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The biological significance and clinical utility of emerging blood biomarkers for traumatic brain injury

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Abstract

HUIBREGTSE, M.E, Bazarian, J.J., Shultz, S.R., and Kawata K. The biological significance and clinical utility of emerging blood biomarkers for traumatic brain injury. NEUROSCI BIOBEHAV REV XX (130) XXX-XXX, 2021.-Blood biomarkers can serve as objective measures to gauge traumatic brain injury (TBI) severity, identify patients at risk for adverse outcomes, and predict recovery duration, yet the clinical use of blood biomarkers for TBI is limited to a select few and only to rule out the need for CT scanning. The biomarkers often examined in neurotrauma research are proteomic markers, which can reflect a range of pathological processes such as cellular damage, astrogliosis, or neuroinflammation. However, proteomic blood biomarkers are vulnerable to degradation, resulting in short half-lives. Emerging biomarkers for TBI may reflect the complex genetic and neurometabolic alterations that occur following TBI that are not captured by proteomics, are less vulnerable to degradation, and are comprised of microRNA, extracellular vesicles, and neurometabolites. Therefore, this review aims to summarize our understanding of how biomarkers for brain injury escape the brain parenchymal space and appear in the bloodstream, update recent research findings in several proteomic biomarkers, and characterize biological significance and examine clinical utility of microRNA, extracellular vesicles, and neurometabolites.

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Declaration of Competing Interest

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Keywords

Blood biomarkers; Traumatic brain injury; Proteomics; Metabolomics; microRNA; Extracellular vesicles

1. Introduction

The heterogeneity of traumatic brain injury (TBI) severity and clinical presentation, combined with the paucity of validated objective tools, hinders optimal immediate and long-term patient care. Unlike routine fluid-based diagnostic tests for heart failure, pregnancy, or diabetes, the clinical use of brain-derived blood biomarkers for TBI is limited to glial fibrillary acidic protein [GFAP] and ubiquitin C-terminal hydrolase-L1 [UCH-L1] or S100B and only to reduce unnecessary CT scanning in a way that circulating levels of GFAP and UCHL1 or S100B possess high negative predictive value for the absence of intracranial injuries (Bazarian et al., 2018; Unden et al., 2013).

Decades of preclinical and clinical research suggest that four major considerations need to be reviewed when profiling blood biomarkers for TBI: (i) time-course; (ii) neurobiological significance, or the relation to specific pathological events and processes; (iii) confounding factors and extracerebral sources; and (iv) utility, usually quantified as sensitivity and sensitivity for dichotomous outcome variables. Understanding the time-course provides an indication for when in the post-injury period a biomarker may be most useful (Papa et al., 2016; Shahim et al., 2020), while understanding the cellular origin and neuropathological underpinnings provides the context needed to interpret alterations in circulating biomarker levels. Further, knowing the neurobiological significance of blood biomarkers is vital to not only the recognition of the clinical implications but also the development and assessment of therapeutic targets (Korley et al., 2021). Next, as a large proportion of TBIs co-occur with other traumatic injuries or during athletic or recreational activities (Haarbauer-Krupa et al., 2018; Taylor et al., 2017), it is important to investigate the potential confounding effects of multitrauma, physical activity, and other factors such as age and sex on biomarkers (Gardner et al., 2018; Mondello et al., 2020b), particularly when markers are known to be expressed in the periphery (Rogatzki et al., 2018). Finally, large studies are required to determine how sensitive and specific a blood biomarker is for certain outcome measures, such as TBI diagnosis, injury severity, mortality, the need for neurosurgical intervention or neuroimaging, recovery trajectory, and resolution of injury (Bazarian et al., 2018; Papa et al., 2016). The heterogenous nature of both the injury itself and the populations and situations in which TBI are sustained can explain why a “silver bullet” biomarker has not yet been discovered and incorporated into clinical practice; it seems much more likely that eventual clinical assessment of TBI will include a panel of biomarkers to provide a comprehensive snapshot of post-injury processes. In the past decade, several novel categories of blood biomarkers, for example, extracellular vesicles, microRNAs, and cellular metabolites, have emerged and have the potential to accurately gauge the severity of neural injury and neurodegenerative progression.

First, this review will summarize the two major pathways for biomarkers to escape from the CNS and enter the peripheral bloodstream. Second, while there are many excellent reviews on proteomic biomarkers (Kawata et al., 2016; Wang et al., 2018; Zetterberg et al., 2013), recent studies exploring the utility of S100B, tau, neurofilament light (NfL), GFAP, UCH-L1, and autoantibodies as biomarkers for TBI merit review, particularly in light of the fact that Banyan Biomarkers received FDA approval in February 2018 for the use of serum levels of GFAP and UCH-L1 to predict which patients with mTBI require a CT scan and Abbott has developed a rapid version of this test to be used on their i-STAT™ Alinity™ platform which received FDA approval in January 2021. These selected proteomic markers and several others are summarized in Table 1. The central and final section of this review will focus on the question, "what are we measuring and why?" for the emerging categories of extracellular vesicles [EVs], microRNA, and neurometabolomics by addressing cellular origins, neurobiological significance, and clinical associations.

2. Escaping the CNS: blood-brain barrier disruption and glymphatic clearance

In the aftermath of TBI, biomarkers (e.g., proteins, nucleic acids, metabolites) can be released into the extracellular space from neurons and glia. These factors either aggregate within the CNS or follow one of two pathways to reach the peripheral bloodstream (Fig. 1).

The blood-brain barrier (BBB) refers to the unique functions of the neurovascular unit, including regulating the movement of ions, solutes, and other molecules between the CNS and the periphery and protecting the CNS from peripheral pathogens, toxins, and injury (Abbott et al., 2010). Blood vessels are formed by contiguous endothelial cells (ECs): multiple endothelial cells are necessary to form the larger arterioles, while single ECs form the circumference of capillaries. Endothelial cells are linked together by the formation of tight junctions (TJs) and adherens junctions (AJs), comprised of claudin, occludin, junction adhesion molecules, and vascular endothelial cadherin, which reduce paracellular permeability (Abbott et al., 2010; Daneman and Prat, 2015). The endothelial cells and their molecular "gatekeepers" are surrounded by astrocytic endfeet, smooth muscle cells, and pericytes, collectively functioning as the neurovascular unit to regulate BBB permeability (Abbott et al., 2010; Daneman and Prat, 2015).

In the event of TBI, mechanical forces coupled with neurometabolic and inflammatory cascades can disrupt BBB integrity and allow for biomarkers to enter the bloodstream. After sustaining cranial impacts, rats exhibited increased BBB permeability, as assessed by extravasation of Evans Blue dye, with maximal disruption at 4 h post-injury and differences compared to sham lasting up to four days post-injury (Shapira et al., 1993). Accordingly, in vitro models reveal the various mechanisms contributing to increased BBB permeability after TBI. Cultured rat brain microvascular ECs subjected to high magnitudes of stretch injury exhibited increased concentrations of lactate dehydrogenase and activated caspase-3 and -7 (indicative of necrosis and apoptosis, respectively) and decreased expression of TJ proteins (Rosas-Hernandez et al., 2018). When human brain endothelial cells were cultured with basement membrane matrices and treated with reactive oxygen species to mimic

post-TBI oxidative stress, the cells exhibited higher activity of matrix metalloproteinases which contributed to the degradation of the basement membrane, higher protein tyrosine kinase activity, and increased phosphorylation and dissociation of TJ proteins, resulting in increased BBB permeability (Haorah et al., 2007). Corresponding evidence has been reported in human TBI by calculating the CSF-serum albumin quotient (Q_A), the gold standard measurement of human BBB integrity, such that a large proportion of patients with moderate and severe TBI exhibited abnormal Q_A within 24 h of injury (Blyth et al., 2011).

Recently, an alternative pathway for clearance of brain-derived biomarkers into the bloodstream has been documented. The glymphatic clearance pathway is characterized by para-arterial influx of CSF from the subarachnoid space into the interstitial space, followed by a paravenous efflux of interstitial fluid (ISF) from the brain parenchyma, where neural factors travel through the dural venous sinuses and drain into the cervical lymphatics via the meningeal lymphatic system before entering the peripheral bloodstream (Bolte et al., 2020; Iliff et al., 2012). This clearance of fluid and solutes is facilitated by transglial water movement via astrocytic aquaporin-4 (AQP4) channels, driving a convective bulk flow of ISF. AQP4 is essential to glymphatic and lymphatic systems function, as demonstrated by considerably reduced clearance rates in *Aqp4*-knockout compared to wild-type mice (Iliff et al., 2012).

In fact, while the exact path of clearance is difficult to determine in human TBI studies, preclinical data suggests that the glymphatic pathway is an essential exit route for biomarkers of brain injury. Glymphatic function in adult mice can be manipulated through AQP4 deletion, surgically draining or pharmacologically reducing CSF, or sleep deprivation. When mice with suppressed glymphatic function sustained a TBI, serum levels of S100B, neuron-specific enolase (NSE), and GFAP at 18 h post-TBI remained comparable to controls (Plog et al., 2015), signifying that these proteins were trapped within the brain parenchymal space. Conversely, mice with TBI alone showed significantly higher S100B, NSE, and GFAP levels as compared to controls and mice with TBI and suppressed glymphatic functions (Plog et al., 2015). In fact, pre-existing meningeal lymphatic dysfunction, and therefore a reduction in capacity to drain CSF and clear debris from the CNS, pre-disposed mice to exaggerated neuroinflammation and cognitive deficits after TBI (Bolte et al., 2020). These mechanistic data de-emphasize the involvement of the pathway across a disrupted BBB, instead underscoring the significance of the glymphatic and lymphatic systems to permit the peripheral appearance of neural factors.

