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Traumatic Brain Injury: Classification, Models and Markers

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Abstract

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality worldwide. Due to its high incidence rate and often long-term sequelae, TBI contributes significantly to increasing costs of medicare expenditures annually. Unfortunately, advances in the field have been stifled by patient and injury heterogeneity that pose a major challenge in TBI prevention, diagnosis and treatment. In this review, we briefly discuss the causes of TBI, followed by its prevalence, classification and pathophysiology. The current imaging detection methods and animal models used to study brain injury are examined. We discuss the potential use of molecular markers in detecting and monitoring the progression of TBI, with particular emphasis on microRNAs as a novel class of molecular modulators of injury and its repair in the neural tissue.

Keywords

Animal Models, Biomarkers, Imaging, microRNA, TBI

Causes of Brain Injury

Brain injury has been recognized as one of the leading causes of disability and death in both developed and developing countries. Depending on the cause, damage to the brain can be classified into two broad categories: traumatic and non-traumatic brain injuries. In traumatic brain injury (TBI), the damage to the brain is generally caused by external forces, either direct or transmitted, caused by falls, motor vehicle collisions, sport-related injuries, abuse/assault or pressure blasts. On the other hand, in non-traumatic brain injury, damage to the brain is mainly caused by infections, brain tumors, ischemia or stroke (Table 1). In some surgical cases, injuries to the brain tissue occur unintentionally through invasive procedures. Regardless of its cause, brain injury can result in physical, cognitive and behavioral impairments, leading to temporary or permanent dysfunction (Reis et al. 2015). The patient and injury heterogeneity associated with TBI, along with the wide spectrum of its clinical manifestations, continue to pose a significant challenge in its prevention, diagnosis, and treatment (Young et al. 2015). Therefore, improved detection methods for milder injuries, identification of reliable prognostic markers to guide clinical decision making, and development of effective therapies are critical steps towards repairing the damaged brain (Menon and Maas 2015).

Traumatic Brain Injury Statistics

In Canada, approximately 160,000 people sustain brain injuries each year, leading to 11,000 deaths and over 6,000 cases of permanent disability ("Brain Injury Association of Waterloo-Wellington; Stats" n.d., "Brain Injury Canada; Acquired Brain Injury" 2015). Nearly half of all TBI cases in Canada result from falls and motor vehicle collisions ("Brain Injury Canada; Acquired Brain Injury" 2015). A review of TBI data obtained from Canadian provinces shows

incidence rates of about 16,000 and 22,000 people per year for Ontario and British Columbia, respectively ("Brain Injury Association Sudbury & District; ABI Basics" n.d., "Northern Brain Injury Association; Brain Injury Statistics" 2014). In addition, First Nation peoples are considered as the highest risk group with a brain injury rate 4-5 times greater than that of the general population ("Northern Brain Injury Association; Brain Injury Statistics" 2014). Together, it is estimated that the direct and indirect costs associated with TBI in Canada are about 3 billion dollars annually ("Brain Injury Canada; Acquired Brain Injury" 2015), further emphasizing the need for more efficient prevention strategies.

Parallel studies in the United States estimate that approximately 1.5 million people suffer from TBI each year, where 50,000 individuals die and an additional 85,000 individuals suffer from long term disabilities ("Traumatic Brain Injury; Understanding TBI" 2006). Similar to Canada, a large percentage of brain injury cases in the U.S. are caused by falls and motor vehicle collisions ("Traumatic Brain Injury; Understanding TBI" 2006). The rate of TBI has also been increasing worldwide due to increased numbers of motor vehicle collisions in developing countries and a greater rate of falls in more developed countries as the population ages (Maas et al. 2008).

The occurrence of TBI peaks during development with nearly 30% of all cases afflicting children and youth ("Northern Brain Injury Association; Brain Injury Statistics" 2014), and it has been recorded as the most common cause of morbidity and mortality in people under the age of 45 (Langlois et al. 2006). Children are at increased risk of TBI due to physiological susceptibility during development, including a more pliable skull, larger head, weaker neck muscles, increased cervical ligament laxity, greater brain water content, less myelin, greater excitation relative to inhibition, greater cerebral blood flow and higher cerebral metabolic rate (Morrison et al. 2013).

Youth and young adults are at greater risk for TBI by virtue of their activities (e.g. sports) and risk-taking behaviors (e.g. inconsistent usage of safety devices, distracted driving, etc.).

The incidence and prevalence of TBI outnumbers those of spinal cord injury, breast cancer, multiple sclerosis and HIV/AIDS combined ("Brain Injury Canada; Acquired Brain Injury" 2015). Furthermore, it is considered as a dominant cause of death and disability, and a major health and socioeconomic concern throughout the world (Cole 2004; Reis et al. 2015; Soares de Souza et al. 2015).

Traumatic Brain Injury Classification, Pathophysiology and Detection Methods

The physical insult is what initiates the biochemical and pathological consequences in TBI. Thus, the clinical manifestations of TBI depend significantly on the nature of the insult (Young et al. 2015). For instance, many open head injuries are caused by objects such as bullets or knives penetrating through the skull and damaging the brain. In contrast, closed head injuries are associated with blunt, overpressure or accelerative forces (Young et al. 2015). TBI classification follows clinical severity and is assessed primarily using the Glasgow Coma Scale (GCS) (Menon and Maas, 2015; Teasdale et al. 2014; Teasdale and Jennett 1974). GCS (range 3-15) consists of the sum of three component scores (eye, motor and verbal scales) and offers a rapid assessment of brain injury severity (Table 2). A score of 13-15, 9-12, and $\leq$ 8 classify mild (mTBI), moderate, and severe TBI, respectively (Bodanapally et al. 2015; Teasdale et al. 2014). In addition to GCS, TBI is evaluated by various imaging modalities to determine the severity of structural damage in the brain.

TBI can be classified into primary and secondary injuries (Besenski 2002; Kubal 2012; Maas et al. 2008). Primary injuries occur at the time of the injury as a direct result of traumatic impact,

resulting in epidural or subdural hematomas, microvascular injuries, cortical contusions and axonal shearing. Secondary injuries occur over hours and days, and generally result from a complex biochemical cascade of events often manifesting as cerebral edema and elevated intracranial pressure (Besenski 2002; Faden 1996; Puntis and Smith 2017). Secondary injuries include the non-mechanical damage that may result from complex metabolic cascades set off by cell membrane disruption (Giza 2001). Subsequently, neuronal membrane deformity leads to ionic flux, release of excitatory neurotransmitters, depletion of cellular energy stores, and activation of apoptosis, resulting in neuronal death. Impairment of the cerebrovascular tissue also leads to decreased cerebral blood flow, metabolic uncoupling, as well as inflammatory responses such as activation of microglia and release of free radicals, further contributing to tissue damage during the secondary phase (Werner and Engelhard 2007). In contrast to primary injuries, secondary injuries can be decreased or delayed by supportive care with sedation, analgesia and ventilation, and by lowering intracranial pressure with intravenous osmotic agents such as mannitol or hypertonic saline, controlling cerebral metabolic rate and by cerebrospinal fluid drainage, offering opportunities to reduce their impact on brain structure and function (Haddad and Arabi 2012; Puntis and Smith 2017). Furthermore, the delayed nature of secondary injuries provides a window of opportunity for using neuroprotective agents to interrupt cell death cascades in vulnerable tissue, which has become a major focus of drug development research in TBI. However, despite promising results in preclinical testing, a wide variety of neuroprotective agents have failed to provide benefit in clinical trials (Puntis and Smith 2017; Kochanek et al. 2017).

Various imaging techniques are routinely used to help characterize TBI severity, rule out surgical lesions and provide prognostic information. Computed tomography (CT) is the most common

imaging technique as it is readily available, fast, allows access to susceptible trauma patients and quickly identifies surgical lesions that require immediate intervention (Marehbian et al. 2017) (Figure 1). Thus, CT imaging is commonly used for initial evaluation and management of intracranial problems following injury (Mechtler et al. 2014). CT uses computer-processed combination of multiple x-ray images taken from different angles to produce cross-sectional images of specific regions of the brain. It can detect cerebral edema, infarctions, hemorrhage and bone fractures. Several CT scores have been developed to aid TBI severity determination and to predict mortality, including the Marshall CT score and the Rotterdam CT Score, respectively (Bodanapally et al. 2015; Marshall et al. 1991; Maas et al. 2005).

As a complementary technique, magnetic resonance imaging (MRI) uses magnetic fields and radio waves to produce high resolution two- or three-dimensional images of the brain. It provides improved structural sensitivity over CT scanning, including better tissue contrast, fewer artifacts, and the non-use of ionizing radiation or radioactive tracers (Gallagher et al. 2007; Hollingworth et al. 2000; Smith-Bindman et al. 2012). Due to its increased sensitivity, particularly for certain types of lesions such as axonal injury which can greatly impact outcomes, MRI provides better prognostic information than CT (Marehbian et al. 2017). More frequent use of MRI, however, is hindered by its availability, cost, length of scanning time, the magnetic field that limits use of certain metallic equipment, and the inability to access the patient quickly in the event of acute deterioration. Finally, the timing of MRI may be important for identification of certain pathologies, such as vasogenic versus cytotoxic edema.

Another imaging modality occasionally used to monitor TBI is color Doppler ultrasound (CD-US). This technique uses high frequency sound waves that reflect off circulating red blood cells to gather information about cerebral blood flow. One potential use of CD-US is in screening for

post-traumatic cerebral vasospasm, which can worsen TBI outcome via cerebral infarction (Kramer et al. 2013; LaRovere et al. 2016). CD-US can be advantageous when patients have metallic implants such as pacemakers, and they are not suitable or immediate candidates for MRI. CD-US is also non-invasive and less expensive than MRI, and it does not require a special type of room setting.

Approximately 75% of all TBI are mTBI patients, including concussions (National Center for Injury Prevention and Control, 2003). Using traditional imaging modalities, such as head CT scanning, patients with mTBI usually show no abnormalities in their neural tissue (National Center for Injury Prevention and Control 2003; Rees 2003; Shin et al. 2017). While most mTBI patients become asymptomatic within days to weeks of the injury, many experience persistent cognitive, neuropsychological and somatic symptoms that are referred to as "persistent post-concussive syndrome"(Alexander 1995; Hillary et al. 2010; Johansson et al. 2009; Makley et al. 2008). Recently, it has been suggested by some that post-concussive symptoms may be more common than previously reported (Grool et al. 2016; Young and Tsao 2017; McInnes et al. 2017). The failure of conventional imaging methods to detect abnormalities in the brains of patients who subsequently suffer persistent symptoms represents a major challenge in predicting clinical outcomes in mTBI (Mechtler et al. 2014). In some cases, more advanced neuroimaging techniques, such as diffusion tensor imaging (DTI), functional MRI (fMRI), magnetoencephalography (MEG), magnetic resonance spectroscopy (MRS) and Single-Photon Emission Computed Tomography (SPECT) are utilized to better characterize the injury. These techniques have been reviewed previously (Mechtler et al. 2014; Reis et al. 2015; Shin et al. 2017).

Recent advances in neuroimaging have accelerated and improved the accuracy of TBI diagnosis and prognosis (Reis et al. 2015; Manning et al. 2017). However, these investigations are lengthy, expensive, and do not take into account the influence of the patient's genetic background. Therefore, improved TBI care remains dependent on novel approaches to better assess TBI.

Animal Models of Traumatic Brain Injury

Over the last few decades, a number of animal TBI models have been developed to closely reflect the morphological, biochemical, molecular and behavioral changes associated with brain injury in patients. Using rodent TBI models, injury has been introduced to the brain in three complementary settings: focal impact, diffuse impact and non-impact (Table 3) (Rostami 2012). Focal impact brain injury animal models are further divided into the weight drop (WD), fluid percussion (FP) and controlled cortical impact (CCI) models. In the WD model, the injury is produced by a free falling weight guided in a tube that is made to hit the skull, producing a contusion (Dail et al. 1981; Feeney et al. 1981; Morales et al. 2005). The severity of the injury can be adjusted by the height and mass of the weight dropped. Simulation of injury using this model is fast and easy, but limitations exist such as unintentional skull fracture, risk of a second rebound injury, and inaccuracy with regards to the impact site (Rostami 2012, Briones 2015). In the FP and CCI injury models, a craniotomy is performed between bregma and lambda. For the former, a pendulum strikes a piston of a fluid reservoir, creating a pressure pulse in the fluid that is transmitted to the exposed, intact dura, while in the latter a pneumatic or electromagnetic impact device is used to drive a rigid impactor to deliver mechanical force onto the dura (Dixon et al. 1987; Dixon et al.1991; Lighthall 1988; McIntosh et al. 1989; McIntosh et al. 1987; Morganti-Kossmann et al. 2010; Xiong et al. 2013). All three techniques cause a deformity in the

underlying cortex, resulting in cortical tissue loss, hemorrhage, axonal injury, concussion, contusion and BBB dysfunction similar to those seen in patients (Chen et al. 1996; Schmidt and Grady 1993; Whalen et al. 1998; Xiong et al. 2013). CCI is considered a superior focal impact model, because it provides better control over factors such as the duration and velocity of impact and the depth of resulting damage in the brain, and also eliminates the risk of a rebound injury (Rostami 2012; Briones 2015). Similar to WD, FP and CCI, diffuse impact and non-impact brain injury animal models have been commonly used and well-cited in the literature (Rostami 2012). A limitation of several TBI models is the fact that injuries are commonly induced by direct contact with the brain through a craniotomy while the animal's head is immobilized, conditions which typically do not characterize human brain injury. The recently developed nonsurgical CHIMERA model (closed-head impact model of engineered rotational acceleration), which involves impact to the intact unrestrained head, overcomes this limitation (Namjoshi et al. 2014). The chimera model has been used to produce precisely controlled injuries, and allows for kinematic analysis of head movement at the time of impact, which can be correlated with behavioural, histological and biochemical outcomes (Namjoshi et al. 2017).

After injury, damage to the brain tissue can be evaluated with various markers to determine injury severity and its progression over time. For instance, the expression of cell apoptosis markers (Figure 2) can be monitored in parallel with behavioural deficits within hours-weeks of the injury to develop potential treatments for inhibiting cell death and enhancing functional recovery in the animals. While each model has its own unique advantages, it is important to note that no injury model accurately reproduces the complete spectrum of pathologies observed in human TBI. Also, animal TBI models require the use of anesthetic agents at the time of injury for ethical reasons. Since certain anesthetics have been shown to be neuroprotective, improving

functional and histological outcomes in TBI models when present at the time of injury (Rowe et al. 2013; Statler et al. 2000; Statler et al. 2006; Statler et al. 2006b), this approach may contribute to reduced clinical translation as patients are devoid of anesthetic agents at the time of injury.

