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Review

Ferroptosis-relevant mechanisms and biomarkers for therapeutic interventions in traumatic brain injury

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Summary. Traumatic brain injury (TBI) is one of the most significant health care problems worldwide, causing disability and death especially among young individuals. Although a large range of agents and therapies have been proved beneficial to lesions post-TBI to some extent, effective treatments have not been translated to the clinic. As a newly discovered form of iron-dependent regulated cell death, ferroptosis has been implicated in TBI. In this review, we update the current state of knowledge related to second injuries post-TBI, including ferroptosis, oxidative stress, mitochondrial dysfunction, neuroinflammation and so on, which often lead to chronic symptoms and long-term disability. This review systematically summarizes the latest progress in the pathophysiological mechanisms of TBI, with a focus on providing references for proposing new multi-molecular targets for comprehensive therapeutic strategies based on ferroptosis-relevant mechanisms. In addition, biomarkers are essential diagnostic and prognostic tools in TBI. Several biomarkers associated with the outcome of TBI have been listed in this article, such as Pde10a, MDA, UCH-L1, S100A9, S100B, ALDOC, ACSL4, MBP and F2-Isoprostane. Therefore, the understating of ferroptosis-relevant mechanisms and biomarkers may contribute to development of promising therapies for TBI clinical trials.

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Introduction

Traumatic brain injury (TBI) is an injury caused by external force to the head, often causing either temporary or persistent damage according to the part and degree of the damage (Schimmel et al., 2017). Annually, TBI leads to many deaths, disabilities and consumes a large amount of healthcare resources due to hospitalization and long-term treatment (Bhatti et al., 2017). Primary injury initiated by damage (mechanical) to neurons, axons, glia and blood vessels occurs within hours or days following the initial injury and leads to cell death and brain parenchyma loss, while TBI patients suffer more from symptoms lasting for years or the rest of their lives called secondary injury. Primary injury then triggers a series of biochemical reactions that often cause secondary cell death or subcellular organelle dysfunction, resulting in prolonged neurodegeneration or even worse outcomes. Secondary injury includes ferroptosis, oxidative stress, mitochondria dysfunction, neuroinflammation and so on. These relevant mechanisms of TBI ordinarily interact with each other to promote the progression of neurodegeneration and contribute to further adverse outcomes. Researchers are devoting themselves to the investigation of ferroptosis-relevant mechanisms and the role of biomarkers in order to seek out new research lines and potential therapies for TBI patients, and eventually to find effective, safe and

stabilized approaches to improve outcomes.

Relevant mechanisms post-TBI

Ferroptosis

Ferroptosis is a kind of new-found non-apoptotic cell death which depends on iron and reactive oxygen species (ROS) and is characterized by lipid peroxidation (Latunde-Dada, 2017). This process was first characterized *in vitro* in cancer cells using synthetic small molecules, but it has been linked to cell death events following acute injury to brain and other tissues. Previous research about brain injury and neurodegeneration contains many descriptions of various pathological cell death events related to iron accumulation and oxidative stress, hence it is very probable that the previous observations associated with iron accumulation and oxidative stress following brain injury can be explained by the induction of ferroptosis (Magtanong and Dixon, 2018). Ferroptosis is different from apoptosis, necrosis, and autophagy morphologically, biochemically, and genetically (Weiland et al., 2019). This process does not depend on the caspase pathway and the only obvious alteration is the smaller size and disorganized cristae of mitochondria (Magtanong and Dixon, 2018).