3. Conventional proteomic biomarkers for brain injury

3.1. S100B

Belonging to the S100 calcium-, copper-, and zinc-binding protein family, S100B is a 21 kDa homodimeric protein primarily expressed in the CNS by astrocytes but also by extracerebral sources such as adipocytes, Schwann cells, and skeletal myofibers (Michetti et al., 2019). The primary intracellular function of S100B is to regulate calcium homeostasis and enzyme activity through calcium-dependent substrate binding. The extracellular effects of S100B appear to be concentration-dependent: neurotrophic at low concentrations (e.g., modulation of long-term potentiation and promotion of neuroplasticity) and neurotoxic at

higher concentrations. For example, in response to stress, activated astrocytes overexpress S100B, which is then released at nanomolar levels into the extracellular space where it can act as a damage-associated molecular pattern (Michetti et al., 2019). Then, extracellular S100B activates the neuronal receptor for advanced glycation end-products (RAGE), which triggers a complex cascade of intracellular events, including ROS production, neuroinflammation, and apoptosis. Elevations of this protein have been implicated in a wide variety of pathological conditions, from melanoma to perinatal asphyxia (Michetti et al., 2019).

As a biomarker for brain injury, acute elevations of S100B in the blood have been associated with TBI severity and prognosis (Ingebrigtsen et al., 1999; Pelinka et al., 2004), in addition to possessing strong predictive value for the detection of neuroradiological abnormalities (Thelin et al., 2013). For this reason, serum S100B levels within 6 h of trauma are included in the Scandinavian guidelines for TBI management—a threshold of <0.1 μg/L for mild TBI patients without risk factors indicates that a CT scan and neurosurgical intervention may be unnecessary (Unden et al., 2013). However, both multitrauma in the absence of head injury and physical exertion have been tied to acute elevations in peripheral S100B (Anderson et al., 2001; Hasselblatt et al., 2004; Rogatzki et al., 2018). Therefore, the clinical utility of circulating S100B as a biomarker for brain injury is undermined by its lack of specificity to cerebral sources and short half-life in the bloodstream of approximately 90 min (Townend et al., 2006), as S100B is quickly eliminated from circulation via degradation in the kidney.

S100B has also been investigated as a blood-based biomarker for exposure to subconcussive head impacts. Circulating levels of S100B have consistently been shown to increase in an impact-dependent manner in field studies of American football players and soccer players (Kawata et al., 2017; Marchi et al., 2013; Stalnacke et al., 2004; Zonner et al., 2019). However, using a laboratory model of subconcussive head impacts, performing 10 controlled soccer headers was not a sufficient stimulus to trigger an increase in plasma S100B relative to a control group (Huibregtse et al., 2020). In the absence of a clinically diagnosed TBI, physical exertion is similarly a confounding factor when using peripheral levels of S100B to ascertain damage due to repetitive head impact exposure (Rogatzki et al., 2018; Straume-Naesheim et al., 2008). Therefore, caution is needed to interpret S100B results especially in the field setting.

3.2. Tau and p-tau

Bound to both α- and β-tubulin subunits, tau regulates microtubule assembly via site-specific phosphorylation and enhances microtubule viscoelasticity, allowing microtubules to endure low, but not high, levels of mechanical strain (Ahmadzadeh et al., 2014). Excessive tau phosphorylation is due to a shift in the balance between protein kinase and phosphatase activity (Shultz et al., 2015; Wang et al., 2018), leading to decreased tubulin binding affinity and, subsequently, reduced microtubule stability. Hyperphosphorylation of tau induces abnormal folding and aggregation, as observed in Alzheimer's disease and other tauopathies.

Within the context of TBI, tau is disrupted from microtubules through mechanical stress, proteolytic cleavage by calpains and caspases, and calcium-dependent protein kinase activation, which results in decreased microtubule binding and increased tau

phosphorylation (Kawata et al., 2016; Rubenstein et al., 2017). Total tau (t-tau) assays measure the biofluid concentration of tau protein with a range of post-translational modifications, including phosphorylation. Conversely, phosphorylated tau (p-tau) assays use specific antibodies to assess the concentration of tau phosphorylated at precise amino acids, such as threonine (Thr) 181. As a biomarker for brain injury, the support in the literature generally varies with the form of the protein that is assayed. Plasma levels of t-tau and p-tau-Thr231, in addition to the p-tau-Thr231/t-tau ratio, were quantified in samples collected within 24 h of injury in a cohort of 196 patients with TBI, revealing significant elevations in all three tau measurements which accurately distinguished patients with acute mild, moderate, and severe TBI from healthy controls (Rubenstein et al., 2017). However, t-tau levels did not differ between TBI severities. P-tau-Thr231 levels, while elevated in patients with moderate and severe TBI compared to mTBI, were not significantly different between the two more severe injury categories (Rubenstein et al., 2017). While all three indices were significantly higher in CT + compared to CT− patients, the discriminatory accuracy ranged from excellent for the p-tau-Thr231/t-tau ratio (AUC [area under the curve] = 0.923) to poor for t-tau (AUC = 0.646) (Rubenstein et al., 2017). When serum levels of t-tau was examined from one-month until 5-years post-injury, t-tau levels were not associated with clinical outcome measures, such as TBI severity, functional outcomes as assessed by the Glasgow Outcome Scale – Extended, or neuroimaging measures, nor were t-tau levels significantly different between the TBI group and a control of healthy volunteers (Shahim et al., 2020). Likewise, in a longitudinal study of mTBI patients, Clarke et al. (2021) found elevated plasma levels of t-tau in patients with mild TBI compared to controls only at the acute (<24 h) time point, with an AUC of 0.70, not detecting significant group differences at 72 h, 2 weeks, 3 months or 12 months post-injury. Gill et al. (2017) detected lower t-tau in athletes with sports-related concussion at 24 h and 72 h post-injury compared to uninjured teammates; however, athletes with longer recovery times (≥10 days) had higher t-tau levels compared to those with shorter recovery times at 6 h, 24 h, and 72 h post injury. It should be cautioned that (i) the diagnostic utility of serum t-tau for concussion has been shown to diminish after 6d post-injury (Wallace et al., 2018), and (ii) non-injured control athletes can show significant elevations in serum t-tau, pointing to the confounding effects of physical exertion and subconcussive head impacts (Gill et al., 2017). While t-tau remains one of the more commonly studied biomarkers in TBI research, circulating p-tau and the p-tau/t-tau ratio may provide superior diagnostic and prognostic information, warranting continued investigation.

3.3. GFAP and UCH-L1

GFAP and UCH-L1 are discussed together in this review, despite their differing origins, due to the Food and Drug Administration approval in 2018 as a dual-marker assay to predict the absence of intracranial lesions in mTBI. GFAP is an intermediate filament protein in astrocytes, while UCH-L1 is an enzyme predominantly expressed by neurons and is involved in the process of tagging cytosolic proteins for degradation or ubiquitination (Diaz-Arrastia et al., 2014). Elevations in GFAP and UCH-L1 reflect two distinct injury processes. Thus, examining these two biomarkers in tandem provides a more comprehensive snapshot of the neurobiological sequelae after TBI. GFAP is upregulated during astrogliosis, resulting in the

hypertrophy of astrocytic processes and the “reactive” astrocyte phenotype. UCH-L1 is also upregulated in response to cellular damage due to its role in ubiquitination.

Serum levels of GFAP and UCH-L1 peak at 20 h and 8 h, respectively, after mild-to-moderate TBI and were higher than those observed in general non-TBI trauma patients (Papa et al., 2016). Acute serum levels of both UCH-L1 and GFAP also correlate with injury severity (Czeiter et al., 2020), with excellent sensitivity and specificity for the discrimination between TBI compared to healthy controls (AUC = 0.94) and between CT + and CT− TBI cases (AUC = 0.88) (Diaz-Arrastia et al., 2014). The high negative predictive value for the absence of intracranial injury detected by CT scan of the combination of serum GFAP and UCH-L1 after mild or moderate TBI suggests that CT scanning for this population could be reduced substantially, decreasing both medical costs and radiation exposure (Bazarian et al., 2018). Relative to S100B with a half-life of about 90 min, the longer half-lives of these proteins in the bloodstream (UCH-L1: 12 h; GFAP: 1–2 d) augment their clinical utility as biomarkers for brain injury (Czeiter et al., 2020; Diaz-Arrastia et al., 2014). The dual-marker test using serum samples collected within 12 h of injury can reliably predict the absence of intracranial neuroradiological abnormalities on a CT scan, with a negative predictive value of 99.6 % (Bazarian et al., 2018).

3.4. Neurofilament light (NfL)

Neurofilaments are intermediate filament proteins abundant in long myelinated axons and, together with microtubules, form the axonal cytoskeleton. Neurofilament light (NfL; ~70 kDa), along with the other neurofilament subunits, promotes axonal stability, supports optimal nerve conduction, and facilitates bidirectional axonal transport (Yuan et al., 2012). In the case of TBI, damage to the axolemma and cytoskeleton leads to the release and calpain-mediated proteolysis of NfL into the extracellular space. Circulating NfL can now be detected at subfemtomolar concentrations using single-molecule array (Simoa®) technology (Rissin et al., 2010; Shahim et al., 2016). Serum NfL was elevated in patients with severe TBI compared to healthy controls at ICU admission and continued to rise through 12d post-injury (Shahim et al., 2016). Higher NfL levels during this acute and subacute period were also associated with worse outcomes, in terms of 12-month Glasgow Outcome Scale scores and mortality rates. Across the entire spectrum of TBI, serum NfL levels are associated with severity at both acute (Czeiter et al., 2020) and chronic (Shahim et al., 2020) time-points. NfL levels are able to accurately distinguish patients with TBI from controls up to six months post-injury and, even more impressively, between patients with mild, moderate, and severe TBI at 30d post-injury (Shahim et al., 2020). Further, elevated NfL levels were tied to significant reductions in grey and white matter volumes and alterations to white matter integrity (Shahim et al., 2020). NfL has also been shown to be a sensitive biomarker for repetitive, subconcussive head impacts in both laboratory and field settings (Oliver et al., 2016; Shahim et al., 2017; Wirsching et al., 2018). NfL remains one of the more promising biomarkers for brain injury due to its specificity to neuronal axons and strong associations with TBI severity and outcome.

