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INVITED REVIEW

MicroRNAs as diagnostic markers and therapeutic targets for traumatic brain injury

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Abstract

Traumatic brain injury (TBI) is characterized by primary damage to the brain from the external mechanical force and by subsequent secondary injury due to various molecular and pathophysiological responses that eventually lead to neuronal cell death. Secondary brain injury events may occur minutes, hours, or even days after the trauma, and provide valuable therapeutic targets to prevent further neuronal degeneration. At the present time, there is no effective treatment for TBI due, in part, to the widespread impact of numerous complex secondary biochemical and pathophysiological events occurring at different time points following the initial injury. MicroRNAs control a range of physiological and pathological functions such as development, differentiation, apoptosis and metabolism, and may serve as potential targets for progress assessment and intervention against TBI to mitigate secondary damage to the brain. This has implications regarding improving the diagnostic accuracy of brain impairment and long-term outcomes as well as potential novel treatments. Recent human studies have identified specific microRNAs in serum/plasma (miR-425-p, -21, -93, -191 and -499) and cerebro-spinal fluid (CSF) (miR-328, -362-3p, -451, -486a) as possible indicators of the diagnosis, severity, and prognosis of TBI. Experimental animal studies have examined specific microRNAs as biomarkers and therapeutic targets for moderate and mild TBI (*e.g.*, miR-21, miR-23b). MicroRNA profiling was altered by voluntary exercise. Differences in basal microRNA expression in the brain of adult and aged animals and alterations in response to TBI (*e.g.*, miR-21) have also been reported. Further large-scale studies with TBI patients are needed to provide more information on the changes in microRNA profiles in different age groups (children, adults, and elderly).

Key Words: *traumatic brain injury; microRNAs; diagnostic markers; therapeutic targets: humans; animal models*

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Introduction

Traumatic brain injury (TBI) due to a violent blow to the head is the leading cause of death and disability in adults under the age of 45 in Western countries (Jennett, 1998; Maas et al., 2008; Ribbers, 2010) with a very high number of brain injuries resulting from motor vehicle accidents and sporting activities. There has also been a marked increase in the incidence of fall-related TBI in persons aged ≥ 75 (Adekoya et al., 2002). The severity of TBI is determined by the nature, speed and location of the impact, and by complications such as hypotension, intracranial hemorrhage or increased intracranial pressure. TBI is characterized by primary damage to the brain from the external mechanical force and by subsequent secondary injury due to various molecular and pathophysiological responses that eventually lead to neuronal cell death. These responses include brain edema, intracranial hypertension, and subsequent neurological dysfunction (Zweckberger et al., 2006). Secondary brain injury events may occur minutes, hours, or even days after the trauma (Ribbers, 2010), and provide valuable therapeutic targets to prevent further neuronal degeneration. However, treatment options for secondary brain damage have failed to show efficacy in clinical trials (Maas et al., 1999), thereby emphasizing a need for new therapeutic targets.

MicroRNAs are short non-coding RNAs (20–24 nucleotides) that negatively regulate gene expression at the post-transcriptional level by binding to the 3′-untranslated region (UTR) of target mRNAs which leads to degradation of the mRNA or direct inhibition of mRNA translation (Baek et al., 2008). They control a range of physiological and pathological functions such as development, differentiation, apoptosis and metabolism (Ambros, 2004). We have searched the PubMed database for recently published studies of microRNAs during the first few days or weeks after TBI in humans and experimental animals, and whether they can serve as potential targets for progress assessment and intervention against TBI to mitigate secondary damage to the brain. This has implications regarding improving the diagnostic accuracy of brain impairment and long-term outcomes as well as potential novel treatments.

MicroRNAs in TBI

The initial severity and prognosis of TBI in humans is classified on the Glasgow Coma Scale (GCS) that is based on three tests: ocular, verbal, and motor responses. The sum of the three values is the GCS score. The injury is classified as severe with GCS 3–8, moderate GCS 9–12, or mild GCS 13–15 (Bilgin et al., 2012). The GCS covers a broad severity of

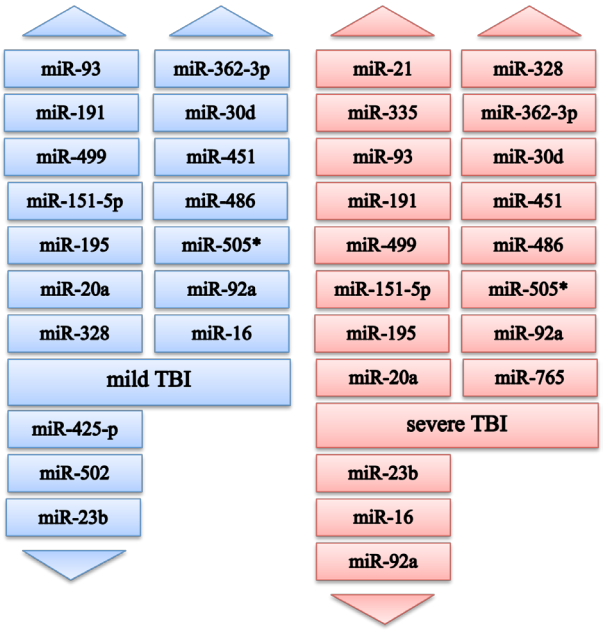

Figure 1 Altered expression of circulating microRNAs in human patients with mild and severe TBI (based on the studies reviewed).
For mild TBI: Increased levels of miR-93, -191, -499 at 0–24 hours, days 2–7 post-injury; miR-151-5p, -195, -20a, -328, -362-3p, -30d, -451, -486, -505*, -92a at 0–24 hours post-injury; miR-16 at 0-10 hours post-injury; decreased levels of miR-425-p, -502 at 0–1 hour, 4–12 hours post-injury, miR-23b at 0–24 hours post-injury. For severe TBI: Increased levels of miR-21 at 4–12 hours, 48–72 hours post-injury; miR-335 at 0–1 hour, 4–12 hours, 48–72 hours post-injury; miR-93, -191, -499 at 0–24 hours, days 2–7 post-injury; miR-151-5p, -195, -20a, -328, -362-3p, -30d, -451, -486, -505* at 0–48 hours post-injury; miR-92a at 0–48 hours (mean 26 hours) post-injury; miR-765 mean 68 hours post-injury; decreased levels of miR-23b at 0–24 hours post-injury; miR-16, -92a at mean 68 hours post-injury.

symptoms from subtle to more serious conditions in some cases accompanied with temporary loss of consciousness (Gennarelli, 2014). Sports-related and other minor traumatic brain injuries with GCS scores in the 13–15 range occur in 1.6 to 3.8 million young healthy individuals annually (Leo and McCrea, 2016). Mild TBI is characterized by loss of consciousness for < 30 minutes and post-traumatic amnesia for < 24 hours and accounts for approximately 75–90% of the total TBI cases (Fischer, 2013). Computed tomography and magnetic resonance imaging have limited sensitivity to detect mild TBI (Kesler et al., 2000; Yuh et al., 2012). Mild TBI is often unnoticed or misdiagnosed due to the absence of specific diagnostic markers.

In experimental animal models, the extent of brain injury is classified according to the parameters used, together with behavioral testing and histological examination of brain sections. For example, in the controlled cortical impact (CCI) procedure which uses a pneumatic controlled cortical impact device to deliver mechanical energy to the intact dura, the amount of brain deformation is a function of the impactor tip size, contact velocity and duration time (Lighthall, 1988; Davis et al., 2008; Gilmer et al., 2009). By varying these parameters different severity grades of TBI injury have been produced in mice and rats. Another procedure is the weight drop injury (WDI) method in which a metal rod is dropped from a known height above the exposed skull of the animal to create a brain injury (Marmarou et al., 1994). Different severity grades of TBI injury have been produced in mice by varying the weight, diameter of tip, and fall height of the impactor rod. The weight drop method was found to recreate the cognitive and behavioral deficits seen in human patients with mild TBI (Yang et al., 2013; Sharma et al., 2014). A third method is the fluid percussion injury (FPI) method, which involves craniotomy to expose the dura and placement of a female Luer lock or plastic cap that is cemented in place over the craniotomy site; this is followed by transient compression and/or deformation of the underlying brain using a fluid-mediated pressure pulse, with a pulse duration time of approximately 20 ms (Kabadi et al., 2010; Alder et al., 2011).