System x_c^- is a heterodimer consisting of the SLC3A2 (4H2hc, CD98) regulatory subunit and the SLC7A11 (xCT) 12-pass transmembrane protein. The key to the induction of ferroptosis is the inhibition of System x_c^-, importing extracellular cystine into cells for exchange of intracellular glutamate and the following inactivation of the lipid hydroperoxidase glutathione peroxidase 4 (GPX4), finally leading to oxidation of membrane polyunsaturated fatty acids (PUFAs) (Latunde-Dada, 2017). System x_c^- is ATP-independent and sensitive to concentration of extracellular glutamate which always increases in many kinds of brain injuries due to excitotoxicity and many other mechanisms. When the above situation occurs, system x_c^- is inhibited and cystine cannot enter into cells, and then glutathione cannot be generated due to the deficiency of cysteine (i.e., the downstream production of cystine). The *bis*-allylic carbons of PUFAs are easily oxidized compared to saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs). GPX4 which is GSH-dependent is regarded as the key to initiation of ferroptosis, using GSH as a cofactor to transform PUFA lipid peroxides (L-OOH) which can be oxidized by Fe^{2+} from highly reactive alkoxy radicals (L-O•) into lipid alcohols (L-OH). Therefore, when GPX4 inactivates, the level of PUFA lipid peroxides (L-OOH) and reactive alkoxy radicals (L-O•) will rise to a dangerous level, eventually leading to catastrophic membrane damage (Doll and Conrad, 2017). On the other hand, iron is transported into labile iron pool in cells by transferrin through the carriage of transferrin receptor 1 (Tang et al., 2018). Critically, cellular iron is stored in a non-toxic form in ferritin, which is a cytosolic heteropolymer made of 24 subunits of ferritin heavy and light chains (FTH1/FTL) that can store up to 4500 iron atoms. Iron is stored in ferritin during times of iron excess and must be released under periods of iron demand. The predominant pathway for iron release from ferritin is via nuclear receptor coactivator 4 (NCOA4)-mediated selective autophagy whereby NCOA4 binds ferritin to traffic it to the lysosome where ferritin is degraded and iron is released for use by the cell (Quiles Del Rey and Mancias, 2019). Therefore, iron, ROS generation, excess glutamate and cysteine deficit contribute to ferroptosis collectively (Latunde-Dada, 2017).

Until now, only a few studies have shown that ferroptosis is implicated in TBI. In a controlled cortical impact injury (CCI) mouse model, ferrostatin-1 (a specific inhibitor of ferroptosis) was administrated and was able to significantly reduce iron deposition and neuronal degeneration while attenuating tissue damage and improving long-term outcomes (Xie et al., 2019). Notably, baicalein (12/15-lipoxygenase inhibitor) can decrease ferroptotic phosphatidylethanolamine oxidation and improve outcome after CCI in mice (Kenny et al., 2019). Recent work also showed that miR-212-5p may protect against ferroptotic neuronal death and behavioral deficits partially by targeting prostaglandin-endoperoxide synthase-2 (Ptgs2) in CCI mice (Xiao et al., 2019). Together, the above evidence suggests that ferroptosis contributes to TBI-induced neuronal death and behavioral outcome.

Oxidative stress

Oxidative stress is a key player in the complex cascade of secondary injury and prominently contributes to neurodegeneration and neuroinflammation (Ma et al., 2018). According to past research, oxidative stress is mainly caused by abnormal levels of two free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Schimmel et al., 2017). Once formed, these reactive radicals can start a chain reaction, like dominoes (Cornelius et al., 2013). Normally, the levels of the two radicals stay low by enzymes and antioxidants due to a dynamic balance between production and consumption. While this balance is broken following TBI, causing various subcellular organelle dysfunctions. The ROS regulators become damaged post-TBI, leading to an increased production of ROS from the electron transport chain. Excessive ROS cause lipoperoxidation of cell membrane, damaging various organelles and cellular structures, hence mitochondria are damaged due to lipoperoxidation of mitochondrial membrane (Halstrom et al., 2017). Antioxidants are molecules that can interact with free radicals and terminate the chain reaction before vital molecules are damaged (Cornelius et al., 2013). Antioxidant defense capacity depending on the redox and energetic state of mitochondria and normality of mitochondrial electron transport chain then decreases after the above reactions (Abdul-Muneer et al.,

2015), contributing to an imbalance between the production and consumption of ROS (Frati et al., 2017). Therefore, a vicious cycle is formed where higher levels of ROS cause damage to mitochondria and mitochondria dysfunction leads to more production of ROS in return, resulting in extensive cell death and subsequent neurodegeneration after TBI (Fig. 1).

