Proteomic identification of biomarkers of traumatic brain injury

Kevin KW Wang, Andrew K Ottens, Ming Cheng Liu, Stephen B Lewis, Colleen Meegan, Monika W Oli, Frank C Tortella and Ronald L Hayes

Traumatic brain injury (TBI) is a major national health problem without a US Food and Drug Administration-approved therapy. This review summarizes the importance of discovering relevant TBI protein biomarkers and presents logical rationale that neuroproteomic technologies are uniquely suited for the discovery of otherwise unnoticed TBI biomarkers. It highlights that one must make careful decisions when choosing which paradigm (human vs. animal models) and which biologic samples to use for such proteomic studies. It further outlines some of the desirable attributes of an ideal TBI biomarker and discusses how biomarkers discovered proteomically are complementary to those identified by traditional approaches. Lastly, the most important sequela of any proteomically identified TBI biomarker is validation in preclinical or clinical samples.


Traumatic brain injury: a human disease in need of diagnostic biomarkers & new drug development

Utility of biomarkers for TBI

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Keywords:
biomarkers, neuroproteomics, proteomics, TBI, traumatic brain injury

Utility of biomarkers for TBI

TBI patients are usually initially assessed by the Glasgow Coma Score (GCS) on hospital admission and during routine neurologic examinations in the acute phase of care. The three categories of TBI that are used based on the GCS are: mild (GCS 13–15), moderate (GCS 9–12) and severe (GCS ≤ 8) [6]. GCS can be further complemented by the use of the Glasgow Outcome Score (GOS), the Glasgow Outcome Score-Extended (GOS-E) and other neuropsychologic testing. Analysis of specific biochemical markers is a mandatory component of diagnosing dysfunction in a number of organs such as the heart, as in myocardial infarction. However, there are no biomarkers of proven clinical utility for TBI and stroke. TBI is difficult to assess and clinical examinations are of restricted value during the first hours and days after injury, at a time when therapeutic intervention is most likely to be effective. Conventional diagnosis of TBI is based on neuroimaging techniques such as computed tomography (CT) scanning, magnetic resonance imaging (MRI) and single-photon emission CT scanning [7,8,9]. However, CT scanning has low sensitivity to diffuse brain damage,
and the availability of MRI is limited [10–12]. Single-photon emission CT scanning detects regional blood-flow abnormalities that are not necessarily related to structural damage. In addition, these brain scans are highly costly – MRI (US$1100–1500) or CT (US$700–1000) – and scheduling is often delayed (subject to availability) and cannot be carried out in a short time interval (due to potential adverse effects and patient safety issues). Collectively, the current TBI diagnostic tools are valuable in the assessment of the TBI patient’s general brain functional status, yet they are lacking in the ability to precisely define and quantify the actual intensity of the brain injury. This current review proposes that protein biomarkers detected and monitored in biologic fluids could provide confirmation of the severity of injury related to the levels of clinical markers, monitor progress of treatment and identify drug therapy targets. Temporal profiles of changes in biomarkers could guide timing of treatment. Biomarkers could also provide clinical trial outcome measures obtainable in a more cost-effective and ready manner than conventional neurologic assessments, thereby significantly reducing the risks and costs of human clinical trials. Despite disappointments in therapy development, management of patients with severe TBI in accordance with evidence-based guidelines has reduced mortality and the number of poor outcomes. Early detection of TBI biomarkers could facilitate further improvements in patient management in emergent and intensive care environments by providing more accurate early diagnosis and prognosis, which is currently lacking. By providing more accurate early diagnosis, treatment is more precise with respect to the extent of injury, and being aware of prognosis directs clinicians toward more specific and appropriate long-term planning for the patient. This most assuredly conducts patient management in a more appropriate and cost-effective manner with the use of critical care beds, ward hospital beds and rehabilitation facilities.