Human studies

Six studies were found and mostly comprised both male and female patients with ages ranging from 14 to 65 years (**Table 1**). The two largest studies were performed with 76 patients (59 males/17 females, mean age 49 years; Yang et al., 2016) and 70 patients (51 males/19 females, mean age 41 years; Di Petro et al., 2017). Of the six studies, five had patients with mild TBI and severe TBI classified on the GCS and two of these also included patients with moderate TBI. The remaining study comprised only patients with severe TBI. These studies showed that serum or plasma levels of specific microRNAs could serve as potentially valuable indicators of the diagnosis, severity, and prognosis of TBI. Serum/plasma levels of miR-93, -191, -499, -23b, -21, -335, -425, -502, -16, -92a, -765 could be used to distinguish between patients with severe, moderate or mild TBI (Redell et al., 2010; Sun et al., 2016; Yang et al., 2016; Di Petro et al., 2017). Moreover, cerebro-spinal fluid (CSF) levels of miR-141, -257, -1297, -228, -362-3p, -451, -486 were identified as distinguishing severe TBI patients from controls (Bhomia et al., 2016; You et al., 2016). In addition, serum levels of miR-93, -191, -499, -425-p were indicative of outcome in TBI patients (Yang et al., 2016; Di Petro et al., 2017). MicroRNAs with upregulated and downregulated expression in patients with mild and severe TBI are shown in **Figure 1**.

Animal studies

Fifteen studies were found which had mostly used male mice and rats (**Table 2**). Ten of these studies had used the CCI method, three the WDI method, and two the FPI method to create the brain injury. The ages of the mice ranged from 8 to 24 weeks (adult) and 22–24 months (aged), and for the rats the ages ranged from 7 to 12 weeks (adult).

CCI studies

The CCI parameters ranged from 3 to 5 mm for diameter of impactor tip, 0.8 to 6 m/s for impact velocity, 85 to 200 ms for duration time, and deformation depth of cortex 1 to 2 mm. By comparison with the parameters used by Liu et al. (2014) to induce a moderate TBI by CCI (equivalent to

Table 1 MicroRNAs in human patients with traumatic brain injury (TBI)

Reference	No. of patients, gender, ages	Comparison	Changes in miRNAs in patients	Functional outcomes	Conclusion
Di Petro et al. (2017)	27 males (M)/8 females (F) mild TBI with extracranial (EC) injury, 40 years (mean); 24 M/11 F, severe TBI with EC injury, 41 years (mean). Serum was collected from patients. Patient outcomes assessed at 6 months post-injury by Glasgow Outcome Scale (GOS).	22 M/13 F healthy volunteers (HV), 15 M/15 F EC injury patients.	Initial screening of serum microRNAs performed on 5 mild TBI + EC and 5 severe TBI + EC patients and compared to controls at days 1 and 15 post-injury by microarray analysis and RT-PCR. Ten differentially expressed microRNAs were chosen as potential candidate biomarkers with the aim to discriminate mild from severe TBI at very early time points. Among the candidate biomarkers of mild TBI + EC, miR-425-p was significantly downregulated at 0–1 hour and 4–12 hours compared to controls or severe TBI + EC, and miR-502 was downregulated at 0–1 hour and 4–12 hours compared to controls or severe TBI + EC. After 48 hours from mild injury, miR-425-p and miR-502 returned to normal levels. MiR-21 and miR-335 were analysed as potential biomarkers of severe TBI since they both were upregulated at both time points in the initial screening and also in the second set of patients. MiR-21 was significantly upregulated in severe TBI + EC at 4–12 hours, 48–72 hours and day 15 after injury compared to controls and mild TBI + EC. MiR-335 was upregulated in severe TBI + EC at 0–1 hour, 4–12 hours, 48–72 hours and day 15 compared to HV and mild TBI + EC, but not EC.	MiR-425-p at 0–1 hour was strongly predictive of 6-month outcome. However, miR-502, -21 and -335 did not show any prognostic value at this time point. At a later time point of 4–12 hours, miR-425-p retained prognostic value, and miR-21 also became highly predictive, while miR-502 and miR-335 remained non-significant.	MiR-425-p and miR-502 could be considered the most promising candidate biomarkers for the early diagnosis and monitoring of mild TBI. MiR-21 and miR-335 are valid biomarkers for the diagnosis of severe TBI. In addition, miR-425-p is a strong predictor of 6-month outcome at 0–1 hour and 4–12 hours, while miR-21 is predictive of outcome at 4–12 hours.
You et al. (2016)	14 M/12 F, severe (coma) TBI, 49 years (mean). Cerebro-spinal fluid (CSF) samples collected by lumbar puncture.	12 M/9 F, 50 years (mean) with headache, vertigo, spinal pain, tinnitus as controls. CSF samples collected.	By microarray analysis of CSF, expression of 14 microRNAs in severe (coma) TBI group differed from that of controls: 10 had higher and 4 had lower expression compared with controls. Of the upregulated microRNAs, miR-141 and miR-257 showed the greatest fold changes of 4.62 and 3.05 that of controls, with the other microRNAs increased from 2.0 to 2.5-fold. Of the down-regulated microRNAs, miR-1297 had the greatest fold change of –3.44 that of controls, with the others decreased by 2.1 to 2.6 fold.	One single nucleotide polymorphism (SNP) (rs11851174 allele: C/T) was identified in the motif area of microRNA has-miR-431-3p gene promoter region.	The altered microRNA expression levels in CSF of TBI patients together with SNP identified within the microRNA gene promoter area provide a new perspective on the mechanism of impaired consciousness after TBI.
Yang et al. (2016)	59 M/17 F, 49 years (mean): 25 mild TBI, 26 moderate TBI, 25 severe TBI. Serum samples collected within 24 hours and then daily for up to 21 days after injury. Patient outcomes assessed at 9 months post-injury by GOS.	29 M/9 F, 47 years (mean) with no evidence of disease.	Serum levels of miR-93, -191 and -499 measured by RT-PCR were significantly increased in TBI patients compared to controls over the study period. The levels of these microRNAs were markedly increased within 24 hours after injury and reached peak levels from days 2 to 7. Subsequently they decreased slightly from days 8 to 14 but remained significantly elevated compared to controls. The levels of these microRNAs were higher in severe TBI patients than in the moderate and mild TBI patients.	Serum levels of miR-93, -191 and -499 were higher in patients with poor clinical outcome (GOS 1–2) than in those with good outcome (GOS 3–5).	Serum miR-93, -191 and -499 are potentially valuable indicators of the diagnosis, severity, and prognosis of TBI.
Sun et al. (2016)	28 M/20 F, 18–50 years: 16 mild TBI, 16 moderate TBI, 16 severe TBI with injury occurring within the preceding 24 hours. Plasma collected from patients.	20 age-matched healthy subjects.	Plasma level of miR-23b measured by RT-PCR was downregulated in TBI patients compared to controls. The miR-23b level was significantly decreased in severe TBI patients compared to mild or moderate TBI patients.	MiR-23b specifically targeted to 3'-UTR of ATG12 mRNA and modified its translation.	MiR-23b might be a potential therapeutic target for TBI.
Bhomia et al. (2016)	7 M/1 F, 75% mild TBI and 25% moderate TBI, 36 years (mean); 8 M severe TBI, 39 years (mean). Serum samples of mild/moderate TBI were collected within 24 hours from injury. Serum and CSF samples were taken from an archived set of a completed severe TBI study. Serum samples were collected within 48 hours (mean 26 hours) from injury and CSF samples collected within 48 hours of injury.	Normal healthy control serum (n = 8) and normal control CSF (n = 6) samples.	By microarray analysis, 39 and 37 microRNAs were significantly upregulated in serum samples of mild/moderate TBI and severe TBI, respectively. A comparison was performed between the serum microRNAs upregulated in both mild/moderate TBI and severe TBI which identified 10 microRNAs (miR-151-5p, -195, -20a, -328, -362-3p, -30d, -451, -486, -505*, -92a) with increased expression in both mild/moderate and severe TBI. RT-PCR demonstrated that all the selected microRNAs were significantly upregulated in serum TBI samples; however, the fold upregulation changes were not the same as in the initial profiling. These 10 microRNAs were validated in CSF from severe TBI and 4 microRNAs (miR-328, -362-3p, -451, -486) were found to be upregulated in CSF.	The mature mRNAs predicted to be targeted by the upregulated microRNAs are involved in major TBI related pathways such as erythropoietin signaling, G protein coupled receptor signaling, GABA receptor signaling and neuropathic pain signaling in dorsal horn neurons.	A panel of 10 unique microRNAs was identified for the diagnosis of mild/moderate TBI and severe TBI.
Redell et al. (2010)	11 mild TBI, > 14 years; 10 severe TBI, 14–65 years. Plasma was collected from mild TBI patients within 10 hours of injury. For severe TBI patients, plasma was obtained at the earliest possible time after admission and patient stabilization.	26 non-trauma healthy volunteers.	By microarray analysis, 108 microRNAs were detected in control plasma and 8 unique microRNAs in severe TBI plasma at 68 ± 8 hours post-injury. Five candidate microRNAs (miR-16, -26a, -92a, -638, -765) were chosen for further analysis by RT-PCR. The plasma levels of miR-16, -92a and -765 were significantly altered in severe TBI patients compared to controls (the first two were decreased, the third increased). The levels of miR-26a and miR-638 were not significantly altered at the time points examined (0–24 hours, 25–48 hours, 49–72 hours). RT-PCR of plasma samples collected from mild TBI patients within 10 hours of the time of injury showed the levels of miR-16 and miR-92a were significantly increased in mild TBI patients.	Combining plasma levels of miR-16, -92a and -765, 100% sensitivity and 100% accuracy could be achieved in identifying severe TBI patients. Poor diagnostic accuracy was found for miR-16 and miR-92a in differentiating between mild TBI patients and orthopedic injury patients.	Plasma microRNAs have the potential to improve TBI patient classification and possibly clinical management.

