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Review

Osteopontin in post-subarachnoid hemorrhage pathologies

Reona Asada[1,†], Hidenori Suzuki[1,*,†]

[1]Department of Neurosurgery, Mie University Graduate School of Medicine, Tsu, 514-8507 Mie, Japan
*Correspondence: suzuki02@med.mie-u.ac.jp (Hidenori Suzuki)
[†]These authors contributed equally.

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Abstract

Rupture of intracranial aneurysms causes subarachnoid hemorrhage (SAH), of which the treatment remains the most difficult among cerebrovascular disorders even in this modern medical era. Following successful surgical ablation of ruptured intracranial aneurysms, other conditions may be encountered including delayed cerebral ischemia and chronic hydrocephalus, in addition to early brain injury. Osteopontin (OPN) is one of matricellular proteins that have cytokine-like effect on various cells and act as secretory extracellular matrix proteins between cells. The complexity of OPN functions is attributed to its several isoforms, cleavage sites and functional changes determined by its differing isoforms following various cleavages or other post-translational modifications. Notably, OPN functions beneficially or harmfully in accordance with the context of OPN upregulation. In the field of aneurysmal SAH, OPN has exerted neuroprotective effects against early brain injury and delayed cerebral ischemia by suppressing apoptosis of neurons, disruption of blood-brain barrier, and/or cerebrovascular constriction, while excessive and prolonged secretion of OPN can be harmful through the occurrence of chronic hydrocephalus requiring shunt surgery. This is a review article that is focused on OPN's potential roles in post-SAH pathologies.

Keywords: Cerebral infarction; Cerebral vasospasm; Early brain injury; Osteopontin; Shunt-dependent hydrocephalus; Subarachnoid hemorrhage

1. Introduction

A subarachnoid hemorrhage (SAH) is one of stroke types [1]. The incidence of SAH is about 5% of all strokes, but its death rate reaches as high as 50% and it occurs in relatively younger people compared to other strokes such as cerebral infarction and cerebral bleeding [1,2]. Most of spontaneous SAH is caused by rupture of cerebral aneurysms [1,2]. After the onset of aneurysmal SAH, multiple pathologies occur including early brain injury (EBI), delayed cerebral ischemia (DCI) and chronic shunt-dependent hydrocephalus, and make its clinical course complicated, while little clinical evidence has been established as to treatments against the pathologies after SAH [3,4]. EBI is any type of brain injury occurring within three days of SAH onset and is believed as a major causative factor of poor outcomes in aneurysmal SAH patients [5]. EBI is also considered to lead to the occurrence of DCI, resulting in cerebral infarct in severe cases, which is another important causative factor for unfavorable outcomes [6]. Delayed cerebral infarction is caused by DCI due to cerebrovascular spasm and vasospasm-irrelevant pathologies developing 4-14 days or later after SAH [6–9]. Chronic shunt-dependent hydrocephalus develops at day 14 and thereafter and is considered as an additional prognosticator for aneurysmal SAH patients [10].

A matricellular protein is an inducible, polyfunctional, secretory and nonstructural protein that acts like cytokines on various cells and as extracellular matrix proteins between cells [11]. Osteopontin (OPN), which consists of about 314 amino acids and has molecular weights of 44–75 kD, is a representative of matricellular proteins [12,13]. OPNs are present on various organs such as the brain [12,13], and have diverse effects from beneficial to harmful, depending on the circumstances or according to various post-translational modifications including cleavage, glycosylation, sulfation, transglutamination and phosphorylation [13,14]. However, it has been considered to be neuroprotective in aneurysmal SAH [14,15]. Recently, it was reported that excessive and highly sustained OPN levels could be harmful in a clinical setting of aneurysmal SAH due to the occurrence of chronic hydrocephalus requiring shunt surgery [16]. In this review, therefore, the focus is on the potential from-bench-to-bedside problems in the use of OPN as a therapeutic molecular target against brain injuries after aneurysmal SAH.

2. OPN

2.1 General characteristics of OPN

OPN is a non-classical extracellular matrix glycoprotein referred to as a matricellular protein. It is different from a classical extracellular matrix protein and has the following common characteristics: it undergoes transient upregulation of expression at a specific stage of development or under a pathological condition, can be present as a soluble protein, induces cell motility, and can interact with a variety of biologically active substances such as growth fac-

tors, chemokines and proteases to modulate the function of the bioactive substances and to act as a reservoir of the bioactive substances. *OPN* gene disruption produces no apparent phenotype, although OPN knock-out mice manifest diverse phenotypes responsive to numerous insults [17,18]. In contrast, a classical extracellular matrix protein is constitutively expressed, present as a structural component and provides scaffolds for the stability of cell–cell adhesions [17]. Transcription of OPN is accelerated by transforming growth factor-β, platelet-derived, epidermal or basic fibroblast growth factors, cytokines (interleukin-1α, interleukin-2, or tumor necrosis factor-α) and endothelin [18]. OPN is known to have 16 signal peptides in the N-terminus, in addition to Ser/Thr phosphorylation sites and a Gly-Arg-Gly-Asp-Ser motif [19].