Choosing the right TBI samples for biomarker studies

For the purpose of this review, the focus is restricted to proteomic biomarker studies for TBI performed in either human or animal studies. When comparing human versus animal studies, there are pros and cons in each scenario. Regarding the animal sample types that can be exploited for proteomic analysis, they will include brain tissues, cerebrospinal fluid (CSF) and blood (serum and plasma) (Table 1). For human TBI studies, samples that are the easiest to obtain are blood samples (which are further fractionated into plasma or serum). For severe TBI, ventriculostomy is routinely performed for patient care, so CSF drawn straight from the ventricle is readily accessible for biomarker studies. CSF is attractive as its status usually reflects that of the CNS itself. Nevertheless, one of the major challenges of using clinical sample-based proteomic studies is that it is extremely difficult to control individual (biologic and genetic) and environmental variables (Table 1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Availability</th>
<th>Amount</th>
<th>Biomarker signal-to-noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human brain tissue</td>
<td>Low</td>
<td>Small</td>
<td>High</td>
</tr>
<tr>
<td>Human CSF</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium/low</td>
</tr>
<tr>
<td>Human plasma/serum</td>
<td>High</td>
<td>Large</td>
<td>Low</td>
</tr>
<tr>
<td>Animal brain tissue</td>
<td>High</td>
<td>Large</td>
<td>High</td>
</tr>
<tr>
<td>Animal CSF</td>
<td>Low</td>
<td>Small</td>
<td>Medium</td>
</tr>
<tr>
<td>Animal plasma/serum</td>
<td>High</td>
<td>medium</td>
<td>Low</td>
</tr>
</tbody>
</table>

CSF: Cerebrospinal fluid.

Brain samples

Brain tissue from human TBI studies would almost inevitably come from deceased TBI patients. These brain samples could be subjected to postmortem artifacts, compounded by various issues and significant time delays before samples can be obtained. The biggest advantage of animal neuroproteomic studies over human counterparts is the ability to obtain brain tissues in a controlled laboratory environment. Furthermore, it is possible to harvest samples from defined anatomic regions. For example, for the TBI studies, the authors often focus on cortical and hippocampal samples [13]. This is important, as different brain regions might be selectively more vulnerable to traumatic or ischemic insults. One significant point is the genetic variability of humans (polymorphisms) that complicate differential proteomic analyses.

CSF samples

CSF can be collected from the cisterna magna from laboratory animals, such as rat and mouse. CSF can contain rich brain proteome information that is relevant to disease diagnosis [14]. However, only approximately 50–100 µl can be withdrawn from rats and 25–30 µl from mice. Care must also be taken not
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Blood samples (serum & plasma)

Blood can be routinely collected in both human and animal TBI studies. Blood is generally further processed into either serum or plasma. Similar to CSF, as pointed out in the schematic in Figure 2, biomarkers generated in the brain tissue after TBI and present in CSF will ultimately find their way into the circulating blood, although in much lower levels. Consequently, information in the blood that would reflect the status of the brain, particularly after TBI due to possible blood-brain barrier (BBB) compromise, could be obtained [15,16].

One potential challenge when analyzing blood or plasma samples is the diversity in protein abundance in these blood fluids. A total of 90% of the whole serum proteome is distributed to approximately a dozen proteins (e.g., albumins and immunoglobulins), making the discovery of low-abundant TBI biomarker proteins almost impossible. Accordingly, it might be more feasible to perform biomarker discovery using brain tissue and CSF. Once potential biomarkers are identified, one can then employ specific enrichment methods (e.g., immunoprecipitation) or sensitive assay (e.g., sandwich enzyme-linked immunosorbent assay [ELISA]) for their quantification in serum or plasma.

Discovery of useful TBI biomarkers by neuroproteomic methods

While there are numerous proteomic methods for proteomic and biomarkers studies, this review focuses on two major approaches that are currently being used to discover useful TBI biomarkers in an unbiased fashion. The first method, protein separation tandem mass spectrometry (MS/MS), separates complex protein mixtures into discrete differential protein displays, allowing for robust MS/MS-based protein identification by comparing tryptic peptide spectra with proteome databases. The second method is a high-throughput (HTP) antibody-based microarray or panel, which requires minimal or no protein separation and relies on antibody-based selective detection to quantify changes of certain biomarkers in a treatment group over control. These two complementary methods are discussed below.