Oxidative stress plays a vital role in the pathophysiological mechanisms of TBI, hence agents and therapies targeting oxidative stress are of great significance to the treatment for TBI. The use of targeted endothelial nanomedicine, with conjugates of the antioxidant enzyme catalase linked to anti-ICAM-1 antibodies, has efficacy in reducing oxidative stress at the BBB and attenuating neuropathological outcomes following TBI (Lutton et al., 2019). Li et al reported L-733,060, a tachykinin NK1 receptor antagonist, exerted neuroprotection by inhibiting oxidative stress and cell death after TBI *in vivo* (Li et al., 2019). In a model of TBI, dexmedetomidine (DEX) treatment relieved encephala edema and neuronal apoptosis and attenuated behavioral outcome. These protective effects were accompanied by upregulation of peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1α) expression, implying that the DEX may protect neurons against oxidative stress, mitochondrial damage and disintegration via the PGC-1α pathway (Li et al., 2018). Garcia-Gonzalez and co-workers analyzed the correlation between post-injury oxidative stress distribution and cognitive deficits in rats following blast-induced TBI. They found that the blast event was reconstructed in silico to provide mechanistic thresholds that best correlate with cognitive damage, indicating that this work is a tool for preventive and therapeutic innovations against blast traumatic brain injury (Garcia-Gonzalez et al., 2018).

Isoliquiritigenin (ILG) has neuroprotective effects and alleviates injuries induced by oxidative stress via the Nrf2-ARE signaling pathway after TBI in a mouse model of controlled cortical impact injury (Zhang et al., 2018). This study demonstrates that Nrf2 is an important protective factor against injuries induced by TBI via a mechanism that involves the promotion of Nrf2 protein transfer from the cytoplasm to the nucleus. Moreover, emerging evidence has demonstrated that activation of Nrf2 and its target genes may protect the brain against ischemia/reperfusion injury, and therapies aimed at increasing Nrf2 activity appear to be beneficial to alleviate brain injury through the suppression of oxidative stress (Zhang et al., 2017). In another similar

