Acute brain injury is the leading cause of human death and disability worldwide, which includes intracerebral haemorrhage, subarachnoid haemorrhage, cerebral ischaemia, traumatic brain injury and hypoxia-ischaemia brain injury. Currently, clinical treatments for neurological dysfunction of acute brain injury have not been satisfactory. Osteopontin (OPN) is a complex adhesion protein and cytokine that interacts with multiple receptors including integrins and CD44 variants, exhibiting mostly neuroprotective roles and showing therapeutic potential for acute brain injury. OPN-induced tissue remodelling and functional repair mainly rely on its positive roles in the coordination of pro-inflammatory and anti-inflammatory responses, blood-brain barrier maintenance and anti-apoptotic actions, as well as other mechanisms such as affecting the chemotaxis and proliferation of nerve cells. The blood OPN strongly parallel with the OPN induced in the brain and can be used as a novel biomarker of the susceptibility, severity and outcome of acute brain injury. In the present review, we summarized the molecular signalling mechanisms of OPN as well as its overall role in different kinds of acute brain injury.

**KEYWORDS**
apoptosis, intracerebral haemorrhage, neuroprotection, osteopontin, stroke, subarachnoid haemorrhage, traumatic brain injury

1 | INTRODUCTION

Acute brain injury, exemplified by stroke, traumatic brain injury (TBI) and hypoxia-ischaemia brain injury, is the leading cause of human death and disability worldwide. Stroke, which represents the primary reason for permanent disability in adults, can be divided into two types: ischaemic stroke, typically occurring in the setting of atherothrombosis, and haemorrhagic stroke, mainly due to the
rapture of cerebral arteries. The latter further consists of subarachnoid haemorrhage (SAH) and intracerebral haemorrhage (ICH) and accounts for approximately 10%-20% of strokes yet has higher mortality vs the former. TBI refers to sudden damage caused by mechanical force, occurring in traffic accidents, blast, wars, violence, terrorism, falls and sporting activity. TBI is currently the major source of fatality in young adults, with an annual global economic loss of approximately US$ 400 billion. Hypoxic-ischaemic brain injury is another frequent, fatal and crippling neurologic disease, particularly perinatal hypoxia-ischaemia remains the dominating cause of acute brain injury in the neonate. These acute brain injuries impose a heavy socio-economic burden, whereas effective therapies are still scarce. Notably, acute neurologic disorders share many common features and processes within the pathophysiology. Although pathogenic mechanisms involved in acute brain injury have been studied extensively, which include cellular apoptosis, neuroinflammation, blood-brain barrier (BBB) disruption, the prognosis of patients remains poor under current therapeutic strategies. New treatments targeting acute brain injury are urgently needed.

Osteopontin (OPN), a highly phosphorylated glycoprotein, is a complex adhesion protein and cytokine that interacts with multiple receptors including integrins and CD44 variants. OPN has been found in various tissues, including the brain, and plays an important role in cellular processes such as adhesion, motility and survival. Altered expression patterns of OPN have been observed in pathological conditions such as multiple sclerosis, atherosclerosis, myocardial infarction and cancers. Under normal conditions, OPN expression is weak in the brain, while under pathological conditions including Alzheimer’s disease, Parkinson’s disease, TBI, stroke and hypoxia-ischaemia brain injury, it is significantly increased in macrophages/microglia and astrocytes and exerts neuroprotective effects. In this review, we will highlight the molecular signalling pathways involved in neuroprotective part of OPN as well as its value as a potential therapeutic target, biomarker and predictor; we will also discuss the potential reason why exogenous OPN is not effective in some experimental models and put forth the limitations of current OPN research.

2 | GENERAL FEATURES OF OPN

Osteopontin is a highly phosphorylated extracellular matrix glycoprotein that is rich in aspartic acid and has acidic characteristics consisting of approximately 314 amino acids with a molecular weight ranging between 44 and 75 kD. OPN is initially found in osteoblasts and is later independently identified as secreted phosphoprotein 1 associated with neoplastic transformation and early T lymphocyte activation. The multiplicity of functions ascribed to OPN may reflect the presence of various isoforms, post-translational modifications, proteolytic processing, and diversity of cell types that OPN can interact with.