3.5. Role of circulating proteases and clinical implications

Many of the other commonly studied blood biomarkers for TBI are also proteomic markers, such as inflammatory cytokines and chemokines (e.g., IL-6, IL-8, IL-10, IL-1β, CCL2, TNF-α) and markers of cerebrovascular injury and BBB disruption (e.g., matrix metalloproteinases, TJ proteins, fibrinogen, von Willebrand factor), with varying degrees of diagnostic and prognostic utility. Proteomic biomarkers are vulnerable to proteolytic degradation by proteases (e.g., calpains, caspases, cathepsins, matrix metalloproteinases) present intra- and extracellularly or in the bloodstream. These markers often have brief half-lives, requiring blood sample collection in the acute period following injury on a timescale of hours to a couple days, which are not necessarily a negative quality. Acute levels of S100B or the combination of GFAP and UCH-L1 can provide valuable clinical information about the need for CT scanning when injured individuals present to an emergency department, reducing both medical costs and radiation exposure. Furthermore, certain markers are known to be chronically released into the bloodstream from the CNS, particularly after moderate or severe TBI (e.g., NfL, GFAP), allowing elevated levels to be detected months and years post-injury (Shahim et al., 2020).

Examining proteomic markers and their breakdown products (BDPs; denoted by the molecular mass in kDa) can reveal the specific proteases that have been activated following TBI. In fact, there is an entire field dedicated to the system-wide study of proteases and protease-substrate pairings, termed “degradomics.” While proteases are active under physiological conditions and necessary for processes such as synaptic plasticity and cell turnover, the cascade of neurochemical and neurometabolic events that occur following TBI provoke dysregulated and uncontrolled protease activation (Abou-El-Hassan et al., 2017). For instance, membrane depolarization and the indiscriminate release of glutamate, an efflux of potassium ions, and an influx and accumulation of calcium ions lead to the overactivation of a group of calcium-dependent proteases called calpains. Calpain-1 can cleave αII-spectrin, resulting in αII-spectrin BDP150 and -145; tau, resulting in tau BDP42, -15, and -35; and GFAP, yielding GFAP-BDP48 (Abou-El-Hassan et al., 2017). Specific BDPs can provide evidence of the biochemical pathways triggered following injury; investigating the degradomes of TBI, or the proteolytic signatures of proteomic biomarkers may reveal therapeutic targets to combat the deleterious effects of protease hyperactivation (Cagmat et al., 2015).

3.6. Autoantibodies

Proteins released by cells following brain injury that enter the peripheral bloodstream can trigger autoimmunity, or the development of autoantibodies against the proteins perceived as antigens. In the case of disrupted BBB, these autoantibodies can enter the CNS and become neuropathogenic. In addition to the antigens themselves, autoantibodies can serve as blood-based biomarkers for brain injury, although the evidence is sparse. Patients with severe TBI exhibited increased levels of autoantibodies for GFAP and GFAP- GFAP-BDPs) in the sub-acute period following injury (Zhang et al., 2014). Critically, Zhang et al. (2014) emphasize that since autoimmunity takes several days to develop, samples collected from patients acutely following TBI can be used to establish baseline levels of circulating autoantibodies and then be compared to samples collected a few days later. Additionally,

serum levels of autoantibodies for GFAP-BDP 38 kDa were significantly higher in patients with worse long-term outcomes, as assessed by the Glasgow Outcome Scale-Extended at 6-months post-injury (Zhang et al., 2014). A similar finding was reported by the TRACK-TBI investigators: in the 24 h following injury, plasma levels of autoantibodies for GFAP and GFAP-BDPs in patients with severe TBI without a history of prior TBI were not different from healthy controls (Wang et al., 2016). However, patients with severe TBI with a history of prior TBI (with or without loss of consciousness) had significantly elevated autoantibody levels relative to acute TBI patients without previous TBI (Wang et al., 2016), suggesting that prior history of TBI could result in chronic autoimmunity. Correspondingly, plasma levels of autoantibodies for GFAP and GFAP-BDPs in patients with chronic TBI admitted to inpatient TBI rehabilitation were significantly elevated compared to healthy controls (Wang et al., 2016). Increased circulating levels of serotonin 5-HT2A receptor autoantibodies were detected in a cohort of veterans with a history of TBI, and when these autoantibodies were cultured with murine N2A neuroblastoma cells, neurite retraction and a dose-dependent neuronal cell loss revealed a possible link between a history of TBI and later neurodegeneration (Zimering et al., 2020).

Additionally, in a cohort of college football players exposed to repetitive head impacts during games Marchi et al. (2013) highlighted that an increase in serum S100B and a concurrent increase in serum S100B autoantibodies may have been driven by greater exposure to head impacts. Further, increased S100B autoantibody levels were correlated with alterations in mean diffusivity from pre- to post-season DTI scans, and post-season S100B autoantibody levels were associated with higher impulse control scores (i.e., more errors on a neurocognitive assessment) and decreased postural stability, as assessed by the Balance Error Scoring System (Marchi et al., 2013). More investigation is needed to characterize the severity-dependent response of autoimmunity to TBI; however, evidence to date indicates that autoantibodies may provide a unique window into immunological changes following brain injury.

4. Emerging biomarkers for brain injury

4.1. MicroRNA

microRNAs are a class of small single-stranded RNAs (21–23 nucleotides) that do not code for proteins, but each microRNA can regulate translation of hundreds of messenger RNAs (mRNA). Therefore, microRNA controls a range of biological and pathological processes such as cell cycle, cell metabolism, apoptosis, and immune responses (Ha and Kim, 2014). Although the timing of expression, cellular origin, and functional overlap in microRNA warrant further exploration, basic and clinical scientists have jointly begun characterizing circulating microRNAs as diagnostic and therapeutic targets for TBI. For example, combined plasma levels of miR-92a, -16, and -765 achieved 100 % sensitivity and specificity in identifying severe TBI patients (Redell et al., 2010). After a synopsis of microRNA biogenesis and function, we will review the role of microRNA as biomarkers for TBI. We centralize the clinical correspondence of a select panel of circulating microRNA in conjunction with their biological significance in Table 2.

4.1.1. Brief overview of microRNA biogenesis and function—microRNAs undergo a pruning process to become mature microRNAs, which starts from the generation of a long primary transcript (pri-microRNA) by RNA polymerase II (Ha and Kim, 2014). Pri-microRNAs are made of thousands of nucleotides that are separated into 60 to 90-nucleotide hairpin-loop precursors referred to as pre-microRNAs by the microprocessor complex (Drosha-DGCR8) in the nucleus. The pre-microRNA is transported into the cytoplasm and then cleaved to the microRNA duplex by Dicer, a ribonuclease III endonuclease. The microRNA duplex is unwound, and one of the strands is loaded into a protein from the Argonaute protein family, forming the microRNA-induced silencing complex (miRISC) that, in most cases, interacts with the 3′ untranslated region (UTR) of target mRNAs to suppress protein expressions (Ha and Kim, 2014). microRNAs have been recognized to play important roles in brain growth in the context of synaptic pruning and formation, neurogenesis, differentiation, and maturation. Conversely, abnormal expression of microRNAs is associated with neurodegenerative diseases and TBI. These changes in microRNA expression have potential to serve as potent clinical biomarkers for TBI (Fig. 2).

4.1.2. Clinical implications of microRNAs for TBI—microRNAs can be released into extracellular space and to the bloodstream via two different modes. Some microRNA can be found within EVs, whereas others are bound to proteins, such as Argonaute-2 (Gallo et al., 2012). Because of these protections, microRNAs in blood samples are resilient to degradation from conditions like boiling, freeze-thaw cycles, pH changes, and even at room temperature for several days (Chen et al., 2008; Mitchell et al., 2008). While microRNA biomarker research has been spearheaded by the field of oncology, microRNA has shown remarkable utility in TBI diagnosis and prognosis.

For example, earlier small-scale studies showed altered blood levels of microRNAs in patients with severe TBI, such ways that: (i) miR-21 and miR-335 were significantly elevated at acute (4 h – 72 h) and subacute (<15d) time windows compared to healthy controls (Di Pietro et al., 2017); (ii) miR-93, miR-191 and miR-499 were elevated in TBI in a severity-dependent manner within 24 h after injury and remained elevated for up to two weeks compared to controls (Yang et al., 2016a); (iii) reductions in miR-16 and -92a and elevation in miR-765 were identified in patients with severe TBI within 10 h of injury compared to controls, with 100 % sensitivity and 100 % specificity when 3 microRNAs are combined (Redell et al., 2010); and (iv) within 6 h of injury, the highest levels of miR-423–3p and miR-124 were detected in patients with severe TBI, followed by patients with both polytrauma and severe TBI, and lowest levels in polytrauma patients without TBI, with fair diagnostic accuracy (AUC = 0.79) (Schindler et al., 2020).