Table 2 MicroRNAs in animal models of traumatic brain injury (TBI)

Reference	No. of animals, gender, ages	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Controlled cortical impact (CCI)					
Sabirzhanov et al. (2016)	C57B/6 mice, male, 12 weeks. A 4-mm craniotomy made on central aspect of left parietal bone. The tip of the injury device was set to impact the cortical surface. CCI parameters were diameter of impactor tip 3.5 mm, impact velocity 6 m/s, deformation depth 2 mm to produce moderate TBI. At 15 minutes post-injury mice received a single intracerebroventricular (i.c.v.) injection of 0.5 nmol of miR-711 hairpin inhibitor or negative control hairpin inhibitor. Drugs were injected into the left ventricle. Behavioral testing was performed post-injury. Mice were sacrificed, brains removed and sections cut and stained to assess lesion volume.	Sham animals underwent same procedures but without CCI and served as controls.	By microarray analysis, the level of miR-711 was upregulated in the cortex of TBI animals compared to uninjured controls. Using RT-PCR, there was a rapid upregulation of miR-711 starting as early as 1 hour post-injury, peaking at 6 hours, and persisting through to 72 hours.	Using RT-PCR, there was rapid downregulation of Akt expression in the injured cortex starting as early as 1 hour post-injury. Treatment of TBI mice with a miR-711 hairpin inhibitor significantly upregulated Akt, phosphorylated Akt, GSK3 and phosphorylated GSK3. TBI resulted in increased levels of pro-apoptotic proteins PUMA and two isoforms of Bim large and small, which are regulators of apoptosis upstream of mitochondrial release of AIF-1 and cytochrome c. Treatment with the miR-711 hairpin inhibitor significantly decreased PUMA and Bim after TBI. Treatment with miR-711 hairpin inhibitor ameliorated TBI-induced deficits in motor and cognitive functions. At day 28 post-injury, treatment of TBI mice with miR-711 hairpin inhibitor significantly reduced the lesion volume compared with the negative control group, and significantly reduced TBI-induced neuronal loss in the cortex and hippocampus.	Changes in miR-711 contribute to neuronal cell death after TBI, in part by inhibiting Akt, and may serve as a novel therapeutic target.
Meissner et al. (2016)	C57Bl6 mice, male, 9–12 weeks. A craniotomy made over the right parietal cortex leaving the dura intact. CCI parameters were diameter of impactor tip 3 mm, impact velocity 0.8 m/s, duration 150 ms, deformation depth 1 mm to produce mild-moderate TBI. Mice were sacrificed at 1, 6 and 12 hours after TBI (n = 6/group) and brains removed.	Shan-operated animals that did not receive CCI (n = 6) and healthy untreated animals (n = 6) served as controls.	Microarray analysis of right parietal cortex at 1, 6 and 12 hours after TBI and corresponding brain region of sham mice (sacrificed 6 hours) and healthy untreated animals was performed. Of the 780 microRNAs represented in the array, 410 were detected in all samples. Two-way hierarchical clustering was applied to the normalized expression values of the 50 most differentially expressed microRNAs across the whole cohort of samples. The two main clusters reflect the difference between the control groups and samples obtained after TBI. Principal component analysis revealed that the 6 hours group was closer to the 12 hours group than to the 1 hour group. 158 microRNAs were differentially expressed between sham and TBI groups. Of these microRNAs, 66 were upregulated and 92 were down-regulated in TBI groups compared to sham. The microarray analysis was validated by RT-PCR. This confirmed the upregulated expression of miR-2137, -451, -21*, -144, -184, and the down-regulated expression of miR-190, -137, -541, -107 after TBI. The up-regulation of miR-1971 could not be confirmed by RT-PCR. RT-PCR profile of miR-31, -767, -409-5p, -191 did not correspond to the expression profile of the microarray. Accordingly, five of six of the upregulated and four of eight down-regulated microRNAs validated by RT-PCR.	MicroRNA-2137 showed the highest upregulation after TBI (up to 25-fold). *In situ* hybridization for miR-2137 on frozen brain sections revealed a constitutive cytoplasmic expression of miR-2137 with a neuronal staining pattern throughout the brain in healthy and sham animals. At 6 hours after TBI, the neuronal staining pattern increased in the penumbral tissue of the contusion and in the adjacent hippocampus. No changes were found in the ipsilateral brain areas not directly affected by TBI and in the contralateral hemisphere.	The microRNA expression levels in the contused cortex after TBI may aid the identification of novel targets for the management of brain trauma.
Miao et al. (2015)	C57B/6 mice, male, 17–19 weeks housed in cages equipped with a running wheel (RW). After 3 weeks, exercised animals divided into two groups: sham procedure (sham-runners) and TBI (TBI-runners). A 3.5-mm craniotomy made on left parietal bone leaving the dura intact. CCI parameters were diameter of impactor tip 3 mm, impact velocity 4.5 m/s, deformation depth 1.5 mm to produce moderate TBI. There were 30 mice in each group: 3 used for microRNA analysis and the remainder for behavioral and survival analyses. Mice were sacrificed and cerebral cortex ipsilateral to injury removed.	Animals exposed to an immobilized RW. Unexercised animals divided into two groups: sham-non-runners and TBI-non-runners.	Effects of exercise on microRNA expression profiles in sham and TBI mice were detected. 87 microRNAs were differentially expressed between the sham non-runner and sham-runner groups: 61 and 26 microRNAs were up-and down-regulated, respectively. Also 33 microRNAs were differentially modulated between TBI-non-runner and TBI-runner groups: 20 microRNAs were up- and 13 down-regulated, respectively. Expressions of miR-21, -92a, -138, -124, -874, let-7c were measured by RT-PCR. In the TBI-non-runner group compared to sham non-runners, miR-21, -92a and -874 were increased while miR-138, -124 and let-7c were significantly decreased. However, in the TBI-runner group the levels of miR-21, -92a and -874 decreased while those of miR-138, -124 and let-7c increased compared with the TBI-non-runner group.	After 14 days, there was 55% survival in TBI-runners group and 30% survival in TBI-non-runners group. RW prior to sham injury did not have an effect on recovery of righting reflex in sham animals, but the righting time was shorter in TBI-runners.	MicroRNAs such as miR-21, -92a, -674, -138, -124 and let-7c could be involved in the prevention and protection afforded by voluntary exercise in TBI model.

