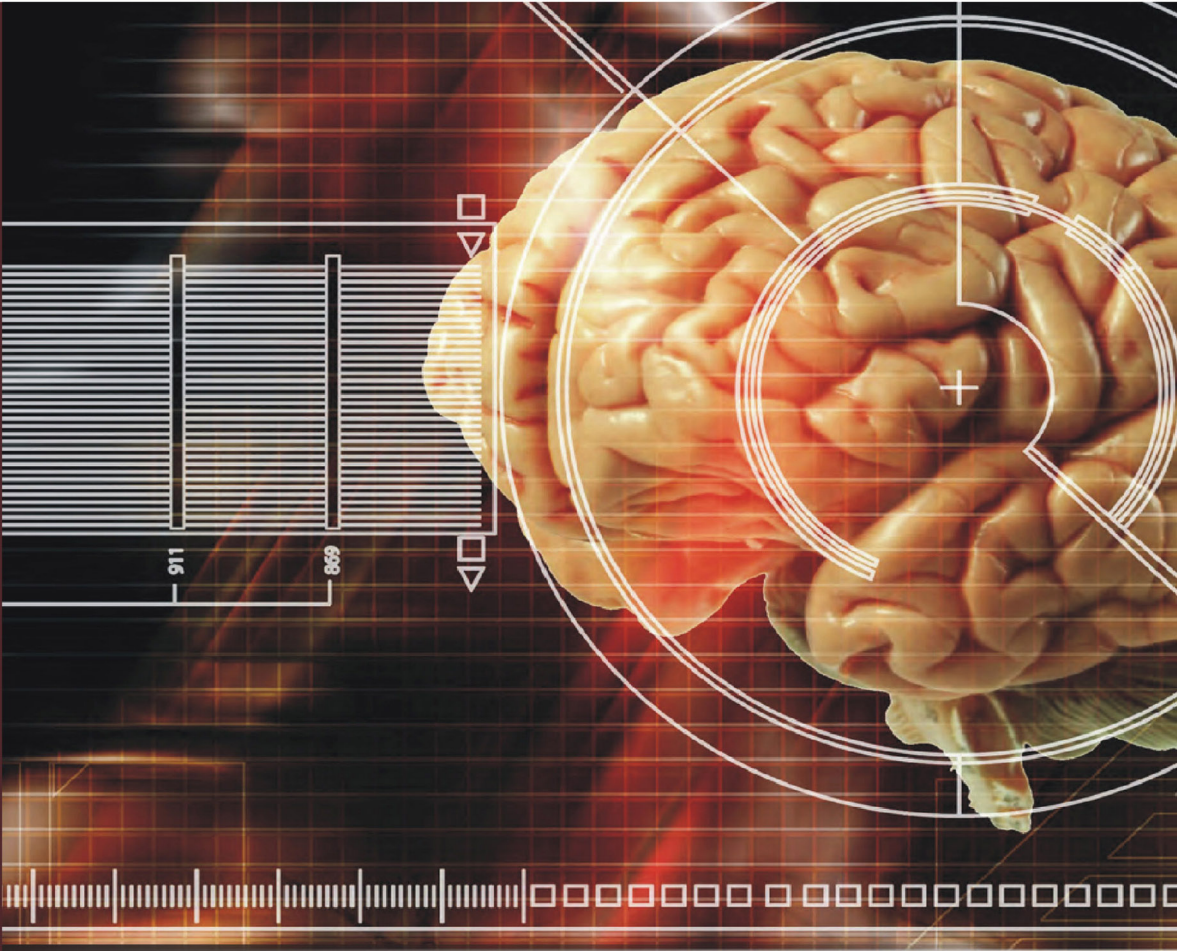

Biomarkers of Brain Injury and Neurological Disorders

Editors
Kevin K.W. Wang
Zhiqun Zhang
Firas H. Kobeissy

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Dedication

To my family, for the love and patience they provide me.

Kevin K.W. Wang

For my husband Jianghui Chao, our wonderful children Ryan and Arnall, my parents, sisters and loved ones who supported and believed in me.

Zhiqun Zhang

To my mom Kawsar; may her soul rest in peace, my dear dad Hosni, and Sister Maha whose love and patience gave me hope in my path. To my mentors, Dr. Kevin K. Wang & Dr. Mark S. Gold whose experience and advice drove my success….

Firas H. Kobeissy

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Foreword

The central nervous system, including the brain and spinal cord, is arguably the most complex human organ system in terms of structure and function. This book stands out in describing the latest biomarker studies in brain disorders relevant to its mechanism, advanced technologies, experimental methods, and clinical trial studies. Diagnostic tests based on biomarkers have already demonstrated proven clinical diagnostic utility in acute care environments. For example, in the area of cardiac injury, cardiac troponin proteins (T and I) and various forms of brain natriuretic peptide (BNP), often in combination with other biomarkers, are routinely used to facilitate accurate diagnosis of congestive heart failure and myocardial infarction, in patients presenting with chest pain. Recently, there has been broad recognition that biomarkers can also play a critical role in drug discovery and development. There are several areas in which biomarkers can facilitate brain injury drug development and eventually personalized medicine. Biomarker research methods may improve our knowledge of the pathobiology that ensues after CNS disorders, and unlock the connections between this pathology, individual variability, and the heterogeneous outcomes experienced by those with central nervous system disorders.

The aim of this book is to draw together the work of leading experts in the fields of neurological disorders, brain injury, and drug abuse and to highlight what is known on a broad range of topics pertaining to biomarkers in central nervous system disorders ranging from traumatic brain injury, spinal cord injury, multiple sclerosis, and alcohol abuse. This elegant volume begins with chapters discussing basic mechanisms and methods of biomarker identification including neuroproteomics analysis, followed by surveying different textures of currently novel biomarkers such as microRNA, proteins as well as genetic fingerprints and their applications in different neurological disorders. This section is then followed by elaborate chapters written by world-renowned experts, who have tackled several neurodegenerative diseases specifically, along with their mechanisms and their associated "known" biomarkers. This work flows smoothly, discussing novel methods such as Deep Brain Stimulation, the endpoint of which can

be used as a neurotherapeutic marker for a number of neuropsychiatric disorders. The book concludes by emphasizing the implications and importance of biomarker research on several fields, such as brain trauma, drug addiction and the need for theranostic (therapeutic and diagnostic) biomarker approaches.

It is my great honor that three of our faculty at the Department of Psychiatry (Wang, Zhang and Kobeissy), who are experts in the areas of biomarkers in brain injury studies and drug abuse–associated neurotoxicity, have gathered in this respected work to deliver one of the most updated presentations in biomarker studies. This collection will have direct implications on neurotherapeutic management, treatment guidance, as well as indicators of injury severity and prognosis.

The book will lay out a foundation for scholars interested in several neurological disorders and related biomarker research. This is currently a unique and timely volume that will provide a comprehensive review for basic scientists, graduate students, medical students, clinical researchers and medical professionals with interest in translational research in the area of brain injury and other brain disorders.

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Preface

Studies in the field of central nervous system (CNS) biomarkers have been evolving at quite a rapid pace especially with the introduction of novel high throughput screening techniques such as proteomics and microarrays coupled with systems biology with its predictive potential of missed targets and their dynamic alteration in respect to disease progression. This in turn has led to the identification of a new generation of biomarkers including signature microRNA biomarkers and even autoantibodies serving as indirect specific indicators of certain brain injury disorders. This exciting advance in biomarker research has encouraged new lines of funding from national and private sectors aiming at identifying novel treatment targets driven via biomarker identification. Among the most challenging and highly demanding fields for biomarker research is the CNS and particularly those related to brain trauma pathology. The CNS, including the brain and spinal cord, is arguably the most complex human organ system in terms of structure and function. Of interest, biomarker identification of CNS disorders is considered the *Holy Grail* that is in constant pursuit by scientists and medical doctors. Identifying these markers in body fluids represents a major challenge due to several factors including their minute levels, turn over/clearance rate, dynamic range in serum/cerebrospinal fluid, etc. which necessitates the use of high throughput technologies such as genomics, neuroproteomics and recently microRNA assessment to decipher these markers dynamics.

In the area of conflicts worldwide, brain injury has been designated the "signature injury" of current military conflicts. CNS trauma primarily from regular accidents or sport associated injury as well as battlefield represents one of the most common causes of morbidity and mortality in our society with enormous societal and economic burdens. Each year, approximately three million people sustain traumatic brain injury; these are often present with neuropsychological deficits that may develop later at chronic phases; coupled with altered emotional, and/or cognitive impairments, exhibiting decreased executive function, depression, and significant drug

abuse problem. Such presentation in some TBI patients has been termed as altered psychological health which overlaps with some symptoms of post-traumatic stress disorders (PTSD) leading to its misdiagnosis and consequently, its treatment. This has driven to the increased funding to identify markers that can distinguish brain injury-induced psychological health problems from traditional PTSD symptoms. Currently, the need for biomarkers identification is of high interest and demand to distinguish neuropsychological disorders due to brain trauma as a pipeline to identify novel effective neurotherapeutics that can guide in treatment management for the cure of these devastating injuries.

In this work we have assembled 21 expert contributors renowned in their scientific work of neurological and neuropsychological disorders and the implication of biomarker research in these disorders as chapter lead authors. The book is divided into three sections **(Biomarker technology, CNS injury biomarkers and Other CNS disorder biomarkers)**.

The first section **(Biomarker technology)** describes experimental concepts and molecular mechanisms of biomarker genesis and biomarker discovery and detection methods such as proteomics applications and microRNAs assessment and the role of the protease systems in generating different potential markers. In the following section **(CNS injury biomarkers)**, the authors describe the utility of different biomarkers in the fields of brain and spinal cord trauma and their use as neurotherapeutic recovery endpoints, diagnostic signatures and rehabilitation markers of different brain insult scenarios such as mild brain injury, spinal cord injury acknowledging the different types of markers including inflammatory and protein biomarkers identified.

In the final section **(Other CNS disorder biomarkers)**, biomarkers of several neurological disorders such as Multiple Sclerosis, Alzheimer's disease, Charcot-Marie-Tooth disease, Parkinson's disease as well as alcohol abuse related markers are fully investigated. These chapters represent a valuable addition in the field of biomarker research and to those interested in the experimental studies for identifying advanced neurotherapeutic treatment for brain injury and other neuropsychological disorders. In this initiative, we would like to thank colleagues and contributors who were enthusiastic about this project and dedicated their precious time and expertise in finalizing this wonderful project. A special recognition goes to the CRC Press/Taylor & Francis editorial team who bear with us all the challenges in delivering this book. Our recognition goes to our talented graphic designer Mr. Hussein Mokdad, who had to go through a lot of

enjoyable unending requests from us—the editors—to reach a final cover art that is decorating our book. To the readers of this book, we hope that you will find this book both informative and stimulating in your own research or clinical area. We also welcome your feedback to us.

