Proteomics-driven design of endothelial stress-based protein array for disease prognostics - applied to plasma and cerebrospinal fluid

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Proteome challenges with EV
Nasty bio fluids & high complexity for proteome scientists..

+1470 proteoforms

+6 proteoforms

(Bellingham et al, 2012)
Stress and treatments

- Etomoxir
- Oxidative stress
- Fe$^{3+}$

Enrichment

CD9, CD44, CD63, CD41, CD81, and MHC2R.

Proteomics

Pros:
- Comprehensive analysis

Cons:
- The sample purity
The EV Array
EV surface exposed membrane protein markers

The EV array principle
Pros:
- Multiplexed up to 70 Ab

Cons:
- Only human samples

Application
Pros:
- Multiple bio fluids

Cons:
- Availability of Ab
- Only surface-exposed biomarkers

Proteome driven design of EVarray

Combined empirical and literature information...

- No enrichment needed
- mAb based capture and detection + 70 parallel detections

**Enrichment imperative**

**Quantitative Identification**

Protein ID
PTM’s
Abundance
Topology
Complexes

**Quantitative Phenotyping**

PubMed
Vesiclepedia
ExoCarta
Endothelial stress in Neuroinflammation

Inside-out or outside-in in MS - That's the BIG question...

- From OUTSIDE IN:
  - Inflammation
  - Demyelination
  - Axonal degeneration

- From INSIDE OUT:
  - Inflammation
  - Demyelination
  - Axonal degeneration
Multiple Sclerosis & MRI correlation
Combined proteomics and Protein arrays (Mesoscale; EVarray)

5 Multiple Sclerosis patients
Weekly MRI scanning (8 weeks)

FLAIR MRI
Gd MRI
Plasma samples

EV Array
33 markers

41 soluble markers
cyto- and chemokines

Proteomics
Multiple Sclerosis & Cytokine, EV correlation

Combined proteomics and Protein arrays (Mesoscale; EVarray)

Quantitative Proteomics at proteome level

Mesoscale

Endothelial stress optimized EVarray

<table>
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<th>Endothelial</th>
<th>Platelet</th>
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Multiple Sclerosis & Cytokine, EV correlation

Combined proteomics and Protein arrays (Mesoscale; EVarray)
Acknowledgements

EVsearch - A Danish research center with focus on extracellular vesicles
Acknowledgements
A wide network of collaborators

Laboratory for Medical Mass Spectrometry, Aalborg University, Denmark
- Tue Bjerg Bennike
- Michael Kruse Meyer
- Kenneth Kastaniegaard

Aarhus University, Denmark
- Gunna Christiansen

Aalborg University Hospital
- Malene M. Jørgensen
- Rikke Bæk
- Kim Varming

Odense University Hospital

Havard Medical School
- Zsolt Illes
- Tobias Sejrbaek
- Francois Cotton
- Charles Guttmann

Aalborg University, Biomedicine
- Torben Moos
- Vladimir Zachar
- Søren Nielsen
- John Nieland

Odense University
- Christian Wiwie,
- Jan Baumbach

Funding sources

[Various logos and institutions]