Surgery is a common procedure to reduce or prevent the progression of damage caused by a hematoma, seizure or tumour in the brain. In particular, glioma evacuation generally causes lesions in the tissue surrounding the extracted tumour. Surgically-induced TBI animal models that mimic this type of damage have been developed (Frontczak-Baniewicz and Walski 2003; Jadhav et al. 2007; Jezierski et al. 2014; Rennie et al. 2013). Typically, these models involve removal of the skull overlying specific regions such as the primary motor cortex, and damaging the neural tissue to a pre-determined depth to evaluate its acute and chronic effects on the brain (Figure 3A-B). Based on our experience, a combination of various staining techniques to detect structural changes in neurons (Figure 3C-D) and biochemical assays to monitor the expression of pro- and anti-apoptotic markers (Figure 3E-F) can be used to assess the severity of damage to the brain.

In addition to these methods, *in vivo* Doppler and Optic imaging techniques can be used to establish a profile of the injury over time. Doppler imaging allows a non-invasive real time quantification of blood flow in specific regions of the brain at high resolution. The changes in blood flow in the injured brain, followed by revascularization as a repair mechanism can be readily monitored using this technology. Furthermore, *in vivo* optical imaging of fluorescent reporters such as green fluorescent protein (GFP) in transgenic mice (Fujiki et al. 2008) can be used to monitor the expression of specific proteins prior to and after injury and treatment (Figure 4). For instance, post-TBI reactive gliosis has been tracked in a spatiotemporal manner, using glial fibrillary acidic protein (GFAP)-GFP transgenic mice (Kim et al. 2012). Similar

methodologies have been used to detect the expression of nestin in the hippocampus (Yu et al. 2008; Wang et al. 2016a) and spinal cord (Matsumura et al. 2010) of Nestin-GFP transgenic mice after injury.

Detection Markers

There is compelling evidence that the knowledge obtained about the aberrant expression of intra- and extra-cellular proteins in the injured brain can contribute to a better understanding of the underlying mechanisms of brain injury. Furthermore, the success of post-TBI interventions heavily relies on targeting the complex molecular signaling pathways associated with the injury. To this end, several markers have been used to assess inflammation, oxidative stress, excitotoxicity and other pathophysiological mechanisms occurring within days to weeks of injury, and these biomarkers have been subjected to systematic review (Yokobori et al. 2013, Daoud et al. 2014; Mondello et al. 2017). Notably, it has been suggested that early biomarkers of structural damage such as S-100β, GFAP, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and tau in cerebrospinal fluid (CSF) or blood may be helpful to determine whether a head CT scan is needed after mTBI (Mondello et al. 2011; Reis et al. 2015). Blood-based biomarkers of intracranial lesions in patients presenting to emergency departments after mild head injury are highly desirable in order to make informed decisions about the need for CT scans. However, recent systematic reviews indicate there is currently only sufficient evidence to support the use of serum S100B as a biomarker of injury, while there is not enough evidence to use serum or plasma GFAP, neuron specific enolase (NSE), UCH-L1, tau and neurofilament proteins in this clinical application (Mondello et al. 2017). At present, CSF remains the most reliable biofluid for providing biomarkers of brain injury (Agoston et al. 2017).

The presence of other factors such as auto-antibodies to brain-specific proteins and their breakdown products in the peripheral circulatory system is suggestive of an increased blood-brain-barrier permeability following TBI (Diamond et al. 2013). TBI patients showed nearly four times increase in anti-GFAP autoantibody levels within the first 10 days after injury (Zhang et al. 2014). Anti-GFAP autoantibodies were also elevated in TBI patients at an average of 6 months post injury compared to healthy controls (Wang et al. 2016b). Anti-N-methyl-D-aspartate (NMDA) receptor and anti-α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor are increased in the serum of children with post-traumatic headache, indicating hyper-stimulation of glutamate receptors and hypoxic ischemic brain lesions (Goryunova et al. 2007). Other markers such as anti-phospholipid correlate with vascular complications following trauma (Ngankam et al. 2011). Further research is needed in order to evaluate the usefulness of these autoantibodies as potential biomarkers of injury severity and prognosis (Reis et al. 2015). In addition, upregulation of cytokines (Galindo et al. 2011), deranged glucose profiles (Ley et al. 2011), changed levels of sex hormones (Wagner et al. 2011), and αII-spectrin breakdown products (SBDPs) are considered as useful biological signatures in assessing TBI (Forde et al. 2014; Mondello et al. 2012). Over the past decade, metabolomic profiling of biological fluids has also been used to identify markers of cerebral metabolic perturbation after TBI that correlate with injury severity or specific outcomes such as cognitive impairments (Daley et al. 2016; Oresic et al. 2016; Posti et al. 2017; Wolahan et al. 2015; Yi et al. 2017).

MicroRNAs

MicroRNAs (miRNAs) are short noncoding regulatory RNA molecules composed of 20-24 nucleotides that are often located within introns, and play key roles in regulating gene/protein expression (Bartel 2004, Ribecco-Lutkiewicz et al. 2016). The human genome encodes over

3500 miRNAs (Bentwich et al. 2005, Londin et al. 2015), where individual miRNAs can target multiple genes, and a group of miRNAs can target the same gene (Lewis et al. 2005; Lim et al. 2005; Xie et al. 2005). In terms of structure, miRNAs originate from regions of RNA transcripts that fold back on themselves to form short hairpins and they function via base-pairing with complementary sequences of mRNA molecules (Bartel 2004; 2009). Once they are paired, mRNA molecules are silenced by cleavage of their strands, destabilization through shortening of their poly(A) tails and repressing translation (Bartel 2009; Fabian et al. 2010). Several laboratories have shown that miRNAs are widely expressed in the brain with many playing key roles in central nervous system (CNS) development and function (Bhalala et al. 2013; Tang et al. 2007; Wang et al. 2008). In recent years, miRNAs have been identified as a novel class of molecular modulators of TBI, based on their roles in regulation of various cellular functions in the brain (Bak et al. 2008; Bhalala et al. 2013; Coolen and Bally-Cuif 2009; Meissner et al. 2016).

miRNAs as Biomarkers

Detection of altered levels of specific miRNAs in peripheral blood and/or cerebrospinal fluid (CSF) after TBI suggests a potential use of miRNAs as injury biomarkers (Bian & Sun, 2011; Meza-Sosa et al. 2012; Redell et al. 2009; Yokobori et al. 2013). For instance, plasma miRNA profiles of severe TBI patients showed a reduction in miR-16 and miR-92a and an increase in miR-765 expression within 48 hours of injury, compared with those of healthy volunteers (Redell et al. 2010). In combination, these three miRNAs provided good diagnostic specificity and sensitivity, effectively distinguishing severe TBI patients from both healthy controls and patients with orthopedic rather than brain injuries. Conversely, plasma levels of miR-16 and miR-92a were increased in patients with mild TBI (Redell et al. 2010). In addition, it was

recently reported that levels of miR-93, miR-191 and miR-499 were all elevated in serum from mild, moderate and severe TBI patients. Notably, levels of all three miRNAs were related to both the severity of the injury (with severe TBI patients exhibiting significantly higher levels than mild or moderate TBI patients) and clinical outcomes several months later (Yang et al. 2016). A panel of 10 serum miRNAs upregulated in the acute phase (within 48 hours) of both mild-moderate and severe TBI patients but not in orthopedic injury patients was reported by Bhomia and colleagues (2016); four of these miRNAs were also found to be upregulated in CSF of severe TBI patients. While Bhomia et al (2016) focused on upregulated miRNAs common to both mild-moderate and severe TBI, a study by Di Pietro et al (2017) aimed to find serum miRNA biomarkers capable of discriminating between mild and severe TBI. They found that downregulation of miR-425-5p and miR-502 at early time points after injury characterized mild TBI patients, while upregulation of miR-21 and miR-335 was observed in serum from patients after severe TBI. Of interest, the ability of the miRNAs to distinguish between mild TBI, severe TBI and orthopedic injury patients was dependent on the interval between injury and serum sampling. Levels of miR-425-5p and miR-21(at 4-12 hours after injury) were also predictive of outcomes on the extended Glasgow Coma Scale 6 months later, suggesting not only diagnostic value but also a potential prognostic use of miRNAs (Di Pietro et al. 2017). Studies of blast-induced TBI in the rat have also identified plasma (or CSF) let-7i as a potential biomarker of brain injury (Balakathiresan et al. 2012), although to our knowledge this has not yet been examined in human blast injury patients. Together, these studies suggest a possible biomarker application of miRNAs, once their roles in TBI are better defined at the molecular and cellular levels (Redell et al. 2010, Hu et al. 2012), and once there is a better understanding of how type of injury and time since injury affect miRNA profiles.

miRNA alterations in animal models of brain injury

A number of studies have used microarrays to examine spatiotemporal patterns of miRNA alterations in injured cortical or hippocampal tissue in rodent TBI models (Lei et al. 2009; Liu et al. 2014; Liu et al. 2009b; Meissner et al. 2016; Redell et al. 2009). Assessment of a selected number of miRNAs in the injured mouse motor cortex shows that different miRNAs exhibit distinct expression profiles over time (Figure 5). In particular, some miRNAs such as miR-132, miR-21 and miR-30a respond within hours of the injury, with miR-21 maintaining upregulation in the injured brain compared with control for at least a week. In contrast, a number of other miRNAs examined in this study were down-regulated three days after injury and maintained lower expression levels compared with the control group after one week. Other studies using mice to model TBI show that changes in the expression of a number of miRNAs can occur as early as 1 h post injury (Sabirzhanov et al. 2014, Meissner et al. 2016). Even after mild TBI (induced by weight drop in the mouse, which resulted in no tissue damage upon histological examination), the expression of several dozen miRNAs in brain tissue was significantly altered (Chandran et al. 2017). Both the severity of the injury and time elapsed since injury influenced the pattern of miRNA changes in the brain (Chandran et al. 2017). Similarly, microarray analyses have revealed dynamic temporal regulation of miRNA expression within both the cortex and hippocampus in rat models of TBI (Bhalala et al. 2013; Lei et al. 2009; Redell et al. 2009).

The above studies demonstrate time-dependent coordinated changes in multiple miRNAs after injury. Although the function of individual miRNAs is not always known, bioinformatics analyses can suggest predicted targets of dysregulated miRNAs in the injured brain. Studies of miRNA expression in traumatically injured brain have revealed that the targets of up- or

359 downregulated miRNAs in injured brain are often clustered into groups linked to distinct cellular
360 processes (Meissner et al. 2016; Reis et al. 2015; and see Figure 6). Time-course studies indicate
361 that while many of the miRNAs affected during the acute post-injury phase are predicted to
362 target genes involved in apoptosis, those affected in the chronic stages of injury regulate genes
363 involved in cytoskeletal organization, intracellular trafficking and other processes related to brain
364 repair (Bhalala et al. 2013; Bhalala, 2015; Hu et al. 2012). Importantly, changes in the
365 expression of miRNAs after injury have been shown to coincide with changes in protein
366 expression of some predicted targets in the damaged tissue (Redell et al. 2011). These types of
367 studies can provide insight into both pathophysiological and endogenous repair processes
368 occurring after injury, may help to explain the efficacy of treatments that protect or promote
369 repair of the injured brain, and can possibly suggest novel therapeutic targets.

370 Similar to TBI, changes in miRNA levels have been observed following stroke and spinal cord
371 injury (SCI). In stroke, miRNAs seem to target angiogenesis, hypoxia, endothelial cell regulation
372 and immune response (Tan et al. 2009), and their expression profiles have been used to
373 differentiate between ischemic and hemorrhagic stroke (Liu et al. 2010a). In SCI, over 35% of
374 the miRNAs expressed in the spinal cord were reported to be significantly affected within the
375 first 7 days of injury (Liu et al. 2009b). Many of the reported miRNAs target the same pathways
376 known to be involved in other CNS injuries, including apoptosis, inflammation, cell proliferation
377 and cell differentiation (Bhalala et al. 2013). Notably, some of these pathways are affected by
378 different sets of miRNAs, depending on the type of injury. For instance, apoptosis is regulated by
379 miR-29b, miR-145 and miR-497 following stroke (Dharap et al. 2009; Shi et al. 2012; Yin et al.
380 2010), and by miR-15b and miR-486 after SCI (Liu et al. 2010b; Moschidou et al. 2012). On the
381 other hand, some miRNAs may play a similar role in diverse types of injury. For example, miR-

21 is known as a common regulator of cell death in TBI, stroke and SCI (Bhalala et al. 2013). MiR-21 level has been shown to increase around the lesion site within the first 2 weeks of injury and is upregulated dramatically in the chronic stages in a murine SCI model (Bhalala et al. 2012). Similar results have also been observed within the first 7 days post injury in a rodent model of stroke (Buller et al. 2010), and miR-21 upregulation has been observed in multiple TBI models including penetrating ballistic-like brain injury (Johnson et al. 2017), CCI (Redell et al. 2010; Sandhir et al. 2014), and FPI (Lei et al. 2009). MiR-21 targets B-cell lymphoma-2 (Bcl2), phosphatase and tensin homolog (PTEN) and cell death protein 4 (CDP4) (Gabriely et al. 2008; Ge et al. 2014; Hashimi et al. 2009; Kim et al. 2009; Lei et al. 2009; Liu et al. 2009a; Lu et al. 2009; Meissner et al. 2016; Papagiannakopoulos et al. 2008; Redell et al. 2009, 2011; Sabatel et al. 2011; Sheedy, 2015; Shi et al. 2013; Van Wynsberghe et al. 2011; Wickramasinghe et al. 2009; Yelamanchili et al. 2010). MiR-21 can also inhibit apoptosis by targeting PTEN, activating Ang-1/Tie-2 and Akt signaling, and promoting outgrowth of neuronal axons in TBI and stroke (Christie et al. 2010; Ding et al. 2013; Ge et al. 2015; Ge et al. 2014; Han et al. 2014; Ohtake et al. 2014; Ohtake et al. 2015; Onyszchuk et al. 2007; Weber et al. 2010; Zhao et al. 2013). Thus, the upregulation of miR-21 in damaged neural tissue may represent a potential mechanism by which the brain attempts to limit neuronal destruction in the aftermath of an injury (Bhalala, 2015). Interestingly, the miR-21 response to TBI in aged mice was shown to be attenuated, possibly contributing to worsened outcomes in older animals (Sandhir et al. 2014).

Changes in miRNA expression may play a role in the positive response to some neuroprotective therapies. While the benefits of therapeutic hypothermia remain controversial in TBI patients (Dietrich and Bramlett 2016; Yokobori and Yokota 2016), hypothermia has a long history as a therapeutic intervention in animal models of brain injury, and several miRNAs with altered

405 expression after TBI are downregulated under hypothermic conditions (Truettner et al. 2011).
406 Hypothermia dampened the upregulation of miR-874 and miR-9 observed in damaged cortex
407 after fluid percussion injury. Since elevation of these miRNAs after injury was predicted to
408 impair intracellular transport of proteins, disrupt membrane organization and damage the
409 cytoskeleton, the authors suggest that the ability of hypothermia to mitigate the miRNA response
410 to brain injury might underlie some of its therapeutic efficacy (Truettner et al. 2011).