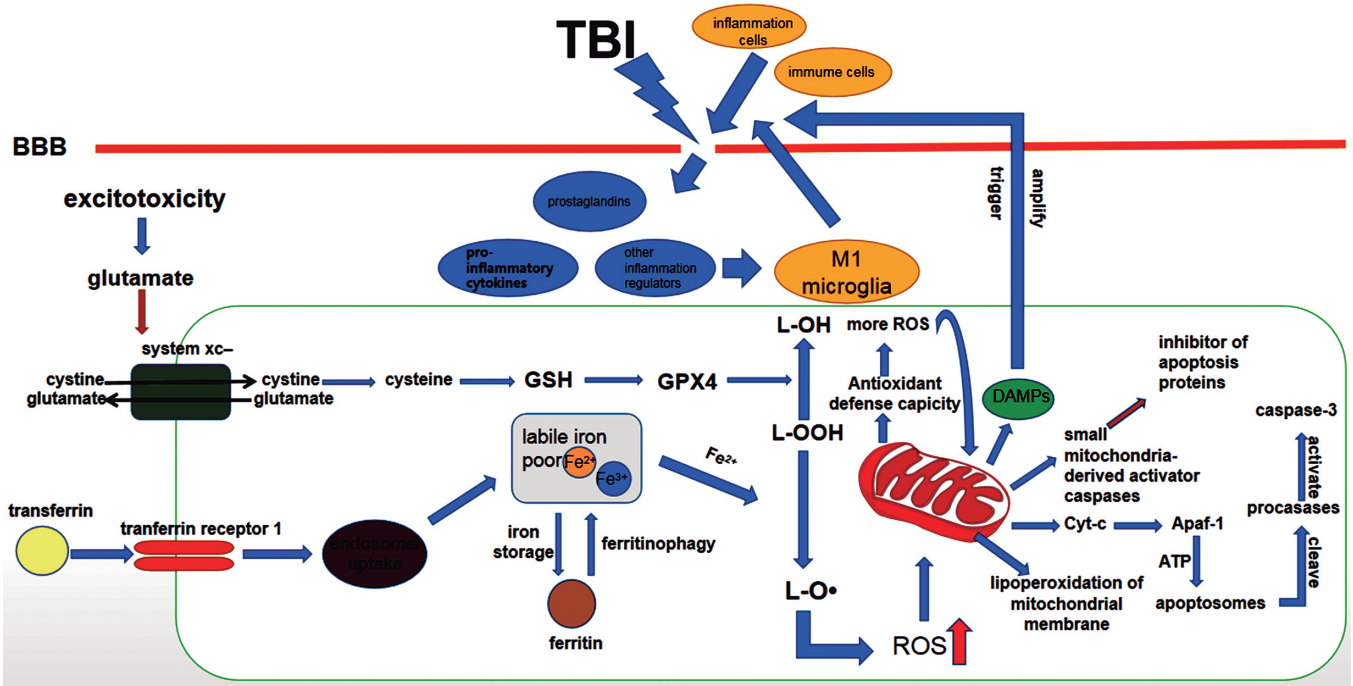

Fig. 1. The relationships among the different mechanisms of TBI, such as ferroptosis, oxidative stress, mitochondrial dysfunction and neuroinflammation. Oxidative stress is mainly caused by the imbalance between production and consumption of ROS post-TBI, and extracellular level of glutamate increases markedly, leading to the inactivation of system X_c- and subsequent GPX4, which can transfer PUFA lipid peroxides (L-OOH) into lipid alcohols (L-OH). Meanwhile, ferritinophagy is activated after TBI, releasing Fe^{2+}, and PUFA lipid peroxides (L-OOH) can be oxidized easily by Fe^{2+} to highly reactive alkoxy radicals (L-O•), causing a higher level of ROS. Excessive ROS damages mitochondria structurally and functionally, resulting in inactivation of inhibitor of apoptosis proteins and activation of AIFs. Moreover, mitochondrial DAMPs can trigger and amplify neuroinflammation.

study, ketamine exhibits neuroprotective effects by attenuating oxidative stress and apoptosis after TBI via Nrf2 pathway (Liang et al., 2018). Ketamine may be an effective therapeutic agent for the treatment of TBI although the use of ketamine is restricted due to its abuse or use as a hallucinogen. Notably, in terms of endogenous cellular mechanisms preventing ferroptosis, the antioxidant transcription factor Nrf2 is exquisitely positioned to modulate the onset and outcomes of ferroptosis and almost all genes thus far implicated in ferroptosis are transcriptionally regulated by Nrf2 (Abdalkader et al., 2018), indicating that the relationship exists among ferroptosis, oxidative stress and Nrf2.