OPN gene is located in the small integrin-binding ligand, N-linked glycoproteins (SIBLING) cluster on chromosome 4 (4q13) in the human genome and on mouse chromosome 5. The gene contains seven exons, six of which are translated in the full-length isoform OPN-a. Alternative translation and splicing result in two splice variants with deletion of exon 5 (OPN-b) or deletion of exon 4 (OPN-c), which correlates with cancer progression and poor prognosis. Besides important isoforms, OPN is subject to significant post-translational modifications, including phosphorylation, sulfation, glycosylation and transglutamination, and regulation of these modifications represents the potential to control OPN function.

When cleaved by thrombin, OPN transforms into two types, the N-terminal fragment (trOPN-N) and C-terminal fragment (trOPN-C). TrOPN-N contains several highly conserved cell adhesive motifs including an Arg-Gly-Asp (RGD) sequence which binds to integrins such as αvβ1, αvβ3, αvβ5, αβ1 and α5β1. As well as a cryptic Ser-Val-Val-Tyr-Gly-Leu-Arg (SVVYGLR)-containing domain that is only exposed after thrombin cleavage and binds to α4β1, α9β1 and α4β7 integrins. whereas trOPN-C binds to CD44 variants. Moreover, mouse OPN cleaved by matrix metalloproteinase (MMP) 3/7 produces LRSKSFSQVSDQY, a novel α9β1 integrin-binding motif in the C-terminal fragment. In the human bone marrow, trOPN-N constitutes the predominant form and acts as a chemotactic factor promoting hematopoietic progenitor cell homing as well as T cell-derived IFN-γ secretion through its binding to α9β1 and α4β1 integrins. In T cells, trOPN-N upregulates IL-17, whereas OPN-C induces IL-10 downregulation by selectively interacting with CD44 isoforms. In carotid specimens, inflammation severity is only associated with trOPN-N expression, not with full-length OPN or trOPN-C. Thus, different terminal fragments may perform different functions.

3 | THE ROLES OF OSTEOPONTIN IN ACUTE BRAIN INJURIES AND OTHER DISEASES

Osteopontin can be secreted by multiple tissues as well as body fluids and serves as a regulator in various biological mechanisms including osteoclast function, wound healing, cell migration, immune response, insulin resistance and cellular processes. Expression levels of OPN vary in different cell types; however, the mechanism underlying the release of OPN is not yet fully understood. In the central nervous system, OPN expression is weak under normal conditions and can be distinctly upregulated in response to either injuries or inflammation. Accumulating evidence has demonstrated that OPN plays a significant role in neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease and multiple sclerosis, as well as acute brain injury including TBI, stroke, and hypoxia-ischaemia brain injury.

Different acute brain injury models share many common features and processes within the pathophysiology. To date, most studies suggest a neuroprotective role of OPN in these neurological diseases. Furthermore, numerous studies have demonstrated that intracerebroventricular injection or intranasal administration...
of exogenous OPN possesses neuroprotective roles and improves neurological outcome following ischaemic stroke and haemorrhagic stroke.35-38 (Table 1). As regards TBI, although studies have reported endogenous OPN positively influenced synapse reorganization and improved functional recovery,39-59 the study by Jullienne et al62 showed exogenous administration of OPN treatment following TBI did not alter lesion characteristics. Moreover, inconsistent therapeutic effects were found in the experimental neonatal hypoxia-ischaemia stroke treated with exogenous OPN.50,61,63,64

The aforementioned experimental hypoxia-ischaemia strokes were performed on neonatal mice or neonatal rats.60,61,63,64 Accordingly, an age-dependent manner of the neuroprotective effects of exogenous OPN has been introduced, in which OPN potentiates injury in the neonatal brain while resisting injury in the adult brain due to the different integrin subunit expression levels and distribution at different neuronal development stages.64 Additionally, the above models of the neuroprotective group60,61 and the non-neuroprotective group63,64 were established on rat pups and mice pups, respectively, and consistently, the animal heterogeneity of OPN efficacy has been proposed.62 Jullienne et al62 were the first to test the function of exogenous OPN after experimental TBI on adult male SD rats (similar ages to the models in cited experimental stroke researches); although several potential beneficial alterations were observed, exogenous OPN did not substantially improve lesion characteristics. It is worthy of note that the protective effects of OPN may be overridden by the hyperacute cerebral injury but sufficient to attenuate delayed thalamic neurodegeneration.65