411 *miRNAs as therapeutic targets*

412 Because of their dynamic involvement in the CNS, the inhibition and restoration of specific
413 miRNAs has been considered a therapeutic opportunity to modulate cellular processes in TBI,
414 SCI and stroke patients. Currently, a few options are being explored, including miRNA
415 amplifiers, miRNA inhibitors, and artificial miRNAs (Bhalala 2015).

416 One strategy is to increase the levels of beneficial miRNAs in the damaged brain by introducing
417 a plasmid containing the sequence of the pri-miRNA or pre-miRNA (precursors to the mature
418 miRNA) into the target tissue. Another strategy is to use miRNA mimics, which are modified
419 double-stranded RNA molecules with one strand containing the same sequence as the mature
420 miRNA (Bhalala 2015), although mimics are not easily introduced into neurons (Zhang et al.
421 2013).

422 Another option is to use miRNA antagonism to mitigate the effect of harmful miRNAs following
423 brain injury. miRNA inhibitors, including antagomiRs, locked nucleic acids (LNAs), and
424 miRNA sponges (Bhalala et al. 2013; Grünweller and Hartmann, 2007; Krützfeldt et al. 2005;
425 Wang et al. 2012), are antisense sequences that bind mature miRNA molecules and block their
426 functions. AntagomiRs are a class of chemically engineered nuclease-resistant oligonucleotides

that prevent miRNAs from binding to a desired site on an mRNA molecule, effectively silencing them (Krützfeldt et al. 2005, Czech 2006, Yue 2011; Zhang et al. 2013); however, because of their relatively low potency, high doses are needed *in vivo* (Broderick and Zamore 2011). LNAs are modified oligonucleotides that contain locked ribose rings to enhance their capacity to bind to target miRNA, resulting in higher potency miRNA inhibition (Grünweller and Hartmann 2007; Stenvang et al. 2008) as well as greater nuclease resistance (Zhang et al. 2013). For longer-term inhibition of miRNAs, sponge constructs that act as competitive inhibitors of miRNAs may be useful. These are artificial RNA transcripts with multiple repeats that are complementary to the target miRNA. Integration of the sponge construct into the genome of CNS cells allows for persistent inhibition of the target miRNA (Bhalala et al. 2012; Gentner et al. 2009; Luikart et al. 2011).

Finally, the generation of artificial miRNAs designed to target multiple mRNAs may be a strategy to achieve coordinated, multi-faceted modulation of the pathophysiological response to brain injury (Bhalala et al. 2013).

While promising, relatively few studies on the ability of miRNA-based molecules to provide neuroprotection or to enhance reparative processes in the experimentally injured brain have been reported. Overexpression of a few miRNAs has been shown to attenuate neural injury in animal models of TBI (Ge et al. 2014; Ge et al. 2015; Sun et al. 2016; Sun et al. 2017). Conversely, administration of a miR-711 hairpin inhibitor after TBI resulted in improved histological and neurological outcomes in a mouse CCI model (Sabirzhanov et al. 2016). However, the utility of these tools as therapeutic agents has yet to be demonstrated.

Exosomes

Exosomes are small membranous microvesicles which carry proteins, lipids and nucleic acids, serving as a communication system between cells and tissues locally or over long distances (Chopp and Zhang 2015; Kalani et al. 2014). Many different cell types secrete exosomes, including multiple CNS cells (Kalani et al. 2014). Because they are accessible and stable in biological fluids but reflect the phenotype and condition of the parent cell, exosomes and their contents, including miRNA, have the potential to serve as biomarkers for CNS pathology (Kalani et al. 2014; Katsuda et al. 2013; Taylor and Gercel-Taylor 2014).

A handful of studies have examined the contents of exosomes released after either human TBI or experimentally induced TBI in animals. Patz et al. (2013) demonstrated that the miR-9 and miR-451 content of CSF-derived microvesicles was altered in TBI patients compared with non-injured controls. In exosomes isolated from the brains of mice subjected to CCI, the level of miR-212 was reduced, while levels of miR-7b, miR-7a, miR-21 and miR-146 were all elevated relative to sham-operated mice (Harrison et al. 2016). It is not yet known which cells might be recipients of this miRNA cargo or what impact exosome-derived miRNA might have on the progression of pathology in the injured brain.

Exosomes derived from mesenchymal stem cells have successfully been used as therapeutic agents in experimental TBI models (Kim et al. 2016; Zhang et al. 2015, 2016), although the role of miRNA in the beneficial outcomes observed following treatment with exosomes is not known. Nevertheless, it may be possible to capitalize on the ability of exosomes to act as delivery vehicles for miRNA by altering the miRNA content of exosomes to modulate specific pathophysiological pathways in TBI (Xiong et al. 2017; Yang et al. 2017).

In conclusion, TBI affects people of all ages and in all geographical areas. Despite the enormous costs of TBI to society, there are no direct therapies. Animal models of TBI may eventually aid development of TBI interventions, but must take into account the significant patient and injury heterogeneity encountered with real world injuries. Here, we review TBI prevalence, classification and pathophysiology, with emphasis on TBI animal models and emerging molecular markers. Once molecular markers are better established, they could improve current TBI diagnostic and prognostic capabilities, as well as be potential therapeutics. Molecular markers may aid TBI diagnostics by improving clinical decision making and by allowing for rapid deployment of rehabilitation services. Molecular markers may improve TBI prognostics that are essential for optimizing patient outcomes, for aiding the next wave of intervention research by stratifying patients for clinical studies, and for use in clinical audits by allowing adjustment for case mix. Also, accurate TBI prognostic tools will be invaluable to the legal guardians of brain injured patients in making informed treatment and compassionate care decisions. While identification of TBI molecular markers is a critical forward step, studies are still in their infancy.

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References:

Agoston, D.V., Shutes-David, A., and Peskind, E.R. 2017. Biofluid biomarkers of traumatic brain injury. Brain Inj. 31(9):1195-1203.

Alexander, M.P. 1995. Mild traumatic brain injury: pathophysiology, natural history, and clinical management. Neurology **45**(7): 1253–1260.

Bak, M., Silahtaroglu, A., Møller, M., Christensen, M., Rath, M.F., Skryabin, B., Tommerup, N., and Kauppinen, S. 2008. MicroRNA expression in the adult mouse central nervous system. RNA **14**(3): 432–44. doi:10.1261/rna.783108.

Balakathiresan, N., Bhomia, M., Chandran, R., Chavko, M., McCarron, R.M., and Maheshwari, R.K. 2012. MicroRNA let-7i is a promising serum biomarker for blast-induced traumatic brain injury. J. Neurotrauma **29**(7): 1379–87. doi:10.1089/neu.2011.2146.

Bartel, D.P. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell **116**(2): 281–297. doi:10.1016/S0092-8674(04)00045-5.

Bartel, D.P. 2009. MicroRNAs: target recognition and regulatory functions. Cell **136**(2): 215–233. doi:10.1016/j.cell.2009.01.002.

Bentwich, I., Avniel, A., Karov, Y., Aharonov, R., Gilad, S., Barad, O., Barzilai, A., Einat, P., Einav, U., Meiri, E., Sharon, E., Spector, Y., and Bentwich, Z. 2005. Identification of hundreds of conserved and nonconserved human microRNAs. Nat. Genet. **37**(7): 766–70. doi:10.1038/ng1590.

Besenski N. 2002. Traumatic injuries: imaging of head injuries. Eur. Radiol. **12**(6): 1237–1252.

Bhalala, O. 2015. The emerging impact of microRNAs in neurotrauma pathophysiology and therapy. *In* Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. CRC Press/Taylor & Francis. Chapter 26.

Bhalala, O.G., Pan, L., Sahni, V., McGuire, T.L., Gruner, K., Tourtellotte, W.G., and Kessler, J.A. 2012. microRNA-21 regulates astrocytic response following spinal cord injury. J. Neurosci. **32**(50): 17935–47. doi:10.1523/JNEUROSCI.3860-12.2012.

Bhalala, O.G., Srikanth, M., and Kessler, J.A. 2013. The emerging roles of microRNAs in CNS injuries. Nat. Rev. Neurol. **9**(6): 328–39. doi:10.1038/nrneurol.2013.67.

Bhomia, M., Balakathiresan, N.S., Wang, K.K., Papa, L., and Maheshwari, R.K. 2016. A panel of serum miRNA biomarkers for the diagnosis of severe to mild traumatic brain injury in humans. Sci. Rep. **6**:28148.

Bian, S., and Sun, T. 2011. Functions of noncoding RNAs in neural development and neurological diseases. Mol. Neurobiol. **44**(3): 359–73. doi:10.1007/s12035-011-8211-3.

Bodanapally U.K., Sours, C., Zhuo, J., and Shanmuganathan, K. 2015. Imaging of traumatic brain injury. Radiol. Clin. North Am. **53**(4): 695–715.

Brain Injury Association of Waterloo-Wellington; Stats. (n.d.). Available from http://www.biaww.com/stats.html.

Brain Injury Association Sudbury & District; ABI Basics. (n.d.). Available from http://biasd.ca/education/abi-basics/.

Brain Injury Canada; Acquired Brain Injury. 2015. Available from http://braininjurycanada.ca/acquired-brain-injury.

Briones, T.L. 2015. Chapter 3 animal models of traumatic brain injury: is there an optimal model that parallels human brain injury? Annu. Rev. Nurs. Res. **33**: 31–73. doi:10.1891/0739-6686.33.31.

Broderick, J.A., and Zamore, P.D. 2011. MicroRNA therapeutics. Gene Ther. **18**(12): 1104–10. doi:10.1038/gt.2011.50.

Buller, B., Liu, X., Wang, X., Zhang, R.L., Zhang, L., Hozeska-Solgot, A., Chopp, M., and Zhang, Z.G. 2010. MicroRNA-21 protects neurons from ischemic death. FEBS J. **277**(20): 4299–307. doi:10.1111/j.1742-4658.2010.07818.x.

Carbonell, W.S., Maris, D.O., McCall, T., and Grady, M.S. 1998. Adaptation of the fluid percussion injury model to the mouse. J. Neurotrauma **15**(3): 217–29. doi:10.1089/neu.1998.15.217.

Cernak, I., Vink, R., Zapple, D.N., Cruz, M.I., Ahmed, F., Chang, T., Fricke, S.T., and Faden, A.I. 2004. The pathobiology of moderate diffuse traumatic brain injury as identified using a new experimental model of injury in rats. Neurobiol. Dis. **17**(1): 29–43. doi:10.1016/j.nbd.2004.05.011.

Chandran, R., Sharma, A., Bhomia, M., Balakathiresan, N.S., Knollmann-Ritschel, B.E., and Maheshwari, R.K. 2017. Differential expression of microRNAs in the brains of mice subjected to increasing grade of mild traumatic brain injury. Brain Inj. **31**(1): 106-119.

Chen, Y., Constantini, S., Trembovler, V., Weinstock, M., and Shohami, E. 1996. An experimental model of closed head injury in mice: pathophysiology, histopathology, and cognitive deficits. J. Neurotrauma **13**(10): 557–68. doi:10.1089/neu.1996.13.557.

551 Chopp, M., and Zhang, Z.G. 2015. Emerging potential of exosomes and noncoding microRNAs
552 for the treatment of neurological injury/diseases. Expert Opin. Emerg. Drugs **20**(4): 523–
553 526. doi:10.1517/14728214.2015.1061993.
554 Christie, K.J., Webber, C.A., Martinez, J.A., Singh, B., and Zochodne, D.W. 2010. PTEN
555 inhibition to facilitate intrinsic regenerative outgrowth of adult peripheral axons. J.
556 Neurosci. **30**(27): 9306–15. doi:10.1523/JNEUROSCI.6271-09.2010.
557 Cole, T.B. 2004. Global road safety crisis remedy sought: 1.2 million killed, 50 million injured
558 annually. JAMA **291**(21): 2531–2. doi:10.1001/jama.291.21.2531.
559 Coolen, M., and Bally-Cuif, L. 2009. MicroRNAs in brain development and physiology. Curr.
560 Opin. Neurobiol. **19**(5): 461–70. doi:10.1016/j.conb.2009.09.006.
561 Czech, M.P. 2006. MicroRNAs as therapeutic targets. N. Engl. J. Med. **354**(11): 1194–5.
562 doi:10.1056/NEJMcibr060065.
563 Dail, W.G., Feeney, D.M., Murray, H.M., Linn, R.T., and Boyeson, M.G. 1981. Responses to
564 cortical injury: II. Widespread depression of the activity of an enzyme in cortex remote
565 from a focal injury. Brain Res. **211**(1): 79–89.
566 Daley M, Bartha R, Brown A, Charyk-Stewart T, Dekaban G, Doherty T, Fischer L, Holmes J,
567 Menon R, Shoemaker KJ, Rupar T, Fraser DD. 2016. Metabolomics Profiling of
568 Concussion Adolescent Hockey Players: Identification of a Novel Diagnostic Plasma
569 Biomarker Panels. Metabolomics, 12: 185. doi: 10.1007/s11306-016-1131-5
570 Daoud, H., Alharfi, I., Alhelali, I., Charyk Stewart, T., Qasem, H., and Fraser, D.D. 2014. Brain
571 injury biomarkers as outcome predictors in pediatric severe traumatic brain injury.
572 Neurocrit. Care **20**(3): 427–35. doi:10.1007/s12028-013-9879-1.
573 Davidsson, J., and Risling, M. 2011. A new model to produce sagittal plane rotational induced
574 diffuse axonal injuries. Front. Neurol. **2**: 41. doi:10.3389/fneur.2011.00041.
575 Dharap, A., Bowen, K., Place, R., Li, L.-C., and Vemuganti, R. 2009. Transient focal ischemia
576 induces extensive temporal changes in rat cerebral microRNAome. J. Cereb. Blood Flow
577 Metab. **29**(4): 675–87. doi:10.1038/jcbfm.2008.157.
578 Diamond, B., Honig, G., Mader, S., Brimberg, L., and Volpe, B.T. 2013. Brain-reactive
579 antibodies and disease. Annu. Rev. Immunol. **31**:345-85. http://dx.doi.org/10.1146/annurev-
580 immunol-020711-075041.
581 Dietrich, W.D., and Bramlett, H.M. 2016. Therapeutic hypothermia and targeted temperature