Mitochondrial dysfunction

Mitochondria are enclosed by an outer membrane and an inner membrane, and the inner membrane has numerous folds called cristae that extend into the matrix. The shape and state of mitochondrial cristae regulates both the mitochondrial efficiency and metabolic functions. Following TBI, mitochondrial dysfunction occurs, with release of excessive ROS and decreasing ATP production (Hiebert et al., 2015). Mitochondrial dysfunction is linked to the production of ROS to a great extent. The ROS regulators become impaired post-TBI, contributing to the increased production of ROS from the electron transport chain. As a result, the excessive radicals lead to lipid-peroxidation of the mitochondrial membrane, disrupting normal mitochondrial function. Meanwhile, the function of mitochondria working as a calcium buffer to maintain homeostasis is damaged by excitotoxicity, and excessive ions cause the calcium-dependent mitochondrial permeability transition pore (mPTP) to stay opened, changing the potential of mitochondrial membrane, thus halting the production of ATP and destroying mitochondria (Schimmel et al., 2017). Moreover, cytochrome c is released from the mitochondria into the external part of the mitochondrial membrane. Cytochrome c binds with apoptotic protease activating factor (Apaf-1) and ATP to generate complex proteins called apoptosomes. These apoptosomes cleave to procaspases that activate caspase-3 (Hiebert et al., 2015). Eventually, faulty mitochondria lead to calpain activation, apoptosis inducing factor (AIF) release and caspase-3 activation, finally resulting in neuro-degeneration and neuronal death (Frati et al., 2017). SS-31, a novel mitochondria-targeted peptide, can directly decrease the ROS content, restore the activity of superoxide dismutase (SOD), and decrease the level of malondialdehyde (MDA) and the release of cytochrome c, thus attenuating neurological deficits, brain water content, DNA damage, and neural apoptosis. Moreover, SS-31 protects mitochondrial function through scavenging ROS from mitochondria and the potential mechanism is the PGC-1α pathway (Zhu et al., 2018). A posttranscriptional activation of an acid sphingo-myelinase (ASM), a key enzyme of the sphingolipid recycling pathway, resulted in a selective increase of sphingolipid in mitochondria during the first week post-TBI, accomplished by reduced activity of mitochondria cytochrome oxidase and activation of NLRP3 inflammasome, while ASM deficiency improved these TBI-induced mitochondrial abnormalities in a model of ASM KO mice (Novgorodov et al., 2019). In a traumatic neuronal injury (TNI) model in primary cultured cortical neurons, TNI decreased markedly the expression of PRDX3, which belongs to a highly conserved family of thiol peroxidases that scavenge peroxides in cells at both mRNA and protein levels (Zhang et al., 2018), and overexpression of PRDX3 by lentivirus (LV-PRDX3) transfection attenuated neuronal apoptosis, preserved mitochondrial membrane potential (MMP) and ATP generation after TNI, demonstrating that PRDX3 protects against TNI insult by preserving mitochondrial function and mitochondrial biogenesis, and may have potential therapeutic value for TBI (Hu et al., 2018). Notably, activation of the endoplasmic reticulum (ER) stress response apoptotic pathway was earlier than the mitochondrial apoptotic pathway, which means the regulation of ER stress is a potent target to protect neuronal mitochondria and promote recovery of mitochondrial function (Tan et al., 2018). Together, the relationship exists among mitochondrial dysfunction, oxidative stress and ER stress.

Neuroinflammation

Neuroinflammation, the major cause of secondary cell death, follows the initial impact and may persist for a long time. The inflammatory reaction to TBI was thought to occur solely through peripheral immune mediators entering via a disturbed blood brain barrier (BBB), it is now recognized as a robust and complex interaction between central and peripheral cellular and soluble components (Crowley et al., 2017). An initial inflammatory response is activated to repair damaged cells and protect the brain from invading pathogens. Pre-treatment of neutral sphingomyelinase inhibitor, such as Altenusin or GW4869 prior to lipopolysaccharide (LPS) stimulation for 4 h or 24 h, significantly downregulated gene expression of the pro-inflammatory mediators TNFα, IL-1β, IL-6, iNOS and CCL2 in microglia and reduced the release of nitric oxide and TNF-α in a controlled cortical injury (CCI) model in mice (Kumar et al., 2019). In another study, dimethyl fumarate (DMF) may be an effective neuroprotectant to attenuate neuroinflammation and neurobehavioral deficits induced by experimental TBI (Casili et al., 2018). The inhibitor of NF-κB kinase (IKK)/NF-κB signaling system is the key regulator of inflammation and is also critically involved in regulation of neuronal survival and synaptic plasticity according to past investigations, using an experimental model of closed-head injury (CHI) in combination with mouse models allowing conditional regulation of IKK/NF-κB signaling in excitatory forebrain neurons. Repression of IKK2/NF-κB signaling

in neurons increases the acute posttraumatic mortality rate, worsens the neurological outcome, and promotes neuronal cell death by apoptosis, thus resulting in enhanced proinflammatory gene expression, indicating that IKK2/NF-κB signaling protects neurons after traumatic brain injury through the regulation of neuroinflammation (Mettang et al., 2018).