Indeed, the regulation and function of OPN may be specific to each pathophysiological condition and may be context-dependent, spatiotemporal-dependent or cell-dependent, which perhaps explain the conflicting results regarding the efficacy of exogenous OPN in distinct disorders.25,67,68,69 Notably, OPN is proposed to play a dichotomous role in the neuroinflammation70,71 (see Section 4.1 for details) and can propel the progress of various autoimmune diseases such as multiple sclerosis.25,67 Thus, the ability to maintain the balance of anti-inflammatory and pro-inflammatory responses and to coordinate these signals with other inputs received by the cell (eg T cell-independent neurodegeneration in ischaemic brain injury vs T cell-mediated aggressive signals in multiple sclerosis contribute to the altered efficacy of OPN65) may define the ultimate role of OPN. Crucially, as mentioned above, different isoforms, different terminal fragments, various post-translational modifications of OPN may yield different impact on acute brain injury.32,43,44

In the present section, we will summarize current research findings concerning the OPN signalling in acute brain injury and integrate knowledge about its underlying mechanisms of neuroprotection and therapeutic potential. Some potential mechanisms for its beneficial effects are summarized in Figure 1, and promoting these signalling pathways may elicit a neuroprotective role of OPN.

4.1 | OPN and neuroinflammation

Inflammation plays a crucial role in the pathogenesis of acute brain injury.9,16,72,73 Potentially exacerbating secondary brain injury via inflammatory cascade in the acute stage, whereas beneficially promoting tissue remodelling and functional repair, inflammatory response induced by acute brain injury is suggested to be a double-edged sword.74 By early inhibition of inflammatory cascade, the coordination of pro-inflammatory and anti-inflammatory responses leads to the alleviation of the brain injury and better patient outcome.75 Interestingly, OPN is also indicated to exert dual roles in neuroinflammation.76 Many studies have highlighted the pro-inflammatory role of OPN in the pathogenesis of various autoimmune diseases, such as multiple sclerosis.25,65,67 During acute brain injury, although some researchers have reported that OPN exacerbates cerebral injury via neuroinflammation,71 OPN is also considered a promising target for anti-inflammation.58,65,77 Thus, considering the important role OPN plays in the initiation of inflammation70 and its overall neuroprotective role,49,53-61 OPN may maintain inflammation homeostasis with a negative feedback mechanism.

An important role of OPN in the inflammatory response is to trigger various leucocytes to cause a functional response and induce cytokine secretion, thereby forming an entire immune response.78 Activated microglia/macrophages and astrocytes are the main cellular sources of OPN induction in the central nervous system.27 Kang et al79 showed that microglia/macrophages and astrocytes induced OPN upregulation at different stages after brain injury. And then, the expression of OPN inversely recruited, activated and polarized additional microglial/macrophages and astrocytes in the lesional and perilesional area, which was based on the interaction of OPN with αvβ3 integrin and/or interaction with CD44.59,80-83 These receptors can also be upregulated after brain injury.52 The activated macrophages/microglia and astrocytes, as well as the induced OPN and its receptors, contribute to the succedent cytokine secretion, removal of the necrotic tissue, extracellular matrix formation, tissue remodelling, angiogenesis and gliosis.52,80,84,85