Gainesville, FL, USA, 2014
Kevin K.W. Wang
Zhiqun Zhang
Firas Hosni Kobeissy

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There are many dear people to thank and acknowledge in the development of this book. Our most abundant gratitude goes to the authors of the chapters in this book. They ultimately made this work possible by providing their top quality manuscripts and comments. Without their extremely valuable and "prompt" contributions, this book would not have been possible. We would like to thank Miss Zeinab Dalloul, MS and Miss Emilia Amrou, BS, for the editing, proof reading and commentary on various components; their help is highly valued.

We would like to take this opportunity to thank our colleagues from the University of Florida, Departments of Psychiatry and Neuroscience, whose help and encouragement have inspired us in completing this book. We want to offer my special thanks to Professor Mark S. Gold, our chairman, who offered his wise advice and guidance that lead to the completion of this book. We feel highly privileged that he agreed to write the Foreword for this book.

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Section A

BIOMARKER TECHNOLOGY

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1

Neuro-proteomics and Neuro-systems Biology in the Quest of TBI Biomarker Discovery

Ali Alawieh,[1] *Zahraa Sabra,*[3] *Zhiqun Zhang,*[2] *Firas Kobeissy*[2] and *Kevin K.W. Wang*[2,*]

INTRODUCTION

Traumatic Brain Injury (TBI) is the leading cause of mortality and disability among the young population in the developed countries, and its worldwide prevalence is sharply increasing (Feigin et al., 2010; Ghajar, 2000; Maas et al., 2008). TBI affects all ages with highest incidence rates among children, young adults and the elderly (Faul et al., 2010; Hemphill III et al., 2012; Koepsell et al., 2011). TBI is associated with increased incidence of disability and premature death along with heightened medical and socioeconomic burden on individuals, families and societies (Leibson et al., 2011). The average annual death from TBI in the US is 53,014, mostly of the young age group (Coronado et al., 2011). This value is only the tip of the iceberg as TBI accounts annually for up to 275,000 hospitalization and 1,365,000 emergency department visits despite those who receive no care or donot appear at the emergency setting (Faul et al., 2010; McCrea et al., 2004)

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(Figure 1.1). The World Health Organization (WHO) predicts that TBI will rise to the third leading cause of global mortality and disability by 2020 (Organization, 2009). The long-term prevalence of disability secondary to TBI in the US is estimated to be 1–2% of the population (Zaloshnja et al., 2008). Even if the incidence of TBI is much less than strokes, but given its early age incidence, the long-term effects and socio-economic costs of TBI can be as high (van Baalen et al., 2003). TBI accounts for about 10% of the health care budget in the US with an estimated annual cost to society of US$ 30 billion (Hoyt et al., 2004).

TBI is often referred to as a "silent epidemic" since the different complications of the disease is not readily apparent, and the general public has limited awareness about this disease (Faul et al., 2010). There are currently no diagnostic techniques that can confirm whether a blow to the brain has resulted in brain injury or not. Clinical symptoms of brain injury may resolve within one or two months, yet axonal injury may persist for years (Johnson et al., 2012; Williams et al., 2010). Around 43.3% of all Americans having TBI had residual disability one year after injury (Corrigan et al., 2010) despite the fact that up to 90% of TBI cases are classified as mild TBI (mTBI) (McCrea, 2007). Therefore, mTBI does not imply a benign or self-limiting condition since it could be associated with neuronal swelling, axonal energy and disconnection of the white matter (Blumbergs et al., 1994; Kirov et al., 2012). Eventually, mTBI patients may have long-term disabling consequences like dizziness, fatigue, headaches and delayed recall of memory (Heltemes et al., 2011).

Traumatic brain injury is a brain injury caused by an external mechanical force like a blow, concussive force or a bullet (Stergiou-Kita et al., 2012). This injury is a dynamic process that starts with a primary injury and initiates a cascade of biochemical and cellular changes of repair and injury (Ottens

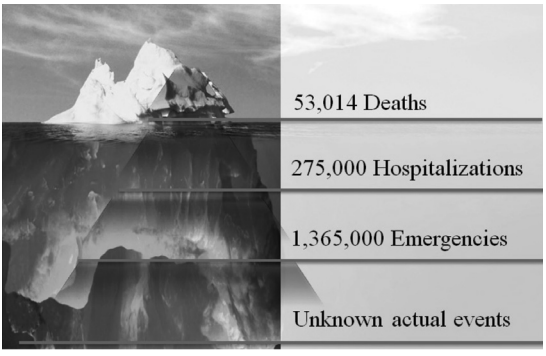

Figure 1.1. Deaths from TBI are only the tip of the iceberg compared to the actual incidence of mild TBI not drawn to medical attention.

Color image of this figure appears in the color plate section at the end of the book.

et al., 2006). These changes contribute to cumulative neuronal death over time resulting in secondary injury and long term complications (Loane et al., 2009). Evidence from pathological studies supported the involvement of several immunological and apoptotic pathways in the progress of this neuronal damage (Raghupathi, 2004) including inflammatory responses (Edwin et al. 2011; Loane and Byrnes, 2010; Ziebell et al., 2012), autophagy and activation of proteases (Clark et al., 2008; Knoblach and Faden, 2005), mitochondrial dysfunction (Lifshitz et al., 2004; Mazzeo et al., 2009), oxidative stress, neurotransmitter release, excitotoxicity and changes in intracranial pressure and cerebrovascular circulation (Cernak and Noble-Haeusslein, 2009; Ghajar, 2000; Maas et al., 2008). Since the early manifestation of these changes is biochemical and molecular in nature; it is in the hands of biochemical and molecular testing to detect and assess the severity of TBI as well as to predict the outcome.

Secondary to the trauma-induced neuronal degeneration, TBI is associated with long-term cognitive deficit (Patterson and Holahan, 2012) that can affect up to 15% of mTBI patients (Røe et al., 2009). Eventually, TBI is considered a risk factor for many neuropsychiatric and neurodegenerative disorders including Alzheimer's disease (Jellinger et al., 2001; Lye and Shores, 2000) where neurofibrillary tangles were detected in the brains of ex-boxers who were subject to mTBI (Tokuda et al., 1991). There is also high comorbidity between TBI and several neuropsychiatric disorders like anxiety, depression, dementia and others (Deb et al., 1999; Rao and Lyketsos, 2000; van Reekum et al., 2000; Whelan-Goodinson et al., 2010).

The aforementioned occult complications of TBI, in the absence of any FDA approved treatment for TBI (Narayan et al., 2002), necessitate the detection of diagnostic and therapeutic biomarkers to improve the quality of life and decrease mortality among patients with TBI. In this chapter we will emphasize the need of biomarker discovery in TBI and highlight the major advances in the field of proteomics as applied to biomarker quest in TBI.

Available Classification and Diagnostic Techniques in TBI

Early classification of acute TBI is of critical importance in the accurate diagnosis, prediction of outcomes (Masel and DeWitt, 2010; Zhu et al., 2009), and; eventually, the clinical workup of patients. TBI is a heterogeneous condition of variable clinical behavior, and a specific targeted therapy for the different subcategories of the disease is essential. In this context, accurate classification is mandatory to discover patients to whom intensive rehabilitation programs are needed and beneficial (van Baalen et al., 2003). The identification of those patients among the heterogeneous population of TBI patients is one of the major challenges in clinical practice (Saatman

et al., 2008) especially that proper management of TBI can significantly alter the clinical progression in the first hours or days after injury (Lee and Newberg, 2005). Therefore, an ideal TBI classification includes a rapid assessment of initial severity that is in accordance with the long-term clinical outcome. Noteworthy, the hyper-metabolic state of the brain post-mTBI may render it susceptible to repetitive mTBI that will have dismal, even fatal, consequences on the outcome (McCrory and Berkovic, 1998). Eventually, there is a sincere need to identify and diagnose those patients for medical and occupational management post-mTBI.

Among the traditional classification modalities, computed tomography (CT) and the assessment of severity of injury by the Glasgow Coma Scale are considered the cornerstones of assessment in neuro-traumatology (Marshall, 2000). Other neuroimaging techniques have been also incorporated including Magnetic Resonance Imaging (MRI), Single Photon Emission Tomography (SPECT), and Positron Emission Tomography (PET) (Bigler, 2001; Le and Gean, 2009).

Glasgow Coma Scale (GCS)

The Glasgow Coma Scale (GCS) is the most common modality for TBI classification among clinicians today. GCS is a 15-point index of neurological injury severity that assesses the level of consciousness after TBI. The scale involves three components; assessment of eye opening, best motor response and best verbal response (Scale, 2001). According to this scale, TBI patients are classified into three broad categories: severe TBI (GCS 3–8), moderate TBI (GCS 9–13) or mild TBI (GCS 14–15) (Maas et al., 2008).

The use of GCS as a diagnostic tool is subject to several limitations. Many confounders may obscure the level of consciousness in patients including medical sedation, paralysis, distracting injuries or intoxication due to alcohol or recreational drugs (Green, 2011; Maas et al., 2008). With the increasing use of early sedation, intubation and ventilation in trauma patients, the value of the Glasgow Coma Scale is decreasing (Zhu et al., 2009); the neurological exam is difficult and the severity maybe over-estimated (Stocchetti et al., 2004). Several epidemiological studies have shown that the prevalence of sedation, drug or alcohol abuse and intoxication among patients with TBI is highly increasing (Lindenbaum et al., 1989). The European Brain Injury Consortium Survey of Head Injuries has shown that the GCS was only fully testable on 77% of the TBI patients admitted (Murray et al., 1999). Other populations of patients also are difficult to assess using GCS including infants, young children and patients with pre-existing neurological impairment (Saatman et al., 2008). GCS performs best for severe TBI; however, for mild TBI cases that constitute 80–90% of all TBI, GCS

has poor performance. Eventually, GCS is strongly associated with acute morbidity and mortality, but not with long-term outcome (Zasler, 1997).

Aside from clinical management, the GCS does not provide any clue about the underlying pathophysiological underlying the neurological deficits and provides less raw material for targeted therapy (Zasler, 1997).

Neuroimaging Techniques

Neuroimaging techniques were implemented in diagnosis and classification of TBI as a supplement to GCS in order to evade some of the limitations of the GCS especially those associated with cases of early sedation and intubation of patients (Maas et al., 2008). Neuroimaging techniques classify patients based on morphological brain changes that can be detected in TBI patients, and the most common used modality is Computed Tomography (CT).

