of Identified Proteins According To MW
CELLULAR LOCALIZATION OF IDENTIFIED PROTEINS

Total Protein Identified: 3688

- Cytoplasm: 56%
- Nucleus: 18%
- Membrane: 13%
- Extracellular: 2%
- Unknown: 12%
Semi-Quantitative Analysis of Differential Proteome Expression


A Model for Random Sampling and Estimation of Relative Protein Abundance in Shotgun Proteomics

Hongbin Liu, Rovshan G. Sadygov, and John R. Yates, III
## Semi-Quantitative Protein Expression Analysis
### Based on Spectra Counts

NP_003968.1 | Aryl hydrocarbon receptor interacting protein; aryl hydrocarbon receptor-interacting protein; HBV-X associated protein [Homo sapiens]

### BCM2 Parent

<table>
<thead>
<tr>
<th>Peptide Sequence</th>
<th>Xcorr</th>
<th>DeltCN</th>
<th>SpeCount</th>
<th>SeqCov</th>
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</thead>
<tbody>
<tr>
<td>* R.EGEIAQFLCDIK.H</td>
<td>4.04</td>
<td>0.243</td>
<td>3</td>
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<tr>
<td>* K.EQPGSPEWIQLDK.Q</td>
<td>3.07</td>
<td>0.160</td>
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<td>* K.AHAAVWNAQEAQADFAK.V</td>
<td>4.78</td>
<td>0.441</td>
<td>4</td>
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<tr>
<td>* K.VLELDPALAPVVS.R.E</td>
<td>4.14</td>
<td>0.495</td>
<td>3</td>
<td></td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
<td></td>
<td></td>
<td><strong>17</strong></td>
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</table>

### BCM2 Bone

<table>
<thead>
<tr>
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<th>DeltCN</th>
<th>SpeCount</th>
<th>SeqCov</th>
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<tr>
<td>* Y.RTLHSDDEGTVLDDSR.A</td>
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<tr>
<td>* K.VLELDPALAPVVVS.R.E</td>
<td>3.59</td>
<td>0.277</td>
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<td><strong>Total</strong></td>
<td><strong>3</strong></td>
<td></td>
<td></td>
<td><strong>9.1</strong></td>
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</tbody>
</table>

### BCM2 Brain

13 peptides identified

| **Total** | **64** | **38.8** |
Aryl Hydrocarbon Receptor Interacting Protein

H.AAVWNAQEAQADFAK.V

Mass: 1620.53  Datfile: PARC_040823_bcm2_brain_frac18_02  Scan number: 16668  Charge: 2  Database:

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<tr>
<th>Rank/Sp</th>
<th>(M+H)+</th>
<th>deltCn</th>
<th>XCorr</th>
<th>Sp</th>
<th>Ions</th>
<th>Reference</th>
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<td>1/1</td>
<td>1620.762</td>
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<td>4.6600</td>
<td>2464.0</td>
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<td>g:45020099refNP_003968.1</td>
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<td>2/57</td>
<td>1621.704</td>
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<td>2.5601</td>
<td>573.7</td>
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<td>3/49</td>
<td>1620.756</td>
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<td>2.5306</td>
<td>579.3</td>
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<td>g:3834838382refNP_940948.1</td>
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<td>4/110</td>
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<td>0.4600</td>
<td>2.5166</td>
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<td>g:4503127refNP_001894.1</td>
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<td>5/14</td>
<td>1622.836</td>
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<td>g:42476209refNP_057711.2</td>
<td>E.KLDAGBQRLMNEAF.Q</td>
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Semi-Quantitative Protein Expression Analysis
Based on Spectra Counts

gi|4504327|ref|NP_000174.1| hydroxyacyl dehydrogenase, subunit B;
3-ketoacyl-Coenzyme A thiolase [Homo sapiens]

BCM2 Parent

<table>
<thead>
<tr>
<th>Peptide Sequence</th>
<th>Xcorr</th>
<th>DeltCN</th>
<th>SpeCount</th>
<th>SeqCov</th>
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</thead>
<tbody>
<tr>
<td>* R.TPFLLSGTSYK.D 2</td>
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<td>* K.DQLLLGPTYATPK.V 2</td>
<td>3.4826</td>
<td>0.2458</td>
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</table>

**total** 10 9.9

BCM2 Bone

<table>
<thead>
<tr>
<th>Peptide Sequence</th>
<th>Xcorr</th>
<th>DeltCN</th>
<th>SpeCount</th>
<th>SeqCov</th>
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</thead>
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<tr>
<td>* R.NVVVVDGVR.T 2</td>
<td>2.6467</td>
<td>0.2107</td>
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<td>* R.AALTGLLHR.T 2</td>
<td>2.8662</td>
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<td>* R.LAAAFAVSR.L 2</td>
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<td>* R.LEQDEYALR.S 2</td>
<td>2.6572</td>
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<td>* M.IVEAYPK.- 1</td>
<td>1.9921</td>
<td>0.183</td>
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**total** 6 9.1

BCM2 Brain

16 peptides identified

**total** 61 29.3

Express in Brain/Neuron but not Breast
SUMMARY

Aim 1: Fractionate complex mammalian cell lysate
Analyze and obtain protein expression profiles

Aim 2: Generate differential protein expression analysis
Build on more specific hypotheses

Aim 3: Biological interrogation
IMPORTANT ISSUES FOR PROTEIN FRACTIONATION

- Starting Material
- Sample Complexity
- High abundant proteins
- Protein Solubility
- Post-translation protein modifications
IMPORTANCE OF PROTEIN FRACTIONATION

- High Abundance
- Medium Abundance
- Low Abundance

Protein Fractionation
- Reduce Sample Complexity
- Increase Protein Identification
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Department of Molecular & Experimental Medicine

Brunhilde Felding-Harbermann, Associate Professor