582 management in traumatic brain injury: Clinical challenges for successful translation. Brain
583 Res. **1640**: 94–103. doi:10.1016/j.brainres.2015.12.034.
584 Ding, J., Guo, J., Yuan, Q., Yuan, F., Chen, H., and Tian, H. 2013. Inhibition of phosphatase and
585 tensin homolog deleted on chromosome 10 decreases rat cortical neuron injury and blood-
586 brain barrier permeability, and improves neurological functional recovery in traumatic brain
587 injury model. PLoS One **8**(11): e80429. doi:10.1371/journal.pone.0080429.
588 Di Pietro, V., Ragusa, M., Davies, D., Su, Z., Hazeldine, J., Lazzarino, G., Hill, L.J., Crombie,
589 N., Foster, M., Purrello, M., Logan, A., and Belli, A. 2017. MicroRNAs as novel
590 biomarkers for the diagnosis and prognosis of mild and severe traumatic brain injury. J
591 Neurotrauma **34**(11):1948-1956.
592 Dixon, C.E., Clifton, G.L., Lighthall, J.W., Yaghmai, A.A., and Hayes, R.L. 1991. A controlled
593 cortical impact model of traumatic brain injury in the rat. J. Neurosci. Methods **39**(3).
594 Dixon, C.E., Lyeth, B.G., Povlishock, J.T., Findling, R.L., Hamm, R.J., Marmarou, A., Young,
595 H.F., and Hayes, R.L. 1987. A fluid percussion model of experimental brain injury in the
596 rat. J. Neurosurg. **67**(1): 110–9. doi:10.3171/jns.1987.67.1.0110.
597 Fabian, M.R., Sonenberg, N., and Filipowicz, W. 2010. Regulation of mRNA translation and
598 stability by microRNAs. Annu. Rev. Biochem. **79**: 351–79. doi:10.1146/annurev-biochem-
599 060308-103103.
600 Faden, A.I. 1996. Pharmacologic treatment of acute traumatic brain injury. JAMA **276**(7): 569–
601 570.
602 Feeney, D.M., Boyeson, M.G., Linn, R.T., Murray, H.M., and Dail, W.G. 1981. Responses to
603 cortical injury: I. Methodology and local effects of contusions in the rat. Brain Res. **211**(1):
604 67–77. doi:10.1016/0006-8993(81)90067-6.
605 Forde, C.T., Karri, S.K., Young, A.M.H., and Ogilvy, C.S. 2014. Predictive markers in traumatic
606 brain injury: opportunities for a serum biosignature. Br. J. Neurosurg. **28**(1): 8–15.
607 doi:10.3109/02688697.2013.815317.
608 Frontczak-Baniewicz, M., and Walski, M. 2003. New vessel formation after surgical brain injury
609 in the rat's cerebral cortex I. Formation of the blood vessels proximally to the surgical
610 injury. Acta Neurobiol. Exp. (Wars). **63**(2): 65–75.
611 Fujiki, Y., Tao, K., Bianchi, D.W., Giel-Moloney, M., Leiter, A.B., and Johnson, K.L. 2008.
612 Quantification of green fluorescent protein by in vivo imaging, PCR, and flow cytometry:

Comparison of transgenic strains and relevance for fetal cell microchimerism. Cytom. Part A **73A**(2): 11–118. doi:10.1002/cyto.a.20533.

Gabriely, G., Wurdinger, T., Kesari, S., Esau, C.C., Burchard, J., Linsley, P.S., and Krichevsky, A.M. 2008. MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. Mol. Cell. Biol. **28**(17): 5369–80. doi:10.1128/MCB.00479-08.

Galindo, L.T., Filippo, T.R.M., Semedo, P., Ariza, C.B., Moreira, C.M., Camara, N.O.S., and Porcionatto, M.A. 2011. Mesenchymal stem cell therapy modulates the inflammatory response in experimental traumatic brain injury. Neurol. Res. Int. **2011**: 564089. doi:10.1155/2011/564089.

Gallagher, C.N., Hutchinson, P.J., and Pickard, J.D. 2007. Neuroimaging in trauma. Curr. Opin. Neurol. **20**(4): 403–9. doi:10.1097/WCO.0b013e32821b987b.

Garner, J.P., Watts, S., Parry, C., Bird, J., and Kirkman, E. 2009. Development of a large animal model for investigating resuscitation after blast and hemorrhage. World J. Surg. **33**(10): 2194–202. doi:10.1007/s00268-009-0105-4.

Ge, X., Han, Z., Chen, F., Wang, H., Zhang, B., Jiang, R., Lei, P., and Zhang, J. 2015. miR-21 alleviates secondary blood–brain barrier damage after traumatic brain injury in rats. Brain Res. **1603**: 150–157. doi:10.1016/j.brainres.2015.01.009.

Ge, X.-T., Lei, P., Wang, H.-C., Zhang, A.-L., Han, Z.-L., Chen, X., Li, S.-H., Jiang, R.-C., Kang, C.-S., and Zhang, J.-N. 2014. miR-21 improves the neurological outcome after traumatic brain injury in rats. Sci. Rep. **4**: 6718. doi:10.1038/srep06718.

Gennarelli, T.A., Thibault, L.E., Adams, J.H., Graham, D.I., Thompson, C.J., and Marcincin, R.P. 1982. Diffuse axonal injury and traumatic coma in the primate. Ann. Neurol. **12**(6): 564–574. doi:10.1002/ana.410120611.

Gentner, B., Schira, G., Giustacchini, A., Amendola, M., Brown, B.D., Ponzoni, M., and Naldini, L. 2009. Stable knockdown of microRNA in vivo by lentiviral vectors. Nat. Methods **6**(1): 63–6. doi:10.1038/nmeth.1277.

Giza C.C., and Hovda, D.A. 2001. The neurometabolic cascade of concussion. J. Athl. Train. **36**(3): 228–235.

Goryunova, A. V, Bazarnaya, N.A., Sorokina, E.G., Semenova, N.Y., Globa, O. V, Semenova, Z.B., Pinelis, V.G., Roshal', L.M., and Maslova, O.I. 2007. Glutamate receptor autoantibody concentrations in children with chronic post-traumatic headache. Neurosci.

644 Behav. Physiol. **37**(8): 761–4. doi:10.1007/s11055-007-0079-3.
645 Grool, A.M., Aglipay, M., Momoli, F., Meehan, W.P. 3rd, Freedman, S.B., Yeates, K.O., Gravel,
646 J., Gagnon, I., Boutis, K., Meeuwisse, W., Barrowman, N., Ledoux, A.A., Osmond, M.H.,
647 Zemek, R.; Pediatric Emergency Research Canada (PERC) Concussion Team. 2016.
648 Association between early participation in physical activity following acute concussion and
649 persistent postconcussive symptoms in children and adolescents. JAMA 316(23):2504-
650 2514.
651 Grünweller, A., and Hartmann, R.K. 2007. Locked nucleic acid oligonucleotides: the next
652 generation of antisense agents? BioDrugs **21**(4): 235–43.
653 Haddad, S.H., and Arabi, Y.M. 2012. Critical care management of severe traumatic brain injury
654 in adults. Scand. J. Trauma. Resusc. Emerg. Med. **20**: 12. doi:10.1186/1757-7241-20-12.
655 Han, Z., Chen, F., Ge, X., Tan, J., Lei, P., and Zhang, J. 2014. miR-21 alleviated apoptosis of
656 cortical neurons through promoting PTEN-Akt signaling pathway in vitro after
657 experimental traumatic brain injury. Brain Res. **1582**: 12–20.
658 doi:10.1016/j.brainres.2014.07.045.
659 Harrison, E.B., Hochfelder, C.G., Lamberty, B.G., Meays, B.M., Morsey, B.M., Kelso, M.L.,
660 Fox, H.S., and Yelamanchili, S. V. 2016. Traumatic brain injury increases levels of miR-21
661 in extracellular vesicles: implications for neuroinflammation. FEBS Open Bio **6**(8): 835–
662 846. doi:10.1002/2211-5463.12092.
663 Hashimi, S.T., Fulcher, J.A., Chang, M.H., Gov, L., Wang, S., and Lee, B. 2009. MicroRNA
664 profiling identifies miR-34a and miR-21 and their target genes JAG1 and WNT1 in the
665 coordinate regulation of dendritic cell differentiation. Blood **114**(2): 404–14.
666 doi:10.1182/blood-2008-09-179150.
667 Hillary, F.G., Genova, H.M., Medaglia, J.D., Fitzpatrick, N.M., Chiou, K.S., Wardecker, B.M.,
668 Franklin, R.G., Wang, J., and DeLuca, J. 2010. The nature of processing speed deficits in
669 traumatic brain injury: is less brain more? Brain Imaging Behav. **4**(2): 141–154. Springer-
670 Verlag. doi:10.1007/s11682-010-9094-z.
671 Hollingworth, W., Todd, C.J., Bell, M.I., Arafat, Q., Girling, S., Karia, K.R., and Dixon, A.K.
672 2000. The diagnostic and therapeutic impact of MRI: an observational multi-centre study.
673 Clin. Radiol. **55**(11): 825–831. doi:10.1053/crad.2000.0546.
674 Hu, Z., Yu, D., Almeida-Suhett, C., Tu, K., Marini, A.M., Eiden, L., Braga, M.F., Zhu, J., and

675 Li, Z. 2012. Expression of miRNAs and their cooperative regulation of the pathophysiology
676 in traumatic brain injury. PLoS One **7**(6): e39357. doi:10.1371/journal.pone.0039357.
677 Jadhav V, Solaroglu I, Obenaus A, Z.J. 2007. Neuroprotection against surgically induced brain
678 injury. Surg. Neurol. **67**(1): 15–20.
679 Jezierski, A., Rennie, K., Zurakowski, B., Ribecco-Lutkiewicz, M., Haukenfrers, J., Ajji, A.,
680 Gruslin, A., Sikorska, M., and Bani-Yaghoub, M. 2014. Neuroprotective effects of GDNF-
681 expressing human amniotic fluid cells. Stem Cell Rev. **10**(2): 251–68. doi:10.1007/s12015-
682 013-9484-x.
683 Johansson, B., Berglund, P., and Rönnbäck, L. 2009. Mental fatigue and impaired information
684 processing after mild and moderate traumatic brain injury. Brain Inj. 23:1027-40.
685 Johnson, D., Cartagena, C.M., Tortella, F.C., Dave, J.R., Schmid, K.E., and Boutté, A.M. 2017.
686 Acute and subacute microRNA dysregulation is associated with cytokine responses in the
687 rodent model of penetrating ballistic-like brain injury. J Trauma Acute Care Surg. 83(1
688 Suppl 1):S145-S149.
689 Kalani, A., Tyagi, A., and Tyagi, N. 2014. Exosomes: mediators of neurodegeneration,
690 neuroprotection and therapeutics. Mol. Neurobiol. **49**(1): 590–600. doi:10.1007/s12035-
691 013-8544-1.
692 Katsuda, T., Kosaka, N., Takeshita, F., and Ochiya, T. 2013. The therapeutic potential of
693 mesenchymal stem cell-derived extracellular vesicles. Proteomics **13**(10-11): 1637–1653.
694 doi:10.1002/pmic.201200373.
695 Kim, D., Nishida, H., An, S.Y., Shetty, A.K., Bartosh, T.J., and Prockop, D.J. 2016.
696 Chromatographically isolated CD63+CD81+ extracellular vesicles from mesenchymal
697 stromal cells rescue cognitive impairments after TBI. Proc. Natl. Acad. Sci. U. S. A. **113**(1):
698 170–5. doi:10.1073/pnas.1522297113.
699 Kim, W.R., Kim, J.Y., Moon, Y., Kim, H.J., Kim, H.J., and Sun, W. 2012. Regional difference
700 of reactive astrogliosis following traumatic brain injury revealed by hGFAP-GFP transgenic
701 mice. *In* Neuroscience Letters. doi:10.1016/j.neulet.2012.02.023.
702 Kim, Y.J., Hwang, S.J., Bae, Y.C., and Jung, J.S. 2009. MiR-21 regulates adipogenic
703 differentiation through the modulation of TGF-beta signaling in mesenchymal stem cells
704 derived from human adipose tissue. Stem Cells **27**(12): 3093–102. doi:10.1002/stem.235.

Kochanek, P.M., Bramlett, H.M., Dixon, C.E., Shear, D.A., Dietrich, W.D., Schmid, K.E., Mondello, S., Wang, K.K., Hayes, R.L., Povlishock, J.T., and Tortella, F.C. 2016. Approach to modeling, therapy evaluation, drug selection, and biomarker assessments for a multicenter pre clinical drug screening consortium for acute therapies in severe traumatic brain injury: Operation Brain Trauma Therapy. J Neurotrauma. 33(6):513-22.

Kramer, D.R., Winer, J.L., Pease, B.A.M., Amar, A.P., Mack, W.J., Kramer, D.R., Winer, J.L., Pease, B.A.M., Amar, A.P., and Mack, W.J. 2013. Cerebral vasospasm in traumatic brain injury. Neurol. Res. Int. **2013**: 415813. doi:10.1155/2013/415813.

Krützfeldt, J., Rajewsky, N., Braich, R., Rajeev, K.G., Tuschl, T., Manoharan, M., and Stoffel, M. 2005. Silencing of microRNAs in vivo with "antagomirs". Nature **438**(7068): 685–9. doi:10.1038/nature04303.

Kubal, W.S. 2012. Updated imaging of traumatic brain injury. Radiol. Clin. North Am. **50**(1): 15–41.

Manning, K.Y., Schranz, A., Bartha, R., Dekaban, G.A., Barreira, C., Brown, A., Fischer, L., Asem, K., Doherty, T.J., Fraser, D.D., Holmes, J., and Menon, R.S. 2017. Multiparametric MRI changes persist beyond recovery in concussed adolescent hockey players. Neurology. 2017 Nov 21;89(21):2157-2166.

Marshall, L.F., Marshall, S.B., Klauber, M.R., van Berkum Clark, M., Eisenberg, H.M., Jane, J.A., Luerssen, T.G., Marmarou, A.and Foulkes, M.A. 1991. A new classification of head injury based on computerized tomography. J. Neurosurg. **75**(1s): S14–S20.

Langlois, J.A., Rutland-Brown, W., and Wald, M.M. 2006. The epidemiology and impact of traumatic brain injury: a brief overview. J. Head Trauma Rehabil. **21**(5): 375–8. doi:00001199-200609000-00001 [pii].

LaRovere, K.L., O'Brien, N.F., and Tasker, R.C. 2016. Current opinion and use of transcranial Doppler ultrasonography in traumatic brain injury in the pediatric intensive care unit. J Neurotrauma. 33(23):2105-2114.

Lei, P., Li, Y., Chen, X., Yang, S., and Zhang, J. 2009. Microarray based analysis of microRNA expression in rat cerebral cortex after traumatic brain injury. Brain Res. **1284**: 191–201. doi:10.1016/j.brainres.2009.05.074.

Lewis, B.P., Burge, C.B., and Bartel, D.P. 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. **120**:15-

20. doi:10.1016/j.cell.2004.12.035.

Ley, E.J., Srour, M.K., Clond, M.A., Barnajian, M., Tillou, A., Mirocha, J., and Salim, A. 2011. Diabetic patients with traumatic brain injury: insulin deficiency is associated with increased mortality. J. Trauma **70**(5): 1141–4. doi:10.1097/TA.0b013e3182146d66.

