Personalized Medicine for Children with TBI

CCCF 2012, Toronto
J. Hutchison, MD
The Honourable Leona Aglukkaq, Minister of Health, announces a $67.5 million dollar investment ($22.5 million from the Canadian Institutes of Health Research, $40 million from Genome Canada and $5 million from the Cancer Stem Cell Consortium) to support funding of research teams in the area of Personalized Health.
Ottawa, Ontario (January 31, 2012)
Acquired brain injury in infants, children and adolescents

• The most common causes of death and acquired disability in the world.
• As many as fifty percent of survivors of severe TBI will have moderate to severe disability or persistent vegetative state.
• These disabilities have a profound effect on the quality of life of the patient and their families.
• There is an urgent need to develop novel methods to monitor the brain and predict and prevent cerebral edema and cell death, the final common pathway for mortality and long term disability among survivors.
External funding at SickKids, Toronto

Annual budget 2011

$ Millions

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<th>TBI</th>
<th>Brain tumor</th>
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<td>$ Millions</td>
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Areas in **urgent** need of further research in Children and Youth with TBI

- Epidemiology
- Neuromonitoring
- Neuroimaging
- Mechanisms of secondary injury and cell death
  - Development of new therapies
- Randomized controlled trials
- Prognosis
- Biomarkers
- Genetics
Gene

mRNA

Protein

Alternative splicing of transcripts

Genes 30,000

Proteins \(> 1 \times 10^6\)

Cleavage

Post-translational modification

Protein
Genes
Microarray analysis of genes affected by hypothermia in human cerebral endothelial cells

Hypothermia (32°C) or Normothermia (37°C)

Cluster analysis

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<tr>
<th>Up-regulated</th>
<th>Down-regulated</th>
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<tr>
<td>MHC class II</td>
<td>Adenosine deaminase</td>
</tr>
<tr>
<td>FK-506 binding protein 12</td>
<td>Runt-related transcription factor 1</td>
</tr>
<tr>
<td>Cystatin B</td>
<td>Peroxyredoxin 1</td>
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<tr>
<td>Interleukin 11</td>
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<tr>
<td>Cathepsin S</td>
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<tr>
<td>Metabotropic glutamate receptor 5</td>
<td></td>
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<tr>
<td>Endothelin receptor, type A</td>
<td></td>
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<tr>
<td>AP1 binding protein</td>
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<tr>
<td>Interferon-regulatory factor 2</td>
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</table>
PROTEOMICS
Proteomics

• Method of identifying and quantifying multiple proteins in body fluids or tissue
  – Eg. Protein changes in response to TBI

• Methods of protein separation, quantification and identification
  – Large number of proteins
  – Evolving

• Bioinformatics methods
  – To analyze large database
  – Evolving
Objectives of Proteomic Studies in Brain Injury

**Discovery**

*Prognostic markers* (e.g., specific neuronal or glial proteins/metabolites in CSF or blood will be correlated with the severity of TBI and long-term outcomes)

*Peripheral markers of blood-brain barrier permeability* or brain vascular pathology after TBI

**Pharmacoproteomics**

Analyses of drug-induced changes in protein profiles and clinical outcomes in individual patients

**Mechanisms of secondary injury**

Lead to drug/therapy discovery
Gel and label-free proteomics: nanoliquid chromatography-mass spectrometry (MS)/MS methods

Compare And Quantify

Identify

Protein biomarkers in serum of pediatric patients with severe TBI identified by ICAT-LC-MS/MS.

Objective

Use advanced gel-free proteomics tools including ICAT and 2D HPLC to analyze and compare low abundant proteins in:

(1) Plasma of pediatric patients with severe TBI

(2) Control serum (no trauma)
ICAT pairs identified in sera from patient #403 and control after nanoLC-MS analysis

Fold change -3.10

Fold change +4.06

SCATTER PLOT

- Differentially expressed (712 pairs)
- Unchanged (1814 pairs)

Patient serum (#403)

Control Serum

Log (Peak intensity of heavy pair)

Log (Peak intensity of light pair)
### Brain-selective proteins identified in sera of Children with TBI