Protein separation MS/MS identification approach

Protein separation

In TBI neuroproteomic biomarker discovery studies, the authors focus on protein expression level or post-translational modification changes that occur in TBI. Consequently, it is important to devise methods in comparing and contrasting the two proteomic data sets, control versus TBI, in order to identify distinctive protein biomarkers uniquely up- or downregulated in TBI. However, the whole proteome in an organ such as the brain is extremely complex. No less than 20,000 proteins are present, not counting the large number of possible post-translational modifications. Hence, it is essential that complex protein mixtures (such as brain samples or biofluids) should be first subjected to multidimensional protein separation. No single protein separation method can satisfactorily resolve all proteins in a proteome, thus multidimensional separation takes advantage of differences in protein size, hydrophobicity, surface charge, abundance, as well as other properties. In this review, two mainstream protein separation methods used for proteomic analysis will be discussed: 2D gel isoelectric focusing (IEF)/electrophoresis and multidimensional liquid chromatography (LC).

2D gel electrophoresis

2D gel electrophoresis (2DE) is the oldest, most established protein separation method for the analysis of a proteome or subproteome [17–19]. It is achieved by subjecting protein mixtures to two protein separation methods under denaturing conditions in the presence of 6–8 M urea and an anionic detergent such as sodium dodecyl sulfate (SDS). Traditionally, proteins
are first separated based on their isoelectric point (pI) value with a tube gel (polyacrylamide) by IEF with the aid of mobile ampholytes with different values. After IEF, the tube gel is placed atop a polyacrylamide gradient gel within which the SDS-bound proteins are separated by size. Due to poor gel consistency, the IEF step (the first dimension) is most variable. However, a recent breakthrough in IEF technology utilizing immobilized pH gradient (IPG) strips provides improved robustness [20,21,22]. An advantage of this approach is that there are several web-accessible 2D gel protein spot databases with previously identified proteins annotated by pI and molecular weight values in X-Y coordinates [23–25]. It is worth noting that when 2D gels are used to contrast two samples (such as control vs. TBI brain samples), analysis is often compromised by the inevitable gel-to-gel variability in overlaying the exact location and pattern of protein spots. Consequently, protein identification only on the basis of the 2D gel pattern is nearly impossible. The recent advances in 2D differential in-gel electrophoresis (DIGE) have resolved this [16,27].

The fluorescent cyanine dyes (Cy2, Cy3 and Cy5) are matched in molecular weight and charge, but have distinct excitation and emission wavelengths [28]. One dye is used to label control and the other dye labels treated samples, which are then mixed and differentially compared in the same gel. Also, for quantitative analysis, a third dye (Cy2) is used for an internal standard. An example of applying this technology to rat TBI versus naive brain comparison is illustrated in FIGURES 3 & 4. A similar approach has been described by others using the rat model of TBI [29] and advantages of using 2D gel in identifying TBI biomarkers include the high resolving power for complex mixtures of proteins, and the capability of resolving post-translationally modified proteins, including acetylation, phosphorylation, glycosylation and protein crosslinking [30,31]. However, there are several weaknesses to the 2D gel approach. Proteins of extreme pI or minute quantity and proteins that are either very small or very large may be missed. Also, integral membrane proteins, of which many are CNS disorder drug targets (membrane-bound receptors or neurotransmitter transporters), are lost due to their extreme hydrophobicity.

**Multidimensional liquid chromatography**

Alternative protein separation methods are needed to overcome some of the shortcomings of 2DE. Recently, there has been a significant movement toward multidimensional LC methods to resolve complex protein mixtures [32]. The general idea draws on classic chromatographic principles, including size-exclusion chromatography (SEC; also known as gel filtration chromatography), ionic interaction (strong cation exchange [SCX] and strong anion exchange [SAX]), hydrophobic interaction (C4- or phenyl-agarose chromatography) and IEF chromatography.