Lighthall, J.W. 1988. Controlled cortical impact: a new experimental brain injury model. J. Neurotrauma **5**(1): 1–15. doi:10.1089/neu.1988.5.1.

Lim, L.P., Lau, N.C., Garrett-Engele, P., Grimson, A., Schelter, J.M., Castle, J., Bartel, D.P., Linsley, P.S., and Johnson, J.M. 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature **433**(7027): 769–73. doi:10.1038/nature03315.

Liu, D.-Z., Tian, Y., Ander, B.P., Xu, H., Stamova, B.S., Zhan, X., Turner, R.J., Jickling, G., and Sharp, F.R. 2010a. Brain and blood microRNA expression profiling of ischemic stroke, intracerebral hemorrhage, and kainate seizures. J. Cereb. Blood Flow Metab. **30**(1): 92–101. doi:10.1038/jcbfm.2009.186.

Liu, G., Keeler, B.E., Zhukareva, V., and Houlé, J.D. 2010b. Cycling exercise affects the expression of apoptosis-associated microRNAs after spinal cord injury in rats. Exp. Neurol. **226**(1): 200–206. doi:10.1016/j.expneurol.2010.08.032.

Liu, L., Sun, T., Liu, Z., Chen, X., Zhao, L., Qu, G., and Li, Q. 2014. Traumatic brain injury dysregulates microRNAs to modulate cell signaling in rat hippocampus. PLoS One **9**(8): e103948. doi:10.1371/journal.pone.0103948.

Liu, M., Wu, H., Liu, T., Li, Y., Wang, F., Wan, H., Li, X., and Tang, H. 2009a. Regulation of the cell cycle gene, BTG2, by miR-21 in human laryngeal carcinoma. Cell Res. **19**(7): 828–37. doi:10.1038/cr.2009.72.

Liu, N.-K., Wang, X.-F., Lu, Q.-B., and Xu, X.-M. 2009b. Altered microRNA expression following traumatic spinal cord injury. Exp. Neurol. **219**(2): 424–429. doi:10.1016/j.expneurol.2009.06.015.

Londin, E., Loher, P., Telonis, A.G., Quann, K., Clark, P., Jing, Y., Hatzimichael, E., Kirino, Y., Honda, S., Lally, M., Ramratnam, B., Comstock, C.E.S., Knudsen, K.E., Gomella, L., Spaeth, G.L., Hark, L., Katz, L.J., Witkiewicz, A., Rostami, A., Jimenez, S.A., Hollingsworth, M.A., Yeh, J.J., Shaw, C.A., McKenzie, S.E., Bray, P., Nelson, P.T., Zupo, S., Van Roosbroeck, K., Keating, M.J., Calin, G.A., Yeo, C., Jimbo, M., Cozzitorto, J.,

767 Brody, J.R., Delgrosso, K., Mattick, J.S., Fortina, P., and Rigoutsos, I. 2015. Analysis of 13
768 cell types reveals evidence for the expression of numerous novel primate- and tissue-
769 specific microRNAs. Proc. Natl. Acad. Sci. U. S. A. **112**(10): E1106–15.
770 doi:10.1073/pnas.1420955112.
771 Long, J.B., Bentley, T.L., Wessner, K.A., Cerone, C., Sweeney, S., and Bauman, R.A. 2009.
772 Blast overpressure in rats: recreating a battlefield injury in the laboratory. J. Neurotrauma
773 **26**(6): 827–40. doi:10.1089/neu.2008.0748.
774 Lu, T.X., Munitz, A., and Rothenberg, M.E. 2009. MicroRNA-21 is up-regulated in allergic
775 airway inflammation and regulates IL-12p35 expression. J. Immunol. **182**(8): 4994–5002.
776 doi:10.4049/jimmunol.0803560.
777 Luikart, B.W., Bensen, A.L., Washburn, E.K., Perederiy, J. V, Su, K.G., Li, Y., Kernie, S.G.,
778 Parada, L.F., and Westbrook, G.L. 2011. miR-132 mediates the integration of newborn
779 neurons into the adult dentate gyrus. PLoS One **6**(5): e19077.
780 doi:10.1371/journal.pone.0019077.
781 Maas, A.I., Stocchetti, N., and Bullock, R. 2008. Moderate and severe traumatic brain injury in
782 adults. Lancet Neurol. **7**(8): 728–741. doi:10.1016/S1474-4422(08)70164-9.
783 Maas, A.I.R., Hukkelhoven, C.W.P.M., Marshall, L.F., and Steyerberg, E.W. 2005. Prediction of
784 outcome in traumatic brain injury with computed tomographic characteristics: a comparison
785 between the computed tomographic classification and combinations of computed
786 tomographic predictors. Neurosurgery **57**(6): 1173–82.
787 Makley, M.J., English, J.B., Drubach, D.A., Kreuz, A.J., Celnik, P.A., and Tarwater, P.M. 2008.
788 Prevalence of sleep disturbance in closed head injury patients in a rehabilitation unit.
789 Neurorehabil. Neural Repair **22**(4): 341–7. doi:10.1177/1545968308315598.
790 Marehbian, J., Muehlschlegel, S., Edlow, B.L., Hinson, H.E., and Hwang, D.Y. 2017. Medical
791 management of the severe traumatic brain injury patient. Neurocrit. Care doi:
792 10.1007/s12028-017-0408-5.
793 Marmarou, A., Foda, M.A., van den Brink, W., Campbell, J., Kita, H., and Demetriadou, K.
794 1994. A new model of diffuse brain injury in rats. Part I: Pathophysiology and
795 biomechanics. J. Neurosurg. **80**(2): 291–300. doi:10.3171/jns.1994.80.2.0291.
796 Matsumura S, Takagi K, Okuda-Ashitaka E, Lu J, Naritsuka H, Yamaguchi M, I.S. 2010.
797 Characterization of nestin expression in the spinal cord of GFP transgenic mice after

peripheral nerve injury. Neuroscience **170**(3): 942–953.

McInnes, K., Friesen, C.L., MacKenzie, D.E., Westwood, D.A., and Boe, S,G. 2017. Mild traumatic brain injury (mTBI) and chronic cognitive impairment: A scoping review. PLoS One 12(4): e0174847.

McIntosh, T.K., Noble, L., Andrews, B., and Faden, A.I. 1987. Traumatic brain injury in the rat: characterization of a midline fluid-percussion model. Cent. Nerv. Syst. Trauma **4**(2): 119–34.

McIntosh, T.K.K., Vink, R., Noble, L., Yamakami, I., Fernyak, S., Soares, H., and Faden, A.L.L. 1989. Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model. Neuroscience **28**(1). doi:10.1016/0306-4522(89)90247-9.

Mechtler, L.L., Shastri, K.K., Crutchfield, K.E., Mechtler LL, Shastri KK, C.K., Mechtler, L.L., Shastri, K.K., and Crutchfield, K.E. 2014. Advanced neuroimaging of mild traumatic brain injury. Neurol. Clin. **32**(1): 31–58. doi:10.1016/j.ncl.2013.08.002.

Meissner, L., Gallozzi, M., Balbi, M., Schwarzmaier, S., Tiedt, S., Terpolilli, N.A., and Plesnila, N. 2016. Temporal profile of microRNA expression in ontused cortex after traumatic brain injury in mice. J. Neurotrauma **33**(8): 713–20. doi:10.1089/neu.2015.4077.

Menon, D.K., and Maas, A.I.R. 2015. Traumatic brain injury in 2014. Progress, failures and new approaches for TBI research. Nat. Rev. Neurol. **11**(2): 71–2. doi:10.1038/nrneurol.2014.261.

Meza-Sosa, K.F., Valle-García, D., Pedraza-Alva, G., and Pérez-Martínez, L. 2012. Role of microRNAs in central nervous system development and pathology. J. Neurosci. Res. **90**(1): 1–12. doi:10.1002/jnr.22701.

Mondello, S., Linnet, A., Buki, A., Robicsek, S., Gabrielli, A., Tepas, J., Papa, L., Brophy, G.M., Tortella, F., Hayes, R.L., and Wang, K.K. 2012. Clinical utility of serum levels of ubiquitin C-terminal hydrolase as a biomarker for severe traumatic brain injury. Neurosurgery **70**(3): 666–75. doi:10.1227/NEU.0b013e318236a809.

Mondello, S., Muller, U., Jeromin, A., Streeter, J., Hayes, R.L., and Wang, K.K.W. 2011. Blood-based diagnostics of traumatic brain injuries. Expert Rev. Mol. Diagn. **11**(1): 65–78. doi:10.1586/erm.10.104.

Mondello, S., Sorinola, A., Czeiter, E., Vámos, Z., Amrein, K., Synnot, A., Donoghue, E.L., Sándor, J., Wang, K.K.W., Diaz-Arrastia, R., Steyerberg, E.W., Menon, D., Maas, A., and

Buki, A. 2017. Blood-based protein biomarkers for the management of traumatic brain injuries in adults presenting with mild head injury to emergency departments: a living systematic review and meta-analysis. J Neurotrauma. doi: 10.1089/neu.2017.5182.

Morales, D.M., Marklund, N., Lebold, D., Thompson, H.J., Pitkanen, A., Maxwell, W.L., Longhi, L., Laurer, H., Maegele, M., Neugebauer, E., Graham, D.I., Stocchetti, N., and McIntosh, T.K. 2005. Experimental models of traumatic brain injury: Do we really need to build a better mousetrap? Neuroscience **136**(4): 971–989. doi:10.1016/j.neuroscience.2005.08.030.

Morganti-Kossmann, M.C., Yan, E., and Bye, N. 2010. Animal models of traumatic brain injury: Is there an optimal model to reproduce human brain injury in the laboratory? Injury **41**: S10–S13. doi:10.1016/j.injury.2010.03.032.

Morrison, G., Fraser, D.D., and Cepinskas, G. 2013. Mechanisms and consequences of acquired brain injury during development. Pathophysiology **20**(1): 49–57. doi:10.1016/j.pathophys.2012.02.006.

Moschidou, D., Mukherjee, S., Blundell, M.P., Drews, K., Jones, G.N., Abdulrazzak, H., Nowakowska, B., Phoolchund, A., Lay, K., Ramasamy, T.S., Cananzi, M., Nettersheim, D., Sullivan, M., Frost, J., Moore, G., Vermeesch, J.R., Fisk, N.M., Thrasher, A.J., Atala, A., Adjaye, J., Schorle, H., De Coppi, P., and Guillot, P. V. 2012. Valproic acid confers functional pluripotency to human amniotic fluid stem cells in a transgene-free approach. Mol. Ther. **20**(10): 1953–67. doi:10.1038/mt.2012.117.

Namjoshi, D.R., Cheng, W.H., Bashir, A., Wilkinson, A., Stukas, S., Martens, K.M., Whyte, T., Abebe, Z.A., McInnes, K.A., Cripton, P.A. and Wellington, C.L. 2017. Defining the biomechanical and biological threshold of murine mild traumatic brain injury using CHIMERA (Closed Head Impact Model of Engineered Rotational Acceleration). Exp. Neurol. 292:80-91.

Namjoshi, D.R., Cheng, W., McInnes, K.A., Martens, K.M., Carr, M., Wilkinson, A., Fan, J., Robert, J., Hayat, A., Cripton, P.A., and Wellington, C.L. 2014. Merging pathology with biomechanics using CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration): a novel, surgery-free model of traumatic brain injury. Mol. Neurodegener. **9**(1): 55. doi:10.1186/1750-1326-9-55.

National Center for Injury Prevention and Control. 2003. Report to Congress on Mild Traumatic

Brain Injury in the United States: Steps to Prevent a Serious Public Health Problem. Atlanta, GA.

Ngankam, L., Kazantseva, N. V, and Gerasimova, M.M. 2011. [Immunological markers of severity and outcome of traumatic brain injury]. Zhurnal Nevrol. i psikhiatrii Im. S.S. Korsakova / Minist. Zdr. i meditsinskoĭ promyshlennosti Ross. Fed. Vserossiĭskoe Obs. Nevrol. [i] Vserossiĭskoe Obs. psikhiatrov **111**(7): 61–5.

Northern Brain Injury Association; Brain Injury Statistics. 2014. Available from http://nbia.ca/brain-injury-statistics/.

Ohtake, Y., Hayat, U., and Li, S. 2015. PTEN inhibition and axon regeneration and neural repair. Neural Regen. Res. **10**(9): 1363–8. doi:10.4103/1673-5374.165496.

Ohtake, Y., Park, D., Abdul-Muneer, P.M., Li, H., Xu, B., Sharma, K., Smith, G.M., Selzer, M.E., and Li, S. 2014. The effect of systemic PTEN antagonist peptides on axon growth and functional recovery after spinal cord injury. Biomaterials **35**(16): 4610–4626. doi:10.1016/j.biomaterials.2014.02.037.

Onyszchuk, G., Al-Hafez, B., He, Y.-Y., Bilgen, M., Berman, N.E.J., and Brooks, W.M. 2007. A mouse model of sensorimotor controlled cortical impact: Characterization using longitudinal magnetic resonance imaging, behavioral assessments and histology. J. Neurosci. Methods **160**(2): 187–196. doi:10.1016/j.jneumeth.2006.09.007.

Orešič, M., Posti, J.P., Kamstrup-Nielsen, M.H., Takala, R.S., Lingsma, H.F., Mattil,a I., Jäntti, S., Katila, A.J., Carpenter, K.L., Ala-Seppälä, H., Kyllönen, A., Maanpää, H.R., Tallus, J., Coles, J.P., Heino, I., Frantzén, J., Hutchinson, P.J., Menon, D.K., Tenovuo, O., and Hyötyläinen, T. 2016. Human serum metabolites associate with severity and patient outcomes in traumatic brain injury. EBioMedicine. **12**:118-126.

Papagiannakopoulos, T., Shapiro, A., and Kosik, K.S. 2008. MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. Cancer Res. **68**(19): 8164–72. doi:10.1158/0008-5472.CAN-08-1305.

Patz, S., Trattnig, C., Grünbacher, G., Ebner, B., Gülly, C., Novak, A., Rinner, B., Leitinger, G., Absenger, M., Tomescu, O.A., Thallinger, G.G., Fasching, U., Wissa, S., Archelos-Garcia, J., and Schäfer, U. 2013. More than cell dust: microparticles isolated from cerebrospinal fluid of brain injured patients are messengers carrying mRNAs, miRNAs, and proteins. J. Neurotrauma **30**(14): 1232–1242. doi:10.1089/neu.2012.2596.

Posti, J.P., Dickens, A.M., Orešič, M., Hyötyläinen, T., and Tenovuo, O. 2017. Metabolomics profiling as a diagnostic tool in severe traumatic brain injury. Front Neurol. **8**:398.

Puntis, M. and Smith, M. 2017.Critical care management of adult traumatic brain injury. Anaesthesia and Intensive Care Medicine. 18(5):233-238.