2.2 Subtypes of OPN

The gene of *OPNs* is located in a small integrin-binding ligand N-linked glycoprotein cluster on chromosome 4 (4q13) in humans [20]. *OPN* genes contain 7 exons, and 6 of the 7 exons are translated into total length of OPNs, which are also called OPN-a [21]. In humans, alternative translation and splicing develop four splicing variants: OPNs-b lacking exon 5, OPNs-c lacking exon 4, OPNs-4 lacking exons 4 and 5, and OPNs-5 containing one extra exon by retaining a portion of intron 3 [22]. OPN can be proteolytically cleaved by thrombin and matrix metalloproteases (MMPs) [22]. Upon thrombin cleavage at the Arg168-Ser169 site, OPN transforms into two types of OPN fragment, one an N-terminal fragment (OPN-N) and the other a C-terminal fragment (OPN-C) [14]. OPN-Ns contain several cell adhesive motifs, which are highly conserved, such as Arg-Gly-Asp (RGD) sequences interacting with integrin receptors including αvβ1, αvβ3, αvβ5, α5β1 and α8β1; and cryptic Ser-Val-Val-Tyr-Gly-Leu-Arg (SVVYGLR)-containing motif sequences binding to α4β1, α4β7 and α9β1 integrin receptors: the latter ones are exposed only after the cleavage by thrombin [23,24]. OPN-C binds to CD44 variants [14]. MMPs-2, -3, -7, -9 and -12 also cleave OPN at various sites, contributing to functional changes of OPN [22]. Additionally, OPNs are subject to a variety of post-translational modifications such as transglutamination, glycosylation, sulfation and phosphorylation, which also potentially control OPN function [25].

OPN exists in two distinct isoforms, one secreted, the other intracellular: the former isoforms exert their functions through activating their receptors on cell surface, while the latter ones bind to myeloid differentiation primary-response protein 88 (MyD88), which is the down-stream of Toll-like receptors, to exert their effects [26]. The coupling of secreted OPN and a specific receptor induces a signal transduction pathway downstream of OPN to secrete cytokine and chemokine, and to cause cellular apoptosis, differentiation, adhesion and migration, and is physiologically or pathologically involved in various processes such as homeostasis, angiogenesis and immune responses in a large number of tissues [11,27]. Another complexity is that the biological role of OPN is highly diverse, ranging from beneficial to harmful, and is often seemingly contradictory in accordance with the biological scenarios on OPN's upregulation [28]. For example, even in the same organ, OPN acts detrimentally for degenerative or demyelinating disorders including Alzheimer's disease, Parkinson's disease and multiple sclerosis, while acts favorably against acute brain injuries after ischemic and hemorrhagic strokes, although OPN is upregulated in all the situations [29].

3. OPN in clinical settings of aneurysmal SAH

3.1 Role as a biomarker

OPN exists in a body fluid and as with other matricellular proteins is available as biomarkers to monitor the progression or regression of tumors and cardiovascular, inflammatory and fibrotic diseases [30]. In patients with aneurysmal SAH, plasma OPN-a levels increased from an acute stage to peak at four to six days post-SAH [31]. In SAH patients with worse admission clinical grades and poor three month outcomes, plasma OPN-a levels were significantly higher at least from day 1 to day 12, while higher OPN-a levels were revealed in patients with delayed-onset non-iatrogenic cerebral infarct at days 1–3 and 10–12, DCI at days 4–9 and chronic shunt-dependent hydrocephalus at days 10–12 [16,31]. OPN-a levels in the peripheral blood at days 1–3 after SAH may reflect the severity of EBIs [30], which have been shown to influence the development of delayed cerebral infarction by experimental and clinical studies [30,32]. Cerebrospinal fluid (CSF) concentrations of OPNs (unknown subtypes) were much higher compared with plasma ones at days one, four and eight in aneurysmal SAH patients, indicating that OPN is primarily produced in the central nervous system after SAH [33]. It was also suggested that an EBI-unrelated cause of DCI was present, because DCI was accompanied with higher plasma levels of OPNs-a not at days 1–3 but 4–9 [31].