Table 2 Continued

Reference	No. of animals, gender, ages	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Hu et al. (2015)	C57B/6 mice, male, 17–19 weeks housed in cages equipped with RW (n = 7). Craniotomy performed and left parietotemporal cortex subjected to CCI using parameters diameter of impactor tip 3 mm, impact velocity 6 m/s, deformation depth 2 mm to produce moderate TBI. TBI-runners were injected i.p. with miR-21 mimic (5 nmol/20 g body wt), miR-21agomir (10 nmol/20 g body wt), or RNA-oligonucleotides negative control (miR-NC) 5 hours post-TBI and twice/week before sacrifice. Behavioral tests performed at 15–20 days post-injury immediately after running. Mice sacrificed and brains removed.	Shan-operated animals did not receive CCI and housed in cages equipped with RW (sham-runners, n = 7). Sedentary control, sham-non-runners, and TBI-non-runners were exposed to immobilized RW (n = 7/group).	By RT-PCR, the level of miR-21 was significantly increased in hippocampus of TBI-non-runners compared with that of sham ones. The increase in miR-21 expression in hippocampus was reversed in TBI-runners but did not reach the level of the sham group.	In Morris Water Maze test, TBI-runners administered miR-21 agomir performed significantly better compared to those treated with miR-21-NC from days 15 to 20 post-injury. Treatment with miR-21 mimic post-injury significantly deteriorated maze performance compared to miR-21 NC treated animals.	The elevated level of miR-21 in hippocampus post-TBI was reduced by spontaneous RW exercise. Overexpression of miR-21 in TBI mice with spontaneous RW induced deteriorations in spatial learning and memory retention. MiR-21 down-regulation reversed these effects. MiR-21 has been involved in neuroprotection induced by voluntary RW exercise post-TBI.
Sandhir et al. (2014)	C57B/6 mice, male, 20–24 weeks (n = 24) and 22–24 months (n = 24). A craniotomy was made lateral to mid-sagittal suture, and animal subjected to CCI with parameters diameter of impactor tip 3 mm, impact velocity 1.5 m/s, duration 85 ms, deformation depth 1 mm to produce mild-moderate TBI. The tip contact area included motor and sensory cortical areas. Animals were sacrificed 1, 3, 5, 7 days post-injury and brains removed. There was no mortality in any of the groups.	Normal healthy control serum (n = 8) and normal control CSF (n = 6) samples.	By RT-PCR, a significantly higher basal expression of miR-21 was found in the aged mice brain compared to adult brain. In addition, miR-21 levels increased following TBI in adult mice with maximum increase at 24 hours post-injury. In the aged mice, miR-21 expression in the brain was decreased after TBI.	TMiR-21 regulates cell survival, invasiveness and apoptosis through specific mRNA targets that include PTEN, PDCD4, RECK and TIMP3. PTEN was upregulated by 3.8-fold in the adult brain 24 hours post-injury. The expression was 98-fold higher in the aged brain. PDCD4 was not upregulated in the adult brain, but was upregulated 28-fold in the aged brain 24 hours post-injury. TIMP3 and RECK were upregulated 20-fold and 6-fold in the aged brain 24 hours post-injury, but no significant effect was observed in adult brain.	MiR-21 response appeared blunted in the aged brain following TBI that resulted in upregulation of mRNA targets. Strategies aimed at upregulation of miR-21 and/or down-regulation of its targets might be useful in improving outcomes in the elderly after TBI.
Sabirzhanov et al. (2014)	C57B/6 mice, male, 12 weeks. A 4 mm craniotomy was made on the central aspect of left parietal bone and subjected to CCI using parameters diameter of impactor tip 3.5 mm, impact velocity 6 m/s, deformation depth 2 mm to produce moderate TBI. At 15 minutes after injury, mice received a single i.c.v. injection of miR-23a-3p, miR-27a-3p, or negative control miR mimics. All drugs made up in CSF and injected into left ventricle at 0.5 mL/min, with a final volume of 5 µL of 0.1 mM mimic solution. Mice were sacrificed and brains removed. Sections of brain were stained with cresyl violet to quantify lesion volume.	Shan-operated animals did not receive CCI.	By microarray analysis, levels of miR-23a and miR-27a in cortex of TBI mice were downregulated at 1 hour, 6 hours, 24 hours post-injury compared to control. These changes were confirmed by RT-PCR with rapid downregulation of miR-23a-3p and miR-27a-3p starting as early as 1 hour after injury and lasting up to 24 hours, followed by recovery at 72 hours after injury. RT-PCR showed no significant changes after TBI in the expression profile of miR-24.By RT-PCR, i.c.v. injection of miR-23a and miR-27a mimics at 15 min post-injury completely reversed the TBI-induced decline in miR-23a and miR-27a, respectively, compared with TBI mice injected with negative control miR mimic. Injection of miR-23a-3p and miR-27a-3p mimics significantly attenuated TBI-induced upregulation of PUMA, Noxa, Bax, and active Bax protein levels in cortex at 24 hours after injury compared with negative control miR mimic.	The mRNA expression levels for various Bcl-2 family proapoptotic proteins in the cortex were analysed at different time points after TBI. By RT-PCR, there was rapid upregulation of BH3-only family members, Noxa, Bid and Bim, with peak levels at 6 hours after injury, followed by progressive decrease toward normal levels at 24 hours after injury. PUMA mRNA reached its peak at 1 hour after injury, and Bak mRNA (a proapoptotic multi-BH domain member) reached its peak at 1 hour after injury and remained elevated thereafter. The mRNA data were confirmed by quantitative measurement of protein levels of several key proapoptotic proteins using Western blot. Injection i.c.v. of miR-23a-3p mimic significantly reduced TBI-induced lesion volume at 28 days after injury compared with negative control miR mimic. Administration of miR-23a-3p mimic significantly attenuated TBI-induced neuronal loss in CA2/3 and dentate gyrus of hippocampus at 28 days after injury compared with negative control miR mimic.	Post-injury decreases in miR-23a and miR-27a contribute to neuronal cell death after TBI by upregulating proapoptotic Bcl-2 family members, thus providing a novel therapeutic target.