Inducible nitric oxide synthase (iNOS) exerts vital roles in inflammation response and is identified to be excitotoxic and neurotoxic.77 Moreover, iNOS-derived nitric oxide is known to activate MMP-9, which is involved in neuroinflammation, cell death and the BBB disruption.86-88 Previous studies have demonstrated that OPN is involved in iNOS pathway.50,89,90 Induced OPN provides a dose-dependent pattern suppressing iNOS expression after acute brain injury65,80 by increasing expression of integrin-β1 and then inhibiting the Janus kinase/signal transducers and activators of transcription.
### TABLE 1 The main findings of OPN-relevant therapeutic potential in acute brain injuries

| Disease                        | Rat models                                      | Agents and methods                                      | Main findings                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | References |
|-------------------------------|------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Ischaemic stroke              | Male SD rats, 250-300 g, MACO                   | R-OPN, injected stereotaxically into the striatum, 1 h post-MACO, 100 ng | Reduced the mean infarct volume, extended the therapeutic window at least to 12 h post-MACO                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Jin et al 58 |
|                               | Male SD rats, 250-300 g, MACO                   | OPN peptide, intranasally, 100 ng                        | Reduced mean infarct volume, ameliorated neurological deficits, suppressed the induction of iNOS; the RGD motif in OPN peptide and endogenous avβ3 integrin are essential for the neuroprotective effects                                                                                                                                                                                                                                                                                                                                                                                                   | Jin et al 50 |
|                               | Male SD rats, 240-280 g, MACO                   | Hyperbaric oxygen preconditioning                       | Improved neurological outcome, promoted expression of OPN, reduced the expression of IL-1β/NFκB and augmented Akt phosphorylation                                                                                                                                                                                                                                                                                                                                                                                  | Hu et al 93 |
|                               | Male C57Bl/6 mice, 8 to 10 wk, transient MACO   | R-OPN, intracerebroventricularly, immediately before and immediately after surgery, 50 ng | Reduced infarct size, increased phosphorylation of Akt and p42/p44 MAPK                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Meller et al 53 |
| Hypoxia-ischaemia brain injury| 7-day-old rat pups, unilateral ligation of the RCA followed by hypoxia | R-OPN, intracerebroventricularly, 1 h post-HI, 0.03 μg and 0.1 μg | Reduced apoptotic cell, cleaved caspase-3 and infarct volume, ameliorated bodyweight loss, improved long-term neurological impairment                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Chen et al 61 |
|                               | Postnatal day 10 SD rat pups, unilateral ligation of the RCA followed by hypoxia | R-OPN, intranasally, 1 h post-HI, 5 μg | Attenuated BBB permeability and brain oedema                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Dixon et al 60 |
|                               | Postnatal day 9 C57BL/6 mice pups, permanent occlusion of the RCA followed by hypoxia | R-OPN peptide, intranasally (350 or 2100 ng), intraperitoneally (10 mg/kg) or intracerebrally (100 ng), at several points in time | Did not exert neuroprotective effects                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Bonestroo et al 65 |
|                               | Postnatal day 5 C57BL/6J mice pups, ligation of the LCA followed by hypoxia | Full-length OPN protein and thrombin-cleaved OPN, intranasally (1.2 μg, immediately before and after HI) and intracerebroventricularly (5 μg, immediately before HI) | Did not exert neuroprotective effects                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Albertsson et al 64 |
| SAH                           | Male SD rats, 300-350 g, endovascular perforation model | Vitamin D3, intranasally | Attenuated BBB disruption through endogenous upregulation of OPN and subsequent CD44 and P-gp glycosylation signals in brain endothelial cells                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Enkhjargal et al 127 |
|                               | Male SD rats, 300-370 g, endovascular perforation model | R-OPN, intracerebroventricularly, 1 h before surgery, 0.1 μg | Improved BBB disruption, increased MAPK phosphatase-1, inactivated MAPK, reduced vascular endothelial growth factor-A                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Suzuki et al 125 |
|                               | Male SD rats, 280-320 g, endovascular perforation model | R-OPN, intranasally, minutes post-SAH, 5 μg | Improved neuronal cell survival, brain oedema and neurological scores, increased p-FAK and p-Akt expressions, decreased caspase-3 cleavage                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Topkoru et al 55 |
|                               | Male SD rats, 250-300 g, endovascular perforation model | Ephedra sinica extract, orally, immediately after the surgery, 15 mg/kg | Upregulated osteopontin signal, reduced the expressions of MMP-9, alleviated the BBB disruption, improved neurological functions                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Zuo et al 132 |
|                               | Male SD rats, 300-370 g, endovascular perforation model | R-OPN, intracerebroventricularly, 1 h pre-SAH, 0.02 and 0.1 g | Ameliorated bodyweight loss, neurologic impairment, brain oedema and BBB disruption, inhibited NFκB and MMP-9, maintained MMP-1 and ZO-1                                                                                                                                                                                                                                                                                                                                                                                                                                 | Suzuki et al 56 |
|                               | Male SD rats, 300-370 g, endovascular perforation model | R-OPN, Intracerebroventricularly, 1 hour pre-surgery or 5 hours post-surgery, 0.01 μg, 0.02 μg or 0.1 μg | Prevented vasospasm, improved neurological impairments, inhibited MAPKs, caldesmon and HSP 27                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Suzuki et al 148 |