![Figure 3. Example of 2D gel protein separation for TBI biomarker identification.](image)

Cy: cyanine; Hb: Hemoglobin; pI: Isoelectric point; TBI: Traumatic brain injury.
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One can envision combining multiple chromatographic approaches in series to achieve multidimensional separations. In the authors’ work, the combined use of SCX and SAX in series has met with some success (FIGURE 4). Incompatibilities of buffer components (e.g., salt concentration and organic components) and the logistics of configuring fraction elution from one column with loading of a second column challenge this method. Often, individual fractions are manually loaded onto the second column, but this is extremely labor intensive and may introduce run-to-run variability.

When selecting chromatographic separation methods, consideration must also be given to take advantage of the size, pI and hydrophobicity differences of the proteins of interests. In addition, when dealing with membrane-bound proteins, the chromatographic method must be compatible with the use of the proper neutral detergent (such as Triton X-100 or CHAPS). Importantly, minute proteins can be concentrated to enhance their detection. One weakness of this approach is that even with 2D-LC separation, it is often not possible to separate all proteins individually. This issue will be addressed under ‘Protein Identification and Quantification’. In summary, when compared with 2DE, tandem LC is more compatible with membrane-bound proteins and can enrich proteins in minute quantity.

Protein identification by MS/MS

Protein separation strategies including LC techniques and gel separations [33] are commonly used to resolve complex protein mixtures. Subsequent LC fractions or gel bands are processed by in-solution or in-gel digestion (most often with trypsin) to form peptides small enough to be effectively analyzed by MS (FIGURE 5). Complex peptide mixtures of protein digests are typically separated by reverse-phase chromatography placed online with electrospray ionization (ESI)-MS, which not only resolves peptides from one another but also concentrates them, providing greater sensitivity. LC-MS is most often performed on high-powered tandem mass spectrometers including the quadrupole-ion-traps, quadrupole time-of-flight and Fourier transform ion cyclotron resonance MS. MS/MS provides the advantage of providing peptide sequence information in addition to the parent peptide mass (FIGURE 5) [34]. Ions of the peptide of interest are isolated (first stage of MS), then fragmented along the peptide backbone by colliding with neutrals. Pairs of b- and y-daughter ions, formed by fragmentation from the N- or C-terminal side of each residue, respectively, will predominantly be generated. The b-and y-daughter ions are then mass analyzed to form a daughter ion spectrum (second stage of MS). Using MS/MS information, the peptide sequence can be reconstructed, which
can be performed rapidly with available bioinformatics software. LC/MS/MS systems work extremely well for protein identification, coupling generated mass and sequence information with database searches, and with the sensitivity to identify picomolar levels of proteins present in complex mixtures such as tissue lysates. One drawback to this approach is that the mass spectrometer has only a limited capacity/duty cycle and therefore only a limited number of peptides can be fragmented in one LC run. Consequently, protein identification results must be carefully verified.

Potentially, differentially expressed proteins in TBI can also be further quantified using a MS-compatible method (see Denslow and coworkers, [35]) such as isotope-coded affinity tags [36] or the use of absolute quantification probes [37].

High-throughput antibody-based approach

As an alternative to MS-based approaches, protein biomarker identification can also be performed using one of the many platforms of antibody arrays or panels (e.g., Zyomyx protein Biochips, BD Powerblot and BD antibody arrays) [38–41]. These methods rely on a HTP format of specific antibody-based capturing of all proteins of interest (i.e., the entire proteome or a subproteome). The quantification of the captured proteins can be performed by prelabeling (including differential labeling) of the protein with fluorescent dye(s) (dye-labeling detection) such as BD antibody arrays, which are similar to the sandwich ELISA (e.g., Zyomyx antibody chips) [42]. A third option is the BD Powerblot, which is a HTP western blotting (immunoblotting) system with two distinct protein samples differentially subjected to a set of five blots (FIGURE 6). Each blot has 39 usable lanes with the use of a manifold system. Each lane is developed with five to six different fluorophore-linked monoclonal antibodies (toward antigen with nonoverlapping molecular weights) [43]. With this method, samples are probed with a total of 1000 monoclonal antibodies. The authors have actually conducted several Powerblot experiments with animal TBI studies (FIGURE 6).

