Proapolipoprotein A1 Demonstrates Improved Potential as Marker for Brain Metastases without Interference from SVI D

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Introduction

• S100β has been identified as a sensitive marker for BBB integrity associated with brain metastases which occur in 2-15% of lung cancer patients.

• Minor leakage of the BBB by SV1D causes elevations in S100β. This limits its usefulness as a reliable marker for brain metastases.

• This talk will characterize a protein marker that is elevated in the presence of brain metastases, yet not significantly elevated by SV1D.
MRI of Metastasis vs. SVI D

SVI D
(Small Vessel I schemic Disease)

Metastasis
Metastases & SVID

- No Met, No SVID: 32 (31%)
- No Met, Yes SVID: 57 (55%)
- Yes Met, No SVID: 14 (14%)
- Yes Met, Yes SVID: 4
Age vs. $S100\beta$
FIGURE 1: Relationship between serum S100β and imaging changes

A. Three groups of patients could be distinguished radiologically (see below for images). A statistically significant difference was found between S100β levels in subjects with normal MRI/CT findings and those either diagnosed with metastatic brain tumor or microvascular changes. No significant difference was found between the two latter groups. * and ** indicate $p<0.05$ and $p<0.01$ by ANOVA.

B. Box plot diagram of the data shown in A. The curve fitting the data point was fitted by a Lorentzian fitting routine in Origin 6.0.
MRI of Converted Patient

LT #1

LT #2
Fluorescent Labeled 2D Gels

Composite Gel (LT#1 + LT#2)

- Proapolipoprotein A1
  - MW: 28.9 kDa, pI: 5.45-5.60
- Transthyretin
  - MW: 15.9 kDa
  - pI: 5.52
2-Dimensional Protein Electrophoresis

R-COOH + OH- → RCOO⁻ + H₂O
At high pH, carboxylic side groups are negatively charged.

R-COO⁻ + H⁺ → R-COOH
At low pH, carboxylic side groups of AAs are neutral.

R-NH₂ + H⁺ → R-NH₃⁺
At low pH, amino-type side groups of AAs are positively charged.

R-NH₃⁺ + OH⁻ → R-NH₂ + H₂O
At high pH, amino-type side groups are neutral.
2D Western Blotting

Western Lightning™
Chemiluminescence Reagent Plus

Separate sample proteins by electrophoresis

Transfer to membrane (PVDF Polyvinylidene Difluoride)

Block non-specific binding sites (5% Blotting Grade Non-Fat Dry Milk)

Incubate with primary antibody

Introduce HRP by:

Direct HRP labeling of primary or secondary antibody

Mix equal volumes of solutions from Bottles 1 and 2 (Bottle 1: Enhanced Luminol Reagent) (Bottle 2: Oxidizing Reagent)

Incubate with membrane

Expose X-ray film to membrane in an autoradiographic cassette

http://www.sciencedirect.com/science
Coomassie 2D Gel Comparison (Lung Metastases Study)

Table 2: Summary of results

<table>
<thead>
<tr>
<th>Investigator sample name</th>
<th>Digest sample number</th>
<th>Identification (NCBI accession number, calculated MW, calculated pI)</th>
<th>Peptides (%sequence coverage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Janigro, 6-14-06 2D gel of LT2, band 176</td>
<td>SB9-71-13</td>
<td>IgG kappa chain (#4176418, 23 kDa, pI 6.9)</td>
<td>11 (63%) 6 (37%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immunoglobulin lambda light chain (#6467839, 23 kDa, pI 6.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgM (#1020013, 11 kDa, pI 9.0)</td>
<td>2 (21%)</td>
</tr>
<tr>
<td>Band 134</td>
<td>SB9-71-14</td>
<td>Transferrin (#339685, 12 kDa, pI 5.3)</td>
<td>9 (95%)</td>
</tr>
<tr>
<td>Band 131</td>
<td>SB9-71-15</td>
<td>Proopiolactoprotein (#178775, 28 kDa, pI 5.4)</td>
<td>27 (81%)</td>
</tr>
<tr>
<td>Area 4</td>
<td>SB9-71-16</td>
<td>Proopiolactoprotein (#178775, 28 kDa, pI 5.4)</td>
<td>10 (34%)</td>
</tr>
<tr>
<td>Area 5</td>
<td>SB9-71-17</td>
<td>Proopiolactoprotein (#178775, 28 kDa, pI 5.4)</td>
<td>3 (12%)</td>
</tr>
</tbody>
</table>
Apolipoprotein vs. Proapolipoprotein

Definition of Apolipoprotein A-I (taken from MedicineNet.com)

**Apolipoprotein A-I: APOA-I.** The major protein component of HDL (high density lipoprotein) and a relatively abundant plasma protein. APOA-I is instrumental in promoting the transfer of cholesterol into the liver where it is metabolized and then excreted via the intestine from the body. The gene locus for APOA-I is on chromosome 11q23. A number of genetic variants of APOA1 are known, including ApoA-I Milano.