While the clinical utility of microRNA in severe TBI is very promising, milder forms of TBI result in heterogeneous response profiles, possibly reflecting the wide degree of injury heterogeneity at the mild end of the TBI severity spectrum. After using a rodent TBI model to identify candidate microRNAs, miR-9a, miR-136, and miR-434–3p were examined in patients with mTBI within 48 h of injury, but less than half of patients showed elevations in miR-9a and miR-136 (Das Gupta et al., 2021). In a similar fashion, microRNA elevations following concussion were not consistently observed in collegiate athletes (miR-153 [n = 12/20], miR-223 [n = 14/20], miR-let-7a [n = 13/20]) (Svingos et al., 2019). This

raises further research questions, such as whether microRNAs are capable of peripherally reflecting concussion pathophysiology, and whether a single concussion is sufficient stimulus to trigger substantial microRNA changes in bloodstream. These questions are partially addressed by recent studies in military personnel. Seventeen exosomal microRNAs (16 upregulated; 1 downregulated) associated with neuroinflammation and cell repair pathways were dysregulated in individuals with a history of TBI during military deployment compared to their uninjured fellow service members (Devoto et al., 2020). In the subset of individuals with a history of 3 or more TBI, the expression of 32 exosomal microRNAs (30 upregulated; 2 downregulated) was altered (Devoto et al., 2020). Further, the relationship between levels of miR-103a-3p and miR-139–5p and persistent post-concussive symptoms was explored, revealing that these microRNAs were associated with not only the total symptom score but also all four sub-domains (somatic, affective, cognitive, and vestibular) (Devoto et al., 2020). Higher levels of miR-139–5p were associated with more severe symptoms, while higher levels of miR-103a-3p were associated with lower symptoms scores (i.e., better functional outcomes) (Devoto et al., 2020). These results were corroborated in veterans who served in Iraq and Afghanistan, whereby those with blast-related chronic mTBI showed aberrant expressions in 32 microRNAs related to vascular remodeling, BBB integrity, and neuroinflammation (Ghai et al., 2020). Taken together, blood biomarker utility of microRNA may be limited for a single concussion, but the microRNA expression profile becomes more pronounced with chronicity. This pattern is further supported by studies of populations at risk for chronic exposure to repetitive head impacts, such as collegiate football players (Papa et al., 2019).

It is important to note that microRNA expressions in saliva may be equally promising for concussion diagnosis/prognosis. For example, 6 microRNAs (miR-182, miR-221, mir-26b, miR-320c, miR-29c, miR-30e) showed altered expressions in both saliva and cerebrospinal fluid with an AUC of 0.852 for identifying concussion in pediatric population (Hicks et al., 2018), whereas acute post-concussion levels of 5 microRNAs combined (miR-320c, miR-133a, miR-769, let-7a, and miR-1307) demonstrated significant prognostic utility in classifying those who develop persistent post-concussive symptoms, with an AUC of 0.856, 80 % sensitivity, and 75 % specificity (Johnson et al., 2018). These promising results fuel the current momentum in microRNA biomarker research. However, biological functions of many microRNAs within the brain parenchyma remain uncertain, warranting additional mechanistic studies followed by clinical validation to establish a biomarker panel of microRNA for TBI diagnosis/prognosis.

4.2. Extracellular vesicles (EVs)

EVs are membrane-bound organelles released from most cells under both physiological and pathological conditions and have been referred to by a plethora of names, including exosomes, microvesicles, and microparticles (Fig. 2). Varying in concentration and size, membrane-bound surface proteins, and cargo, EVs are thought to carry “messages” from cells, making them an ideal category of blood biomarkers for brain injury.

4.2.1. Formation and neurobiology of EVs—The formation of EVs follows two primary mechanisms: budding directly from the cellular plasma membrane or from within

intracellular multivesicular bodies (Fig. 3). In either case, EVs contain the constitutive array of proteins, lipids, and RNAs, yet their contents differ based on the cellular origin and physiological or pathological condition of the cell. Thus, EVs can act as indicators of the health status of the originating cell (Yang et al., 2017). EVs facilitate cell-to-cell communication and waste clearance through the transfer of cargo and surface proteins to nearby cells. Target cell selection depends on the surface proteins, such as adhesion molecules, integrins, tetraspanins, and other membrane-bound proteins (Guedes et al., 2020; Gurunathan et al., 2019). Release from the cells of origin, such as neurons and glial cells, is continuous; however, indicators of cellular damage, such as alterations to ionic homeostasis and presence of neurotransmitters and inflammatory cytokines, can prompt increased EV formation and release (Guedes et al., 2020; Gurunathan et al., 2019). EVs can reach the bloodstream by crossing the BBB or through glymphatic clearance, serving as peripheral surrogates of pathological processes occurring in the brain (Guedes et al., 2020; Gurunathan et al., 2019; Shah et al., 2018).

For instance, if an EV membrane contains EAAT1 (or GLAST1) or EAAT2 (or GLT-1), which are astrocyte-specific transporter proteins vital for the removal of glutamate from synaptic cleft to prevent excitotoxicity, this EV originated from an astrocyte (Ohmichi et al., 2019). Presence of L1 cell adhesion molecule (L1CAM or CD171) or synaptosomal-associated protein 25 (SNAP25) would suggest the EV is derived from neurons, and oligodendrocyte-myelin glycoprotein (OMG) identifies an oligodendrocyte-derived EV (Ohmichi et al., 2019). While cell-specific membrane proteins indicate the source of EVs, microRNA content within EV cargo indicates the potential purpose of EVs. For example, microRNAs packaged within EVs can modify the phenotype and the physiology of the recipient cell, including the processes of proliferation, differentiation, and survival. Mice exposed to repetitive TBI showed significant elevations in miR-124–3p levels within microglia-derived EVs (Huang et al., 2018). When these EVs were co-cultured with scratch-injured neurons, miR-124–3p suppressed mTOR signaling, which resulted in inhibition of neuronal inflammation, supporting the role of EVs in paracrine signaling after TBI (Huang et al., 2018). Indeed, the therapeutic applications of EVs is a rapidly growing research area, and numerous preclinical studies support that EVs generated by extracranial cells may modulate neuroinflammation and neuroplasticity (Yang et al., 2017). For example, when rats with TBI received exosomes derived from mesenchymal cells enriched with microRNA, activation of astrocyte and microglia was attenuated and spatial learning and sensorimotor functions were improved compared to the control treatment (Zhang et al., 2015, 2017). However, the fate of brain-derived EVs in circulation remains unclear of whether they are to influence peripheral cells or designed to clear wastes from the CNS. Nonetheless, EVs carrying cellular messages have the potential to be useful in clinical diagnosis and prognosis of TBI.

4.2.2. Clinical utility of EVs as biomarkers for TBI—Comparison of EV characteristics (e.g., concentration, size, surface proteins, contents; Fig. 3) in patients with varying severities of TBI can provide valuable insights into acute and chronic pathophysiology. A recent study examined the acute time-course expression of EVs containing t-tau, GFAP, UCH-L1, and NfL and compared those expressions to circulating

levels of the same proteomic biomarkers in patients with moderate to severe TBI (Mondello et al., 2020a). Strong time-course correlations between EV and circulating levels were noted for GFAP and NfL, whereas the strength of the correlations declined over time for t-tau and UCH-L1. Acute expressions of EV markers were particularly sensitive to diffuse injury compared to focal injuries (GFAP, NfL), with excellent prognostic utility for early mortality (UCH-L1, 100 % sensitivity and specificity) (Mondello et al., 2020a). When within-EV levels of GFAP and NfL were examined at 1-year post-injury, patients with moderate and severe TBI exhibited higher concentrations of GFAP relative to healthy controls (AUC = 0.858 and 0.876, respectively), while within-EV GFAP levels were not significantly different between patients with mild TBI and controls (Flynn et al., 2021). Additionally, higher levels of EV GFAP and EV NfL were significantly associated with lower Glasgow Outcome Scale – Extended scores (i.e., worse outcomes), suggesting that EV cargo can still provide valuable prognostic utility at chronic post-injury time points; however, the exact mechanisms by which these markers are chronically elevated in the CNS, packaged in EVs, and then translocated to the bloodstream remain unknown.

The clinical utility of circulating EVs extends into concussion research, which was first noted in a case report of two concussion patients in 2016. The study reported a novel finding that plasma levels of neuron- and astrocyte-derived EVs, containing SNAP25, NfL, tau and GFAP, were elevated after concussion (Kawata et al., 2018). Additionally, the time course of EV elevation mirrored symptom recovery trajectory. Similarly, neuron-derived exosomal levels of UCH-L1, amyloid-β 42 (Aβ42), t-tau, p-tau-Thr181, and pro-inflammatory cytokine IL-6 were significantly elevated in collegiate athletes with acute sports-related concussion compared to healthy control subjects with no recent history of TBI (Goetzl et al., 2019). Exosomal levels of t-tau, Aβ42, p-tau-Thr181, p-tau-Ser396, and IL-6 were also elevated in a group of individuals with a history of two or more concussions relative to the healthy control subjects (Goetzl et al., 2019). Likewise, plasma exosomal levels of t-tau, Aβ42, and IL-10 were significantly higher in military personnel with a history of mTBI compared to their peers who did not report a history of TBI (Gill et al., 2018). As mentioned in the previous section, alterations of microRNA content within EVs vary commensurately with TBI history in military personnel, and these dysregulated microRNAs are implicated in the regulation of inflammatory, cell repair, and apoptotic pathways (Devoto et al., 2020). Two of these dysregulated exosomal microRNAs (miR-103a-3p and miR-139–5p) were significantly associated with neurobehavioral symptom scores (Devoto et al., 2020). Altogether, these findings demonstrate that EVs and their cargo can serve as meaningful biomarkers for brain injury during both the acute recovery phase and in cases of persistent symptomology, even years after the most recent TBI. While additional research into the biological role and clinical value of circulating EVs after TBI is imperative, studies indicate the unequivocal potential of EVs. Additionally, modified EVs are being explored as a potential pharmaceutical agent delivery system (Gurunathan et al., 2019) since EVs can cross the BBB; thus, continued investigation into the therapeutic potential of EVs is also merited.

4.3. Metabolomics

Metabolomics, or the study of the biochemical substrates of metabolism, is a promising subfield of brain injury biomarker research, as neurometabolic crisis is a hallmark of concussion (Fig. 2) (Giza and Hovda, 2014). Upon mechanical stress, neurons are subjected to a series of ionic fluxes and excitotoxic events. Neuronal Na^{2+}/K^{+}-ATPase pumps increase activity to restore ionic homeostasis; however, the supply of ATP is quickly diminished after an increase in glycolysis and subsequent accumulation of lactate, which are exacerbated during the post-injury period of reduced cerebral blood flow and oxygen and glucose availability (Giza and Hovda, 2014). Due to this mismatch between metabolic supply and demand, neurons then enter a hypometabolic state. Unlike traditional proteomic biomarkers, circulating levels of metabolites (e.g., N-acetylaspartate [NAA], myo-inositol, glycerophospholipids, and choline) allow researchers to inspect neurometabolism following TBI (Fig. 4).