A neuroimaging descriptive system using CT scan was described by Marshall et al. (1991) and includes criteria such as the presence of mass lesions, and diffuse brain injury assessed by signs of elevated intracranial pressure (ICP) like compression of basal cisterns or midline shift. However, these criteria suffer several limitations. The sensitivity of CT scans to diffuse brain damage is very low and the absence of abnormal finding on CT does not rule out the presence of brain damage especially in case of mild TBI (Güzel et al., 2009; Haydel et al., 2000; Yuh et al., 2012). CT scan, similar to other neuroimaging techniques, can only capture momentary changes in the brain and could not account for the dynamic changes that start at the microscopic level after TBI (Maas et al., 2008). Even though several recommendations have suggested criteria for use of CT scans for high risk patients (Smits et al., 2007) the physicians' lack of confidence in available diagnostic tools have led to the practical consideration of routine use of CT in mTBI patients eventually leading to higher costs and radiation exposure (Stiell et al., 2005).

MRI provided an additional sensitivity to CT scans in terms of detecting diffuse brain damage (Mittl et al., 1994); however, MRI would be impractical to use in the acute phase of the trauma due to limited availability and the physically unstable status of TBI patients. Patients who have metallic items in their body, a common incidence in emergency care, are not candidates for this imaging modality (Wang et al., 2005). Some studies have also associated limited outcome prediction with the use of MRI in TBI (Hughes et al., 2004). The use of other modalities like SPECT and PET also suffers major limitations. Regional blood flow abnormalities as detected by SPECT imaging does not necessarily correlate with a structural brain damage (Wang et al., 2005). SPECT has also low sensitivity in detecting small brain lesions, and the association between abnormalities detected by SPECT and the

neurocognitive outcome is still weak (Hofman et al., 2001). The assessment of regional glucose metabolism in TBI by PET scan is also nonspecific due to the heterogeneous nature of TBI where hyper-metabolism and hypo-metabolism in the same regions across different TBI patients have been reported (Le and Gean, 2009).

Other limitations also circumscribe the use of neuroimaging in TBI including their availability, high cost and the inability to carry them in a repeated manner due to inconvenience and radiation exposure. Therefore, they cannot be used to monitor the occurrence of secondary lesions that may occur in a short period of time.

The challenges associated with a reliable and efficient classification of TBI along with the need for such classification have led the National Institute of Neurological Disorders and Stroke (NINDS) to convene a workshop to study the steps needed for a new valid classification system for TBI (Saatman et al., 2008). In October 2011, the collaborative efforts of the European Commission, the Canadian Institutes of Health Research and the National Institutes of Health set the "International Initiative for Traumatic Brain Injury Research" (InTBIR) to advance clinical traumatic brain injury (TBI) research, treatment and care (European-Commission). An expected alternative to imaging based classification is a biomarker-based classification that can overcome many of those limitations.

Needs for Biomarkers

The unapparent progression of TBI consequences and the rapid resolution of signs and symptoms post-mTBI along with the irreversible disabling complications emphasize the need for accurate specific biomarkers for diagnosis, classification and monitoring of the disease and its progression. These biomarkers once discovered and validated will represent definite diagnostic criteria of TBI and reliable outcome predictors added to the current clinical examination and neuroimaging results.

A biomarker has been defined as a "*characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*" (Biomarkers Definitions Working Group, 2001). In the clinical context of TBI, the traumatic injury to the brain may cause a series of cellular changes including degeneration, protease activation, oxidative stress, metabolic disturbances, etc…, and these changes result in shedding specific proteins into the CSF or serum that can be identified and studied for their association with the disease presence, outcome and progression. These biomarkers reflect the earliest changes that occur in the cell before the evidence of injury appear

on imaging techniques. Therefore, the use of biomarkers could offer a rapid, noninvasive and cost-effective tool for the diagnosis of TBI and subsequent classification and triage. These diagnostic biomarkers would also help monitor disease progression and assess outcomes of therapeutic interventions. Prognostic biomarkers in TBI may help promote early and effective treatment measures.

Biomarkers are of essential importance for successful decisions in the context of serious situations as with TBI. In these conditions, the rapid evaluation of the severity and future progression of disease is very critical especially in the first hours and days after injury where irreversible damage starts to accumulate (Selassie et al., 2008; Stocchetti et al., 2008).

Away from the bedside, biomarkers have a critical bench role of providing an insight into the underlying pathological processes of TBI associated brain damage. Valid and specific biomarkers are believed to be key players in the cascade of events leading to the pathological manifestations and could provide evidence of the involved pathways in the context of TBI and other diseases (Wagner, 2002). Biomarkers can also act as a surrogate endpoint to substitute a clinical endpoint reducing the cost and risks associated with clinical trials (Woods et al., 2012). The role of biomarkers can be summarized into reflecting disease *traits* (indicating susceptibility, predisposition and risk factors of disease), *states* (assisting in disease diagnosis) and *rates* (providing information about the progression and pathophysiology of the disease) (Fox and Growdon, 2004).

Therefore, the aforementioned limitations of current diagnostic and classification techniques in prediction of occult brain injury in TBI can be best surpassed by the discovery, validation and utilization of diagnostic biomarkers in serum or cerebrospinal fluid (CSF) that can allow for rapid assessment, minute-to-minute monitoring of disease progression, and reliable prediction of the outcome. This temporal profile of disease changes that can be reflected by these biomarkers will be essential in the chronic therapy of TBI to identify the treatment target and timing, and to early detect worsening of neurological status before microscopic lesions become apparent. This has been illustrated in Figure 1.2.

Eventually, an ideal biomarker would (1) diagnose TBI with high sensitivity/specificity before neuroimaging manifestations, (2) measure disease extent and severity, (3) predict the outcome, (4) allow for monitoring of treatment and disease progression, (5) give insight about the underlying pathophysiological mechanisms, (6) uncover new targets for therapy, and (7) this marker should be present in detectable amounts in serum and CSF.

Major advances in biomarker research have taken place in the context of several diseases like troponins in cardiovascular disease, C-reactive protein for inflammation, and creatinine for renal failure. These biomarkers,

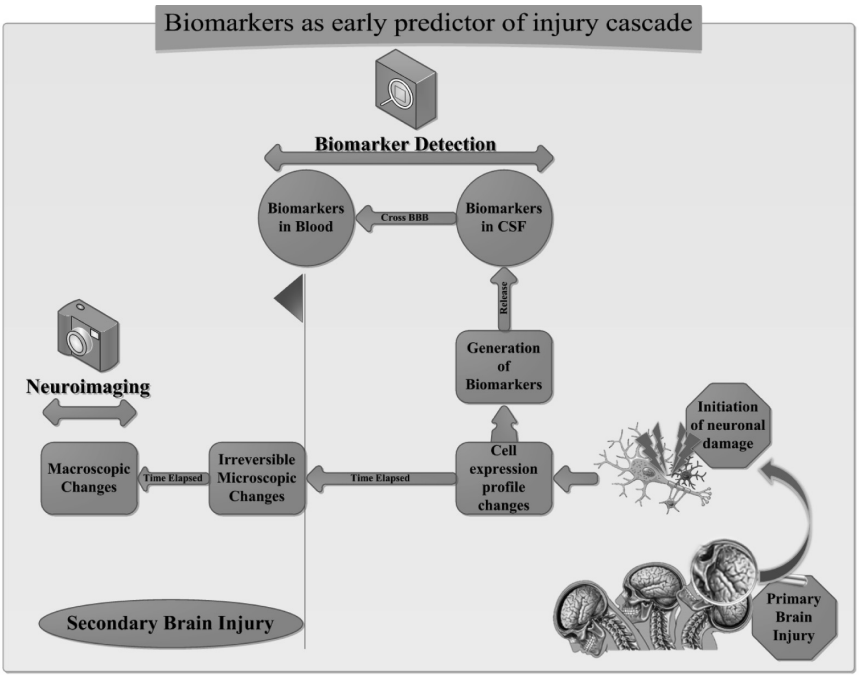

Figure 1.2. The advantage of tissue specific biomarker discovery over current imaging and diagnostic tools is that it can allow for the detection of injury early on before irreversible damage to the brain tissue ensues.

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among others across the medical disciplines, paved the way for therapeutic revolutions in the corresponding fields. However, in the context of neurological disorders like TBI, the quest of biomarker discovery is still in its early phases and awaits profound advances in discovery and verification strategies and techniques (Maas et al., 2008; Wang et al., 2005). Several areas are suggested as fields for the biomarker quest in TBI and other CNS disorders including proteomics (Cadosch et al., 2010; Conti et al., 2004; Wu et al., 2012b; Yang et al., 2009), transcriptomics (Di Pietro et al., 2010), epigenomics (Conley and Alexander, 2011), lipidomics (Bayir et al., 2007; Sparvero et al., 2010), metabolomics (Keller et al., 2011; Viant et al., 2005; Yang et al., 2012), and microRNA analysis (Lei et al., 2009; Redell et al., 2009; Redell et al., 2010). Later we will illustrate a major area of biomarker quest in TBI; namely, proteomics, illustrating the techniques used and the current standpoint of research along with limitations and future challenges.

Proteomics in TBI

The early definition of a proteome was the entire complement of expressed proteins in a biological system. However, the study of proteomics is not limited to the identification of the expressed protein sets. It rather involves the study of protein abundance, activity, localization, isoforms and modifications, as well as protein-protein interactions and functioning within higher complexes (Tyers and Mann, 2003). This is a golden aim for current proteomic practice that is limited to certain aspects of proteins status and function and to a limited subset of the protein complement (MacBeath, 2002).

The study of proteomics involves two major strategies that could be separate or complementary; (1) a discovery-oriented strategy and (2) a hypothesis-driven strategy. The major emphasis in the research community is on the unbiased discovery-oriented strategy. This methodology studies differential global expression of the proteome across varied conditions to discover new proteins and pathways. The hypothesis-driven strategy assesses the behavior of certain subset of candidate proteins to confirm their implication in certain pathophysiological or physiological effects. Both strategies attempt at the ultimate aim of discovering new disease specific biomarkers that can make their way into clinical practice (Blonder et al., 2011; Mehan et al., 2012; Schiess et al., 2009; Sjödin, 2012). In both aspects, proteomics constitute an important tool for biomarker discovery in TBI through its application on brain tissue, body fluids (serum and CSF) as well as through its utilization in different animal models of TBI as described later. In addition to the discovery of few biomarkers, proteomics can help detect expression profiles that could be disease or stage specific and allow monitoring the progression of disease and assessing response to therapy. Table 1.1 illustrates some advantages of the use of proteomics in TBI biomarker discovery.