(Continues)
TABLE 1 (Continued)

| Disease | Rat models | Agents and methods | Main findings | References |
|---------|------------|--------------------|---------------|------------|
| ICH     | Male SD rats, 280-320 g, a collagenase model | R-OPN, intranasally, 1 h after ICH, 1 μg, 3 μg or 9 μg | Attenuated brain inflammation and brain oedema improved neurological functions via integrin-β1 induced inhibition of JAK2/STAT1 pathway | Wu et al81 |
|         | CD-1 mice, 30-40 g, a collagenase model | R-OPN, intracerebroventricularly, 20 min pre-ICH, 10 ng, 50 ng, or 100 ng | Improved neurological scores and brain water content | Wu et al86 |
|         | Male SD rats, 280-320 g, injection of autologous blood into the right basal ganglia | R-OPN, intracerebroventricularly, 1 h post-ICH, 0.1 μg | Reduced neurological deficits, rotarod latencies and brain water content, increased p-Akt expression and decreased p-GSK-3β, Bax/Bcl-2 ratio and cleaved caspase-3 | Zhang et al99 |
| TBI     | Male SD rats, 275-375 g (9 to 12 wks old), moderate-to-severe controlled cortical impact | R-OPN, intranasally, 1 h post-TBI, 5 μg | Did not improve neurological score, lesion volumes, BBB dysfunction, or vascular characteristics; increased the microglial and HO-1 response | Julienne et al142 |
| Healthy rats | Male SD rats, 270-320 g | R-TNC, r-OPN, or both were injected into a cisterna magna | R-OPN had no effect on the vessel diameter but could reverse prolonged contractions of rat basilar arteries induced by r-TNC | Suzuki et al146 |

Abbreviations: BBB, blood-brain barrier; FAK, focal adhesion kinase; GSK-3β, glycogen synthase kinase 3β; HO-1, haem oxygenase 1; ICH, intracerebral haemorrhage; IL, interleukin; JAK2/STAT1, Janus kinase/signal transducers and activators of transcription 1; LCA, left carotid artery; MACO, middle cerebral artery occlusion; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NFκB, factor-κ-gene binding; OPN, osteopontin; RCA, right carotid artery; RGD, Arg-Gly-Asp; r-OPN, recombinant; SAH, subarachnoid haemorrhage; TNC, tenascin-C; ZO-1, zona occludens-1

1 (JAK/STAT1) pathway, which is closely linked to iNOS expression.86,91 Ladwig et al81 suggested that OPN downregulates iNOS by shifting the microglia phenotype towards an M2 to reduce iNOS-expressing M1 cells. In addition to iNOS pathway, OPN also downregulates MMP-9 expression by inhibiting interleukin (IL)-1β/nuclear factor-κ-gene binding (NFκB) pathway, through which IL-1β activates NFκB and subsequently mediates the induction of MMP-9.56,89,92,93