Redell, J.B., Liu, Y., and Dash, P.K. 2009. Traumatic brain injury alters expression of hippocampal microRNAs: potential regulators of multiple pathophysiological processes. J. Neurosci. Res. **87**(6): 1435–48. doi:10.1002/jnr.21945.

Redell, J.B., Moore, A.N., Ward, N.H., Hergenroeder, G.W., and Dash, P.K. 2010. Human traumatic brain injury alters plasma microRNA levels. J. Neurotrauma **27**(12): 2147–56. doi:10.1089/neu.2010.1481.

Redell, J.B., Zhao, J., and Dash, P.K. 2011. Altered expression of miRNA-21 and its targets in the hippocampus after traumatic brain injury. J. Neurosci. Res. **89**(2): 212–21. doi:10.1002/jnr.22539.

Rees, P. 2003. Contemporary issues in mild traumatic brain injury. Arch. Phys. Med. Rehabil. **84**(12): 1885–1894.

Reis, C., Wang, Y., Akyol, O., Ho, W.M., Ii, R.A., Stier, G., Martin, R, and Zhang, J.H. 2015. What's new in traumatic brain injury: update on tracking, monitoring and treatment. Int. J. Mol. Sci. **16**(6): 11903–11965.

Rennie, K., Haukenfrers, J., Ribecco-Lutkiewicz, M., Ly, D., Jezierski, A., Smith, B., Zurakowski, B., Martina, M., Gruslin, A., and Bani-Yaghoub, M. 2013. Therapeutic potential of amniotic fluid-derived cells for treating the injured nervous system. Biochem. Cell Biol. **91**(5): 271–86. NRC Research Press. doi:10.1139/bcb-2013-0019.

Ribecco-Lutkiewicz, M., Ly, D., Sodja, C., Haukenfrers, J., Smith, B., Liu, Q.Y., Sikorska, M., and Bani-Yaghoub, M. 2016. MicroRNA Expression in Amniotic Fluid Cells. *In* Fetal Stem Cells in Regenerative Medicine. *Edited by* D. Fauza and M. Bani. Springer New York, New York. pp. 215–228. doi:10.1007/978-1-4939-3483-6_11.

Ross, D.T., Meaney, D.F., Sabol, M.K., Smith, D.H., and Gennarelli, T.A. 1994. Distribution of forebrain diffuse axonal injury following inertial closed head injury in miniature qwine. Exp. Neurol. **126**(2): 291–298. doi:10.1006/exnr.1994.1067.

Rostami, E. 2012. Traumatic brain injury in humans and animal models. Karolinska institutet.

Rowe, R.K., Harrison, J.L., Thomas, T.C., Pauly, J.R., Adelson, P.D., and Lifshitz, J. 2013.

Using anesthetics and analgesics in experimental traumatic brain injury. Lab Anim. (NY). **42**(8): 286–291. doi:10.1038/laban.257.

Sabatel, C., Malvaux, L., Bovy, N., Deroanne, C., Lambert, V., Gonzalez, M.-L.A., Colige, A., Rakic, J.-M., Noël, A., Martial, J.A., and Struman, I. 2011. MicroRNA-21 exhibits antiangiogenic function by targeting RhoB expression in endothelial cells. PLoS One **6**(2): e16979. doi:10.1371/journal.pone.0016979.

Sabirzhanov, B., Stoica, B.A., Zhao, Z., Loane, D.J., Wu, J., Dorsey, S.G., and Faden, A.I. 2016. miR-711 upregulation induces neuronal cell death after traumatic brain injury. Cell Death Differ. 23(4):654-68.

Sabirzhanov, B., Zhao, Z., Stoica, B.A., Loane, D.J., Wu, J., Borroto, C., Dorsey, S.G., and Faden, A.I. 2014. Downregulation of miR-23a and miR-27a following experimental traumatic brain injury induces neuronal cell death through activation of proapoptotic Bcl-2 proteins. J. Neurosci. **34**(30): 10055–71. doi:10.1523/JNEUROSCI.1260-14.2014.

Sandhir, R., Gregory, E., and Berman, N.E. 2014. Differential response of miRNA-21 and its targets after traumatic brain injury in aging mice. Neurochem. Int. **78**: 117–121. doi:10.1016/j.neuint.2014.09.009.

Schmidt, R.H., and Grady, M.S. 1993. Regional patterns of blood-brain barrier breakdown following central and lateral fluid percussion injury in rodents. J. Neurotrauma **10**(4): 415–30. doi:10.1089/neu.1993.10.415.

Shapira, Y., Shohami, E., Sidi, A., Soffer, D., Freeman, S., and Cotev, S. 1988. Experimental closed head injury in rats: mechanical, pathophysiologic, and neurologic properties. Crit. Care Med. **16**(3): 258–65.

Sheedy, F.J. 2015. Turning 21: Induction of miR-21 as a key switch in the inflammatory response. Front. Immunol. **6**: 19. doi:10.3389/fimmu.2015.00019.

Shi, G., Liu, Y., Liu, T., Yan, W., Liu, X., Wang, Y., Shi, J., and Jia, L. 2012. Upregulated miR-29b promotes neuronal cell death by inhibiting Bcl2L2 after ischemic brain injury. Exp. brain Res. **216**(2): 225–30. doi:10.1007/s00221-011-2925-3.

Shi, Z., Zhang, J., Qian, X., Han, L., Zhang, K., Chen, L., Liu, J., Ren, Y., Yang, M., Zhang, A., Pu, P., and Kang, C. 2013. AC1MMYR2, an inhibitor of dicer-mediated biogenesis of Oncomir miR-21, reverses epithelial-mesenchymal transition and suppresses tumor growth and progression. Cancer Res. 73(17): 5519–31. doi:10.1158/0008-5472.CAN-13-0280.

953 Shin, S.S., Bales, J.W., Edward Dixon, C., and Hwang, M. 2017. Structural imaging of mild
954 traumatic brain injury may not be enough: overview of functional and metabolic imaging of
955 mild traumatic brain injury. Brain Imaging Behav. 11(2):591-610.
956 Smith, D.H., Soares, H.D., Pierce, J.S., Perlman, K.G., Saatman, K.E., Meaney, D.F., Dixon,
957 C.E., and McIntosh, T.K. 1995. A model of parasagittal controlled cortical impact in the
958 mouse: cognitive and histopathologic effects. J. Neurotrauma **12**(2): 169–78.
959 doi:10.1089/neu.1995.12.169.
960 Smith-Bindman, R., Miglioretti, D.L., Johnson, E., Lee, C., Feigelson, H.S., Flynn, M.,
961 Greenlee, R.T., Kruger, R.L., Hornbrook, M.C., Roblin, D., Solberg, L.I., Vanneman, N.,
962 Weinmann, S., and Williams, A.E. 2012. Use of diagnostic imaging studies and associated
963 radiation exposure for patients enrolled in large integrated health care systems, 1996-2010.
964 JAMA **307**(22): 2400–9. NIH Public Access. doi:10.1001/jama.2012.5960.
965 Soares de Souza, R., Pessoa Pinheiro, P., Ferreira de Lima Silva, J.M., Leite Rolim Neto, M.,
966 Machado Filho, J.A., and Filho, J.A.M. 2015. Traumatic brain injury (TBI): morbidity,
967 mortality and economic implications. Int. Arch. Med. **8**(0). doi:10.3823/1672.
968 Statler, K.D., Alexander, H., Vagni, V., Dixon, C.E., Clark, R.S.B., Jenkins, L., and Kochanek,
969 P.M. 2006a. Comparison of seven anesthetic agents on outcome after experimental
970 traumatic brain injury in adult, male rats. J. Neurotrauma **23**(1): 97–108.
971 doi:10.1089/neu.2006.23.97.
972 Statler, K.D., Alexander, H., Vagni, V., Holubkov, R., Dixon, C.E., Clark, R.S.B., Jenkins, L.,
973 and Kochanek, P.M. 2006b. Isoflurane exerts neuroprotective actions at or near the time of
974 severe traumatic brain injury. Brain Res. **1076**(1): 216–24.
975 doi:10.1016/j.brainres.2005.12.106.
976 Statler, K.D., Kochanek, P.M., Dixon, C.E., Alexander, H.L., Warner, D.S., Clark, R.S.,
977 Wisniewski, S.R., Graham, S.H., Jenkins, L.W., Marion, D.W., and Safar, P.J. 2000.
978 Isoflurane improves long-term neurologic outcome versus fentanyl after traumatic brain
979 injury in rats. J. Neurotrauma **17**(12): 1179–89. doi:10.1089/neu.2000.17.1179.
980 Stenvang, J., Silahtaroglu, A.N., Lindow, M., Elmen, J., and Kauppinen, S. 2008. The utility of
981 LNA in microRNA-based cancer diagnostics and therapeutics. Semin. Cancer Biol. **18**(2):
982 89–102. doi:10.1016/j.semcancer.2008.01.004.
983 Sun, L., Liu, A., Zhang, J., Ji, W., Li, Y., Yang, X., Wu, Z., and Guo, J. 2016. miR-23b improves

cognitive impairments in traumatic brain injury by targeting ATG12-mediated neuronal autophagy. Behav Brain Res. pii: S0166-4328(16)30603-9.

Sun, L., Zhao, M., Wang, Y., Liu, A., Lv, M., Li, Y., Yang, X., and Wu, Z. 2017. Neuroprotective effects of miR-27a against traumatic brain injury via suppressing FoxO3a-mediated neuronal autophagy. Biochem Biophys Res Commun. **482**(4):1141-1147.

Tan, K.S., Armugam, A., Sepramaniam, S., Lim, K.Y., Setyowati, K.D., Wang, C.W., and Jeyaseelan, K. 2009. Expression profile of MicroRNAs in young stroke patients. PLoS One **4**(11): e7689. doi:10.1371/journal.pone.0007689.

Tang, X., Gal, J., Zhuang, X., Wang, W., Zhu, H., and Tang, G. 2007. A simple array platform for microRNA analysis and its application in mouse tissues. RNA **13**(10): 1803–22. doi:10.1261/rna.498607.

Taylor, D.D., and Gercel-Taylor, C. 2014. Exosome platform for diagnosis and monitoring of traumatic brain injury. Philos. Trans. R. Soc. B Biol. Sci. **369**(1652): 20130503–20130503. doi:10.1098/rstb.2013.0503.

Teasdale, G., Maas, A., Lecky, F., Manley, G., Stocchetti, N., and Murray, G. 2014. The Glasgow Coma Scale at 40 years: standing the test of time. Lancet Neurol. **13**(8): 844–854.

Teasdale, G., and Jennett, B. 1974. Assessment of coma and impaired consciousness. A practical scale. Lancet **2**(7872): 81–84.

Traumatic Brain Injury; Understanding TBI. 2006. Available from http://www.traumaticbraininjury.com/understanding-tbi/what-are-the-causes-of-tbi/.

Truettner, J.S., Alonso, O.F., Bramlett, H.M., and Dietrich, W.D. 2011. Therapeutic hypothermia alters microRNA responses to traumatic brain injury in rats. J. Cereb. Blood Flow Metab. **31**(9): 1897–907. doi:10.1038/jcbfm.2011.33.

Valiyaveettil, M., Alamneh, Y.A., Miller, S.-A., Hammamieh, R., Arun, P., Wang, Y., Wei, Y., Oguntayo, S., Long, J.B., and Nambiar, M.P. 2013. Modulation of cholinergic pathways and inflammatory mediators in blast-induced traumatic brain injury. Chem. Biol. Interact. **203**(1): 371–375. doi:10.1016/j.cbi.2012.10.022.

Vlachos, I.S., Zagganas, K., Paraskevopoulou, M.D., Georgakilas, G., Karagkouni, D., Vergoulis, T., Dalamagas, T and Hatzigeorgiou, A.G. 2015. DIANA-miRPath v3.0: deciphering microRNA function with experimental support. Nucleic Acids Research, **43**(Web Server issue), W460–W466.

Wagner, A.K., McCullough, E.H., Niyonkuru, C., Ozawa, H., Loucks, T.L., Dobos, J.A., Brett, C.A., Santarsieri, M., Dixon, C.E., Berga, S.L., and Fabio, A. 2011. Acute serum hormone levels: characterization and prognosis after severe traumatic brain injury. J. Neurotrauma **28**(6): 871–88. doi:10.1089/neu.2010.1586.

Wang, H., Chiu, M.M., Xie, Z., Chiu, M.M., Liu, Z., Chen, P., Liu, S., Byrd, J.C., Muthusamy, N., Garzon, R., Croce, C.M., Marcucci, G., and Chan, K.K. 2012. Synthetic microRNA cassette dosing: pharmacokinetics, tissue distribution and bioactivity. Mol. Pharm. **9**(6): 1638–44. doi:10.1021/mp2006483.

Wang, K.K., Yang, Z., Yue, J.K., Zhang, Z., Winkler, E.A., Puccio, A.M., Diaz-Arrastia, R., Lingsma, H.F., Yuh, E.L., Mukherjee, P., Valadka, A.B., Gordon, W.A., Okonkwo, D.O., Manley, G.T., Cooper, S.R., Dams-O'Connor, K., Hricik, A.J., Inoue, T., Maas, A.I., Menon, D.K., Schnyer, D.M., Sinha, T.K., and Vassar, M.J. 2016b. Plasma anti-glial fibrillary acidic protein autoantibody levels during the acute and chronic phases of traumatic brain injury: a transforming research and clinical knowledge in traumatic brain injury pilot study. J Neurotrauma. 33(13):1270-1277.

Wang, W.-X., Wilfred, B.R., Baldwin, D.A., Isett, R.B., Ren, N., Stromberg, A., and Nelson, P.T. 2008. Focus on RNA isolation: Obtaining RNA for microRNA (miRNA) expression profiling analyses of neural tissue. Biochim. Biophys. Acta - Gene Regul. Mech. **1779**(11): 749–757. doi:10.1016/j.bbagrm.2008.01.005.

Wang, X., Seekaew, P., Gao, X., and Chen, J. 2016a. Traumatic brain injury stimulates neural stem cell proliferation via mammalian target of rapamycin signaling pathway activation. eNeuro 3(5). pii: ENEURO.0162-16.2016.

Weber, M., Baker, M.B., Moore, J.P., and Searles, C.D. 2010. MiR-21 is induced in endothelial cells by shear stress and modulates apoptosis and eNOS activity. *In* Biochemical and Biophysical Research Communications. doi:10.1016/j.bbrc.2010.02.045.

Werner, C., and Engelhard, K. 2007. Pathophysiology of traumatic brain injury. Br. J. Anaesth. **99**(1): 4–9. doi:10.1093/bja/aem131.

Whalen, M.J., Carlos, T.M., Kochanek, P.M., and Heineman, S. 1998. Blood-brain barrier permeability, neutrophil accumulation and vascular adhesion molecule expression after controlled cortical impact in rats: a preliminary study. Acta Neurochir. Suppl. **71**: 212–4.

