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Mild traumatic brain injury-induced hippocampal gene expressions: The identification of target cellular processes for drug development

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HIGHLIGHTS

- A single mild concussive traumatic brain injury in mice is associated with many changes in hippocampal gene expressions.
- Changed genes were associated with inflammation and neurological functional gene ontology and molecular pathways.
- Several gene ontology and molecular pathways were associated with neurodegeneration, namely Alzheimer's Disease.
- Knowledge of altered molecular processes induced by mild traumatic brain injury will help to develop novel therapies.

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ABSTRACT

Background: Neurological dysfunction after traumatic brain injury (TBI) poses short-term or long-lasting health issues for family members and health care providers. Presently there are no approved medicines to treat TBI. Epidemiological evidence suggests that TBI may cause neurodegenerative disease later in life. In an effort to illuminate target cellular processes for drug development, we examined the effects of a mild TBI on hippocampal gene expression in mouse.
Methods: mTBI was induced in a closed head, weight drop-system in mice (ICR). Animals were anesthetized and subjected to mTBI (30 g). Fourteen days after injury the ipsilateral hippocampus was utilized for cDNA gene array studies. mTBI animals were compared with sham-operated animals. Genes regulated by TBI were identified to define TBI-induced physiological/pathological processes. mTBI regulated genes were divided into functional groupings to provide gene ontologies. Genes were further divided to identify molecular/cellular pathways regulated by mTBI.
Results: Numerous genes were regulated after a single mTBI event that mapped to many ontologies and molecular pathways related to inflammation and neurological physiology/pathology, including neurodegenerative disease.
Conclusions: These data illustrate diverse transcriptional changes in hippocampal tissues triggered by a single mild injury. The systematic analysis of individual genes that lead to the identification of functional categories, such as gene ontologies and then molecular pathways, illustrate target processes of relevance

Abbreviations: TBI, traumatic brain injury; mTBI, mild traumatic brain injury; ICR, Institute for Cancer Research mouse; AD, Alzheimer's disease; CNS, central nervous system; cDNA, (cDNA) complementary DNA; cRNA, complementary RNA; PAGE, Parametric Analysis of Gene Set Enrichment; GO, gene ontology term; q-RT-PCR, quantitative reverse-polymerase chain reaction; FDA, U.S. Food and Drug Administration.

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to TBI pathology. These processes may be further dissected to identify key factors that can be evaluated at the protein level to highlight possible treatments for TBI in human disease and potential biomarkers of neurodegenerative processes.

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1. Introduction

Traumatic brain injury (TBI) has become a highly prevalent medical cause for concern, an insult soon to exceed traditional ailments as a main cause of death in the United States (Faul et al., 2010). The induction of TBIs may occur via several mechanisms, such as a concussive event or a high pressure shockwave generated by an explosion (Daneshvar et al., 2015). Clinically, TBI can be categorized into several classes such as mild, moderate and severe. These classifications are based upon criteria related to several indices, such as the Glasgow Coma Scale, length of post-traumatic amnesia, results of neuroimaging and whether or not the insult resulted from an open or closed skull injury (Gómez et al., 2014). Clinical cases of TBI in the civilian world are predominantly categorized as 'mild' and concussive in nature, requiring a visit to a hospital emergency department followed by discharge (Korley et al., 2015). In addition to the immediate health related effects of TBI on patients, recent epidemiological studies suggest that there is an association between diverse forms of concussive TBI and the subsequent development of neurodegenerative dementia-related illness in later life (Barnes et al., 2014; Daneshvar et al., 2015; Gupta and Sen, 2015).

Currently, there is no proven effective drug therapy available for the treatment of any form of TBI (Stein et al., 2015; Xiong et al., 2015). This leaves a significant treatment gap for TBI which requires urgent attention from the scientific and medical community. With the multiple types of clinical TBI, no simple animal model best represents clinical injury (Marklund and Hillered, 2011). Appropriate models need to be developed to aid in the evaluation of candidate therapies, including the use of novel or repurposed medicines.