4.2 | OPN and apoptosis

Apoptosis is a highly complex energy-dependent programmed cell death process closely in correlation with neuron development and homeostasis under normal physiological conditions.94,95 Nevertheless, apoptosis also participates in the process of neurological disorders under pathological conditions and is considered as a key player for the progression and prognosis of patients.96-98 Previous studies have suggested that OPN ameliorates apoptosis and promotes cell survival during brain injury through several molecular signalling pathways, which may suggest a potential therapeutic strategy for treating acute brain injury.61,99,100 And accumulating studies indicate that OPN exerts direct anti-apoptotic actions via interaction with integrins or CD44.53,65,101,102

Among all the proteins involved in the initiation and execution of apoptosis, the caspases stand out as being crucial mediators of this process.103-105 Furthermore, of all the caspases, caspase-3 is probably the best understood and required for a large portion of distinct apoptotic processes and cell deaths.104,106,107 And not until caspase-3 zymogen is cleaved by an initiator caspase following the initiation of apoptosis signal does it have activity.106 Thus, suppression of caspase-3 cleavage may be effective to inhibit neuronal apoptosis.55,77,108 Consistently, experimental studies focusing on stroke and hypoxia-ischaemia neonatal brain injury found a remarkable reduction of caspase-3 cleavage following the OPN administration and suggested that OPN-induced neuroprotection was associated with the inhibition of caspase-3 activity.61 However, the underlying mechanisms of OPN-induced caspase-3 inhibition remain not entirely clear. Topkoru et al performed nasal administration of recombinant OPN 30 minutes after SAH induction on rat models, and they not only found a robust decrease of caspase-3 cleavage but also a notable increase of phosphorylated focal adhesion kinase (FAK) and phosphorylated Akt, in line with
the decline of brain oedema and improvement of neuronal cell survival and neurological status. Similarly, Zhang et al intracerebroventricularly injected recombinant OPN 1 h after ICH induced on rat models and found increased phosphorylated Akt expression and decreased phosphorylated glycogen synthase kinase 3 beta (GSK-3β), Bax/Bcl-2 ratio and cleaved caspase-3, consistent with attenuated brain water content and cell death. Moreover, Meller et al and He et al also found a reduction of cleaved caspase-3 accompanied by increased phosphorylated Akt expression and a better neurological function.

The PI3K/Akt signalling pathway is involved in numerous cellular processes, including apoptosis, BBB disruption, neurogenesis and angiogenesis. Inducing the phosphorylation of FAK via integrin receptor or CD44 receptor, OPN subsequently activates the PI3K/Akt signalling pathway. Then, phosphorylated Akt suppresses the pro-apoptotic proteins including Bax and Bad and upregulates the anti-apoptotic proteins such as Bcl-2. These molecules play important roles in the caspase-dependent pathway, in which caspase-3 is considered to be a common downstream protein, and the regulation of phosphorylated Akt results in the suppression of caspase-3 and apoptosis. In addition, phosphorylated Akt induces the phosphorylation and subsequent inactivation of GSK-3β, which is also pro-apoptotic and has been found to aggravate brain injury in experimental ICH, ischaemic stroke and traumatic brain injury. Additionally, inhibitors of FAK and PI3K administrated to the rat models could abolish the protective effects of OPN. Thus, the PI3K/Akt pathway may play a critical role in the OPN-induced anti-apoptotic actions.

Besides, some proteins involved in the process of apoptosis after acute brain injury such as FAK, Bcl-2, PI3K and Akt may overlap with autophagic pathways. Emerging studies suggest that OPN enhances autophagy and reduces apoptosis after experimental SAH through FAK signalling, resulting in the attenuation of early brain injury and improvement of long-term outcome.

### 4.3 OPN and BBB disruption

The BBB is built up by specialized monolayer endothelial cells which are connected by tight junctions without fenestrations. The endothelial barrier is also supplemented with a large number of pericytes, which share the common basal membrane with the endothelial cells. Besides, the abluminal surface of the microvascular basement membranes is covered by astrocytic perivascular end-feet. This major barrier plays a significant role in maintaining brain haemostasis and protecting the brain from disease and injury. Dysfunction of the BBB has been described as one of the independent risk factors for poor prognosis after acute brain injury, which may amplify inflammation and lead to further parenchyma damage and oedema by allowing more blood-borne cells and substances to flow into the brain parenchyma. Previous studies have...
demonstrated that OPN is significantly induced and locates at reactive capillary endothelial cells and astrocytes during the recovery of BBB function, suggesting an important role for OPN in BBB reconstruction. And still, OPN exerts effects of BBB maintenance via interaction with integrins and CD44 receptors.