Primary accession number P02647 (Entry name APOA1_HUMAN)
Length: 267 AA, Theoretical pI/MW: 5.56/30,778 Da

**Serum Prevalent Apolipoprotein**
Length: 243 AA, Theoretical pI/Mw: 5.27 / 28,078.62

**Proapolipoprotein A1: ACCESSI ON   AAA51747**
Length: 249 AA, Theoretical pI/MW: 5.45 /28,961.60

Serum Apolipoprotein Sequence Starting Point
Expanded 2D-Gels for Proapolipoprotein Analysis

2D Coomassie & Western Blot Comparisons (pI 4.7-5.9)

- MRI-, SVID-
  - Western Blot
  - S100β = 0.035 ng/mL
  - FC = -13.32
  - Coomassie 2D Gel
  - FC = +4.47

- MRI+, SVID-
  - Western Blot
  - S100β = 0.211 ng/mL
  - FC = +5.76
  - Coomassie 2D Gel
  - TTRmono
  - FC = +11.17

- MRI+, SVID+
  - Western Blot
  - S100β = 0.106 ng/mL
  - FC = 1.00
  - Coomassie 2D Gel
  - TTRmono
  - FC = 1.00

- MRI-, SVID+
  - Western Blot
  - S100β = 0.055 ng/mL
  - FC = -1.19
  - Coomassie 2D Gel
  - TTRmono
  - FC = +1.43
Apolipoprotein A1 Western Blot Comparison

Western Blot for Apolipoprotein A1

MRI +, SVID-
S100β = 0.211 ng/mL

MRI -, SVID-
S100β = 0.106 ng/mL

MRI -, SVID-
S100β = 0.035 ng/mL

MRI +, SVID+
S100β = 0.055 ng/mL

Proapoplipoprotein A1 (uncleaved CSF protein)
Theoretical pl/Mw: 5.45 / 28961.60

Apolipoprotein A1 (cleaved serum protein)
Theoretical pl/Mw: 5.27 / 28078.62
Imaging and SVI D

Bar Graph

Box Scatter Plot

S\text{100}\beta (\text{ng/ml})

MET-/SV-
MET+/SV-
MET-/SV+
MET+/SV+

n=32
n=9
n=57
n=5

p<0.05
n.s.

S\text{100}\beta (\text{ng/ml})

MET-/SV-
MET+/SV-
MET-/SV+
MET+/SV+

1.128 LT 011305-0900
0.88 LT 031904-1115
0.59 LT 070103-1600

p<0.05
n.s.
**Data Analysis**

**Age Comparison of SVID in Lung Tumor Patients**

- Age distribution for SVID- patients: 62.00, 64.00, 66.00, 68.00 years.
- Age distribution for SVID+ patients: 62.00, 64.00, 66.00, 68.00 years.
- p-value: 0.997

**S100β Comparison of SVID in Lung Tumor Patients**

- S100β levels for SVID- patients: 0.000, 0.050, 0.100, 0.150, 0.200 ng/ml.
- S100β levels for SVID+ patients: 0.000, 0.050, 0.100, 0.150, 0.200 ng/ml.
- p-value: 0.540

**MRI Scan Comparison of SVID in Lung Tumor Patients**

- % MRI Positive for SVID- patients: 10.0%, 15.0%, 20.0%, 25.0%, 30.0%.
- % MRI Positive for SVID+ patients: 10.0%, 15.0%, 20.0%, 25.0%, 30.0%.
- p-value: <0.05
MRI Findings & Proapolipo A1

* p<0.01
Proapolipoprotein A1 & S100β

![Graph showing the relationship between S100β levels and fold-change difference from MRI- to MRI+ conditions. The graph includes data points for MRI-SVID+, MRI-SVID-, MRI+ SVID+, and MRI+ SVID-.]
Conclusions

- Previous studies (Marchi et al., Clinica Chimica Acta 2004 342:1-12 and Vogelbaum et al., Cancer 2005 104(4):817-24) have found protein markers such as S100β and TTR-monomer to be sensitive markers of BBB integrity in evaluating brain metastases, yet they are often elevated with SVI D.

- The Apolipoprotein A1 variant, Proapolipoprotein A1 (NCBI # 178775, MW= 28.9 kDa, pl = 5.45) is not significantly affected by SVI D relative to its elevation in lung tumor patients in which brain metastases are detected, on follow-up, by MRI.