Table 2 Continued

Reference	No. of animals, gender, ages	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Wang et al. (2015)	Sprague-Dawley rats, male, 9–11 weeks. A 6 mm craniotomy was made lateral to sagittal suture and centered between bregma and lambda, keeping the dura intact. CCI parameters were diameter of impactor tip 5 mm, impact velocity 3.5 m/s, deformation depth 2 mm to produce moderate TBI. At 12 hours post-injury, animals sacrificed and hippocampus removed (n = 5). The nuclear, cytosolic and purified total mitochondria fractions were isolated from hippocampus.	Naive uninjured animals as controls (n = 5).	TBI resulted in altered expression levels of miRNAs in both hippocampal mitochondria and cytosol fractions at 12 hours following injury. RT-PCR revealed that the differential overall expression levels of miRNAs in cytosol relative to mitochondria increased from 2.5-fold in naïve samples to 4.2-fold in injured hippocampus. The levels of most mitochondria-associated miRNAs were decreased at this early time point following TBI. In particular, mitochondria-enriched miRNAs such as miR-142-3p, -142-5p, -146a were decreased more than 2-fold following TBI, while these miRNAs increased in cytosol. The levels of several mitochondria-associated miRNAs were found to increase following TBI. Among them, miR-155 and miR-223 levels were significantly increased to 7.3- and 4.63-fold, respectively, in mitochondrial fractions of injured compared to naive (uninjured) rats. RT-PCR was used to detect miR-155, -223, -146a, -142-3p, -142-5p, -150, -124a expression in enriched cortical neuron, astrocyte and microglial cultures. MiR-155, -223, -146a, -142-3p, -142-5p, -150 were detected in all three cell types but showed the highest expression in microglia and astrocytes. Levels of miR-124a were highest in cortical neurons.	MiR-155 is known to participate in the regulation of inflammatory responses.	At 12 hours following TBI, several microRNAs are significantly altered in hippocampal mitochondria and cytoplasm. In addition, levels of miR-155 and miR-223, both of which play a role in inflammation processes, are significantly increased in both the cytoplasm and mitochondria.
Sun et al. (2014)	Sprague-Dawley rats, male/female, 7–8 weeks. A 4 mm × 4 mm craniectomy was performed to expose the dura. CCI parameters were impact velocity 3.5 m/s, duration 200 ms, deformation depth 2 mm to produce moderate TBI. Animals were sacrificed on day 1 (n = 5), day 3 (n = 5), day 5 (n = 5) and ipsilateral hippocampus removed.	Shan-operated animals did not receive CCI (n = 5).	By microarray analysis, a total of 205 microRNAs were identified. At day 1 post-injury, 41 microRNAs were more than 1.5-fold upregulated and 31 microRNAs were more than 1.5-fold downregulated; at day 3 post-injury, 81 microRNAs were more than 1.5-fold upregulated and 11 microRNAs were more than 1.5-fold downregulated; at day 5 post-injury, 52 microRNAs were more than 1.5-fold upregulated and 41 microRNAs were more than 1.5-fold downregulated. RT-PCR was used to validate the microarray results of miR-142-3p and miR-221. A strong correlation was found between the microarray profiling and RT-PCR data. The level of miR-142-3p in hippocampus showed a downregulation at days 1 and 3 followed by upregulation at day 5 post-injury. MiR-221 was persistently upregulated in hippocampus at all three time points post-injury.	MiR-221 has pro-proliferative and pro-migratory roles in different cell types and is also an anti-angiogenic microRNA.	Temporal expression of miR-142-3p may be used as a molecular marker for assessment of TBI progression and inference of damage time. MiR-221 may play a key role in regulating cell proliferation and angiogenesis by PDGF signaling pathway at the early stages after TBI.
Liu et al. (2014)	Sprague Dawley rats, male/female, 7–8 weeks. A 4 mm × 4 mm craniectomy was performed over left parietal bone, keeping the dura intact. CCI parameters were impact velocity 3.5 m/s, duration 200 ms, deformation depth 2 mm to produce moderate TBI of left temperoparietal cortex. Animals were sacrificed at 1 hour, 1 day, 3 days, 5 days and 7 days post-injury (n = 15/group) and ipsilateral hippocampus removed. Two of TBI animals died of injury. Five animals of each group were used for molecular studies.	Sham-operated animals did not receive CCI (n = 15) and sacrificed 1 hour after surgery.	By microarray analysis, 156 microRNAs were detected in sham and the five TBI groups. Of these, 60, 41, 81, 52 and 60 microRNAs were upregulated (> 1.5-fold) while 45, 31, 11, 41 and 35 were downregulated (> 1.5-fold) at 1 hour, 1, 3, 5 and 7 days post-injury. There were 10 microRNAs that were consistently up-/down-regulated (≥ 1.5-fold) at all five time points after injury, among which miR-142-3p, -144, -340-5p, -674-5p, -153, -186, -190, -132*, -138-1* were upregulated whereas let-7b was downregulated. RT-PCR was used to confirm the microarray findings for three selected microRNAs (miR-144, -153 and -340-5p). The expression levels of all three microRNAs in ipsilateral hippocampus were significantly elevated at all five time points after TBI compared to sham controls.	There was no significant lesion or cell death in hippocampus of sham group on H&E staining of sections. Neuronal cell death was observed at 1 hour after TBI and it increased time-dependently from 1 hour to 3 days post-TBI. At 7 days after injury, the hippocampus structures were almost complete and neurons returned to normal. Predicted target genes included *Cask*, *Nfe212* and *Snca*. Western blot results showed a significant decrease in protein expression of CASK in hippocampus at 1 hour, 1 day, and 3 days post-injury compared to control group. A decrease was detected in expression of NRF2, the protein of *Nfe212* gene that is a putative target of both miR-144 and miR-153, in hippocampus post-injury. SNCA, one of the target proteins of miR-153, was also significantly suppressed in hippocampus after TBI.	MiR-144, -153 and -340-5p might serve as potential targets for progress assessment and intervention to mitigate secondary damage after TBI.
Hu et al. (2012)	Sprague-Dawley rats, male, 8–9 weeks. A 4 mm craniotomy was made over left temperoparietal cortex. CCI parameters were diameter of impactor tip 3 mm, impact velocity 3.5 m/s, duration 200 ms, deformation depth 2 mm to produce moderate TBI. Animals were sacrificed at 1 day and 7 days post-injury and brain tissue from dorsal hippocampus collected (n = 3–4/group).	Sham-operated animals did not receive CCI (n = 3–4).	By microarray analysis, 328 microRNAs were identified. RT-PCR was used to validate the results of deep sequencing by measuring the expression change of miR-124, -135, -153, -222. MiR-153 expression was elevated and miR-222 expression was decreased at 24 hours post-injury; expression of both miR-135a and miR-135b was reduced at 7 days post-injury; miR-124 expression was comparable in control and TBI samples at both 24 hours and 7 day time points. These results were consistent with findings from deep sequencing. Using deep sequencing, 13 microRNAs were downregulated and 8 microRNAs were upregulated at 24 hours post-injury. Among the 21 microRNAs altered at 24 hours, 5 (miR-144, -136, -148b-5p, -342-5p, -23a*) showed persistent changes at 7 days post-injury. Number of changed microRNAs was reduced to 10 (7 down-regulated, 3 upregulated) at 7 days post-injury.	Gene ontology enrichment analysis showed that at the early times after brain injury, microRNAs might be involved in inhibition of white blood cell apoptosis to augment the inflammatory response, dampening of transcription and facilitation of protein folding and catabolism to reduce stress-induced denaturation and aggregation of intracellular proteins, and promotion of aerobic respiration to exert protective actions. At the later times after injury, microRNAs preferentially regulate gene expression, intracellular trafficking and metabolism, cytoskeleton and cell adhesion to allow cell structure remodeling and synaptogenesis for repair of neuronal circuitry.	In addition to known pathophysiological changes, other cellular pathways that are subjected to modification by differentially expressed microRNAs in brains of TBI animals have been identified. These can potentially be targeted for development of novel treatment of TBI.

Table 2 Continued

Reference	No. of animals, gender, ages	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Weight drop injury (WDI)					
Chandran et al. (2017)	C57B/6J mice, male, 10–12 weeks. Skull was exposed and injury made over the left parietal lobe. WDI parameters were diameter of tip of rod 1 mm, weight of rod 246 and 333 g, fall height 2 and 3 cm for each of the two different weights of rod to produce increasing grade of mild TBI. Animals were sacrificed at 24 hours and day 7 post-injury (n = 3/group). Brain was exposed and full-depth brain tissue punch biopsies were done using a 4 mm tissue punch. The punch biopsy centered at the injury site of left parietal lobe was taken so that an area covering the injury as well as the surrounding site was included in the profiling analysis. The biopsy punch contained regions of the parietal lobe cortex, hippocampus, thalamus, mid brain, substantia nigra and amygdala. A separate group of animals for each of the injury and sham groups was used for histologic evaluation at 24 hours (n = 4/group) and day 7 (n = 4/group) post-injury.	Sham operated animals did not receive WDI.	By microarray analysis, at 24 hours 96 microRNAs were upregulated and 87 microRNAs downregulated in the injury severity 1 group (246 g, 2 cm). 66 microRNAs were upregulated and 63 microRNAs downregulated in the injury severity 2 group (246 g, 3 cm). 43 microRNAs were upregulated and 47 microRNAs downregulated in the injury severity 3 group (333 g, 2 cm). 46 microRNAs were upregulated and 48 microRNAs downregulated in the injury severity 4 group (333 g, 3 cm). At day 7, 10 microRNAs were upregulated and 6 microRNAs downregulated in the injury severity 1 group. 23 microRNAs were upregulated and 27 microRNAs downregulated in the injury severity 2 group. 11 microRNAs were upregulated and 4 microRNAs downregulated in the injury severity 3 group. 63 microRNAs were upregulated and 59 microRNAs downregulated in the injury severity 4 group. Three microRNAs (miR-296-5p, -154*, -466) were selected for validation by RT-PCR. Expression of all three was upregulated and similar to the expression pattern observed by microarray analysis.	No lesions were observed at and around the injury site in the brains of any of the injury groups. Altered microRNAs common to all the injury groups at both 24 hours and day 7 time points post-injury were analysed using Ingenuity Pathway Analysis for their correlation, with particular reference to acute and sub-acute pathologic processes. At 24 hours post-injury, a total of 81 pathways was predicted to be significantly affected by the combined effect of the significantly modulated microRNAs common to all the injury groups. Of the total of 81 pathways, 26 were relevant to neurological functions. Similar analysis of the significantly modulated microRNAs common to all the injury groups at 7 days post-injury indicated 73 pathways to be targeted by these microRNAs. Of the 73 pathways, 25 were relevant to neurological functions.	Varying intensities of mild TBI induced a differential microRNA expression profile in the brain post-injury. Pathways such as calcium and synaptic signaling were major targets of modulated microRNAs and may play a role in pathophysiology of mild TBI.
Sharma et al. (2014)	C57B/6J mice, male, 11–12 weeks. Skull was exposed and injury made over the left parietal lobe. WDI parameters were diameter of tip of rod 1 mm, weight of rod 246 and 333 g, fall height 2 and 3 cm for each of the two different weights of rod to produce increasing grade of mild TBI. Injury day was day 0. WDI parameters were diameter of tip of rod 1 mm, weight of rod 246 and 333 g, fall height 2 and 3 cm for each of the two different weights of rod to produce increasing grade of mild TBI: IS1 (246 g, 2 cm, n = 32), IS2 (246 g, 3 cm, n = 29), IS3 (333 g, 2 cm, n = 20), IS4 (33 g, 3 cm, n = 6). Baseline measurements for behavioral studies were taken at day 3 before injury. Also measurements at 1, 14 and 30 days post-injury. A separate group of animals for all four injuries were used in the microRNA study (n = 6/group). Serum was collected at 3 hours post-injury, and whole brains were collected. Animal mortality immediately after the impact increased with increase in height of fall and weight of rod.	Sham operated animals did not receive WDI (n = 42). Naïve animals (n = 25). A separate group of sham animals (n = 6) was used for microRNA study.	By microarray analysis of serum at 3 hours, the number of significantly modulated microRNAs increased with increasing grade of injury except in the IS4 injury group where the numbers of significantly modulated microRNAs were marginally less than the IS3 injury group. 23 microRNAs (14 up- and 9 down-regulated) were significantly modulated in IS1 injury group. 53 microRNAs (35 up- and 18 down-regulated) were significantly modulated in IS2 injury group. 116 microRNAs (70 up- and 46 down-regulated) were significantly modulated in IS3 injury group. 106 microRNAs (66 up- and 40 down-regulated) were significantly modulated in IS4 injury group. 13 microRNAs (9 up- and 4 down-regulated) were found to be modulated in all four injury groups and had similar expression patterns. Expression of miR-214, -376a, -199a-3p was validated by RT-PCR.	Staining of brain section at hippocampus level (site of injury) did not show lesion volume in any of the injured groups indicating mild brain injury. Neurobehavioral severity scale-revised (NSS-R) scores increased with increase in fall height and weight of rod. Open field activity and acoustic startle response of the animals was reduced following injury. Several brain function related pathways such as axon guidance, gap junction, dopaminergic synapse, cholinergic synapse, long-term potentiation, tight junction, adherens junction, and long-term depression were targeted by the modulated microRNAs.	13 microRNAs were found to identify mild TBI regardless of its severity within the mild spectrum of injury. The more severe injuries were associated with a greater number of microRNAs involved in brain related functions.