As mentioned above, MMP-9 is involved in aggravation of BBB disruption in addition to facilitating the inflammatory cascade and cell death. The balanced interaction between MMP-9 and its corresponding inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1), determines the severity of BBB disruption. Induced by acute brain injury, oxidative stress and IL-1β then activate NF-κB, which directly upregulates MMP-9 and inhibits TIMP-1 levels in the brain. There is plenty of evidence that MMP-9 plays a role in degrading the extra-cellular matrix of cerebral microvessel basal lamina including laminin, fibronectin, collagen IV and zona occludens-1 (ZO-1) and causing BBB disruption.  ZO-1 belongs to endothelial tight junction-related proteins, loss of which will increase BBB permeability. The inhibition of IL-1β/NFκB pathway and reduction of oxidative stress have been widely reported by exogenous OPN administration, through which OPN downregulates MMP-9 expression and upregulates TIMP-1 to protect cerebral microvessel basal lamina and subsequently maintain BBB integrity. Furthermore, the FAK/PI3K signalling pathway is also involved in BBB dysfunction following experimental brain injury. It has been demonstrated that exogenous OPN promotes the phosphorylation of FAK and subsequently activates the PI3K, leading to the induction of Ras-related C3 botulinum toxin substrate 1 (Rac-1) expression and the preservation of BBB integrity.

Interestingly, endogenous OPN induced after acute brain injury may ameliorate BBB disruption through different mechanisms. Mitogen-activated protein kinase (MAPK) can influence the expression of vascular endothelial growth factor (VEGF)-A, which is a potent inducer of vascular permeability. Endogenous OPN induction after acute brain injury keeps angiopoietin (Ang)-1 levels within the normal range, which inhibits the effect of VEGF-A with a robust anti-permeability property. And blockade of endogenous OPN reduces Ang-1 and MAPK phosphatase-1, an endogenous MAPKs inhibitor, then activates MAPKs including p38, c-Jun N-terminal kinase, and extracellular signal-regulated kinase 1/2, resulting in the induction of VEGF-A and the exacerbation of BBB permeability. In addition, endogenous OPN has been shown to induce reactive astrocyte polarization, which is pivotal to the complete neovessel coverage by astrocyte end-feet and the integrity of BBB. Moreover, Enkhjargal et al demonstrated that brain OPN protected BBB against disruption via the CD44/P-glucoprotein glycosylation pathway in the vascular endothelial cells.

### 4.4 Additional aspects of OPN

Previous studies also revealed that OPN exerted positive roles in the proliferation, survival and differentiation of various cells, including cerebrovascular smooth muscle cells, neural progenitor cells and neural stem cells. Moreover, adhesive and chemotactic properties of OPN exert function in the lateral migration of neuroblasts from the subventricular zone to the injured region following focal cerebral ischaemia and ICH. OPN also reportedly enhances the sensitivity of adult corticospinal neurons to insulin-like growth factor 1, ameliorates cerebral vasospasm and stabilizes smooth muscle cell phenotype following acute brain injury.