Many events triggered by TBI have been described (Choi et al., 1987; Maas et al., 2008; Greve and Zink, 2009; Stoica and Faden, 2010; Barkhoudarian et al., 2011; Cornelius et al., 2013), still further evaluation and description of the molecular events related to the pathology of TBI require study. One powerful approach to address this knowledge gap includes one of a mass screening of central nervous system (CNS) molecular markers to provide insight into the molecular and cellular processes driving the pathology of TBI. The use of large-scale gene array chips and powerful bioinformatics tools to examine an organ or a tissue's transcriptomic profile has opened up an avenue to perform such studies. The identification of molecular pathways initiated by TBI provides a platform for the systematic evaluation of known and novel therapies that may ameliorate or slow the progression of TBI-induced cognitive or neurological disorders.

As mild TBIs may be the more common form of injury in the clinic, we have opted to study molecular events triggered by a well-characterized mild closed head weight drop model of TBI in the mouse. This model, involving a 30–40 g mouse concussed with a 30 g weight, bears face validity to the human condition involving the clash of heads between two similar sized adults in a sports injury. The study described here illustrates the effects of a mild TBI on mouse hippocampal tissue gene expressions 14 days after the initial injury; however, the same methodologies can be applied to other models of TBI, animal species and times post injury. Through the use of sensitive, large-scale gene array chips we identified large numbers of subtle gene regulations driven by a single mTBI event. Additional bioinformatics analysis of the regulated genes indicated that TBI was associated with numerous inflammatory and neurological physiological/pathological processes that provide a basis for targeted drug development programs.

2. Materials and methods

2.1. Animal studies—Housing and induction of mTBI

ICR mice (Institute for Cancer Research (ICR)) were housed five per cage under a constant 12-h light/dark cycle, at room temperature (23 °C). Food (Purina rodent chow) and water were available ad libitum. Each animal was used for one experiment only. The Ethics Committee of the Sackler Faculty of Medicine approved the experimental protocol (M-12-063), in compliance with the guidelines for animal experimentation of the National Institutes of Health (DHEW publication 85–23, revised, 1995). A minimal number of mice were used for the study and all efforts were made to minimize suffering. mTBI was induced as has been described previously (Zohar et al., 2003; Milman et al., 2005; Edut et al., 2011; Baratz et al., 2011), mice (30–40 g) were fully anesthetized by exposure to Isoflurane. After full anesthesia was achieved, the animals were placed under the opening of a weight drop device and a weight (30 g) dropped from a height of 80 cm. Immediately thereafter, the animals were placed in a recovery cage and were observed until full recovery from anesthesia occurred and they could be returned to their home cages. For the sham procedure, animals were anesthetized and placed under the weight drop device; however, no weight was dropped. In the present study the group sizes were as follows: sham $n = 5$ and for mTBI $n = 4$.

2.2. Hippocampal cDNA gene array hybridization

Fourteen days after the induction of mTBI, mice were euthanized and the ipsilateral hippocampus extracted for use in cDNA gene array studies. The entire hippocampus was used to prepare RNA and the Qiagen RNeasy Mini Kit used to prepare total RNA using the manufacturer's specifications (Qiagen, Inc. Valencia CA). The Agilent 2100 Bioanalyzer with RNA 6000 Nano Chips Quantity was used to determine the quality and quantity of the RNA. Biotin-labeled, amplified (aRNA) was created by using the Illumina TotalPrep RNA Amplification Kit (Ambion; Austin, TX, cat # IL1791). A total of 750 ng of aRNA was hybridized at 58 °C for 16 h to Illumina's SentrixMouse Ref-8, v2 Expression BeadChips (Illumina, San Diego, CA). The arrays were washed and then blocked, after which the biotin-labeled probe was detected by staining with streptavidin-Cy3. Arrays were scanned at a resolution of 0.8 μm using Beadstation 500× from Illumina, and data intensity extracted from the array image using Illumina BeadStudio software, V3.

2.2.1. Bioinformatic analysis of array data—Regulated genes

Hippocampal gene expression profiles were compared between mTBI and sham animal samples, as has been described previously (Tweedie et al., 2013a,b, 2015). Raw array chip hybridization image signals were filtered and processed to generate normalized data that was then transformed to create Z-scores for each gene. Z-score transformed data was then utilized to generate a Z-ratio measurement, which allowed for statistical analysis of the gene expression