Techniques

Traditionally, proteomics made use of two major methodologies: (1) 2-D Difference Gel electrophoresis (2D-DiGE) method, and (2) non-gel based Liquid Chromatography coupled/mass spectrometry (LC/MS) method (Becker et al., 2006; English et al., 2011; Rodriguez-Suarez and Whetton, 2013) (Figure 1.3). 2D gel electrophoresis (2D-GE) involves the separation of proteins based on molecular weight and isoelectric point. With the DiGE modification, the new technique allows for the detection of deferentially expressed fluorescent spots across samples, and then the obtained image is quantified and spots are excised to be identified by MS. In the LC/MS technique, proteins are first digested into fragments, separated by LC,

Table 1.1. Advantages and limitations of the use of proteomics in TBI biomarker discovery.

Status of proteomics in TBI biomarker discovery	
Advantages	**Challenges and limitations**
1. Allow for proteome differential analysis in serum, CSF and tissue specimens	1. Limited sensitivity of current techniques and failure to characterize entire proteome
2. Suggest putative diagnostic and prognostic biomarkers for further validation	2. Dyamic range of protein abundance in biofluids spanning 10 orders of magnitude
3. Detection of protein profile signature that adds to the criteria of patient classification	3. Non-reproducibility of the results of several studies
4. Detection of protein profile signature that can act as an outcome predictor post-injury	4. Emphasis on discovery rather than validation of findings
5. Give an idea about the involved cellular pathways in the pathogenesis of TBI	5. Inability to demonstrate the specificity of findings to TBI
6. Allow for the study of protein degradation patterns to monitor disease progression	6. Paucity of well-controlled studies that adjust for age and other confounders
7. Permit an un-biased discovery-oriented strategy for biomarker discovery	7. Non-uniformity of sample acquisition when it comes to CSF samples
8. Adjuvant protein saturation techniques help detect low abundance proteins	8. Absence of translational studies that can translate results in animals to human
9. Detection of location specific differential protein expression in tissue specimens	9. Low sample size reducing the power and reliability of the studies

and then the eluting solvent is introduced into a mass spectrometer for analysis. Hereby, mass analyzers such as Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight (MALDI-TOF) or others are used in tandem (MS/MS) to achieve higher degrees of ion separation (Lai et al., 2012). 2D-GE is more quantitative, robust and more reproducible, especially with the use of fluorescent dyes in 2D-differential-in-gel-electrophoresis (2D-DiGE) (Chen et al., 2007). However, 2DiGE is labor intensive and can cover a small range of high abundance proteins. However, LC/MS has wider protein coverage, but is less reproducible and does not support quantification unless coupled to an isotope labeling technique such as iTRAQ (Isobaric tag for relative and absolute quantitation) (Wiese et al., 2006). Despite these limitations, these two techniques are continuously refined and still play a vital role in the study of proteomics (Angel et al., 2012; Rabilloud, 2012; Sabido et al., 2012). Techniques that are more powerful have been also studied for application in high-throughput proteomics like protein microarrays or high-throughput Immunoblotting analysis (Fung et al., 2001; Hall et al., 2007; Talapatra et al., 2002). A protein microarray chip is made of thousands of different affinity reagents like antibodies, aptamers, recombinant peptides or others (for review (Espina et al., 2004)). These can interact specifically for proteins and allow for detection of large numbers of proteins with high

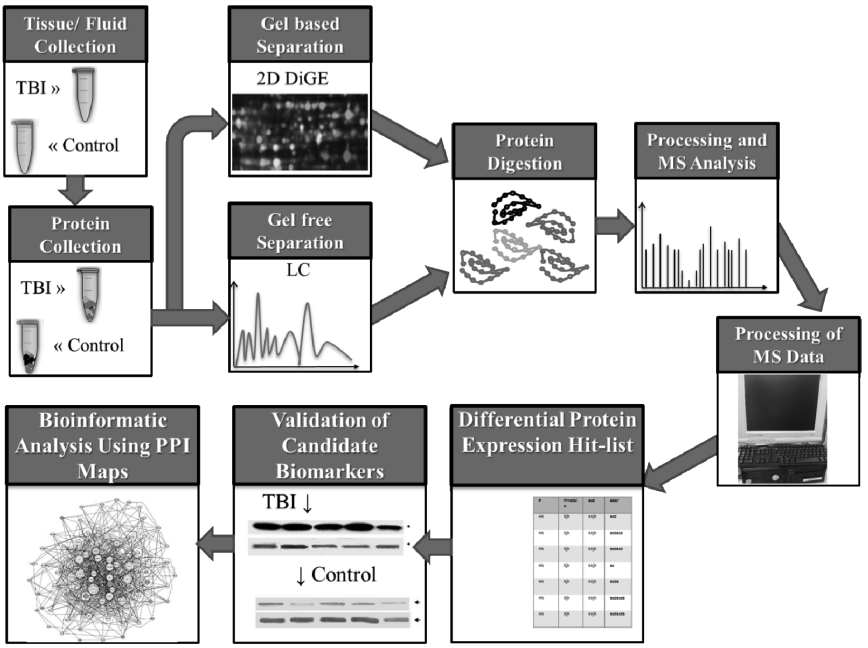

Figure 1.3. Overview of Proteomics-Based Biomarker Discovery: In brief, samples are separated by gel-based or gel-free techniques, digested and then processed into MS that allows for identification of differentially expressed proteins with the help of online databases. The obtained list of differentially expressed proteins provide an insight into candidate biomarkers that can be further validated by traditional molecular biology tools like Western blot. Further use of systems biology protein interaction analysis tools allows for analysis and visualization of resulting data in terms of interacting proteins and protein networks.

Color image of this figure appears in the color plate section at the end of the book.

fidelity through ELISA-style sandwich assays (MacBeath, 2002; Mitchell, 2002). These microarrays can also facilitate the detection of the very high number of proteins, a major limitation of previous techniques, and allows for an extensive study of protein-protein interaction (Melton, 2004; Zhu and Snyder, 2001). Future advances in the utilization and enhancement of protein microarray chips is foreseen with the incorporation of nanotechnology producing miniaturized nano-arrays that can enhance the specificity and sensitivity of current detection technique (Gonzalez-Gonzalez et al., 2012; Mitchell, 2002; Wingren and Borrebaeck, 2007; Yeates, 2011). Linked to the study of proteomics, TBI degradomics play an important role in TBI biomarker detection since protease over activation is a major aspect in brain injury (Knoblach and Faden, 2005; Raghupathi, 2004). Degradomics involves the application of high-throughput genomic and proteomic techniques to identify proteases and the protease-substrate repertoire on organismal scale (López-Otín and Overall, 2002). This allows the study of protein degradation

products of CNS-specific proteins in the serum and CSF that could be important indicators of protease activation upon injury; therefore, these degradation products can be important biomarker candidates for detecting and monitoring the progression of brain injury post-trauma.

Target Sources for Proteomics

The quest for biomarkers involves serum and cerebrospinal fluid (CSF) protein pools as well as brain tissue samples. It also involves intact and proteolytic products of proteins.

For brain tissue proteomics, human brain tissue is not readily available for proteomics tools; alternatively, animal models of brain injury have been used as initial evidence to be translated into human studies. The reason behind the use of proteomic in animal models is that brain tissue is the most abundant source of protein biomarkers (Hergenroeder et al., 2008). Several animal models have been proposed like Controlled Cortical Impact (CCI) (Edward Dixon et al., 1991), closed-head Projectile Concussive Impact (PCI) (Chen et al., 2012), fluid percussion injury (McIntosh et al., 1989), penetrating ballistic-like injury (Boutte et al., 2012; Guingab-Cagmat et al., 2012), etc… These models provide a tissue milieu to investigate relevant protein biomarkers at the site of injury or sometimes at distant sites. The studies not only investigate neurons as the master cells of the brain, but also supporting cells like microglia, astrocytes and oligodendrocyte for biomarkers as they also can be involved in major pathologies in the brain. In addition to direct tissue investigation, animal neuronal and glial cell cultures can also be used to monitor the alteration in protein expression profile post-TBI. Tissue studies as well as cell cultures can allow for spatial study of differential protein expression across different cellular locations through studying synapse-enriched or axoplasm-enriched samples (Garland et al., 2012; Wishart et al., 2012).

Akin to tissue sources that provide information about the pathological brain changes, diagnostic biomarkers suggested for the routine use in emergent conditions are to be sought in the serum, or preferentially, the CSF. Although CSF sampling is with lower convenience, CSF sampling is being more preferred for several reasons. The reasons include its proximity to the tissue of origin, higher abundance of potential biomarkers especially if blocked by the Blood-Brain-Barrier (BBB), and less contamination of other proteins present in the plasma (Alawieh et al., 2012). However, an important limitation of CSF sampling is the dynamic protein gradient between the ventricular and the lumbar cisterns (for review (Reiber, 2001)). This acts as an important confounder while comparing protein samples from patients of severe, moderate and mild TBI. Serum is another more convenient source,

yet the possibility to fish a CNS specific biomarker in the serum is quite low given the dynamic range of abundance in serum proteins and its contact with all body tissues (Alawieh et al., 2012).

These proteomics techniques were also couples to ontologies, databases and bioinformatics tools that can analyze relationships between discovered proteins and relate several proteins to a certain network, pathway or pathophysiological process (Alawieh et al., 2012). We will illustrate recent examples of the application of neuroproteomics tools to research in TBI.

Application of Different Techniques in TBI

2D-DiGE-MS

2-Dimensional differential gel electrophoresis (2D-DiGE) is one the most commonly used tool in proteomics and neuro-proteomics. Several studies have used 2D-DiGE to investigate diagnostic and therapeutic biomarkers for TBI as well as to monitor differential protein profile expression across time post-injury to monitor damage or response to therapy. The majority of reported studies used animal models to simulate TBI.