Table 2 Continued

Reference	No. of animals, gender, ages	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Sun et al. (2016)	Sprague Dawley rats, male, 12 weeks. In the 3 days before TBI preparation, groups of animals received a single injection of 7 μL lentivirus encoding miR-23b (LV-miR-23b) or LV-miR NC into the right lateral ventricle. Skull was exposed and a steel disc 10 mm diameter, 3 mm thickness) was adhered to the skull. WDI parameters were weight of rod 450 g, fall height 150 cm to produce moderate TBI. Neurological impairments were assessed using the modified neurological severity score (mNSS) test at 1, 3, 7 and 14 days post-injury. Also Morris Water Maze test carried out.. Plasma collected and animals sacrificed at 1, 3, 7 and 14 days post-injury, and brains removed.	Sham operated animals did not receive WDI.	By RT-PCR, the levels of miR-23b were decreased in the plasma, hippocampus and cortex of TBI animals compared to sham group at 1, 3, 7 and 14 days post-injury.	Pretreatment of TBI animals with LV-miR-23b significantly reduced lesion volume of the contused hemisphere and brain edema compared with TBI group. Also mNSS score at 3, 7 and 14 days post-injury was decreased in group pretreated with LV-miR-23b. mNSS score in TBI group at 1 day post-injury was 9, indicative of moderate injury. On mNSS scale of severity: a score of 13–18 indicates severe injury, 7–12 indicates mean-moderate injury and 1–6 indicates mild injury (Zhang et al., 2013). LV-miR-23b pretreatment improved cognitive impairments in TBI animals.	MiR-23b may be a potential therapeutic target for TBI.
Fluid percussion injury (FPI)					
Ge et al. (2014)	Sprague-Dawley rats, male, 12 weeks. A 3 mm craniotomy was made over the right parietal bone and Luer-lok end attached over the craniotomy. FPI parameters were pressure applied 1.8–2.0 atm, pulse duration not reported. Mortality rate was approx 5%. i.c.v. infusion was performed 10 minutes after FPI with miR-21 agomir, agomir negative control, miR-21 antagomir, antagomir negative control, and injury control at a rate of 1 μL/min. Animals sacrificed and brains removed..	Sham operated animals did not receive FPI or i.c.v. infusion.	By RT-PCR, miR-21 expression levels were detected in the impacted area with a 7 mm diameter including injured cerebral cortex and ipsilateral hippocampus at different time points from 0 hour to 14 days post-injury. The expression of miR-21 in the injury control group was increased at 6 hours post-injury, reached a peak at 3 days post-injury, and then gradually declined to baseline at 14 days post-injury. The miR-21 expression level was significantly increased (or decreased) in the agomir (or antagomir) group at 6 hours, 1 day and 3 days (or 6 hours and 1 day) post-injury compared to injury control group.	A significant decrease (or increase) in lesion volume was found in the agomir (or antagomir) group compared with the injury control group. The lesion volume was significantly correlated with mNSS score at day 14 post-injury. mNSS score for injury control group was 8 at day 1 post injury indicating moderate TBI injury. Upregulation of miR-21 in the brain improved the neurological outcome after FPI injury. MiR-21 regulated the expression of apoptotic and angiogenesis-related molecules in brain after TBI. The expression of Pten, a miR-21 target gene, was inhibited and Akt signaling was activated by miR-21 after TBI.	MiR-21 could be a potential therapeutic target for intervention after TBI.
Lei et al. (2009)	Wistar rats, gender not reported, 8–9 weeks. A 3 mm craniotomy was made over the right parietal bone and plastic injury cap attached over the craniotomy site. FPI parameters were pressure applied 1.8–2.2 atm, pulse duration not reported, to produce moderate injury. Animals assigned to four groups corresponding to 6 hours, 24 hours, 48 hours, 72 hours post-injury (n = 2/group). Animals were sacrificed and brains removed.	Sham operated animals did not receive FPI (n = 2).	By microarray analysis, of the 136 microRNAs detected at 6 hours post-injury, 13 were upregulated (> 2-fold) and 14 were downregulated (> 2-fold). In the detected 118 microRNAs expressed at 24 hours post-injury, 4 were upregulated (> 2-fold) and 23 were downregulated (> 2-fold). In the detected 149 microRNAs expressed at 48 hours post-injury, 16 were upregulated (> 2-fold) and 11 were downregulated (> 2-fold). In the detected 203 microRNAs expressed at 72 hours post-injury, 19 were upregulated (> 2-fold) and 5 were downregulated (> 2-fold). Upregulation of miR-21 expression occurred at all four time points. MiR-21 was chosen as the candidate microRNA for validation by RT-PCR which confirmed the results of microarray analysis.		MiR-21 could be involved in the process of TBI course.

a GCS score of 9–12 for humans), most of the studies had used parameters to create a moderate TBI. In two of the ten studies (Sandhir et al., 2014; Meissner et al., 2016), the parameters chosen would more likely be associated with a mild-moderate brain injury. Many of the studies identified specific microRNAs as possible biomarkers and therapeutic targets. Meissner et al. (2016) reported the upregulated expression in cortex of miR-2137, -451, -21*, -144, -184, and the downregulated expression of miR-190, -137, -541, -107 at 1, 6 and 12 hours after TBI in mice compared to control. Similarly, miR-23a and miR-27a were downregulated in cortex of TBI mice as early as 1 hour and lasting up to 24 hours post-injury compared to control (Sabirzhanov et al., 2014). Liu et al. (2014) found that miR-142-3p, -144, -340-5p, -674-5p, -153, -186, -190, -132*, -138-1* were upregulated whereas let-7b was downregulated in hippocampus of TBI rats compared to controls. Also, miR-155 and miR-223 levels were significantly increased in mitochondrial fraction of hippocampus of TBI rats compared to controls (Wang et al., 2015). Interestingly, two studies examined microRNA profiles in mice given free access to a running wheel to exercise. The cortical levels of miR-21, -92a and -874 decreased while those of miR-138, -124 and let-7c increased compared with the TBI-non-runner group (Miao et al., 2015). Hu et al. (2015) observed the level of miR-21 was significantly elevated in hippocampus of TBI-non-runners compared with that of sham animals and the increase in miR-21 expression in hippocampus was reversed in TBI-runners. Also Sandhir et al. (2014) showed that basal expression of miR-21 was higher in the aged mice brain compared to adult brain. While miR-21 levels increased following TBI in adult mice with maximum increase at 24 hours post-injury, in the aged mice miR-21 expression in the brain was decreased after TBI. Strategies using microRNA mimics or agomirs to increase the levels of downregulated microRNAs or microRNA antagomirs to decrease the levels of upregulated microRNAs were also examined as possible ways to improve outcomes in TBI (*e.g.* Sabirzhanov et al., 2014, 2016).