The major advantage of the HTP antibody approach is that proteins of interest can be readily identified since all antibodies used have known antigens and their positional assignment on the antibody chip or panel is known. Another advantage is the ease of quantification and subsequent biomarker confirmation and validation with additional samples, since the same or similar antibodies to those used in the array or panel are usually available commercially. However, it is expected that the use of antibodies collected from many different sources will result in uneven sensitivity. Also, as in other immunoassay methods (western blotting, immunostaining or ELISA), it is a given that antibody array methods might likely detect nonspecifically bound proteins or other substances effecting quantification with high chemical noise and leading to false-positive reactions. Another disadvantage of this approach is that it is practically impossible to be exhaustive as one would have high-fidelity antibodies that cover only a subset of the whole human proteome (or other species).

**TBI biomarkers today**

While the focus of the current review is on proteomic-based discovery of TBI biomarkers, it is useful to survey the existing biomarkers identified through more traditional approaches (TABLE 2) [44]. To date, S100β, an astrocyte-glial calcium-binding protein, is perhaps the most recognized brain injury marker studied in cardiac arrest- or surgery-induced brain injury, TBI and stroke [14,45–47]. Its non-neuronal origin might be its greatest shortcoming [48]. Fazio and colleagues suggested that S100β can be released to the perivascular space and extravasate immediately after BBB opening following brain injury. Accordingly, it may be more appropriately considered a BBB-permeability marker [49,50]. Also of interest is that the S100β level in blood has a very good negative predictive value in TBI. For example, the S100β level can potentially be used to discriminate between patients who need radiologic and emergency care versus those who can safely return home. Also,
along with routine neurologic examination, S100β levels may contribute to a reduced number of CT scans, especially in alcohol-intoxicated patients [51]. Neuron-specific enolase (NSE) is the second most studied brain injury biomarker [43,52–54]. Yet, NSE, despite its namesake, is expressed in various tissues other than the CNS [55]. In addition, studies relating these proteins to other pathophysiologic parameters of TBI and stroke have produced conflicting results [56,57–59]. Myelin basic protein (MBP), detected in CSF and serum following TBI in humans [60,61], and glial fibrillary acidic protein (GFAP) have also been considered as potential biomarkers for TBI [62,63].

However, as both S100β and NSE are glial proteins, they might only serve as indirect measures of neural damage at best. Neurofilament-L (NFL) is an axonally enriched protein and is potentially attractive as a TBI biomarker [64–66]; however, very few data are available in either animal or human studies. More recently, there was also identification of two axonally located structural proteins (tau and αII-spectrin) that are protease truncated. Such modified forms, cleaved tau (C-tau) and spectrin breakdown products (SBDPs), were detected in CSF from TBI patients as well as in experimental TBI rat brain tissue and CSF [13,67–70]. In addition, there is a fragmented form of the glutamate-N-methyl-D-aspartate (NMDA) receptor (NR2A/2B subtype), which was found to be released into the circulating blood after ischemic stroke [71]. These neuron-derived proteins appear to induce autoantibodies in the circulation as part of the body’s immune response. The authors proposed that such autoantibodies can also be considered potential biomarkers (TABLE 2). It is of interest to point out that one or more of these previously identified biomarkers might come up as hits in the proteomics-based TBI biomarker discovery studies. Their reappearance could indeed serve as confirmation of the approach that was taken.

The attributes of ideal TBI biomarkers are outlined in BOX 1. These include information on mechanism of neuronal injury, brain-originated, correlation to magnitude of injury severity or lesion size, correlation with other TBI benchmarks (GCS, MRI, CT and neuropsychologic scores), sensitivity to subclinical or mild TBI, selectivity to TBI over other non-brain injury neurologic disorders, prediction of outcome, direct translation from preclinical animal models to clinical environment and prediction of therapy efficacy. For example, S100β will fall short of providing injury mechanism, while C-tau and αII-SBDPs. Some examples of proteomically identified TBI markers by the methods described in this review are listed in TABLE 2. Obviously, novel TBI markers should also be measured against the same attributes (BOX 1).