4.3.1. Neurobiology of metabolites—Metabolic activity in the CNS supports virtually every process by providing energy for active cellular processes and producing substrates for neurotransmission. There are multiple metabolic pathways that can occur in the brain parenchyma, including glycolysis, the TCA cycle, the pentose-phosphate pathway, oxidative phosphorylation, amino acid metabolism, cholesterol biosynthesis and steroidogenesis, and glycogenesis and glycogenolysis. These processes directly or indirectly rely on cerebral oxygenation, supply of glucose from cerebral arteries, ionic homeostasis, and mitochondrial function—all of which can be altered following TBI (Giza and Hovda, 2014).

NAA, myo-inositol, choline, and glycerophospholipids are some of the most common metabolites studied within the context of TBI. NAA is an amino acid synthesized in neurons and hydrolyzed in oligodendrocytes for myelin formation. Its utility as a metabolic biomarker of brain injury is due to the fact that the rate of NAA synthesis has been directly coupled to glucose metabolism in both rodent models and magnetic resonance spectroscopy (MRS) studies in humans (Baslow, 2003). A systematic review of MRS findings in patients with sports-related concussion reported that 9 of 11 studies that met study inclusion criteria detected decreases in NAA after injury (Gardner et al., 2014), consistent with findings from rodent models of TBI.

Myo-inositol is a sugar-alcohol metabolite enriched in glial cells, particularly astrocytes, and participates in the metabolism of membrane phospholipids and osmoregulation (Bittsansky et al., 2012). Concentrations of myo-inositol vary widely across different areas of the brain depending on glial cell concentrations and biochemical demands. Generally, an increase in myo-inositol concentration is linked to astrogliosis, while a decrease has been associated with elevated osmoregulatory activity due to brain edema (Harris et al., 2012). Following a controlled cortical impact to the sensorimotor cortex, myo-inositol concentrations, assessed by *in vivo* MRS, decreased relative to pre-injury on days 1 and 3 but was elevated on days 7 and 14, suggesting that after an acute period of cerebral edema, astrocyte activation intensifies (Harris et al., 2012). Similarly, a study of pediatric TBI showed increased myo-inositol concentrations one week after injury in the occipital cortex relative to healthy

controls, and patients with poor outcomes exhibited higher myo-inositol levels than those with good outcomes (Ashwal et al., 2004).

Under physiological conditions, total choline correlates with the level of membrane-bound phospholipids due to the constant synthesis and breakdown of the phospholipids sphingomyelin and phosphatidylcholine, both of which are metabolized from choline and the choline-containing compounds phosphocholine and glycerophosphocholine (Bittsansky et al., 2012). Under pathological conditions, total choline increases as myelin phospholipids are catabolized (Bittsansky et al., 2012).

Lastly, glycerophospholipids are the most abundant lipid species within the CNS as critical components of cell membranes. Glycerophospholipid structure follows the general form of two fatty acid chains linked by a glycerol backbone attached to a polar phosphodiester group and a “base” group. Glycerophospholipids comprise a large class of lipids and can be further categorized by the identity of the base group (e. g., choline: phosphatidylcholine [PC], inositol: phosphatidylinositol [PI], ethanolamine: phosphatidylethanolamine [PE]). In the event of TBI, calcium influx triggers the activation of phospholipases, enzymes that cleave fatty acids from glycerophospholipids through hydrolysis, further degrading cell membrane integrity. While glycerophospholipids are bound in membranes and therefore not visible in MRS spectra, they can be detected in biofluids using liquid-chromatography/tandem mass spectrometry (LC–MS/MS).

4.3.2. Clinical utility of circulating metabolites as biomarkers for TBI—The potential clinical utility of metabolomics in TBI management is evident. The detection of altered or disrupted neurometabolism could delay return to activity and reduce risk of sustaining a second injury before achieving complete recovery, as hypometabolism can persist for weeks or months after injury—occasionally, even after symptoms have resolved (Gardner et al., 2014; Manning et al., 2017). Blood-based metabolomics offers a minimally invasive and convenient method to probe the altered metabolic state of an injured brain.

In general, blood-based metabolomic investigations of TBI report altered circulating levels of metabolites compared to healthy controls. However, the characterization of these changes varies with duration between injury and blood collection, metabolite(s) of interest, and severity of injury. Plasma samples taken from male youth ice hockey players with and without acute concussion and assayed for a panel of metabolites using LC–MS/MS revealed that 10 components of 9 metabolites each could explain over 80 % of the between-group variance (Daley et al., 2016). A smaller panel of 17 metabolites with excellent diagnostic accuracy (AUC = 0.91) was generated; notably, 12 of the 17 selected metabolites were glycerophospholipids—specifically PCs (Daley et al., 2016). The direction of metabolite level change within the TBI cohorts varied across the 6 metabolites, which the authors speculated reflected the alterations in metabolic demands and processes after injury. An investigation of changes in plasma glycerophospholipids within 72 h of concussion in adolescent male ice hockey players found that 26 of the 71 examined glycerophospholipids were significantly reduced compared to uninjured age- and sex-matched control athletes; the remaining glycerophospholipids were not significantly different between groups (Miller et al., 2021). When the four glycerophospholipids (PCaeC36:2, PCaaC32:0, PCaaC42:6,

and PCaeC36:0) with the highest individual AUCs were combined, the diagnostic accuracy increased to excellent (AUC = 0.96) (Miller et al., 2021). The authors hypothesized that these findings, which corroborated their MRS data from the same cohort (Manning et al., 2017), suggest that decreases in choline and choline-containing PCs could indicate slowed cell membrane and myelin turnover after TBI (Miller et al., 2021).

5. Conclusion

Brain-derived blood biomarkers for brain injury, ranging from traditional proteomic biomarkers (e.g., tau, GFAP, UCH-L1, NfL) to emerging biomarker categories (e.g., microRNA, EVs, metabolites), can indicate injury severity and prognosis, provide evidence of molecular and cellular responses, and serve as objective evaluations for treatment efficacy. As blood biomarker detection methods evolve, we can continue to expand our understanding of TBI beyond subjective symptom reporting, with the goal of using biomarkers in routine clinical practice to diagnose TBI and improve management across the spectrum of injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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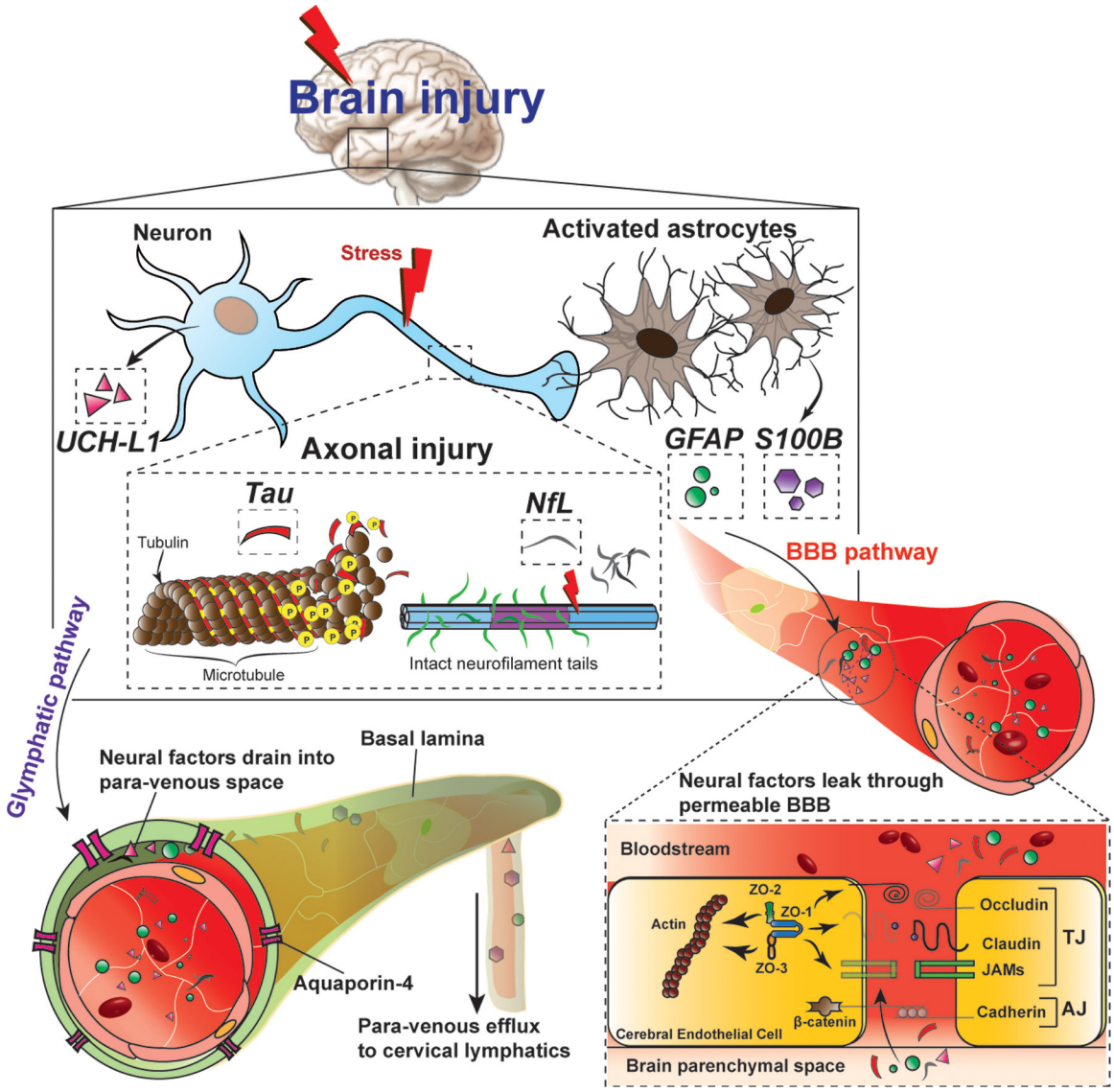

Fig. 1. Conventional proteomic biomarkers and clearance pathways.