WDI studies

The parameters used ranged from 246 g/333 g (mice) to 450 g (rats) for weight of rod 1 mm diameter, and 2 cm/3 cm (mice) to 150 cm (rats) for fall height. No lesions were observed in stained sections of injured mouse brains, consistent with creating a mild TBI in these animals (Sharma et al., 2014; Chandran et al., 2017). A modified neurological severity score (mNSS) (Zhang et al., 2013) of 10 in injured rats indicated a moderate TBI (Sun et al., 2016). Varying intensities of mild TBI induced a differential microRNA expression profile in the mouse brain post-injury (Sharma et al., 2014; Chandran et al., 2017). A set of 13 microRNAs was found to identify mild TBI regardless of its severity: mmu-miR-376a, hsa-214, mmu-214, mmu-337-5p, mmu-574-3p, mmu-434-3p, mmu-671-3p, mmu-218, mmu-199a-3p were all upregulated, whereas hsa-miR-106b*, mmu-106b, mmu-31, mmu-196c were all downregulated (Sharma et al., 2014). In the rat study, levels of miR-23b were decreased in the plasma, hippocampus and cortex of TBI animals post-injury compared to sham. Pretreatment of TBI animals with lentivirus-miR-23b significantly reduced the lesion volume of the contused hemisphere and brain edema compared with TBI group. Also mNSS score at 3, 7 and 14 days post-injury was decreased in TBI animals pretreated with lentivirus-miR-23b (Sun et al., 2016).

FPI studies

In one of the rat studies the pressure applied was 1.8–2.0 atm while in the other it was 1.8–2.2 atm. The duration of pulse was not reported in either study. Based on mNSS score of 8 on day 1 post-injury (Ge et al., 2014) and histological sections of cerebral cortex after injury (Lei et al., 2009) both studies had utilized animals with moderate TBI. Expression of miR-21 was increased in the impacted brain area (Lei et al., 2009; Ge et al., 2014) and was significantly increased or decreased in the agomir- or antagomir-injected animals post-injury compared to controls (Ge et al., 2014). Upregulation and downregulation of specific microRNAs in brains of TBI animals occurred at different time points post-injury (Lei et al., 2009). MiR-21 could be a potential therapeutic target for intervention after TBI.

The alteration of microRNAs in different body fluids and tissues from the described human and animal studies is summarized in **Table 3**.

MicroRNA Diagnostic and Therapeutic Strategies for TBI

The human studies have identified specific microRNAs in serum/plasma (miR-425-p, -21, -93, -191 and -499) and CSF (miR-328, -362-3p, -451, -486a) as possible indicators of the diagnosis, severity, and prognosis of TBI (**Table 1**). To date, there have been only a limited number of studies involving patients with TBI. These studies have included patients with mild, moderate, and severe TBI. However, such studies are important to increase the accuracy of diagnosis and outcomes, and refine treatments of injured patients. The early identification of biomarkers measured in samples collected within 1 hour from injury by the first responders (ambulance crew) at the injury scene before patients were admitted to hospital would allow clinicians to recognize and treat patients at risk of secondary brain injury and optimize early management. While the levels of specific microRNAs in serum or plasma may be developed into biomarkers for TBI, it is likely that other organs contribute to microRNAs in the blood (Sierzega et al., 2017) so that the circulating levels may not accurately reflect the levels of specific microRNAs in the brain itself. The microRNA profile in CSF would more reliably indicate alterations in the brain caused by TBI, since it is in direct contact with the extracellular matrix in the brain and its composition better reflects biochemical changes that occur in this organ. However, collection of CSF involves an invasive procedure and may not be easily accomplished in the elderly due to narrowing of the lumbar intervertebral space due to disc degeneration. Measurement of specific

Table 3 Summary of microRNA alterations in traumatic brain injury (TBI) of human patients and animal models

			Alteration of microRNA expression	
Reference	Species	Sample	Upregulated	Downregulated
Di Petro et al. (2017)	Human	Serum	miR-21 and miR-335 at 4–12 hours, 48–72 hours and day 15 in severe TBI	miR-425p and miR-502 at 0–1 hour and 4–12 hours in mild TBI
You et al. (2016)	Human	Cerebro-spinal fluid (CSF)	miR-141, -257, -181a*, -27b*, -483-5p, -30b, -1289, -431*, -193b*, -499-3p of which miR-141 and miR-257 had the greatest change in severe TBI (coma)	miR-1297, -33b, -933, -449b of which miR-1297 had the greatest change in severe TBI (coma)
Yang et al. (2016)	Human	Serum	miR-93, -191 and -499 in TBI over 24 hours to day 14 with the levels highest in severe TBI	
Sun et al. (2016)	Human	Plasma		miR-23b was decreased in TBI with the levels lowest in severe TBI
Bhomia et al. (2016)	Human	Serum, CSF	miR-151-5p, -195, -20a, -328, -362-3p, -30d, -451, -486, -505*, -92a in serum; miR-328, -362-3p, -451, -486 in CSF	
Redell et al. (2010)	Human	Plasma	miR-16 and miR-92a at 0–10 hours in mild TBI; miR-765 at 68 hours in severe TBI	miR-16 and miR-92a at 68 hours in severe TBI
Sabirzhanov et al. (2016)	Mouse	Cerebral cortex	miR-711 in moderate TBI as early as 1 hour post-injury, peaking at 6 hours, and persisting through to 72 hours.	
Meissner et al. (2016)	Mouse	Cerebral cortex	miR-2137, -451, -21*, -144, -184 in mild-moderate TBI	miR-190, -137, -541, -107 in mild-moderate TBI
Miao et al. (2015)	Mouse	Cerebral cortex	miR-21, -92a and -874 in moderate TBI-non-runners	miR-138, -124 and let-7c in moderate TBI-non-runners
Hu et al. (2015)	Mouse	Hippocampus	miR-21 in moderate TBI-non-runners	
Sandhir et al. (2014)	Mouse	Cerebral cortex	miR-21 in mild-moderate TBI with maximum increase at 24 hours post-injury (adult)	miR-21 in mild-moderate TBI (aged)
Sabirzhanov et al. (2014)	Mouse	Cerebral cortex		miR-23a-3p and miR-27a-3p in moderate TBI at 1 hour, 6 hours, 24 hours post-injury
Wang et al. (2015)	Rat	Hippocampus	mitochondrial-associated miR-155 and miR-223 in moderate TBI at 12 hours post-injury; miR-155, -223, -146a, -142-3p, -142-5p, -150 expression highest in enriched microglia and astrocytes, miR-124a expression highest in cortical neurons	
Sun et al. (2014)	Rat	Hippocampus	miR-142-3p in moderate TBI at day 5 post-injury; miR-221 in moderate TBI at 1, 3 and 5 days post-injury	miR-142-3p in moderate TBI at 1 and 3 days post-injury
Liu et al. (2014)	Rat	Hippocampus	miR-142-3p, -144, -340-5p, -674-5p, -153, -186, -190, -132*, -138-1* in moderate TBI at 1 hour, 1, 3, 5 and 7 days post-injury	let-7b in moderate TBI at 1 hour, 1, 3, 5 and 7 days post-injury
Hu et al. (2012)	Rat	Dorsal hippocampus	miR-153 in moderate TBI at 24 hours post-injury	miR-222 in moderate TBI at 24 hours post-injury; miR-135a and miR-135b in moderate TBI at 7 days post-injury
Chandran et al. (2017)	Mouse	Brain tissue biopsy	miR-296-5p, -154*, -466 in mild TBI in mild TBI at 24 hours and 7 days post-injury	
Sharma et al. (2014)	Mouse	Serum	number of microRNAs increased with increasing grade of injury except for IS4 injury group in mild TBI at 3 hours post-injury	Number of microRNAs increased with increasing grade of injury except for IS4 injury group in mild TBI at 3 hours post-injury
Sun et al. (2016)	Rat	Plasma, cerebral cortex, hippocampus		miR-23b in moderate TBI at 1, 3, 7 and 14 days post-injury
Ge et al. (2014)	Rat	Cerebral cortex/ hippocampus	miR-21 in moderate TBI at 6 hours and 3 days post-injury	
Lei et al. (2009)	Rat	Brain tissue	miR-21 in moderate TBI at 6 hours, 1, 2 and 3 days post-injury	Plasma microRNAs have the potential to improve TBI patient classification and possibly clinical management.

microRNAs in the serum/plasma or CSF of human patients enables mild TBI to be recognized and distinguished from severe TBI. At present, many cases of mild TBI go unnoticed or misdiagnosed, as the current diagnostic tests are neither sensitive nor specific enough to identify individuals who have sustained a mild TBI (Belanger et al., 2007; Dash et al., 2010).