One of the most recent studies done by Yang et al. (2013) used impact accelerated model of TBI in rats, and used 2D-DIGE to detect the differential abundance of serum proteins between injured rats and controls (Yang et al., 2013). Five hundred protein spots were detected of which five proteins were identified by Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF). Of interest was Haptoglobin, a serum protein produced by the liver, whose levels were elevated post TBI and confirmed by mRNA-PCR to be over-expressed by mediation of serum IL-6 that is also elevated post-TBI. As such, serum Haptoglobin is putative biomarker for monitoring damage post-TBI especially that mediated by cytokines and acute phase reactants. Boutte et al. (2012) used a rat model of penetrating ballistic-like brain injury to investigate changes in brain tissue protein expression profile post-TBI and search for CSF biomarkers for brain injury (Boutte et al., 2012). The group used 2D-DIGE coupled to MS to discover 321 differentially expressed proteins in brain tissue that were analyzed by systems biology approach using the Ingenuity Pathway Analysis (IPA) tool or the biological functions. These proteins were involved in common cellular pathways including protein metabolism, signal transduction and cell development. Three proteins were noted to be significantly elevated in CSF and brain tissue, namely ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1), tyrosine hydroxylase, and syntaxin-6 (UCH-L1) was suggested as a potential biomarker for TBI in this model given that it has been suggested to be elevated in serum and CSF of human TBI patients by

several previous studies (Berger et al., 2012; Brophy et al., 2011b; Mondello et al., 2012b). Opii et al. (2007) used CCI model of TBI in young adult rats to investigate the role of mitochondrial oxidation abnormalities in TBI (Opii et al., 2007). Mitochondria were isolated and differential proteomics analysis was performed using 2D-DIGE-MS. The group identified several proteins that were oxidatively modified in the hippocampi and cortices of rats post-TBI including pyruvate dehydrogenase, voltage-dependent anion channel, fumaratehydratase 1, ATP synthase, and prohibitin in the cortex and cytochrome C oxidase Va, isovaleryl coenzyme A dehydrogenase, enolase-1, and glyceraldehyde-3-phosphate dehydrogenase in the hippocampus. Kochanek et al. (2006) studied the change in protein expression 2 weeks post-TBI in rats subject to CCI using 2D-DIGE-MS. Protein identification and function analysis using bioinformatics tools showed significant changes in proteins involved in glial and neuronal stress, oxidative metabolism, calcium uptake and neurotransmitter function. These proteins were further investigated for their possible implication in hippocampal plasticity and cognitive dysfunctions post-TBI (Kochanek et al., 2006). In an attempt to investigate pathways implicated in neuronal damage and degeneration post-TBI, Jenkins et al. (2002) used gel-based MS proteomics in a rat model of moderate CCI studying the changes in protein expression profile of hippocampal neurons after TBI (Jenkins et al., 2002). Their investigation using conventional and functional genomics revealed the implication of protein kinase B (PKB) signal transduction pathway in the pathogenesis of TBI as the substrates of PKB showed altered levels of phosphorylation after injury.

Knowing that aging is an important factor that influences neurodegeneration (Williams, 1995), it can act as a confounder in the study of patients' response to TBI and account for the variable response to TBI with age. Mehan et al. (2011) used a rat model of CCI using rats of all age groups (juvenile, adult and geriatric), and performed proteomics investigation for differential protein expression using 2D-DIGE-MS on neocortical tissue (Mehan and Strauss, 2011). Results have shown the involvement of 13 gene products in the age-related response to TBI including T-kininogen 1, β-actin higher in the geriatric group, collapsin response mediating protein-2 (CRMP2) that were higher in adults than the elderly, and serine protease inhibitors that were higher in the adults than juvenile and elderly. However, apolipoprotein E was upregulated post-TBI in all age groups. These results could provide an insight into the underlying mechanism of differential vulnerability of age groups to TBI, as well as to investigate universal markers of TBI that are not age-related.

The implication of oxidative stress in the pathogenesis of TBI brought up the possibility of anti-oxidant therapy using gamma-glutamylcysteine ethyl ester (GCEE) (Reed et al., 2009). Proteomics tool, namely 2D-DIGE-

MS, was used by Reed et al. (2009) to assess the therapeutic value of GCEE in the treatment of TBI through assessing the effect of GCEE on the protein expression profile difference between rats subject to brain injury with or without GCEE treatment. Results showed that the untreated group showed 19% increase in protein nitration in the brain; however, protein nitration was reduced to below controls with the administration of GCEE. Proteins protected from nitration included synapsin 1, gamma enolase, guanosine diphosphate dissociation inhibitor 1 (GDP), phosphoglyceratemutase 1, heat shock protein 70, ATP synthase, and α-spectrin. This suggests that GCEE could be of a potential therapeutic value in TBI and warrants further investigations.

Rat cortical and glial primary cultures were also used to study the changes in the cellular proteome after TBI (He et al., 2010; Siman et al., 2004). He et al. (2010) used primary astrocyte cultures from rats subject to fluid percussion injury and identified through 2D-DIGE-MS identified five proteins to exhibit significant dynamic changes after injury. These proteins were cofilin 1, destrin, phosphoglyceratemutase 1, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10, annexin 1.

In addition to animal models of TBI that suffer the limitations of validation and translation to man, 2D-DIGE-MS was also used in human studies of protein expression changes in both tissue and bio-fluids after TBI. Yang et al. (2009) studied the differential expression of human brain sample from the prefrontal cortex (PFC) of 11 injured patients compared to glioma patients (Yang et al., 2009). Seventy one proteins were identified and investigated for their functions. The main functions attributes to these discovered proteins were cell cytoskeleton, metabolism and oxidative stress, protein turnover, signal transduction and electron transport. Further investigation of these detected proteins can reveal major pathways involved in the pathogenesis of TBI and can give an insight about the identity of corresponding serum or CSF degradation products. Despite this study by Yang et al. 2009, the use of brain tissue from patients with TBI for research investigation is a rare occasion in the literature. Invariably, human neuroproteomics, in contrast to other tissue proteomics, targets patients' bio-fluids (serum and CSF) for protein biomarker discovery. Cadosch et al. (2010) studied the serum and CSF of patients with TBI compared to controls using 2D-DIGE that showed differential expression of serum and CSF proteins that included proteins that can bind to human osteo-progenitor cells (Cadosch et al., 2010). The study identified unique proteins in the serum and CSF of TBI patients, and demonstrated that some of these proteins are osteo-inductive and may be involved in fracture healing. Gao et al. (2007) used 2D-DiGE-MS to investigate the difference between inflicted and non-inflicted injury in pediatric TBI (Gao et al., 2007). Comparison of the CSF from samples of the two groups revealed a four-fold increase in acute phase

reactants and Haptoglobin in the non-inflicted group; however, the levels of prostaglandin D2 synthase and cystatin C were 12-fold more elevated in the inflicted group. Moreover, Conti et al. (2004) investigated markers of severe TBI using 2D gel electrophoresis followed by MS, and found that two proteolytic degradation products of fibrinogen beta were present only in TBI patients (Conti et al., 2004).

Gel Free Mass Spectrometry

As mentioned previously, Gel free MS is usually associated with LC separation for protein detection and identification. As 2D-DIGE, this proteomics tool has been widely used in animal models, cell cultures and human samples. In a model of mouse cortical lesion, Wishart et al. (2012) used gel-free MS with iTRAQ on synapse-enriched brain tissue samples to detect differential synaptic protein expression in TBI (Wishart et al., 2012). The investigation revealed 47 proteins six of which are potential regulators of synaptic and axonal degeneration *in vivo* and maybe involved in the pathophysiology of the disease. Protein enrichment techniques are important adjuvants to proteomics tools to overcome the limitations of masking low abundance proteins. Garland et al. (2012) also used this concept to prepare axoplasm-enriched samples of rodent optic nerve post-injury (Garland et al., 2012). Sample was analyzed by MS/MS assisted by iTARQ for quantification and results showed altered expressions of more than 300 proteins. The highest frequency of increased expression was for actin cytoskeleton proteins that showed increased expression 24 and 48 hours post-injury introducing actin cytoskeleton as a novel point of regulation in axon degeneration. Analysis of these proteins also incriminated RhoA pathway that regulates actin cytoskeleton in pathogenesis of brain injury.

MS/MS coupled to iTRAQ was also used by Crawford et al. (2012) on a mouse CCI model of mild and severe TBI (Crawford et al., 2012), and results were further analyzed for their function using Ingenuity Pathway Analysis (IPA). Results showed a temporal difference in plasma protein levels between mild and severe TBI with acute elevation of serum protein levels in mild TBI possibly indicating a reparatory process. Further investigation compared transgenic mice with favorable outcome, i.e., has human APOE3 genotype and mice with unfavorable outcome, i.e., has human APOE4 genotype. This technique allows for the detection of prognostic biomarkers for TBI that can distinguish the two outcomes.

Since different areas of the brain can be the site of TBI sequel as already mentioned, hippocampal tissue was investigated in addition to tissues from neocortex. Wu et al. (2012a) investigated the altered regulation of proteins in the CA3 sub-region of the hippocampus using a fluid percussion model

of TBI in rats. They used LC-MS/MS and discovered 1002 dysregulated proteins. An interesting finding was the involvement of calcineurin regulatory subunit, CANB1, and its catalytic binding partner PP2BA, that were decreased in TBI without changes in other calcineurin subunits. Tandem cation-anion exchange chromatography–gel electrophoresis, followed by reversed-phase LC-MS/MS was used by Ottens et al. (2010) to detect common protein markers of traumatic and ischemic brain injury. Comparison of protein profiles between a CCI and middle cerebral artery occlusion rat models revealed common upregulated proteins like albumin, degraded spectrin, and fetuin β, and common downregulated proteins like enolase α, GAPDH, aconitase 2, transgelin-3, aldolase, and MAP2 (Ottens et al., 2010). Less recently, Haskins et al. (2005) used the same technique applied to ipsilateral hippocampal tissue of rats following CCI (Haskins et al., 2005). Haskins et al., 2005 identified a protein profile for TBI that contains more than 100 differentially regulated proteins to be investigated for biomarker discovery. They also confirmed previously established biomarkers like alphaII-spectrin, brain creatine kinase, and neuron-specific enolase. Putative biomarkers like CRMP-2, synaptotagmin, and alphaII-spectrin were also suggested by (Kobeissy et al., 2006) while studying differentially expressed proteins in CCI rat model using the same technique.

Gel free MS techniques were applied to different types of CNS primary cell cultures in search for differential protein expression in response to cytotoxic and apoptotic challenges that are hypothesized to occur post-TBI. For example, Guingab-Cagmat et al. (2012) subjected rat neuronal glial culture to excitotoxic and apoptotic challenges, and identified, using MS, several proteins to be differentially expressed post injury. Of note was GFAP that was proposed as a surrogate biomarker which is degraded post-injury and its degradation products can be detected in serum. In addition, Kim et al. (2012) used gel-free LC-MS/MS to assess the changes in protein profile in response to moderate hypothermia. In their study, moderate hypothermia appeared to have a significant influence on protein profile expression of brain glial cells especially those related to inflammation. Identified proteins may have a possible role in the protective mechanism of hypothermia in response to brain injury.