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Tissue specificity</th>
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<tbody>
<tr>
<td>Spectrin alpha chain, <strong>brain</strong></td>
<td>Non-erythroid; cleaved by calpain and caspase-3; detected in CSF after TBI</td>
</tr>
<tr>
<td><strong>Neuron-specific</strong> enolase (NSE)</td>
<td>The alpha/gamma heterodimer and the gamma/gamma homodimer found in neurons</td>
</tr>
<tr>
<td>Neurofilament triplet H protein (200 kDa neurofilament protein)</td>
<td>Neuron-specific cytoskeletal protein</td>
</tr>
<tr>
<td>Amyloid beta A4 protein precursor (APP) (ABPP)</td>
<td>Highly enriched in the brain</td>
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<tr>
<td>Prostaglandin-H2 D-isomerase precursor (PDG2 synthase)</td>
<td>Abundant in the brain and CNS where it is expressed in the <strong>blood-brain barrier</strong></td>
</tr>
<tr>
<td>Microtubule-associated protein tau (Neuro fibrilary tangle protein)</td>
<td>and secreted into the CSF</td>
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<tr>
<td>Contactin 3 precursor (Brain derived immunoglobulin superfamily protein- BIG-1)</td>
<td>Expressed in neurons. Isoform PNS-tau – in peripheral nervous system; others are expressed in the CNS</td>
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<tr>
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<td>Expressed in the brain – frontal and occipital lobe, cerebellum and amygdala</td>
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Hierarchical clustering of 98 differentially expressed proteins in comparison to admission GCS and S100B
CONCLUSIONS

- Adaptation of gel-free, ICAT-based proteomics that included depletion of abundant serum proteins and use of common control serum allowed identification of a large number (>500) of differentially expressed peptides/proteins in each patient from samples taken within 24 hours after injury.

- Analyses of differentially expressed peptides/proteins reveal novel brain proteins released into serum.

- Systematic ICAT-based proteomics analyses of patient sera can be used to identify peripheral ‘biomarkers’ of TBI.
Safar Resuscitation Research Centre, Pittsburgh

- CSF from external ventricular drain within 24 hours of injury
- Children ≤ 4 years
  - N=13 children with inflicted TBI
  - N=13 children with non-inflicted TBI
- Pooled CSF – 2 Dimensional gel electrophoresis comparison between the two groups
- Protein changes confirmed by Westerns
Haptoglobin (HP) and their precursors in CSF of children with non-inflicted (n) compared to inflicted (i) TBI.
Prostaglandin D2 Synthase in CSF of children with non-inflicted (n) compared to inflicted (i) TBI
Cystatin C in CSF of children with non-inflicted (n) compared to inflicted (i) TBI
Conclusions

• Haptoglobins and their precursors – acute phase reactants – are increased 3 to 5-fold in nTBI compared to iTBI CSF

• Prostaglandin D2 Synthase and Cystatin C are increased 12 and 7-fold in iTBI compared to nTBI CSF
Metabolomics
Objective: Determine if serum amino and organic acids at day 1 post-injury are associated with outcome at 6 months post-TBI in children

Methods:
- Measured 36 different amino and organic acids using mass spectrometry
- Compared 4 children with bad outcome to 14 children with good outcome

Results:
- 6 amino acids ↓ in those with bad outcome
- 3 – methyl-histidine ROC AUC 1.0
Lipidomics
Novel brain trauma biomarkers: Significant post-traumatic changes in serum levels of eicosanoids and leukotrienes

Lo T et al. PCCM 2011 A113

Objective: To determine if eicosanoids and leukotrienes are elevated in the serum of children with severe TBI compared to healthy controls

• Methods:
  – Measured 22 lipids in serum using mass spectrometry
  – Compared 6 severe TBI to 8 healthy controls

• Results:
  – 8/22 (36%) lipids were elevated in the serum of children with TBI compared to controls
The Molecular Fingerprint of Traumatic Brain Injury

MOLECULAR BIOMARKERS → BLOOD

BRAIN

CSF

MOLECULAR BIOMARKERS
Systematic review: 24 biomarkers which are associated with outcome in children with TBI?
Shibata ARO, Ide K, Augustinavicius J, Lo T-M, Nickel C, Hutchison JS, Guerguerian A-M

- Brain specific
  - GFAP, myelin basic protein, nerve growth factor, NSE, phosphorylated NF H, S100B, α spectrin breakdown product 145

- Inflammatory
  - high mobility group box-1, Interleukin (IL)-1β, IL-6, IL-8, IL-10, L-selectin

- Apoptotic
  - Bcl-2

- Other
  - Doublecortin, fibrin degradation products (FDP), Heme oxygenase 1 (HO-1), pancreatic enzymes, quinolinic acid and ubiquitin carboxyl-terminal esterase L1

- Amino acids
  - aspartate, glutamate and glycine

Papers published by 1) Haqqani A et al. and 2) Fraser D. et al. on behalf of the CCCTBG
Overall Conclusion

• ‘Omics provide powerful tools for discovery of biomarkers for diagnosis, prognosis and to provide insights into mechanisms of brain injury.
Acknowledgements

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- Lipidomics: Martin Post, SickKids, Toronto, ON
- Metabolomics: J. Suh, Children’s Hospital Oakland Research Institute, Oakland, CA