The animal studies have examined specific microRNAs as biomarkers and therapeutic targets for moderate and mild TBI (*e.g.*, miR-21, miR-23b) (**Table 2**). No studies were found on microRNA expression levels in animals subjected to severe TBI, presumably because of the likely high mortality rate and ethical concerns. Several of the studies had examined the effect of voluntary exercise on microRNA profiling and shown to be altered by exercise. In addition, differences in basal microRNA expression in the brain of adult and aged animals and alterations in response to TBI (*e.g.*, miR-21) have been reported. The latter is very relevant as persons aged ≥ 75 have an increasing incidence of fall-related TBI.

Downstream targets of several important microRNAs have been indicated in the studies reviewed. For example, miR-23b specifically targeted to 3′-UTR of autophagy-related protein 12 (ATG12) mRNA in human TBI patients (Sun et al., 2016). Also upregulation of miR-711 inhibited Akt expression in the injured mouse cortex as early as 1 hour post-injury. TBI resulted in increased levels of pro-apoptotic proteins PUMA and two isoforms of Bim large and small, which are regulators of apoptosis upstream of mitochondrial release of allograft inflammatory factor 1 (AIF-1) and cytochrome c (Sabirzhanov et al., 2016). MiR-21 regulated cell survival, invasiveness and apoptosis through specific mRNA targets that included PTEN, which was upregulated by 3.8-fold in the mouse adult brain 24 hours post-injury (Sandhir et al., 2014). Post-injury decreases in miR-23a and miR-27a contributed to neuronal cell death in mouse brain after TBI by upregulating proapoptotic Bcl-2 family members (Sabirzhanov et al., 2014). In rat hippocampus, upregulated miR-221 may regulate cell proliferation and angiogenesis by PDGF signaling pathway at the early stages after TBI (Sun et al., 2014). A decrease was detected in expression of NRF2, the protein of *Nfe212* gene that is a putative target of both the upregulated microRNAs, miR-144 and miR-153, in rat hippocampus post-TBI injury. SNCA, one of the target proteins of miR-153, was also significantly suppressed in the hippocampus after TBI (Liu et al. 2014). In injured rat cerebral cortex and hippocampus, upregulated miR-21 modulated the expression of apoptotic and angiogenesis-related molecules in brain after TBI. The expression of *Pten*, a miR-21 target gene, was inhibited and Akt signaling was activated by miR-21 after TBI (Ge et al., 2014). The mature mRNAs predicted to be targeted by the upregulated microRNAs in human patients with TBI are involved in major TBI related pathways such as erythropoietin signaling, G protein coupled receptor signaling, GABA receptor signaling and neuropathic pain signaling in dorsal horn neurons (Bhomia et al., 2016). Several brain function related pathways such as axon guidance, gap junction, dopaminergic synapse, cholinergic synapse, long-term potentiation, tight junction, adherens junction, and long-term depression were targeted by the modulated microRNAs in injured mouse brain (Sharma et al., 2014).

Wang et al. (2015) examined miR-155, -223, -146a, -142-3p, -142-5p, -150, -124a expression in enriched cortical neuron, astrocyte and microglial cultures of injured rats. MiR-155, -223, -146a, -142-3p, -142-5p, -150 were detected in all three cell types but showed the highest expression in microglia and astrocytes, whereas levels of miR-124a were highest in cortical neurons. While not included in this review, other studies have examined the actions of specific microRNAs in cultures of cortical neurons (Han et al., 2014; Ma et al., 2016).

Future Perspectives

At the present time, there is no effective treatment for TBI due, in part, to the widespread impact of numerous complex secondary biochemical and pathophysiological events occurring at different time points following the initial injury (Rosenfeld et al., 2012). These secondary injury events include edema, glutamate excitotoxicity, inflammation, oxidative stress damage, activation of apoptosis, necrosis and autophagy, and impaired mitochondrial function (Raghupathi, 2004; Singh et al., 2006; Ziebell and Morganti-Kossmann, 2010). The earliest and most severe neuropathological changes occur in the hippocampus after TBI (Orrison et al., 2009; Fanselow and Dong, 2010). The hippocampus, a key brain structure for learning and memory, is particularly vulnerable to TBI. The molecular mechanisms underlying hippocampal alterations and cognitive impairments following TBI are unclear and further studies are warranted.

A single microRNA can regulate the expression of hundreds of target genes, so alterations in a panel of microRNAs could greatly affect the pathophysiology and outcome of TBI. Identification of specific genes and signal transduction pathways directly involved in TBI is essential for development of novel therapeutic strategies.

Evidences have also shown that several aspects of the response to brain injury are altered in aged animals. These include worsening of behavioral outcomes, increase of inflammatory responses to injury, and decrease of neuroprotective responses (Sandhir et al., 2004, 2008; Anderson et al., 2009; Sandhir and Berman, 2010). In the aged brain following injury there is increased blood-brain barrier (BBB) permeability accompanied by increased matrix metalloproteinase-9 activity and decreased BBB repair processes (Lee et al., 2012). These changes are all likely to result from altered gene and protein expressions after injury. Voluntary exercise by individuals with TBI (*e.g.*, treadmill, exercycle) could aid recovery of TBI patients.

Plasma microRNAs have the potential to improve TBI patient classification and possibly clinical management. Combining plasma levels of miR-16, -92a and -765, 100% sensitivity and 100% accuracy could be achieved in identifying severe TBI patients (Redell et al., 2010). It is possible that a panel of specific circulating microRNAs could achieve 100% sensitivity and 100% accuracy in identifying mild TBI.

Bhomia et al. (2016) identified a panel of 10 unique microRNAs for the diagnosis of mild/moderate TBI and severe TBI. Temporal expression of specific microRNAs may be used as a molecular marker for assessment of TBI progression and inference of damage time. At the present time there are concerns about the specificity of human biofluid biomarkers such as S100β, glial fibrillary acid protein, neuron-specific enolase, and tau protein for TBI. Many investigators have failed to detect associations between serum levels of S100β and CT abnormalities. The utiity of S100β in the setting of multiple trauma remains controversial because it is also elevated in trauma patients without head injuries (Papa et al., 2015). A study by Papa et al. (2012) indicated that serum glial fibrillary acid protein had good specificity for brain injury acutely after injury, although it is also detected in patients with non-TBI injuries. Studies with the serum biomarker neuron-specific enolase concluded that it had limited utiliiy as a marker of neuronal damage (Fridriksson et al., 2000). A proteolytically cleaved form of tau protein, c-tau, was significantly elevated in CSF in TBI patients and the levels correlated with clinical outcome (Shaw et al., 2002). Although levels of c-tau were also elevated in plasma from patients with severe TBI, there was no correlation between plasma levels and clinical outcome (Chatfield et al., 2002). There clearly is a need for identifying and innovating sensitive and reliable new TBI biomarkers.

A strong association has been shown between TBI and later development of ischemic stroke that remained significant even after several potential confounders, such as vascular risk factors and comorbidities, were taken into consideration. Specific biomarkers for ischemic stroke have been reported (Martinez and Peplow, 2016). Further large-scale studies with TBI patients are needed to provide more information on the changes in microRNA profiles in different age groups (children, adults, and elderly). Future studies should assess the values of the altered microRNAs in TBI to other measures of injury severity according to assessments other than the GCS such as duration of coma and the duration of post-traumatic amnesia. Also as hypertension is a common comorbidity in persons with TBI (Holcomb et al., 2012), and a risk factor for ischemic stroke, studies with hypertensive animals subjected to a brain contusion injury should be performed.

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