In the event of brain injury, the axonal cytoskeleton is disrupted. Increased tau phosphorylation contributes to further microtubule disassembly. Neurofilaments are compacted and NfL is dislodged through calpain-mediated proteolysis. UCH-L1 is upregulated and released from neurons. Astrocytes assume their reactive phenotypes, exhibiting hypertrophy of the astrocytic processes and upregulated GFAP and S100B. These neural factors can then leave the brain parenchyma through a leaky BBB or through clearance via the glymphatic pathway. Permeability of the BBB is tightly regulated by TJ and AJ molecules (e.g., cadherin, claudin, occludin, JAMs). After TBI, the junctions between the cerebral endothelial cells can become disrupted, increasing BBB permeability. The other major clearance pathway, the glymphatic system, is characterized by a para-arterial influx of CSF from the subarachnoid space into the interstitial space, followed by a paravenous efflux of interstitial fluid (ISF) from the brain parenchyma. This convectional bulk flow of fluid pulls neural factors into the para-venous space, from where they travel

through the dural sinuses and then drain the cervical lymphatics. The glymphatic system may serve as an important exit pathway for neural factors and debris to be cleared from the CNS.

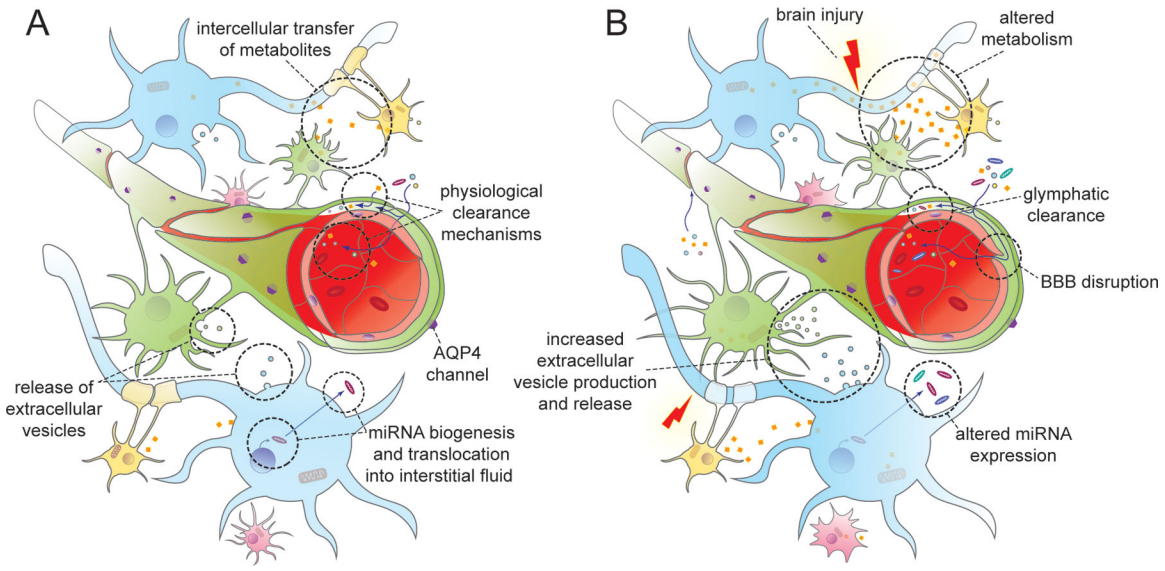

Fig. 2. Emerging categories of biomarkers (microRNA, EVs, and neurometabolites) and their presence within the CNS.

microRNA, EVs, and neurometabolites are shown (A) under physiological conditions and (B) after traumatic brain injury. Metabolites, microRNA, and EVs are expressed and released under physiological conditions, yet after brain injury, the expression of microRNA (up- and down-regulation) is altered to control post-injury mRNA translation and subsequent protein expression. TBI has been shown to also alter EV formation, resulting in different sizes and concentrations of EVs, in addition to varied contents. Additionally, neurometabolic processes are altered as ionic homeostasis is disrupted and glucose availability and CBF are reduced.

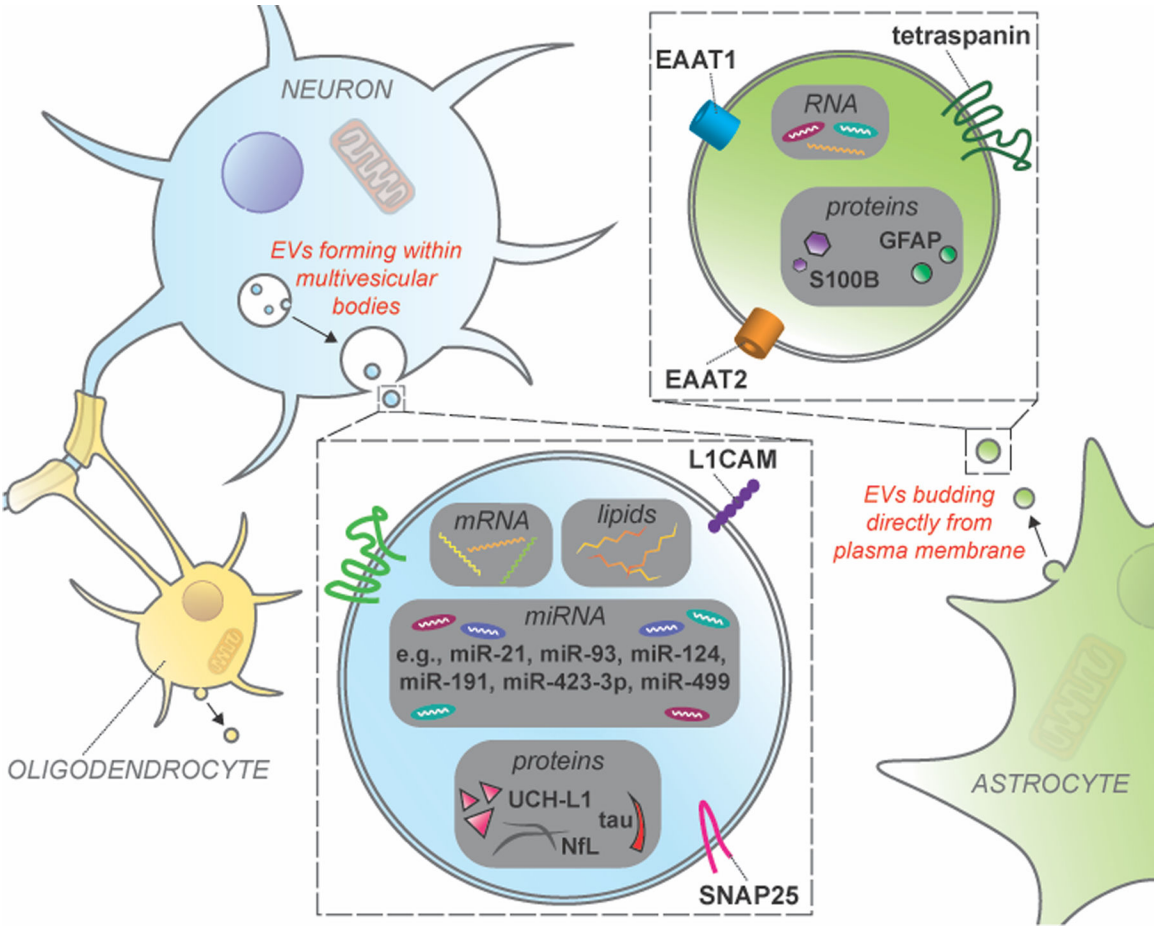

Fig. 3. Extracellular vesicles: formation, secretion, surface proteins, and cargo.

EVs can form within multivesicular bodies or directly bud from the plasma membrane. Surface proteins embedded in the EV membrane are indicative of the cellular origin (e.g., EAAT1 and EAAT2 on astrocyte-derived EVs, SNAP-25 and L1CAM on neuron-derived EVs). The cargo contained within EVs can also signal the cellular origin, in addition to the ongoing cellular processes, as EVs are used as mediums for intercellular communication and waste clearance.