Expert commentary

The current review summarizes the importance of discovering clinically relevant protein biomarkers for TBI. It also presents logical rationale that applications of state-of-the-art proteomic technologies (both separation MS-based and antibody-based) are uniquely suited for the discovery of otherwise overlooked TBI biomarkers. It also highlights that one must make careful decisions when choosing what paradigm (human vs. various animal models of TBI) and what biologic samples to use for such proteomic studies. Some of the desirable attributes of an ideal TBI biomarker are also outlined, and the idea that neuroproteomics-based biomarkers will be complementary to those that were identified by traditional approaches is put forward.

In the future, it would be of great interest to concurrently assess the efficacy of all the available biomarkers (discovered by traditional and proteomic approaches) on the same patients. Panels of biomarkers will be powerful tools in better management and treatment of TBI patients.

Five-year view

Given the current intense industrial race to secure a piece of the proteomic technology and instrument market as well as government-funded academic efforts to push the technology barrier envelope, one can expect that there will be rapid improvement in both the separation MS and HTP antibody-based approaches. On the MS side, it is expected that significant improvement in the hardware of MS/MS instrumentation (e.g., better mass accuracy, better compatibility with biologic
samples, higher reproducibility of protein identification), as well as bioinformatic (data-mining) software (e.g., better protein hit scoring system, compressed multispecies–protein hits into single protein hit from species of interest, automatic protein function and protein–protein pathways/networks information). These predicted advances in MS and related bioinformatic technologies will greatly enhance the separation MS-based neuroproteomic capability as a tool for TBI biomarker discovery. Similarly, for the HTP antibody-based approach, several competing platforms of antibody arrays or panels will continue to be pursued. Inevitably, the rate-limiting step of the antibody-based TBI biomarker discovery approach will be the availability of a complete set of antibodies that covers the whole human proteome. With the involvement of Human Proteome Organization (HUPO) [72] and Human Brain Proteome Project (HBPP) [73], one should be optimistic that that day will come even sooner than expected.

Therefore, one should not be surprised that the first generation of TBI biomarker(s) will be completed within 5 years. Within this period, we will also begin to learn how neuroproteomically discovered biomarkers or panel of markers for TBI (and other related disorders) fares in deconvoluted analysis using samples from animal models as well as in validation studies with clinical samples.

**Information resources**

**2D gel databases**
- Nobel Foundation
  www.noble.org/2DPage/intro.asp
  (Viewed July 2005)
- Swiss-2D PAGE
  http://au.expasy.org/ch2d
  (Viewed July 2005)
- Siena 2D Page
  www.bio-mol.unisi.it/2d/2d.html
  (Viewed July 2005)

**MS-based proteomics**
- DTAselect & Contrast Software
  http://fields.scripps.edu/DTASelect
  (Viewed July 2005)
- European Bioinformatics Institute
  www.ebi.ac.uk
  (Viewed July 2005)
- Human Protein Reference Database
  www.hprd.org
  (Viewed July 2005)

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**Table 2. Examples of traumatic brain injury biomarkers identified by traditional and proteomic approaches.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Approach</th>
<th>Method used</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Traditional</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raabe et al. (2002)</td>
<td>S100β</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ross et al. (1996)</td>
<td>Neuron-specific enolase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas (1978), Yamazaki et al. (1995)</td>
<td>Myelin basic protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norgren et al. (2003)</td>
<td>Neurofilament proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pike et al. (2000), Ringer et al. (2004)</td>
<td>αII-spectrin breakdown product</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zemlan et al. (2002), Shaw et al. (2002)</td>
<td>Cleaved tau</td>
<td></td>
<td></td>
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<tr>
<td>Dambinova et al. (2003)</td>
<td>Autoantibodies to NMDA-R</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proteomic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jenkins et al. (2002)</td>
<td>PKB substrate proteins</td>
<td>2D gel/MS/MS</td>
<td></td>
</tr>
<tr>
<td>Haskin et al. (2005)</td>
<td>αII-spectrin enolase 2, γ</td>
<td>PAGE/MS/MS</td>
<td>[74]</td>
</tr>
<tr>
<td>Ottens et al. (2005)</td>
<td>αII-spectrin</td>
<td>LC-PAGE/MS/MS</td>
<td>[75]</td>
</tr>
<tr>
<td>Kobeissy et al. (2005)</td>
<td>Ceruloplasmin</td>
<td>LC-PAGE/MS/MS</td>
<td>[76]</td>
</tr>
<tr>
<td>Wang et al. (2003, 2004)</td>
<td>βII-spectrin</td>
<td>High-throughput immunoblotting</td>
<td></td>
</tr>
<tr>
<td>Wang et al. (2003, 2004)</td>
<td>nNOS</td>
<td>High-throughput immunoblotting</td>
<td></td>
</tr>
</tbody>
</table>