Studies using Gel free MS on human samples and fluids were also reported. For instance, Lakshmanan et al. (2010) analyzed the microdialysate of patients with severe TBI 96 hours after their injury. Proteins were enriched by magnetic beads and phospho-peptides and analyzed by LC-MS/MS. Differential protein expression was detected among those patients where higher levels of cytoskeletal proteins were expressed in patients with severe metabolic distress. Alternatively, Haqqani et al. (2007) studied the serum protein profile of pediatric patients with severe TBI using ICAT followed by tandem MS. Ninety five differentially expressed proteins were identified,

and many of which were compared to the expression pattern of S100B, an established biomarker for brain injury. Eventually, several proteins were identified to exhibit comparable expression profile to S100B such as β-2 Glycoprotein I precursor and Neurofilament triple H protein.

Protein Arrays

The application of protein arrays and high-throughput immunoblotting (HTPI) in neuroproteomics is still limited to few studies. Shu et al. (2011) studied a closed brain injury rate model to identify differential protein expression in serum and hippocampus post-injury. Shu et al. (2011) utilized two types of protein arrays; a weak cationic exchanger (WCX2) chips and immobilized metal affinity capture arrays-Cu (IMAC-Cu) chip. WCX2 chips allowed the identification of 10 differentially expressed proteins and the IMAC-Cu identified 13 differentially expressed proteins; those proteins are suggestive putative biomarkers for TBI. Similarly, Kwon et al. (2011) used protein microarrays applied to both serum and brain tissue to blast injury rat model that were subjected to stress. The study revealed a protein expression profile that allows distinguishing stressed samples from samples with both TBI and stress. An interesting study done by Liu et al. (2006) aimed to compare rat hippocampal lysate in rat post-TBI to the *in vitro* calpain-2 and caspase-3 degradation profile. Liu used HTPI with 1000 monoclonal antibodies and identified 48 proteins to be downregulated in TBI, 42 of which overlapped with the calpain/caspase degradation profile. These identified proteins may serve as proteolytic targets for proteases post-TBI. To compare the difference in protein expression profile between traumatic and ischemic brain injury, Yao et al. (2008) utilized HTPI to identify the differential expression of proteins across two models of acute brain injury, ballistic-like brain injury that mimics TBI and middle cerebral artery occlusion that mimics stroke. Nine hundred and ninety eight proteins were screened; of which, 23 proteins were different across the two groups, and one was differentially expressed. The group verified the results of five proteins through Western blot, namely STAT3, Tau, PKA RIIβ, 14-3-3ϵ and p43/EMAPII.

Kovesdi et al. (2011) used protein microarrays to assess the effect of physically and socially enriched environment on the neurogenesis after TBI. The group used a blast injury model of TBI, and results have shown that such an environment was successful to ameliorate the long-term effects of TBI; reducing the levels of IL-6 and IFNγ in ventral hippocampus and normalizing the levels of tau and VEGF in dorsal hippocampus.

Nanoproteomics

With the increasing interest in incorporating nanotechnology into "-omics" approaches, nanoproteomics was incorporated in the study of TBI. Sjödin et al. (2010) used hexapeptide ligand libraries (HLL) for the enrichment of low abundant proteins then Shotgun proteomics, in combination with isoelectric focusing (IEF) and nano-LC-MS/MS, to characterize the CSF proteome of two TBI patients. The group of 339 proteins, 130 had overlap in the two studied TBI patients, and 45% of the proteins that had been previously associated with changes post-TBI were also verified. These included NSE, GFAP, S100B, and CK-B. Similarly, Hanrieder et al. (2009) used shotgun proteomics based on nano-LC in conjugation with MALDI-TOF-MS/MS to analyze the temporal profile of protein expression in three patients post-TBI. A series of ventricular CSF samples were analyzed and detected the upregulation of GFAP and NSE in addition to acute phase reactants post-injury.

In addition, new techniques have been studied more recently for the analysis of protein contents of CSF microdialysate for biomarker detection. In the study done by Dahlin et al. (2012) proteins were adsorbed to a surface-modified catheter, followed by on-surface enzymatic digestion in order to identify and quantitate proteins using isobaric tags (iTRAQ) and nanoLC-MS/MS analysis.

Evaluation of the Results of Proteomics Studies

As has been extensively reviewed before in the literature (Lista et al., 2012; Reinders et al., 2004), proteomics techniques still lag behind the promising aims that proteomics wishes to attain. General imitations include (1) inability of these techniques to identify the entire proteome complement in the body and create a unified proteome database (Perez-Riverol et al., 2013), (2) the dynamic range of protein abundance in body fluids and tissue (Zubarev, 2013), (3) interference of various other analytes like lipids, electrolytes and others, (4) absence of robust validation techniques for the findings of these studies, and non-reproducibility of findings (Alawieh et al., 2012). Table 1.1 illustrates the different challenges in proteomics application in TBI biomarker research.

Nevertheless, studies utilizing proteomics in neuro-trauma suffer additional limitations. Many of the reported studies above did not validate the proteomics findings via Western blot or other techniques. Despite the need for validation, these studies still provide a pool of putative biomarkers

for others to assess and validate. Another major limitation is the inability of the majority of reported studied to demonstrate that the findings are specific for TBI and not associated with other sequelae like inflammation and bone fracture. Human subjects that were studied for CSF and tissue samples were not always compared to healthy controls but to subjects with other diseases (Yang et al., 2009). There is also a non-uniformity of sample acquisition when it comes to CSF studies. Samples can be withdrawn from ventricles or lumbar puncture, the former usually used for patients with severe injury and the latter for mTBI patients. However, the CSF has differential protein abundance between ventricles and the lumbar levels (Hu et al., 2005). Therefore, this differential abundance if not controlled or corrected for may be the source of the difference in protein profiles and not the disease itself. For example, tau protein was reported by several studies to be differentially expressed comparing ventricular CSF samples of severe TBI and lumbar puncture CSF of control groups; however, this could be due to the normal difference in Tau levels between ventricular samples and lumbar puncture as reported by other studies (Zetterberg et al., 2013). In addition, many of these studies did not account for age-related response where age can act as an important determinant of response to injury post-trauma (Williams, 1995). Once applied to animal models, most of these studies did not demonstrate the translation potential of the findings to human; consequently, a huge effort still resides in animal-to-human translation. Statistically, the majority of these studies has lower sample size due to availability and cost eventually resulting in reduced power of the study and increased incidence of type one errors.

Despite that, studies tend to report differential protein expression in patients with TBI vs. controls; however, few of these studies dwell on the clinical value of the results and their possible prognostic or diagnostic applications in clinical practice. This scientific enthusiasm in putative biomarker discovery rather than validation is still a major limitation in proteomics research specifically as well as in the entire field of "-omics" research (Alawieh et al., 2012).

Data Mining and Neurosystems Biology Analysis

Systems biology is an approach to build a holistic, systematic and unbiased understanding (Feala et al., 2013) of the structural and behavioral model pertaining to biological networks. The exploration and analysis of the different models are based on bioinformatics data collected from genomics, proteomics, transcriptomics and metabolomics studies. The collected data cannot be manually treated and studied due to its abundance, thus data

mining using computational tools is a must in order to handle the huge amount of the collected datasets. From the exhaustive work on the collected data, dynamic models are proposed to reflect the genetic regulatory networks, the protein-protein interaction networks, the metabolic networks and canonical biological networks (Alawieh et al., 2012). The developed model is suggestive of expected cellular physiology, with candidate components and networks that can optimize experimental search and investigation and provide new data to be further incorporated in the model and holistic approach (Alawieh et al., 2012).

In vitro experiments on brain biopsy to simulate TBI or performing clinical trials for developing treatments for TBI is not an option. An ideal biomarker would be a perfect solution, but since such a biomarker has not yet been proven to exist, the best of what we can do is to identify a panel or signature of markers (Feala et al., 2013) and try to find out accurate networks that can describe and simulate the pathophysiology of TBI.

The challenge of treating and anticipating secondary damage in TBI can be handled by systems biology through constructing a system scale model to detect and treat TBI, to handle the observations and come up with hypothesis that can be tested and fed back to the system to optimize the results of the model and get insight into the underlying pathophysiology and possible diagnostic and therapeutic modules.

In the context of proteomics, systems biology tools can be incorporated in several ways to overcome many of the limitations of simple proteomics studied. Discovered proteins through common proteomics approaches can be mapped, using protein databases. Many online systems biology resources are available to help building the proteomics and canonical networks of systems biology model into the associated pathways in the cell including cell division, apoptosis, energy metabolism, oxidative stress and others. For instance, Boutte et al. (2012) used IPA software to map 321 differentially expressed proteins obtained by mass spectrometry into relevant cellular pathways.

The importance of the integration that systems biology apply to form hypothesis and propose candidate biomarkers is that the system level would give value to some biomarkers that a human would find of no value at a small scale and may be proven to be of essential role at a system scale. Such a finding could not be realized without application of systems biology. Furthermore, the complexity of the pathways and protein-protein interactions that occurs in the secondary stage, along with the presence of a cascade of injury over a wider area of the brain (diffuse axonal damage). All of these secondary phase TBI diseases are difficult to be studied and interpreted to suggest hypotheses and new biomarkers without a systematic model that takes into accounts the dynamicity of the system along with the different interactions among components. It is through systems biology

network analysis also that we can find major players in relevant pathogenic pathways to be identified as putative targets of novel biomarker based theranostics.

Clinical Relevance of Suggested Biomarkers

The immense investigation of protein biomarkers in TBI has resulted in several putative diagnostic and prognostic markers; however, none of these biomarkers is yet approved by FDA for adoption in clinical practice (Robinson et al., 2009). Most of the application of putative biomarkers is restricted to clinical trials, and several algorithms were proposed to incorporate their use in clinical practice. The clinical status of a group of significant biomarkers for TBI will be reviewed here and are summarized in Table 1.2.

Table 1.2. The clinical relevance of major TBI biomarkers.