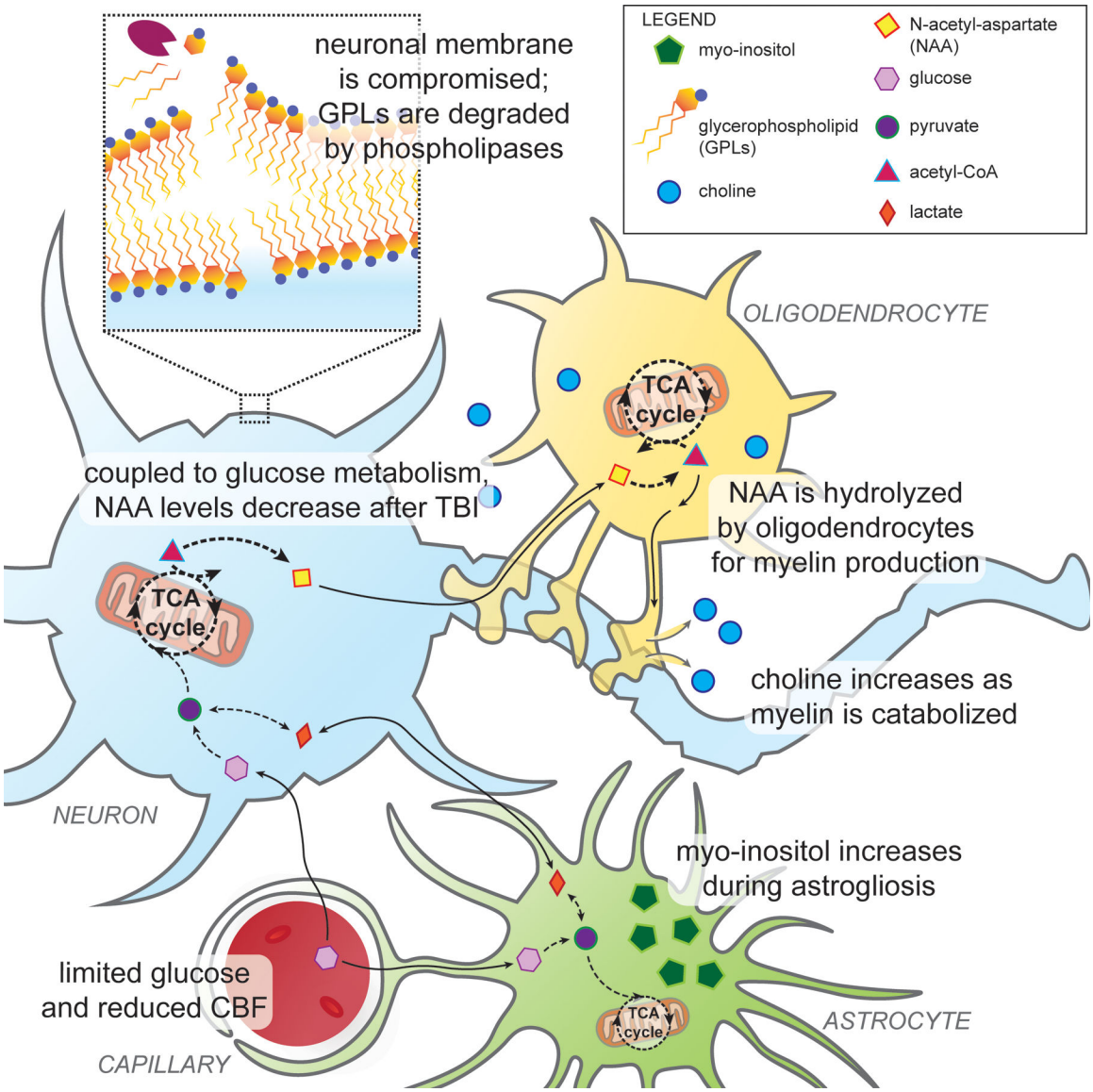

Fig. 4. Alterations in neurometabolism after traumatic brain injury.

Cell membranes are disrupted in the event of TBI, and glycerophospholipids are degraded by phospholipases, releasing the fatty acids from the phosphodiester and base groups. NAA is coupled to glucose metabolism and transferred from neurons to oligodendrocytes to be converted to myelin through hydrolysis. After TBI, when CBF is reduced and glucose is limited, NAA concentration decreases as the neuron's capacity for mitochondrial phosphorylation and the rate of the tricarboxylic acid (TCA) cycle is reduced. Levels of choline, a component of several membrane phospholipid species and myelin, are linked membrane and myelin integrity due to the constant breakdown and reformation of cell membranes and myelin under physiological conditions. After brain injury and resulting axonal damage, myelin can degrade, and choline increases with myelin catabolism. Myo-inositol is predominantly expressed in glial cells, particularly astrocytes, and increases with astrogliosis.

Table 1

Proteomic blood biomarker profiles.

Biomarker	Associated pathophysiology	CNS origin	Extracerebral sources	Half-life in bloodstream	Used clinically?
S100B	Astrogliosis	Astrocytes Oligodendrocytes	Schwann cells Ganglion cells Adipocytes Skeletal myofibers	90 min (Townend et al., 2006)	Yes – to rule out the need for a CT scan
NSE	Neuronal damage	Neurons	Neuroendocrine cells Red blood cells	1–3 d (Thelin et al., 2017)	No
Tau, p-tau	Axonal disruption	Neurons	Smooth muscle cells Pancreatic islets	10 h (Randall et al., 2013)	No
GFAP	Astrogliosis	Astrocytes	Osteocytes Leydig cells Chondrocytes	1–2 days (Thelin et al., 2017)	Yes – to rule out the need for a CT scan
UCH-L1	Neuronal damage	Neurons	Oocytes Spermatogonia	8–12 h (Thelin et al., 2017)	Yes – to rule out the need for a CT scan
NfL	Axonal disruption	Neurons	Peripheral nerves	Unknown	No
αII-spectrin and SBDPs	Axonal disruption Cell death mechanisms	Neurons	Red blood cells Cardiomyocytes	Unknown	No
MAP2	Dendritic injury	Neurons	n/a	Unknown	No
MMP-9	BBB disruption Degeneration	Neurons	Schwann cells Macrophages Fibroblasts T-cells	Unknown	No
MCP-1 (CCL2)	Inflammation	Microglia Astrocytes Cerebral endothelial cells	Macrophages Fibroblasts Peripheral endothelial cells	Unknown	No
MCP-4 (CCL13)	Inflammation		Chondrocytes	Unknown	No
Occludin	BBB disruption	Cerebral endothelial cells	Peripheral endothelial cells Epithelial cells Sertoli cells Hepatocytes	Unknown	No
TNF-α	Inflammation	Neurons Microglia	Macrophages	Minutes (Zahn and Greischel, 1989)	No
Fibrinogen	BBB disruption	n/a	Hepatocytes	Several days (Weisel, 2005)	No
Von Willebrand factor	BBB disruption	Cerebral endothelial cells	Peripheral endothelial cells Megakaryocytes	Up to 24 h (Lenting et al., 2015)	No
IL-1β	Inflammation	Microglia Astrocytes Oligodendrocytes Neurons	Monocytes Macrophages	1–2 h (Kudo et al., 1990)	No

NSE, neuron-specific enolase. P-tau, phosphorylated tau. GFAP, glial fibrillary acidic protein. UCH-L1, ubiquitin carboxyl-terminal hydrolase L1. NfL, neurofilament-light. SBDP, spectrin breakdown product. MAP2, microtubule-associated protein-2. MMP-9, matrix metalloproteinase-9. MCP-1, monocyte chemoattractant protein-1. MCP-4, monocyte chemoattractant protein-4. TNF-α, tumor necrosis factor alpha. IL-1β, interleukin-1 beta. IL-6, interleukin-6.

Table 2

List of miRNAs implicated in TBI with biological functions and known expression in neurological conditions. References can be found in Supplemental File 1.

miRNA	Biological function	Reported changes in expression in human neurological conditions
miR-9a	Most abundant brain miRNA (Schonrock et al., 2012). Participates in neurogenesis and angiogenesis by regulating VEGF expression through upstream control of transcription factors TLX and ONECUTs (Madelaine et al., 2017). Represses expression of SIRT1, which interferes with Aβ formation and TGF-β (Schonrock et al., 2012).	↓ AD [serum]: compared to controls (Geekiyanage et al., 2012) ↑ Mild and severe TBI [plasma]: collected within 2 d post-injury, compared to age-matched controls (Das Gupta et al., 2021)
miR-16	Induces apoptosis through targeting BCL2 (Cimmino et al., 2005). Downregulates pFAk, pAkt, NF-κB, IL-6, IL-8, and TGF-β (Wang et al., 2014a). Inhibits expression of BACE1, thus downregulation of miR-16 can contribute to Aβ neurotoxicity (Zhong et al., 2018).	↓ Severe TBI [plasma]: <24 h post-injury, compared to orthopedic injury patients, and 25–72 h post-injury compared to healthy controls (Redell et al., 2010) ↑ Mild TBI [plasma]: collected <10 h post-injury, compared to healthy controls (Redell et al., 2010) ↓ AD [cortical tissue]: postmortem brain samples from AD patients compared to control samples (Zhong et al., 2018)
miR-21	Protects against ischemic neuronal death and promote neuronal regeneration by down-regulating PDCD 4 and FasL (Buller et al., 2010) and contributes to anti-inflammatory cascade (decrease IL-6, increase IL-10, interacts with Smad7 and Spry1 to modulate neuroinflammation) (Ji et al., 2018). miR-21 can reduce neuronal apoptosis after TBI through activation of the PTEN-Akt signaling pathway (Ji et al., 2018).	↑ Severe TBI [serum]: at 1d and 15d post-injury compared to healthy controls (Di Pietro et al., 2017) ↑ Concussion [saliva]: collected 48–72 h post-injury, compared to non-injured age-matched peers (Di Pietro et al., 2021) ↑ Stroke [whole blood]: at 6–18 months post-injury, compared to healthy controls (Tan et al., 2009) ↑ Relapsing remitting MS [peripheral mononuclear cells from whole blood]: compared to matched healthy controls (Fenoglio et al., 2011) ↓ Progressive MS [peripheral mononuclear cells from whole blood, enriched for CD4 + T cells]: compared to healthy controls (Sanders et al., 2016)
miR-34a	Participates in regulation of energy metabolism and inflammation by mediating the crosstalk between NF-κB pathway and SIRT1 enzyme: NF-κB upregulates miR-34a expression which in turn inhibits SIRT1 expression (Kauppinen et al., 2013). Inhibits expression of Bcl-2, a protein that inhibits caspase-3 and -9 activation and prevents apoptosis (Wang et al., 2009). Increased miR-34a expression in cerebral cortex was observed in mouse model of AD (Wang et al., 2009).	↑ MDD [CSF and serum]: compared to healthy controls (Wan et al., 2015) ↑ AD [peripheral mononuclear cells from whole blood]: compared to matched healthy controls (Schipper et al., 2007) ↓ AD [plasma and CSF]: compared to healthy controls (Kiko et al., 2014)
miR-92a	Modulates PTEN-Akt-NF-κB pathway by binding to PTEN, controlling the inflammatory response (Fu et al., 2018). Regulates AMPA receptor expression by inhibiting mRNA translation of GluA1 subunit (Letellier et al., 2014).	↓ Severe TBI [plasma]: <24 h post-injury, compared to orthopedic injury patients, and 25–72 h post-injury, compared to healthy controls (Redell et al., 2010) ↑ Severe TBI [serum]: collected within 48 h post-injury; compared with healthy controls (Bhomia et al., 2016) ↑ Mild TBI [plasma]: <10 h post-injury, compared to healthy controls (Redell et al., 2010)
miR-93	Decreases the expression of MMP2, a matrix metalloproteinase that degrades protein components of the extracellular matrix (Wu et al., 2019). Targets TLR-4/NF-κB signaling pathway, contributing to inflammatory response and apoptosis after injury (Shang et al., 2019).	↑ TBI, all severities [serum]: elevated at all time points (<24 h, 2–7 d, 8–14 d, and 15–21 d), compared to matched controls (Yang et al., 2016a) ↑ AD [exosomal miRNA in serum]: compared to healthy controls (Cheng et al., 2015) ↓ Glioma [tissue specimens collected during enlarged tumor resection]: relative to adjacent tissue specimens (Wu et al., 2019)
miR-103a-3p	Inhibits expression of HMGB1which regulates a cascade of cytokine production (e.g., IL-1β, TNF-α, and IL-6) during an inflammatory response (Li et al., 2021).	↓ History of multiple mild TBI [exosomal miRNA in plasma]: compared to control group (Devoto et al., 2020) ↓ AD [whole blood]: compared to healthy controls (Chang et al., 2017; Leidinger et al., 2013)
miR-107	Modulates cellular glucose metabolic pathway by interacting with granulin. Decrease in neuronal miR-107 in the hippocampus was noted acutely after TBI in mouse, allowing transcription of granulin to trigger inflammation and healing (Wang	↓ AD [whole blood]: compared to healthy controls (Chang et al., 2017; Leidinger et al., 2013) ↑ Concussion [saliva]: collected 48–72 h post-injury, compared to non-injured age-matched peers (Di Pietro et al., 2021)