Inflammatory cells, neutrophils, monocytes, and lymphocytes then cross the BBB and release prostaglandins, pro inflammatory cytokines, and other inflammation regulators, recruiting M1 microglia which can promote inflammation and immune cells to the brain by increasing the expression of chemokines and cell adhesion molecules (Schimmel et al., 2017). Microglia have two major polarization states, M1 phenotype and M2 phenotype. The M1 phenotype is related to the release of proinflammatory cytokines, while the M2 phenotype has been proved to be responsible for the release of anti-inflammation cytokines and for central nervous system (CNS) repair (Xu et al., 2017). Attenuation of proinflammatory M1 macrophage polarization and increase of anti-inflammatory M2, reducing edema development, enhancing cerebral blood flow, and improving neurobehavioral outcomes were observed after TBI (Fig. 1).

Astrocytes are also involved in injury sites post-TBI by working with neurotrophic factors to increase cell proliferation, aid in neuronal survival and inhibit programmed cell death (Schimmel et al., 2017). TLR4 knockdown attenuates brain injury and neuro-inflammation after TBI in rats by inhibiting neuronal autophagy and astrocyte activation (Jiang et al., 2018). Phospholipid cardiolipin is translocated from the inner to outer mitochondrial membrane post-TBI, tags damaged mitochondria for mitophagy but may also be a final pathway for inflammasome activation. Failure of mitophagy and resultant cell death can lead to release of mitochondrial DAMPs, which can trigger and amplify neuroinflammation (Crowley et al., 2017). It is noteworthy that inflammasomes can be activated by oxygen species, oxidative stress and lipid peroxidation, which can also lead to ferroptosis (Morris et al., 2018), indicating the relationship exists among neuro-inflammation, oxidative stress and ferroptosis.

Other mechanisms post-TBI

Other mechanisms post-TBI consist of excitotoxicity, the breakdown of blood-brain barrier (BBB), ES stress, mitophagy and so on. These mechanisms interact with each other, eventually contributing to cell death, neurodegeneration and a worse outcome. Therefore, further investigation into these mechanisms is crucial for discovery of new research lines and potential therapies.

Biomarkers

Biomarkers of TBI can be diagnostic (the identification of the cause and nature of a condition) or prognostic (predicting the likelihood of a patient's survival or outcome). The identification of several important biomarkers could play a vital role in diagnosing, and treating the underlying individual pathophysiological changes of TBI.

Pde10a

Phosphodiesterase 10A (Pde10a) is considered to be the node protein in the cAMP-PKA signaling pathway using Fuzzy c-means (FCM) clustering analysis, functional bioinformatics analysis and protein-protein interaction (PPI) network mapping of these FCM clusters. Pde10a modulates cAMP/PKA signaling cascade due to its higher affinity for cAMP in brain tissue and hence regulates phosphorylation of downstream protein kinase A (PKA). Pde10a was up-regulated at 6 and 24 hours and Pde10a mRNA level was significantly increased at 30 min post FPI (fluid-percussion brain injury) (Oliva et al., 2012). Moreover, Pde10a was acutely up-regulated in severity-dependent manner by mTBI (Song et al., 2018). In summary, Pde10a may serve as therapeutic target for mTBI by regulating the cAMP/PKA signaling pathway.