Prominent TBI biomarkers in scientific literature			
Biomarker	**Location**	**Findings**	**Process involved**
S100B	Serum, CSF	Increased in TBI patients, high negative predictive value toward CT findings, yet not specific for TBI	Astroglial injury/BBB damage
Neuron Specific Enolase (NSE)	Serum, CSF	Increased in serum and CSF of TBI patients; low sensitivity and specificity; limited utility due to effect of hemolysis	Neuronal Injury
Glial Fibrilllary Acidic Protein and BDPs	Serum, CSF	Increased in serum and CSF of TBI patients; controversy toward specificity as biomarker; CNS specific protein; best if used in combination with NSE and S100B	Astroglial injury
Tau Protein	Serum, CSF	Good outcome prediction in patients with severe TBI; limited utility in mTBI	Axonal Injury
AlphaII-Spectrin-SBDP	CSF, Serum	Possible predictor of outcome in severe TBI; not studied in mild TBI	Axonal Injury/ Cell death
UCH-L1	CSF, Serum	Useful in assessment of patients with severe TBI; better outcome prediction if measured with α-II spectrin; limited studies in mTBI	Neuronal Injury
MAP2a/2b	Serum	Recently studied; limited evidence; reported to increase in the sera of patients with severe TBI	Neuronal Injury

S100B

S100B is a low molecular weight calcium-binding protein important in intracellular calcium regulation. It was thought to be specific to astrocytes but later discovered to be present in oligodendrocyte and other extra-cerebral cell types such as adipocytes, chondrocytes, skeletal muscles and bone marrow cells (Berger et al., 2006; Donato, 2001; Olsson et al., 2011). S100B is the earliest and most extensively studied biomarker for TBI, and most of published studies examined its increased level in serum as a putative marker of TBI. However, S100B donot cross the Blood Brain Barrier (BBB), and its presence in the serum is dependent on disruption of the integrity of BBB (Herrmann et al., 2000). Elevated levels of S100B have been highly linked to astroglial injury. The first study to emphasize the role of serum S100B in mTBI patients was done by Ingebrigtsen et al. (1995). The study showed that elevated serum S100B levels in patients with negative CT findings is associated with the occurrence of post-concussive symptoms (Ingebrigtsen et al., 1995). Several other studies, since then, have investigated the clinical prognostic value of elevated serum S100B levels in TBI patients with conflicting evidence (Bazarian et al., 2006; De Kruijk et al., 2001; Egea-Guerrero et al., 2012; Ingebrigtsen et al., 2000; Ingebrigtsen et al., 1999; Mercier et al., 2013; Muller et al., 2007; Schiavi et al., 2012; Spinella et al., 2003; Unden et al., 2007; Vos et al., 2010; Vos et al., 2004). Noteworthy, (Undén and Romner, 2010), did a meta-analysis of articles studying mild head injury comparing CT findings and S100B in the acute phase of injury. The group found 12 eligible articles with a total 2466 patients, and discovered a high sensitivity of low levels of S100B in the prediction of negative CT findings. They suggested that a low serum S100B level (<0.10 µg/L) in the first three hours after injury has more than 90% negative predictive value of the presence of clinically relevant CT findings. Similar findings were also reported by other studies using large samples of patients suggesting the use of serum S100B as a substitute for CT in assessment of mTBI patients (Biberthaler et al., 2006; Zongo et al., 2012).

Even if those studies demonstrate the sensitivity of the use of S100B as a biomarker for TBI, the main limitation towards its use is the lack of specificity to brain trauma especially that S100B can be released by cells other than astrocytes (Berger et al., 2006). Several studies have demonstrated the elevation of S100B in bone fractures without head injury (Anderson et al., 2001; Routsi et al., 2006; Undén et al., 2005).

Despite the abundance of studies reporting serum S100B elevation, studies of CSF levels of S100B in TBI is still limited (Zetterberg et al., 2013). In the study of Neselius et al. (2012) brain injury was found to trigger the

release of some biomarkers into the CSF including S100B and other proteins. This; however, does not defy the use of S100B as a screening agent due to its high sensitivity.

Neuron Specific Enolase

Neuron-Specific Enolase (NSE) is an isozyme of glycolytic enzyme enriched in neuronal cell body (Olsson et al., 2011). After its isolation in brain tissue and peripheral neurons, NSE was found to be also expressed in erythrocytes, platelets neuroendocrine cells and oligodendrocyte (Kövesdi et al., 2010). Yet, it was investigated as a biomarker indicating neuronal damage and a possible predictor of TBI outcome.

The levels of NSE were studied in both serum and ventricular CSF. NSE was found to be a predictor of outcome after TBI, especially in patients with severe injury, yet unsatisfactory specificity and sensitivity have been reported (Böhmer et al., 2011; Fridriksson et al., 2000; Geyer et al., 2009; Meric et al., 2010; Skogseid et al., 1992; Topolovec-Vranic et al., 2011). In the studies reporting the utility of NSE as a biomarker for brain trauma, lower sensitivities and specificities than S100B were detected (Herrmann et al., 2000; McKeating et al., 1998; Meric et al., 2010; Topolovec-Vranic et al., 2011). Eventually, it is proposed that NSE is not to be used as a standalone screening biomarker for brain injury (Topolovec-Vranic et al., 2011). The limited utility of NSE as a biomarker of brain trauma may also be related to the high sensitivity of NSE to hemolysis (Ramont et al., 2005).

Glial Fibrillary Acidic Protein

Glial Fibrillary Acidic Protein (GFAP) is an intermediate filament that is believed to be exclusively expressed by astroglia (Olsson et al., 2011). GFAP was studied in both CSF and sera of patients with TBI (Böhmer et al., 2011; Honda et al., 2010; Nylen et al., 2006; Vos et al., 2010; Zetterberg et al., 2013).

Several studies have reported a range of predictive value and specificity. This is likely due to different in ELISA methods used (Honda et al., 2010; Metting et al., 2012; Vos et al., 2010). More recently, GFAP have been reported to be processed into breakdown products (Guingab-Cagmat et al., 2012; Zoltewicz et al., 2012). Detection of GFAP and its breakdown products with a new ELISA format has been reported to detect both mild-moderate TBI (Papa et al., 2012a) and the full spectrum of TBI (TRACK-TBI cohort) (Okonkwo et al., 2013) in two independent studies. Another recent follow up paper with the TRACK-TBI cohort shows that the combination of UCH-L1 with GFAP/BDP further improves its diagnostic utilities (Diaz-Arrastia et al., 2013).

Tau is an axonally enriched microtubule associated protein and one of the best established CSF biomarkers of axonal damage (Zetterberg et al., 2013). TBI was reported to cause the cleavage of tau protein and elevation of levels of cleaved-tau (C-tau) in CSF and serum (Gabbita et al., 2005). The level of C-tau protein in serum and CSF was studied similar to other biomarkers and was associated with both disruption of BBB and cleavage of tau protein post injury (Gabbita et al., 2005). Studies by Zemlan et al. (2002) and Franz et al. (2003) have demonstrated the significance of C-tau in prediction if outcome in patients with severe TBI (Öst et al., 2006). Similarly, other studies reported the utility of C-tau in the prediction of outcome in mTBI (Bulut et al., 2006; Wuthisuthimethawee et al., 2013). However, other studies have reported the poor ability of tau protein to predict outcome and post-concussion syndrome in mTBI (Bazarian et al., 2006; Ma et al., 2008). This limits the utility of C-tau in the outcome prediction of patients with mTBI.

AlphaII-spectrin Breakdown Products (SBDPs)

αII-spectrin degradation products is among the novel biomarkers studied for their clinical relevance in TBI (Zetterberg et al., 2013). αII-spectrin is a cytoskeletal protein enriched in neuronal axons and presynaptic terminals (Berger et al., 2012). While αII-spectrin is present in various nucleated cells, and most tissues, but its high abundance and enrichment of brain still make it a candidate biomarker, especially if used in combination with another more brain-specific marker (Zhang et al., 2011). The breakdown products of αII-spectrin (SBDPs) is thought to be due to the activation of calpain and caspase in the brain after TBI, and thus reflects axonal damage (Pike et al., 2004). SBDP150 and SBDP145 are characteristics of calpain activation, often associated in acute necrotic neuronal cell death while SBDP120 is generated by action of caspase-3 and is affiliated with delayed apoptotic neuronal death (Wang, 2000; Zhang et al., 2009).

Mainly, αII-spectrin was studied in the context of severe rather than mild TBI. Elevation of levels of αII-spectrin degradation products SBDP150 and/or SBDP145 in CSF was reported as a possible outcome predictor in patients with severe TBI (Cardali and Maugeri, 2006; Mondello et al., 2010; Pineda et al., 2007).

Ubiquitin C-terminal Hydrolase (UCH-L1)

Ubiquitin C-terminal Hydrolase (UCH-L1) is a deubiquitinase highly expressed in neuronal cells that is another recently discovered candidate biomarker from a rat TBI model-based differential proteomic study

(Kobeissy et al., 2006). In addition, its high brain specificity and abundance in brain tissue makes it an attractive candidate marker (Brophy et al., 2011a). Similar to αII-spectrin, UCH-L1 CSF and serum levels were found to be elevated in patients with severe TBI correlating with the severity and outcome of injury (Brophy et al., 2011a; Mondello et al., 2012c; Papa et al., 2010; Siman et al., 2009). The elevation of levels of UCH-L1 post-TBI is proposed to be secondary to BBB dysfunction (Blyth et al., 2011). In addition, several recent studies also demonstrated the detectability of UCH-L1 in blood following mild TBI (Diaz-Arrastia et al., 2013; Papa et al., 2012b) and mild TBI injury (Siman et al., 2008).

Together with the breakdown products of α-II spectrin, the serum levels of UCH-L1 was found to change in a similar manner to S100B and GFAP post-injury and to be a an important predictor of outcome in patients with moderate to severe brain injury (Berger et al., 2012; Mondello et al., 2011).

The study of UCH-L1 utility in mTBI is still limited; one study by Papa et al. (2012b) reported that UCH-L1 was identified in the sera of patients with mild to moderate TBI, and its levels correlated with traditional clinical assessment. It was reported that using a cutoff level of 0.09 ng/ml, 100% sensitivity was achieved with 21% specificity demonstrating the negative predictive potential of the test. Nevertheless, the utility of UCH-L1 in mTBI still needs further clinical assessment.

Microtubule Associated Protein-2 (MAP-2)

MAP-2 is dendritically enriched neuronal cytoskeletal protein with two isoforms (MAP-2a and MAP-2b) (Conde and Cáceres, 2009). MAP-2 was reported to be degraded in hippocampal tissue post-TBI so that its degradation products would be suggestive biomarkers for TBI (TAFT et al., 1992). Recently, Mondello et al. (2012a) studied the levels of MAP-2 in patients with severe TBI. This study demonstrated the elevation of the levels of MAP-2 degradation products in patients with severe TBI six months after injury suggesting that MAP-2 could be involved in the chronic neuronal changes that occur post-TBI. However, this remains a solitary study that reported elevation of levels of MAP-2; thus, MAP-2 is still early on the road of application in clinical practice.