Neurosci Biobehav Rev. Author manuscript; available in PMC 2021 November 01.

miRNA	Biological function	Reported changes in expression in human neurological conditions
	et al., 2010). miR-107 targets mRNA of BACE1—a decrease in miR-107 results in an increase in BACE1, accelerating AD progression (Wang et al., 2008).	↑ Ischemic stroke [plasma]: collected <24 h after admission, compared to matched, healthy controls (Yang et al., 2016b) ↓ ALS [serum]: compared to healthy controls (De Luna et al., 2020)
miR-124	A brain-specific miRNA, miR-124 is transferred from neurons to astrocytes in EVs, to regulate expression of GLT-1 (Morel et al., 2013). Inhibits neuroinflammation by targeting and suppressing mTOR signaling activity (Huang et al., 2018). Promotes neurite outgrowth through downregulation of RhoA, APP, and p-tau after in vitro injury (Huang et al., 2018).	↓ Stroke [serum]: <24 h after stroke onset, compared to healthy controls (Liu et al., 2015) ↑ Severe TBI [serum and plasma]: collected <6 h after injury, compared to healthy controls (Schindler et al., 2020) ↓ MS [peripheral mononuclear cells from whole blood]: compared to healthy controls (Amoruso et al., 2020)
miR-136	Modulates inflammatory response through inhibition of IL-18, NLRP5, caspase-1, and IL-1β expression; overexpression promotes the expression of NF-κB (Gao et al., 2020).	↑ Mild TBI [plasma]: collected <2 d post-injury, compared to age-matched controls (Das Gupta et al., 2021)
miR-139–5p	Downregulated after fluid percussion induced TBI, miR-139–5p inhibits the expression of NOTCH1 (Puhakka et al., 2017), a transcription factor associated with synaptic plasticity.	↓ History of mild TBI [exosomal miRNA in plasma]: compared to control group (Devoto et al., 2020) ↓ AD [exosomal miRNA in plasma]: compared to matched, healthy controls (Lugli et al., 2015)
miR-153	Inhibits Ptc expression, activating the Shh signaling pathway and promoting angiogenesis through regulation of VEGF and ANG-1/-2 expression.(Wang et al., 2019) Inhibits SNAP-25 expression, thus regulating pre-synaptic neurotransmitter release of glutamate, ACh, and glycine (Wei et al., 2013).	↑ Ischemic stroke [plasma]: collected <24 h after admission, compared to matched, healthy controls (Yang et al., 2016b) ↑ Concussion [serum]: compared to individual baseline (Svingos et al., 2019)
miR-191	Targets BDNF, a neurotrophin involved in long-term potentiation and cell survival through binding to Trk receptors (Mohammadipoor-Ghasemabad et al., 2019), and tropomodulin 2, an essential actin regulator protein in neurons and involved in spine plasticity during long-term depression (Hu et al., 2014).	↑ TBI, all severities [serum]: elevated at all time points (within 24 h, 2–7 d, 8–14 d, and 15–21 d), compared to matched, healthy controls (Yang et al., 2016a) ↓ AD [plasma; serum]: compared to controls (Kumar et al., 2013; Tan et al., 2014)
miR-223	Inhibits aberrant NLRP3 inflammasome activity and expression of IL-1β, IL-18, and caspase-1 (Bauernfeind et al., 2012).	↑ Epilepsy [serum]: compared to healthy controls (De Benedittis et al., 2021) ↑ Concussion [serum]: compared to individual baseline (Svingos et al., 2019) ↑ Symptomatic HD [plasma]: compared to healthy, matched controls (Diez-Planelles et al., 2016) ↑ Ischemic stroke [leukocytes from whole blood]: collected <72 h after stroke onset, compared to controls (Wang et al., 2014b) ↑ MS [peripheral mononuclear cells from whole blood]: compared to healthy controls (Amoruso et al., 2020)
miR-335	Inhibits expression of caspase-7, preventing apoptosis (De Luna et al., 2020). Spatial learning induces down-regulation of miR-335 and overexpression of miR-335 impairs spatial memory (Capitano et al., 2017).	↑ Severe TBI [serum]: at 1 d and 15 d post-injury compared to healthy controls (Di Pietro et al., 2017) ↑ AD [exosomal miRNA in serum]: compared to healthy controls (Cheng et al., 2015) ↓ ALS [serum]: compared to healthy controls (De Luna et al., 2020)
miR-423–3p	Regulates cell cycle through inhibition of p21 expression (Liu et al., 2021).	↑ Severe TBI [serum and plasma]: collected <6 h after injury, compared to healthy controls (Schindler et al., 2020) ↓ ALS [serum]: compared to healthy controls (De Luna et al., 2020; Dobrowolny et al., 2021)
miR-434–3p	Enriched in rodent neurons, miR-434–3p increases after fluid percussion injury (Das Gupta et al., 2021).	Not detected in humans (Mainieri and Haig, 2019)
miR-499	Inhibits expression of c-reactive protein,(Jia et al., 2020) reducing the inflammatory response, and PDCD4 (Li et al., 2016), protecting against apoptosis.	↑ TBI, all severities [serum]: elevated at all time points (<24 h, 2–7 d, 8–14 d, and 15–21 d), compared to matched controls (Yang et al., 2016a) ↑ Mild TBI [serum]: compared to healthy controls, (Polito et al., 2020)
miR-765	Inhibits BCL2L13, Bcl-2-like protein involved in regulating mitochondrial apoptosis and oxidative phosphorylation (Lu et al., 2020).	↑ Severe TBI [plasma]: 25–48 h post-injury, compared to healthy controls (Redell et al., 2010)

Notes: All mechanistic and functional descriptions are derived from preclinical studies. The summaries of changes in miR expression are based on clinical studies and are presented as: “direction of change + condition + [sample type]: sampling time-frame OR synopsis of findings.”.

ACh, acetylcholine. **AD**, Alzheimer's disease. **Akt**, protein kinase B. **ALS**, amyotrophic lateral sclerosis. **AMPA**, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid. **ANG-1/-2**, angiopoietin-1/-2. **APP**, amyloid precursor protein. **Aβ**, amyloid beta. **Bcl-2**, B-cell lymphoma 2 protein. **BCL2L13**, Bcl-2-like 13. **BDNF**, brain-derived neurotrophic factor. **BACE1**, β-site amyloid precursor protein-cleaving enzyme 1. **CSF**, cerebrospinal fluid. **FasL**, factor associated suicide ligand. **GLT-1**, glucose transporter 1 (known as excitatory amino acid transporter 2 in humans). **HMGB1**, high mobility group box 1. **IL-10**, interleukin 10. **IL-18**, interleukin 18. **IL-1β**, interleukin 1 beta. **IL-6**, interleukin 6. **IL-8**, interleukin 8. **MDD**, major depressive disorder. **MMP2**, matrix metalloproteinase 2. **MS**, multiple sclerosis. **mTOR**, mechanistic target of rapamycin. **NF-κB**, nuclear factor kappa light chain enhancer of activated B cells. **NLRP3**, NOD-like receptor pyrin domain-containing protein 3. **NLRP5**, NOD-like receptor pyrin domain-containing protein 5. **ONECUT**, a family of transcription factors with one cut domain. **p21**, cyclin-dependent kinase inhibitor. **pAkt**, phosphorylated Akt. **PDCD4**, programmed cell death protein 4. **pFAk**, phosphorylated focal adhesion kinase. **Ptc**, Patched protein. **PTEN**, phosphatase and tensin homolog deleted on chromosome 10. **RhoA**, Rho-GTPase. **Shh**, Sonic hedgehog. **SIRT1**, sirtuin 1. **Smad7**, mothers against decapentaplegic homolog 7; inhibits TGF-β. **SNAP-25**, synaptosomal-associated protein, 25 kDa. **Spry1**, sprouty homolog 1. **TBI**, traumatic brain injury. **TGF-β**, transforming growth factor beta. **TLR-4**, toll-like receptor 4. **TLX**, orphan nuclear receptor. **TNF-α**, tumor necrosis factor alpha. **Trk**, tyrosine kinase. **VEGF**, vascular endothelial growth factor.