### 5 OSTEOPONTIN AS A BIOMARKER OF ACUTE BRAIN INJURY

Considering the upregulation and multifarious effects of OPN following acute brain injury, it may reflect the occurrence and severity of neurological damage. And the OPN induced in the brain strongly parallels increased OPN protein in the blood, which may owe to the leakage of OPN produced in the brain or at the interface between brain and blood into the circulation. Thus, the plasma/serum OPN can be used as a novel biomarker of the susceptibility, severity and outcome of acute brain injury. Nakatsuka et al demonstrated that plasma OPN levels ≥ 915.9 pmol/L at days 10-12 was the most useful predictor of poor outcome and that plasma OPN levels ≥ 955.1 pmol/L at days 1-3 was an independent predictor of poor outcome after SAH. Li et al suggested that plasma OPN levels at 48 h after hypoxic-ischaemic encephalopathy were accordant with the severity of brain damage at day 7 recovery. Carbone et al showed serum OPN levels (cut-off value, 30.53 ng/mL) were the best predictor of poor outcome at day 90 after ischaemic stroke, which is analysed by receiver operating characteristic curve, while Jing et al showed the thrombin-cleaved OPN levels were significantly correlated with the clinical outcome 12 months after hospital discharge and could discriminate ischaemic stroke patients from healthy individuals at a cut-off of 166.8 ng/mL. Plasma OPN levels also predict the atherosclerotic plaque destabilization. Ozaki et al revealed that plasma thrombin-cleaved OPN N-terminal levels of >5.47 pmol/L were independent predictors of atherothrombosis even within 3 h from stroke onset, thus exhibiting the early diagnostic value. The sensitivity and specificity of these cut-off values are shown in Table 2.

### 6 CONCLUSIONS AND FUTURE PROSPECTS

In the present review, we mainly focused on the roles and therapeutic potential of OPN in acute brain injury including ICH, SAH, cerebral ischaemia, TBI and hypoxia-ischaemia brain injury. OPN plays a bidirectional role in neuroinflammation. Following acute brain injury, OPN participates in the initiation of inflammation, meanwhile maintaining inflammation homeostasis with a negative feedback mechanism. In addition, BBB maintenance and anti-apoptotic actions are involved in the OPN-induced neuroprotection. Moreover, OPN exerts positive roles in the chemotaxis, proliferation, survival and differentiation of various cells, including cerebrovascular smooth
muscle cells, neural progenitor cells, neural stem cells and neuroblasts. Collectively, OPN is a pleiotropic extracellular matrix glycoprotein that is mainly believed to be neuroprotective during acute brain injury. These beneficial effects of OPN are induced through its interaction with integrins and CD44. Therefore, OPN represents a potential therapeutic target for acute brain injury. Furthermore, the blood OPN strongly parallels the OPN induced in the brain and can be used as a novel biomarker of the susceptibility, severity and outcome of acute brain injury.

Notably, although endogenous OPN is believed to be neuroprotective, studies regarding TBI and neonatal hypoxia-ischaemia brain injury suggest that exogenous OPN treatment may be ineffective or even harmful. Thus far, the reason for this discrepancy remains unclear. The efficacy of OPN may depend on the region, injury pattern and stage of brain lesions, and the age and species of victims. Additional experiments based on these variables need to be conducted to determine whether exogenous OPN can indeed activate neuroprotective signalling pathways and consequently improve neurological outcome. Furthermore, the diversity of receptors, various isoforms, post-translational modifications and different proteolytic fragments contribute to the multiplicity of functions ascribed to OPN, which makes things more difficult to target this molecule or its putative receptors. On the contrary, precise regulation of these different types of OPN and receptors may represent the potential to control its function. Therefore, in future studies, forms of OPN and corresponding receptors should also be specified, which is not satisfactory in previous studies.

Currently, no OPN-relevant clinical trials are being conducted. Numerous intracerebroventricular or intranasal administrations of recombinant OPN or OPN peptide in experimental models have been carried out. These administration routes, however, limit the clinical application value of OPN-related agents, although intranasal administration of vitamin D, oral administration of Ephedra sinica extract and hyperbaric oxygen preconditioning have been shown to confer their neuroprotection through endogenous OPN signalling pathways. Besides, there is no agreement about the time window and the appropriate dose for administration hitherto. Studies concerning the protocols that are more applicable to the clinical application of OPN are warranted.

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### CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

### AUTHOR CONTRIBUTIONS

Yunxiang Zhou: Data curation (equal); Writing-original draft (equal); Writing-review & editing (equal). Yihan Yao: Writing-review & editing (equal). Lesang Shen: Validation (equal); Writing-review & editing (equal). Jianmin Zhang: Writing-review & editing (equal). John H.
Zhang: Conceptualization (equal); Writing-review & editing (equal).
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DATA AVAILABILITY STATEMENT
No data, models or code were generated or used during the study.

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