MDA

As the main components of cellular membranes, lipids have an indispensible role in maintaining the structural integrity of cells. Excessive oxidation of lipids alters the physical properties of cellular membranes and can cause covalent modification of proteins and nucleic acid (Gaschler and Stockwell, 2017). Malondialdehyde (MDA) is produced from polyunsaturated fatty acids (PUFAs) both by chemical reactions and enzyme-catalyzed reactions, and MDA is one of the most frequently measured biomarkers of oxidative stress, namely of lipid peroxidation (Tsikas, 2017). MDA can also represent an early indicator of neurosurgery-related brain injury within the cerebrospinal fluid (CSF) as shown in a non-randomized open prospective trial of 36 children (Piastra et al., 2020).

S100A9

Pro-inflammatory and amyloidogenic S100A9 protein is an important contributor to Alzheimer's disease (AD) pathology (Lee et al., 2017). Massive accumulations of amyloid-β peptide (Aβ) toxic oligomers and plaques are among the major AD pathological hallmarks and targets for therapeutic interventions (Ittner and Gotz, 2011). Previously TBI was linked to AD via the amyloid cascade and aggregation of Aβ peptide. S100A9 is a multifunctional calcium-binding protein with diverse roles in the inflammatory signaling pathways. An abundance of S100A9 was observed both extracellularly in the precursor plaques and in neurons or microglial cells.

Moreover, S100A9 can be a common denominator in inflammation-associated conditions in TBI and AD as a driving component of the amyloid-neuroinflammatory cascade, demonstrating that S100A9 may serve as a mechanistic link between TBI and AD (Wang et al., 2018). Taken together, S100A9 can be viewed as a prospective therapeutic target during various post-TBI stages.

S100B

S100B, an astroglial 11 kDa calcium-binding protein, is perhaps the most investigated biomarker for brain injury to date (Schulte et al., 2014). S100B was originally believed to directly relate to the extent of brain damage after insult and it is a reliable tool that can be used as a surrogate of imaging by contrast MRI and CT (Adrian et al., 2016). Nanomolar S100B concentrations enhance hippocampal progenitor cell proliferation, neuronal differentiation and cognitive recovery, while micromolar concentrations may foster inflammatory effects and counteract neuroplasticity (Baecker et al., 2019). It is probable that S100B is first released in the brain extracellular space, then transported to the cerebral spinal fluid (CSF) where a passive diffusion from CSF to blood. S100B is already present in the CSF and interstitial fluid, which when in communication with blood can elevate the marker's levels in the absence of release or ex novo synthesis (Dadas et al., 2018).

UCH-L1

UCH-L1 is an enzyme predominantly expressed in neuronal cytoplasm, removing abnormal proteins from the cell through ubiquitination under normal neuropathologic conditions and is imperative for healthy neuron function (Mahan et al., 2019). Human ubiquitin C-terminal hydrolase (UCHL1) is a well-accepted serum biomarker for severe TBI and can be used to detect the severity of a head injury (Singh et al., 2018). Elevated serum levels of UCHL1 during the acute phase following brain injury have been correlated with injury severity (Agoston et al., 2017).

ALDOC

Aldolase C (ALDOC), a brain type isozyme of a glycolysis enzyme, is expressed heterogeneously in subpopulations of cerebellar Purkinje cells (PCs) that are arranged longitudinally in a complex striped pattern in the cerebellar cortex, a pattern which is closely related to the topography of input and output axonal projections (Pamidimukkala et al., 2018). ALDOC levels were markedly high and stable in the first week after TBI (Halford et al., 2017). In another study, ALDOC was also detected at higher levels on the first day after trauma (Thelin et al., 2018). Overall, ALDOC may have significant value in early diagnosis of TBI.