Wickramasinghe, N.S., Manavalan, T.T., Dougherty, S.M., Riggs, K.A., Li, Y., and Klinge,

C.M. 2009. Estradiol downregulates miR-21 expression and increases miR-21 target gene expression in MCF-7 breast cancer cells. Nucleic Acids Res. **37**(8): 2584–95. doi:10.1093/nar/gkp117.

Williams, A.J., Hartings, J.A., Lu, X.-C.M., Rolli, M.L., Dave, J.R., and Tortella, F.C. 2005. Characterization of a new rat model of penetrating ballistic brain injury. J. Neurotrauma **22**(2): 313–31. doi:10.1089/neu.2005.22.313.

Williams, A.J., Hartings, J.A., Lu, X.-C.M., Rolli, M.L., and Tortella, F.C. 2006. Penetrating ballistic-like brain injury in the rat: differential time courses of hemorrhage, cell death, inflammation, and remote degeneration. J. Neurotrauma **23**(12): 1828–46. doi:10.1089/neu.2006.23.1828.

Wolahan, S.M., Hirt, D., and Glenn, T.C. 2015. Translational metabolomics of head injury: exploring dysfunctional cerebral metabolism with ex vivo NMR spectroscopy-based metabolite quantification. In: Kobeissy, F.H., editor. Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. Boca Raton (FL): CRC Press/Taylor & Francis.

Van Wynsberghe, P.M., Chan, S.-P., Slack, F.J., and Pasquinelli, A.E. 2011. Chapter 8 – Analysis of microRNA Expression and Function. *In* Methods in Cell Biology. pp. 219–252. doi:10.1016/B978-0-12-544172-8.00008-6.

Xie, X., Lu, J., Kulbokas, E.J., Golub, T.R., Mootha, V., Lindblad-Toh, K., Lander, E.S., and Kellis, M. 2005. Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. Nature **434**(7031): 338–45. doi:10.1038/nature03441.

Xiong, Y., Mahmood, A., and Chopp, M. 2013. Animal models of traumatic brain injury. Nat. Rev. Neurosci. **14**(2): 128–42. doi:10.1038/nrn3407.

Xiong, Y., Mahmood, A., and Chopp, M. 2017. Emerging potential of exosomes for treatment of traumatic brain injury. Neural Regen Res. **12**(1):19-22.

Yang, T., Song, J., Bu, X., Wang, C., Wu, J., Cai, J., Wan, S., Fan, C., Zhang, C., and Wang, J. 2016. Elevated serum miR-93, miR-191, and miR-499 are noninvasive biomarkers for the presence and progression of traumatic brain injury. J. Neurochem. **137**(1): 122–9. doi:10.1111/jnc.13534.

1076 Yang, Y., Ye, Y., Su, X., He, J., Bai, W., and He, X. 2017. MSCs-derived exosomes and
1077 neuroinflammation, neurogenesis and therapy of traumatic brain injury. Front Cell
1078 Neurosci. **11**:55.
1079 Yelamanchili, S. V, Chaudhuri, A.D., Chen, L.-N., Xiong, H., and Fox, H.S. 2010. MicroRNA-
1080 21 dysregulates the expression of MEF2C in neurons in monkey and human SIV/HIV
1081 neurological disease. Cell Death Dis. **1**: e77. doi:10.1038/cddis.2010.56.
1082 Yi, .L, Shi, S., Wang, Y., Huang, W., Xia, Z.A., Xing, Z., Peng, W., and Wang, Z. 2016. Serum
1083 metabolic profiling reveals altered metabolic pathways in patients with post-traumatic
1084 cognitive impairments. Sci Rep. **6**:21320.
1085 Yin, K.-J., Deng, Z., Huang, H., Hamblin, M., Xie, C., Zhang, J., and Chen, Y.E. 2010. miR-497
1086 regulates neuronal death in mouse brain after transient focal cerebral ischemia. Neurobiol.
1087 Dis. **38**(1): 17–26. doi:10.1016/j.nbd.2009.12.021.
1088 Yokobori, S., Hosein, K., Burks, S., Sharma, I., Gajavelli, S., and Bullock, R. 2013. Biomarkers
1089 for the clinical differential diagnosis in traumatic brain injury--a systematic review. CNS
1090 Neurosci. Ther. **19**(8): 556–65. doi:10.1111/cns.12127.
1091 Yokobori, S., and Yokota, H. 2016. Targeted temperature management in traumatic brain injury.
1092 J. intensive care **4**(1): 28. BioMed Central. doi:10.1186/s40560-016-0137-4.
1093 Young, L., Rule, G.T., Bocchieri, R.T., Walilko, T.J., Burns, J.M., and Ling, G. 2015. When
1094 physics meets biology: low and high-velocity penetration, blunt impact, and blast injuries to
1095 the brain. Front. Neurol. **6**: 89. doi:10.3389/fneur.2015.00089.
1096 Young, G.R., and Tsao, J.W. 2017. Rate of persistent postconcussive symptoms. JAMA
1097 317(13):1375.
1098 Yu, T.-S., Zhang, G., Liebl, D.J., and Kernie, S.G. 2008. Traumatic brain injury-induced
1099 hippocampal neurogenesis requires activation of early nestin-expressing progenitors. J.
1100 Neurosci. **28**(48): 12901–12. doi:10.1523/JNEUROSCI.4629-08.2008.
1101 Yue, J. 2011. miRNA and vascular cell movement. Adv. Drug Deliv. Rev. **63**(8): 616–622.
1102 doi:10.1016/j.addr.2011.01.001.
1103 Zhang, Y., Chopp, M., Meng, Y., Katakowski, M., Xin, H., Mahmood, A., and Xiong, Y. 2015.
1104 Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional
1105 recovery and neurovascular plasticity in rats after traumatic brain injury. J. Neurosurg.
1106 **122**(4): 856–867. doi:10.3171/2014.11.JNS14770.

Zhang, Y., Chopp, M., Zhang, Z.G., Katakowski, M., Xin, H., Qu, C., Ali, M., Mahmood, A., and Xiong, Y. 2016. Systemic administration of cell-free exosomes generated by human bone marrow derived mesenchymal stem cells cultured under 2D and 3D conditions improves functional recovery in rats after traumatic brain injury. Neurochem. Int. doi:10.1016/j.neuint.2016.08.003.

Zhang, H., Shykind, B., and Sun, T. 2013. Approaches to manipulating microRNAs in neurogenesis. Front. Neurosci. **6**:196.

Zhang, Z., Zoltewicz, J.S., Mondello, S., Newsom, K.J., Yang, Z., Yang, B., Kobeissy, F., Guingab, J., Glushakova, O., Robicsek, S., Heaton, S., Buki, A., Hannay, J., Gold, M.S., Rubenstein, R., Lu, X.-C.M., Dave, J.R., Schmid, K., Tortella, F., Robertson, C.S., and Wang, K.K.W. 2014. Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products. PLoS One **9**(3): e92698. doi:10.1371/journal.pone.0092698.

Zhao, J., Qu, Y., Wu, J., Cao, M., Ferriero, D.M., Zhang, L., and Mu, D. 2013. PTEN inhibition prevents rat cortical neuron injury after hypoxia–ischemia. Neuroscience **238**: 242–251. doi:10.1016/j.neuroscience.2013.02.046.

Figure Legends

Figure 1: Example of traumatic brain injury detected by MRI in a teenager, following a motor vehicle collision. A,B) CT imaging shows injury-induced hemorrhage (arrow). C,D) Brain tissue abnormalities in the left frontal lobe are observed in fluid attenuated inversion recovery (FLAIR)- and T2-based MRI axial sections. In both imaging modalities, acute blood appears dark. Notice the heterogeneous increased signal intensity consistent with evolving blood products with surrounding edema in the frontal lobe. All images courtesy of Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

Figure 2: Caspase 3 expression in the mouse primary motor cortex in a controlled cortical impact (CCI) model. A-E) Peri-lesion cortex; A) sham, B) 3 hours, C) 1 day, D) 3 days and E) 1 week after injury. 10 week old male C57Bl/6 mice were anesthetized with isoflurane and mounted in a stereotaxic apparatus. A 5mm diameter disc centred 0.5mm posterior and 2mm lateral to Bregma was removed from the skull. An ImpactOne device (Leica Biosystems, Concord, ON) with 3mm diameter flat-tipped impactor tip was used to produce an injury 1mm deep (impact velocity was 3.25m/s with dwell time 100ms). Sections from formalin fixed brains were sectioned on a vibratome at 50 µm and processed for immunohistochemistry using anti-Caspase 3 (1:200; Cell Signaling Technology, Danvers, MA), and visualized using Vector Elite ABC reagent and Vector NovaRed (Vector Laboratories, Burlington, ON). Scale bar: 200 µm

Figure 3: Morphological and biochemical changes in the primary motor cortex of a surgically-induced brain injury mouse model. Injuries were induced in 8-10 week old male C57Bl/6 mice as

described in Rennie et al. (2013). Briefly, the skull overlying the motor cortex was removed, and cortical tissue was removed to a depth of 1mm. Images of whole brain in (A) sham and (B) 3 hours post-injury mice; lesion site (*). Scale bar: 1mm (A,B). Representative Golgi-Cox staining of (C) sham and (D) 3 hours post-injury brain sections show extensive tissue loss (*) in the damaged region. Here, the damage extends through layer V of the cortex. Scale bar: 200 µm (C, D). The image in (C) is a portion of an image previously published in Rennie et al. (2013). Western blot shows the expression of pro- and anti-apoptotic proteins 3 hours, 3 days and 1 week after injury. In particular, changes in the expression of Bax1 and Bcl-xL are notable. For western blots, the protein was extracted by homogenizing the tissue in 100µl of Ripa buffer with protease inhibitors on ice using a hand held homogenizer. The homogenate was then incubated on ice for 30 to 45 minutes, and centrifuged for 20 minutes at 16 000g. 100µg of protein was loaded per well.

Figure 4: In vivo detection of GFP in control and transgenic GFP-expressing mice. GFP expression was monitored in the head of both A) control and B) hemizygous C57Bl/6-Tg (CAG-EGFP)131Osb/LeySopJ mice expressing GFP under the control of a beta actin promoter using an eXplore Optix imager (ART Advanced Research Technologies Inc, Saint-Laurent, Qc) with 470nm emitting laser and 1.5mm step size. Abundant levels of reporter protein can be detected in the GFP-expressing mouse.

Figure 5: miRNA expression in the primary motor cortex of a mechanically-induced brain injury mouse model. Surgically induced brain injury was performed in 8-10 week old male C57Bl/6 mice as described in Rennie et al. (2013). Q-RT-PCR analysis shows an upregulation of miR-

132, miR-21 and miR-30a three hours after injury. In particular, the expression of miR-21 was significantly increased in the three hour post-injury samples and remained higher three days and one week after injury, compared with the control group. In contrast to miR-21, other miRNAs showed reduction three days and one week after injury. Expression levels of miR-21 were significantly different from sham at all investigated time points; sham/3h p=0.0011, sham/3day p=0.0002, sham/1week p=0.0003. **Foot note:** Total RNAs were extracted from the primary motor cortex of sham and injured mice, using Tri-reagent. Taq-Man qRT-PCR experiments were performed in duplicate, using 10 ng total RNA samples from each of sham and injured mice (n=6 for each group). SnoRNA202 expression was used to normalize miRNA expression of all data points. Following normalization, the injury groups were scored against the sham group, set at 1 fold. Fold changes were calculated by mean $2^{-\Delta Ct}$, where ΔCt is the normalized cycle number difference between the injury groups and the sham group.

Figure 6: KEGG pathways affected by miRNAs up/downregulated by brain injury. An example of bioinformatics analysis of cellular pathways and processes predicted to be affected by miRNAs modulated in injured brain. DIANA mirPath v.3 software (Vlachos et al. 2015) was used to predict KEGG pathways affected by a panel of 8 miRNAs whose expression was altered in injured mouse brain (see Figure 5 legend for experimental details). The number of miRNAs (of the 8) predicted to target genes in each pathway is shown in light grey, and the number of genes in the pathway predicted to be targeted by the altered miRNAs is shown in black. From left to right, pathways are shown in decreasing order of significance (increasing p-value), but all are significant at the $p<0.05$ level using the modified Fisher's Exact test and False Discovery Rate correction built into mirPath software.

Table 1: Traumatic and non-traumatic brain injuries

Brain injury	**Traumatic brain Injury**		**Non-traumatic brain injury**				
Definition	**Damage to the brain caused by an external force.**		**Damage to the brain caused by infection, brain tumors, ischemia or stroke.**				
Types	Penetrating head injury	Closed-head injury	Ischemia	Toxicity	Infection	Tumors	Stroke
Description	Open head injury occurring when object penetrates the skull	Closed head injury occurring when the skull is not significantly damaged, but the brain is damaged	Anoxic injury where the brain does not receive adequate oxygen	Occurs after exposure to unsafe substances	Often caused by a virus	Caused by either malignant tumor by spreading throughout the brain, or by benign tumors inducing pressure on the brain tissue and damaging the tissue.	Hypoxic injury results when the blood supply to the brain is interrupted
Examples	Gunshot. Knife wounds.	Blunt force (e.g. a fall). Overpressure (e.g. blast force). Accelerative force (e.g. car accidents). Shaking or strong rotation of the head (e.g. Shaken Baby Syndrome).	After a cardiac arrest.	Substances such as lead. After kidney failure and buildup of body's own chemicals.	Meningitis, Encephalitis	Brain cysts, Glioma	Blood clot. Low blood pressure.

Table 2: The Glasgow Coma Scale*

	Score					
Response	**1**	**2**	**3**	**4**	**5**	**6**
Eye	Does not open eyes	Opens eyes in response to painful stimuli	Opens eyes in response to voice	Opens eyes spontaneously	N/A	N/A
Verbal	Makes no sound	Incomprehensible sounds	Utters inappropriate words	Confused, disoriented	Oriented, converses normally	N/A
Motor	Makes no movements	Extensions to painful stimuli	Abnormal flexion to painful stimuli	Flexion/Withdrawal to painful stimuli	Localizes painful stimuli	Obeys commands

*Based on Teasdale and Jennett 1974.

Table 3: TBI models

Focal Impact	**Diffuse Impact**	**Non-Impact**
• Weight drop [1,2,3,4] • Fluid percussion injury o Midline [5,6] o Lateral [7,8] • Controlled cortical impact [9, 10, 11] • Penetrating and ballistic injury [12,13]	• Diffuse injury [14] • Impact acceleration [15]	• Inertial acceleration [16,17] • Rotational TBI [18] • Blast injury [19, 20]

Numbered citations in the above table refer to the following references: 1: (Feeney et al. 1981); 2:(Dail et al. 1981); 3:(Shapira et al. 1988); 4:(Chen et al. 1996); 5:(McIntosh et al. 1987); 6:(Dixon et al. 1987); 7:(McIntosh et al. 1989); 8:(Carbonell et al. 1998); 9:(Lighthall 1988); 10:(Dixon et al. 1991); 11:(Smith et al. 1995); 12:(Williams et al. 2005); 13:(Williams et al. 2006); 14:(Cernak et al. 2004); 15:(Marmarou et al. 1994); 16:(Gennarelli et al. 1982); 17:(Ross et al. 1994); 18:(Davidsson and Risling 2011); 19:(Long et al. 2009); 20:(Garner et al. 2009).