Conclusion

Currently, the applications of proteomics in the field of neuro-critical care have failed to provide new FDA-approved biomarkers. However, future work aiming at overcoming the aforementioned limitations of the applied techniques together with better statistical regulation of the analysis and

reporting of results, and a more reproducible and robust approaches, still hold a lot of capacity to step up our current understanding of TBI, suggest new therapeutic approaches, and provide a bases for personalized medicine. In this context, basic science researchers, clinicians, epidemiologists, biostatisticians, engineers and mathematics experts together with authoritarian agencies should bring hands together in order to provide a new platform for cooperative work that will ultimately lead to new discoveries.

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2

Protein Biomarkers in Traumatic Brain Injury: An Omics Approach

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INTRODUCTION

Traumatic brain injuries (TBIs) pose a great health concern. In the United States alone, nearly 2 million people per year will have suffered a TBI. In the civilian population, most TBIs result from falls (35.2%), motor vehicle accidents (17.3%) and assault (10%). Both youth and adult athletes may suffer multiple concussions (http://www.cdc.gov/traumaticbraininjury/statistics.html). They are often exposed to a variety of combat traumas. More than 30,000 military personnel suffered a TBI in 2012. Another 13,000 or more people had a TBI in 2013 (http://www.dvbic.org/dod-worldwide-numbers-tbi). TBI is highly variable and characterized by several severities

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(mild, moderate, severe) as well as multiple injury types (concussive, non-penetrating, penetrating). Mild TBIs are of particular interest to the military, as explosions or "blast"-induced injuries are the most common TBI among active duty personnel (Mondello et al., 2013). More recently, mTBI is a key topic among persons involved in athletic programs (Omalu et al., 2010; Stern et al., 2011). Both youth and adult athletes may suffer multiple concussions (http://www.cdc.gov/traumaticbraininjury/statistics.html).

Defining specific mechanisms for which therapeutic approaches would be effective for TBI, which is heterogeneous, remains elusive. In addition, mild TBIs are often difficult to detect with cognitive tests alone and cannot be resolved using clinical imaging techniques, such as Magnetic Resonance Imaging (MRI) (Yuh et al., 2013). Thus, the use of protein biomarkers to define, diagnose, monitor or treat TBI would greatly enhance efforts to manage patient care. Biomarkers may lead to understanding mechanisms of injury and recovery. Basic and clinical research efforts are currently determining the mechanism of TBI with many methods, including analysis of targeted biomarkers coupled to clinical and/or behavioral tests (Diaz-Arrastia et al., 2013; Okonkwo et al., 2013). The main goal of many biomarkers studies is to provide correlations to severity and/or duration of injury. In addition, biomarkers could potentially be used to evaluate the therapeutic response (Mondello et al., 2013). TBI biomarker research is poised to greatly impact wound healing, recovery, and increased survival with improved quality of life.

The Role of Proteomics in TBI Biomarker Discovery

Biomarkers are naturally occurring molecules found in tissues or bio-fluids that uniquely identify an abnormal/pathological state, such as TBI. They are often (and ideally), indicators of very specific processes, events or conditions (Guingab-Cagmat et al., 2013; Martins-de-Souza, 2010). "Omics" technologies encompass any study that considers all parts of a system or group of analytes collectively. Omics may study any macromolecule or metabolite, e.g., DNA or RNA for genomics fatty acids in lipidomics, and metabolites in metabolomics. Proteins are the molecules of interest in proteomics, which has a plethora of methodologies available to TBI research.

The proteome includes all proteins in the cell, tissue, or organ of interest and proteomics is the investigation of all proteins in a particular physiological state and may also encompass metrics such as TBI type, time lapsed or severity. Proteomics has become one of the most sophisticated and sensitive tools employed in biological studies. It has become more popular due to, in part, (1) the ability to transfer studies across species due to redundancy of the mammalian genome, (2) the ability to detect brain-specific proteins in biofluids in TBI, and (3) the need for novel markers of injury

that are readily detectable in biofluids. Furthermore, proteomics may be a preferred method, as opposed to genomics. Genomics assays infer changes in protein abundance; the correlation of gene based studies to protein confirmation is low (Pradet-Balade et al., 2001). In addition, messenger RNA studies are not readily translated to bio-fluid analyses. Proteomics analyses have also expanded from beyond studying groups of proteins to understanding the protein complexes, gaining knowledge of isoforms, as well as discerning distinct roles that certain protein-protein interactions and intact pathways may play within the milieu systems biology.

Proteomics is becoming a well-established approach to discover and validate protein biomarkers in TBI. The collective number of published reports and citations utilizing proteomics in brain injuries is increasing: there were more than 500 in 2012 alone compared to less than 50 a decade ago. In addition, there is an increased interest in clinical and/or deployment related TBI, for the number of military TBI publications and citations has more than doubled since 2010 (Thompson Reuters, Web of Knowledge). Both tissue and biofluid-based biomarkers have been used to determine severity of injury (Zoltewicz et al., 2013) and may be able to determine therapeutic response (Zhang et al., 2010).

Proteomics-based technologies could potentially be used in-theatre (on or near the battlefield) or during triage to aid diagnoses, specifically in mild injuries where greater sensitivity and specificity is key. Omics technologies may be used to determine a variety of endpoints in the realm of protein markers: intact proteins, products of proteolysis and post-translational modifications. This chapter presents an overview of current technologies, sample sources (e.g., cell types, brain tissue regions and biofluids), and possible future directions in TBI proteomics. First the role of animal models is briefly introduced.

Animal (e.g., rodent) models can serve as surrogates to understand injury mechanisms and define treatments based on biomarkers and the vast majority of proteomics research is performed with experimental animal models of TBI. Several types of TBIs are explored in animal models (Xiong et al., 2013), some of which are:

1. Controlled Cortical Impact (CCI)—an open skull injury that uses a piston to penetrate the brain at a specific velocity and distance (Dixon et al., 1991).
2. Projectile Concussive Impact (PCI)—a helmet shielded closed skull injury (Chen et al., 2012).
3. Fluid Percussion Injury (FPI) or Lateral Fluid Percussion (LFP)—an open skull injury that rapidly inject fluid into the intracranial cavity (Alder et al., 2011; Dixon et al., 1987).

4. Penetrating Ballistic-like Brain Injury (PBBI)—a skull breaching gunshot waveform projectile model that forms a permanent, but non-lethal, cavity in the brain (Williams et al., 2006).
5. Middle Cerebral Artery occlusion (MCAo)—a model of stroke, ischemia and infarct that produces a brain lesion (Dave et al., 2009; Carmichael, 2005).
6. Blast or Blast Over-Pressure (BOP)—a model of an explosive charge wherein a whole body pressure wave is administered (Ahlers et al., 2012; Elder et al., 2010).

Many of these models are well defined in terms of post-mortem histopathology; however, there are currently few reliable markers that fully characterize these injuries and harbor clinical utility. These studies may allow definition of injury-specific mechanisms among individuals or groups of patients and facilitate or improve therapeutic regimens. The status of protein biomarkers used in TBI research is introduced next and extensively summarizes applicable proteomics approaches for discovery and confirmation.

Protein Biomarkers and Their Origins

Overview

Proteomics studies may be used to discover biomarkers from many sources. The current convention is to perform proteomics discovery or confirmation with brain tissues or cells containing the lesion induced by TBI. Brain proteins are then assayed in cerebral spinal fluid (CSF) or in the blood (serum or plasma) as a consequence of blood brain barrier (BBB) breakdown and leaky cellular membranes of apoptotic/necrotic cells (Boutte et al., 2012; Guingab-Cagmat et al., 2013). These approaches have proven successful as brain specific proteins have been detected across multiple models and clinical studies.

The Milieu of Protein Biomarkers

Intact proteins, Proteolytic Fragments and Post-translational Modifications

A class of neuronal and glial proteins has been reproducibly identified across animal models and clinical cases of TBI. Some models or clinical samples often identify these same proteins within the proteome, particularly proteins from neurons, astrocytes and oligodendrocytes. In both neurons

and glia, proteins are released from necrotic and/or apoptotic cells and are detectable in Cerebral Spinal Fluid (CSF) as well as in blood plasma or serum. These biomarkers have been detectable in the CSF and blood of some patients with severe TBI, including those suffering stroke/cerebral ischemia or penetrating injuries (Hergenroeder et al., 2008; Mondello et al., 2012; Morochovic et al., 2009; Rundgren et al., 2012; Stein et al., 2011a). Defining biomarkers with greater sensitivity and specificity is imperative to determine severity of injury, recovery or response to therapy and to differentiate injuries from one another.

The abundance of neuronal proteins like Neuron Specific Enolase (NSE), ubiquitin C-terminal lyase (UCH)-L1, amyloid precursor protein (APP), neurofilament protein (NF), non erythroid α-II spectrin, and Tau are differentially abundant in tissues and biofluids of TBI models including MCAo, PBBI, LFP and CCI (Aikman et al., 2006; Bohmer et al., 2011; Brophy et al., 2009; Bulut et al., 2006; Magnoni et al., 2012; Papa et al., 2012; Park et al., 2007; Zurek and Fedora, 2012). Microglia, astrocytes and oligodendrocytes also play a key role in TBI as neuro-glial inflammation peaks in response to injury (Ramlackhansingh et al., 2011; Yu et al., 2010). These cells express and release intracellular proteins, such as Glial Fibrillary Acidic Protein (GFAP), and Myelin Basic Protein (MBP), respectively. Microglial cells also secrete greater proportions of cytokines and chemokines when activated after TBI (Ghirnikar et al., 1998). Infiltrating immune cells from the periphery also play a role. Derived from infiltrating immune cells/leukocytes, endothelial monocyte-activating polypeptide II precursor (p43/pro-EMAPII), has been identified as a potential biomarker that is differentially regulated in models of hemorrhagic vs. non-hemorrhagic TBI. P43/pro-EMAPII was upregulated following PBBI which is hemorrhagic and downregulated following MCAo which is non-hemorrhagic (Yao et al., 2009).

In addition to intact proteins, fragments or Break-Down Products (BDPs) may be considered markers of TBI. BDPs are greatly increased after TBI as a consequence of cell death pathway activation and increased activity of pro-apoptotic enzymes (like caspase-3) and pro-necrotic enzymes (like calpain-2). In fact, the 2-dimensional proteomic map of protein fragments isolated from the brain after rodent CCI overlapped with that of brain lysates subjected to caspase-2 or calpain-2 degradation (Liu et al., 2006). This "degradome" introduced several potential targets of study, but several specific protein fragments or BDPs have been the focus of TBI. GFAP-BDPs and non-erythroid α-II spectrin BDPs (SBDPs) have been the focus of many animal models and clinical studies. Both have been detected in biofluids and are correlated to injury severity, poor outcome