LC: Liquid chromatography; MS: Mass spectrometry; MS/MS: Tandem mass spectrometry; PAGE: Polyacrylamide gel electrophoresis
Antibody-based proteomics

- BD Powerblot
  (Viewed July 2005)
- Biosite Protein Array
  www.biosite.com/discovery/arrays.aspx
  (Viewed July 2005)
- Zyomyx
  www.zyomyx.com
  (Viewed July 2005)
- Clinical Micro Arrays
  www.clinicalmicroarrays.com
  (Viewed July 2005)

Protein functions & pathways

- Protein function, sequences, tissue distribution
  http://harvester.embl.de
  (Viewed July 2005)
- SOURCE
  http://genome-www5.stanford.edu/cgi-bin/source/sourceSearch
  (Viewed July 2005)

Current TBI markers

- Banyan biomarkers
  www.banyanbio.com
  (Viewed July 2005)

Selected TBI & neuroproteomics centers/institutes

- Human Brain Proteome Project
  www.hbpp.org
  (Viewed July 2005)
- Human Proteome Project
  www.hupo.org
  (Viewed July 2005)
- University of Florida Center for Traumatic brain injury studies (CTBIS)
  www.mbi.ufl.edu/ctbis
  (Viewed July 2005)
- University of Florida: Center For Neuroproteomics and Biomarkers Research (CNBR)
  http://cnbr.mbi.ufl.edu
  (Viewed July 2005)
- University Kentucky Spinal Cord and Brain Injury Research Center (SCoBIRC)
  www2.mc.uky.edu/scobirc
  (Viewed July 2005)
- University of Pittsburgh Brain Trauma Research Center
  www.neurosurgery.pitt.edu/trauma
  (Viewed July 2005)
- UCLA Brain Injury Research Center
  http://neurosurgery.ucla.edu/Birc1/index.htm
  (Viewed July 2005)
- Miami Project to Cure Paralysis
  www.miamiproject.miami.edu
  (Viewed July 2005)
- Baylor College of Medicine Center for Neurosurgical Intensive Care
  www.bcm.edu/neurosurgery/faculty/robertson.htm
  (Viewed July 2005)
- Pecs University/Pannonian Symposium on CNS Injury
  http://neurosurgery.pote.hu/pannsymp
  (Viewed July 2005)

Conflict of interest

Kevin KW Wang and Ronald L Hayes are inventors of technology to discover and use brain-injury biomarkers discussed in this publication, and hold equity in Banyan Biomarkers, Inc., a company commercializing the technology. Wang and Hayes may benefit from this technology by receiving royalties and equity growth.

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996.

Key issues

- Deciding which paradigm (human vs. various animal models) of traumatic brain injury (TBI) to use.
- Optimization of separation mass spectrometry-based proteomic studies.
- Availability of complete sets of antibodies to detect all human gene products (for antibody-based approach).
- Preclinical and clinical validation of proteomically identified TBI biomarkers.
- Defining attributes of an ideal TBI biomarker.
References

Papers of special note have been highlighted as:

- of interest
- of considerable interest


- Important paper highlighting the utility of biomarkers for drug development.


- Emphasizes translational research.


- 2D difference in-gel electrophoresis methods paper.


- Important isotope-coded affinity tag mass spectrometry quantification methods paper.
Proteomic identification of TBI biomarkers


**Website**

101 Centers for Disease Control and Prevention – TBI Outcomes and Consequences www.cdc.gov/node.do/id/09003ec8000dbdc/aspectId/A0400027 (Viewed July 2005)

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