Figure 1

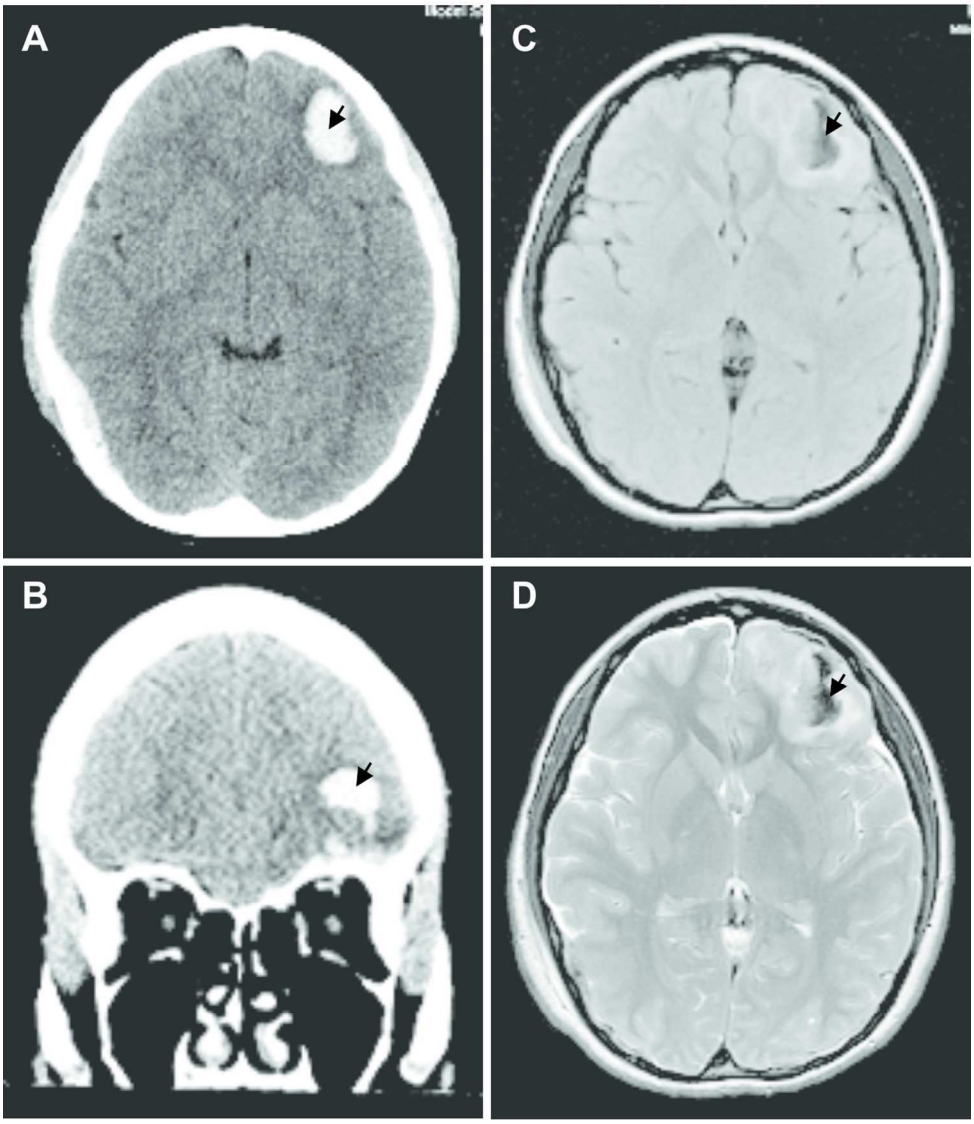

Figure 1

113x140mm (300 x 300 DPI)

Figure 2

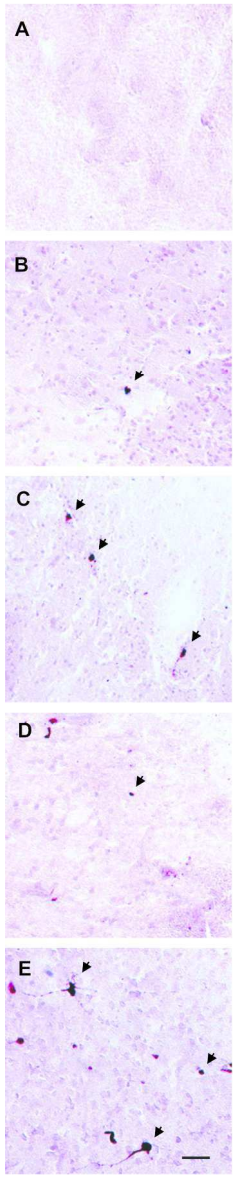

Figure 2

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Figure 3

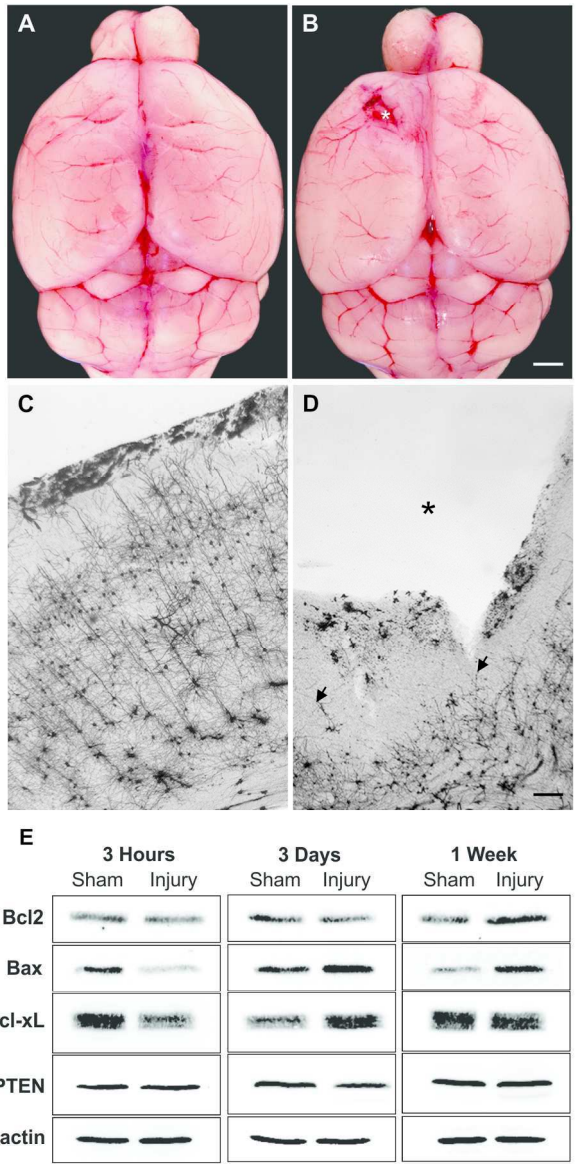

Figure 3

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Figure 4

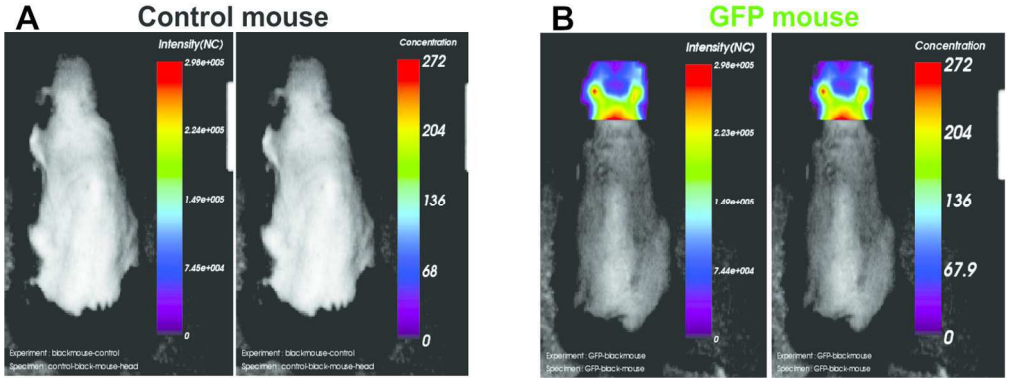

Figure 4

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Figure 5

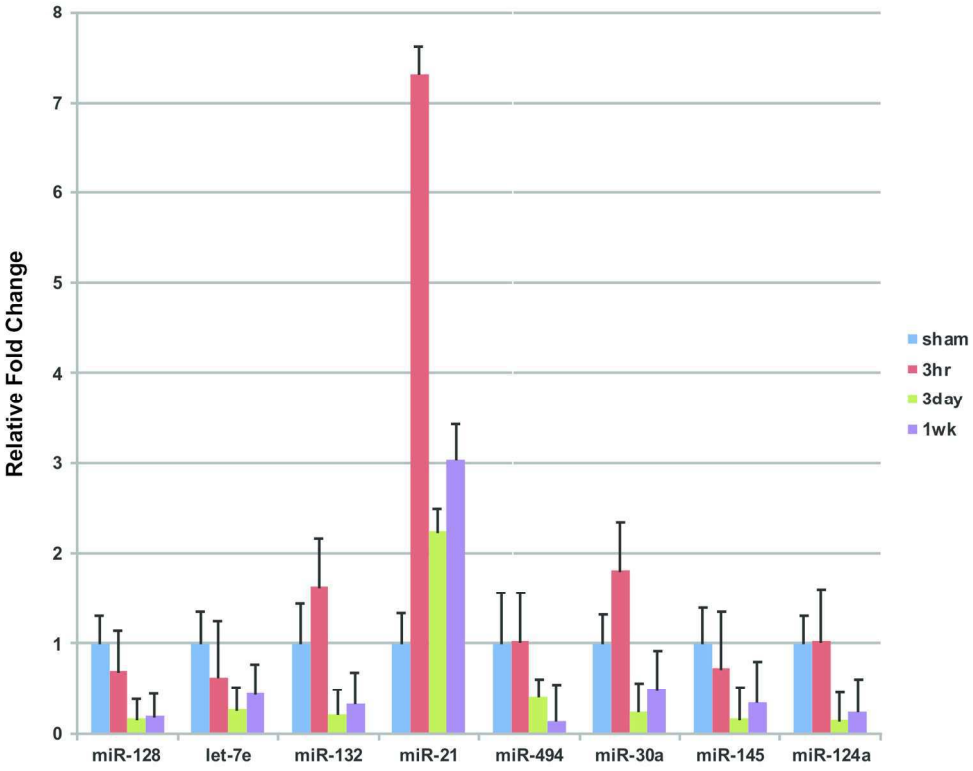

Figure 5

158x137mm (300 x 300 DPI)

Figure 6

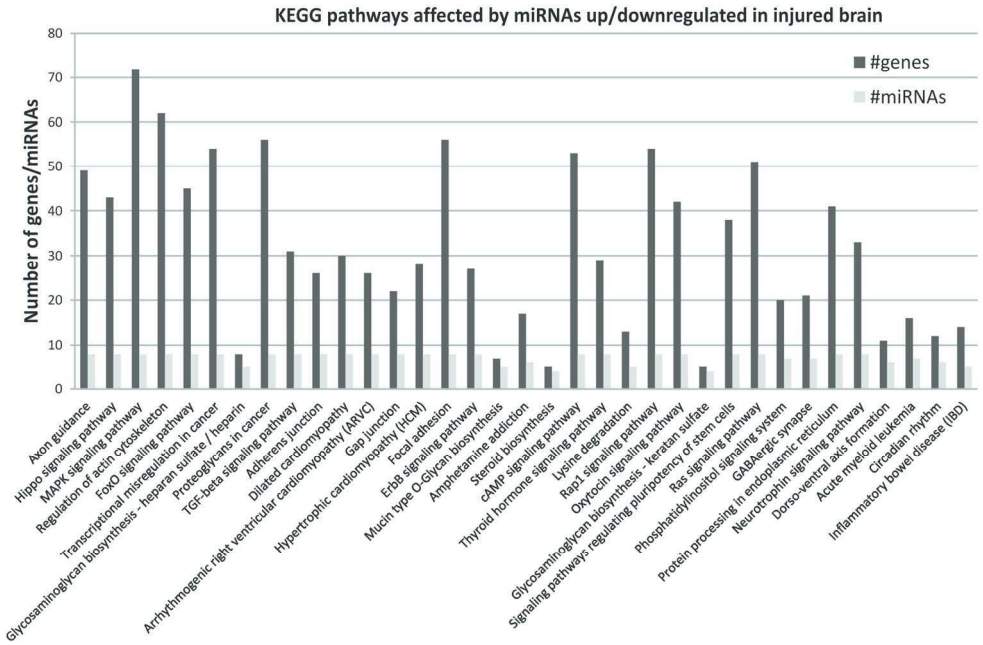

Figure 6

152x102mm (300 x 300 DPI)

The scientific community is mourning the loss of the young colleague, Dr. Mahmud Bani-Yaghoub (1964-2016), a Senior Research Officer and Team Leader at the National Research Council (NRC) of Canada. Mahmud was Adjunct Professor at the University of Ottawa since 2006, member of Editorial board of several journals, including American Journal of Stem Cells, and member of the Ontario Institute for Regenerative Medicine.

Mahmud was a champion of multidiciplinary science and innovation. He led many collaborative projects with national and international centres, including the Centre for Commercialization of Regenerative Medicine (CCRM).

He was a recipient of the NRC's Outstanding Achievement Award for scientific breakthrough, NRC-Institute for Biological Sciences Outstanding Group Achievement and Industrial Partnership Awards, and NRC-Human Health Therapeutics Portfolio Leadership Award.

Mahmud made a lasting impact in advancing stem cell models and applications in regenerative medicine. His most recent achievement is the development of human stem-cell based models for accelerating the development of novel therapies for brain diseases, including a unique industrial-grade blood-brain barrier model, used in partnership with many biopharmaceutical companies. The book on 'Fetal Stem Cells in Regenerative Medicine' co-edited by Dr. Mahmud Bani and Dr. Dario Fauza, was published by Humana Press in the spring of 2016, a few months before his sudden departure.

Mahmud leaves a legacy of uncompromising commitment to science and innovation, outstanding scientific advances and inspirational leadership that embodies scientific entrepreneurship and innovation. NRC lost one of its rising stars, prematurely, at the peak of his carrier. Dr. Mahmud Bani will be missed dearly by everyone who worked with him for his boundless energy, sharp inquiring mind and genuinely caring personality.

169x112mm (300 x 300 DPI)