MBP

As an oligodendrocyte protein, myelin basic protein (MBP) is a key structural component of the multi-layered myelin sheath covering nerve fibers, maintaining the correct structure of myelin, and interacting with the lipids in the myelin membrane (Liu et al., 2017). If MBP is degraded in myelinated fiber tracks of the white matter by proteases, degradation of axons and the myelin sheath (demyelination) occur subsequently. Therefore, MBP or its fragmented forms might be released into biofluid such as CSF (cerebrospinal fluid) and serum post-TBI (Agoston et al., 2017). Using cases of head injury and cases of sudden death (cardiopulmonary failure, no injuries of the head as control group), elevated MBP level was observed in the CSF of the deceased from the head injury group irrespective of whether the sustained head injury was fatal (severe) or moderate (not assumed to be the cause of death) (Olczak et al., 2018), hence MBP should be considered as early markers of severe and moderate TBI in post-mortem examination and may also be useful in diagnosis of forensic cases.

PEA-15

PEA-15, a small phosphoprotein, is prominently expressed in astrocytes, modulating essential cellular functions, including apoptosis and proliferation, and containing a death effector domain (DED) (Sung and Koh, 2017). PEA-15 binds the DED of both the Fas-associated death domain (FADD) and caspase-8, and inhibits apoptosis initiated by the Fas ligand. PEA-15 exerts anti-apoptotic effects also by inhibiting formation of the death-inducing signaling complex (DISC) and preventing activation of the caspase cascade. Moreover, its anti-apoptotic functions are modulated by the phosphorylation of PEA-15, hence the down-regulation of astrocytic phosphoprotein PEA-15 indicates astroglial injury (Koh, 2012). Some researchers have found that estradiol attenuates down-regulation of PEA-15 in ischemic brain injury (Koh, 2015). Another study by the same research group demonstrated that melatonin prevents down-regulation of astrocytic phosphoprotein PEA-15 in ischemic brain injury (Koh, 2011). Although PEA-15 has not been implicated in TBI, the above evidence indicates that PEA-15 may be a candidate therapy target for astroglial injury in TBI.

ACSL4

ACSL4, which belongs to a family of enzymes called acyl-coenzyme A synthetases long-chain isoform (ACSL), is a unique isozyme that preferentially catalyzes several polyunsaturated fatty acids (PUFAs) and is of great significance to PUFA metabolism (Kuwata and Hara, 2019). Overexpression of ACSL4 promotes ferroptosis due to its regulation of PUFAs, and if PUFAs reach to a dangerous level, ferroptosis occurs.

Moreover, knockdown of ACSL4 by specific shRNA inhibited erastin-induced ferroptosis in HepG2 and HL60 cells (Yuan et al., 2016).

F2-Isoprostane

As a family of prostaglandin F2-like compounds, F2-Isoprostane is produced when arachidonic acid-containing lipids are oxidized by free radicals (Tyurin et al., 2000). F2-Isoprostane is initially formed *in situ* on membrane lipids, and released by phospholipases, and can then be detected in biological fluids, including CSF (Varma et al., 2003). Enhanced oxidative damage in the brain of TBI patients and the association of higher CSF levels of F2-Isoprostane with a poor outcome have been observed (Yen et al., 2015). Overall, F2-Isoprostane is a reliable marker of oxidative stress post-TBI *in vivo*.

Perspectives

Until now, although many experiments have been conducted and a large range of agents and therapies have proved beneficial to lesions post-TBI to some extent, no effective and safe therapies for TBI have been translated to the clinic. Researchers have been devoting themselves to find the relative mechanisms post-TBI and biomarkers within the past several decades, trying to find new research lines and potential therapies. We will move forward to investigate the specific link and interaction between mechanisms post-TBI, such as the contact sites that the ER form with mitochondria, a hot topic in research (van Vliet and Agostinis, 2018). Moreover, we should further make use of proteomics, metabonomics and transcriptomics to find more candidate targets for TBI research. Lastly, large sample size, accurate experimental design and correction of selection biases are necessary for translation from trials to clinic. A complete understanding of TBI and disease progression will improve the clinical outcome for TBI patients.

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