It has been reported that OPNs are upregulated in injured brain tissues and cerebral arteries in a delayed manner after experimentally induced SAH and induce intrinsic defense mechanisms on EBIs, cerebral vasospasm and therefore DCI [11,34]. If the findings in experimental studies can be applied to clinical practice, plasma OPN-a may show a delayed increase by reflecting the severity of preceding tissue injuries associated with EBI, cerebral vasospasm and DCI and/or the extent of the resultant inflammatory reactions [17,31]. OPN that is upregulated in injured tissues may exert healing effects, but intrinsically induced OPN levels may be insufficient to recover the injury immediately. However, in the case that exogenous OPNs are administered, OPNs may exert neuroprotective effects [11,34]. Nevertheless, it should also be remembered that when OPN remains highly induced from days

4–6 to days 10–12 post-SAH, chronic hydrocephalus requiring CSF shunting surgery may develop, possibly via an undue OPN's healing effect that promotes fibroses in the subarachnoid and other CSF spaces [16]. Despite this, plasma OPN-a levels could be used as a biomarker for EBI, cerebral vasospasm, DCI, chronic hydrocephalus requiring shunt surgery or poor outcomes on the basis of measurement time points [16,30]. Recently, machine learning analyses have been developed and constructed early prediction models for the occurrence of DCI, angiographic cerebrovascular spasm, and delayed cerebral infarct using admission clinical factors and plasma levels of three types of matricellular proteins (OPN-a, periostin, and galectin-3) at post-SAH days one to three in a clinical setting [35]. Periostin and galectin-3 have been implicated in EBI and DCI or delayed cerebral infarction after SAH [36–44]. However, a clinical study demonstrated that plasma OPN-a levels at days one to three were also one of the three most important features in all of the three prediction models with high accuracy and sensitivity [35].

3.2 Therapeutic application

OPN plays diverse roles in neuroinflammation, blood-brain barrier (BBB) disruption and cellular apoptosis and has a positive effect on chemotaxis, proliferation, differentiation or survival of a variety of cells such as smooth muscle cells in the cerebral artery, neural progenitor or stem cells and neuroblasts in the central nervous system by interacting with integrin and CD44 receptors [14]. Collectively, OPNs are believed to be neuroprotective during an early phase of SAH and represent potential therapeutic targets against post-SAH cerebral tissue injuries. However, clinical trials relevant to OPNs have never been conducted. Recombinant OPN (r-OPN) or OPN peptide has been administered intrathecally or intranasally to animal models of SAH, but the administration route limits the clinical applications of OPNs [14]. Orally administrated *Ephedra sinica* (a Chinese herb extract) and preconditioning with hyperbaric oxygen conferred protection on acute brain injuries by enhancing endogenous OPN's signaling [14,45], offering an alternative possibility of the therapeutic application. Also, computer-aided drug design methods have advanced to minimize ligands that should be screened in bioassays, saving the cost, time, and efforts required for developing a new drug [46]. Such technologies will help develop new OPN-relevant drugs in future studies.

4. OPN in EBI after experimental SAH

4.1 Possible mechanisms of EBI development after SAH

EBI is defined in clinical settings as post-ictus cerebral damage that occurs within 72 hours following rupture of an intracranial aneurysm and before the development of DCI [47]. In experimental SAH studies, endovascular perforation models in mice or rats have shown an acute metabolic change similar to clinical conditions and have been established as the most suitable models for studies of EBI after SAH [5,48,49]. Cerebral tissue injuries that occur under ischemic or hemorrhagic stroke are characterized by imbalances of metabolism and energy within cerebral tissue cells and the structural damages are followed by a decrease in cerebral blood flow and the disruption of cellular membranes [50]. This leads to mitochondrial impairment, inflammatory or oxidative reactions, ionic gradient breakdown, glutamate-mediated excitotoxicity, stress signaling and finally to cell deaths [50]. After SAH, decreasing CSF glucose-to-lactate ratios were reported to be accompanied by unfavorable outcomes [51], but the role of extracellular lactate is still controversial if the lactate supports basal metabolism in neurons or contributes to neurotransmission activity per se [52,53]. Recently, it has been revealed that neurons and glia orchestrate a metabolic reaction to sustain energy demands and the redox balance of neuronal activities or cause post-stroke brain injuries such as aquaporin 4-medated brain edema and neuronal apoptosis, which may be reduced by hypothermia-induced suppression of metabolic activity [50,53–56]. However, in the models, EBI has been studied in the same time frame of 72 hours as the time course of cerebrovascular spasm occurrence: therefore, it is impossible to differentiate brain injuries by EBI from those caused by cerebral vasospasm or DCI [5,48,49]. Nevertheless, it has been revealed that EBI involves various pathophysiological processes including a surge of intracranial pressure, subsequent transient occurrence of global cerebral ischemia, mechanical brain injuries by associated intracerebral hemorrhage and acute hydrocephalus, and subarachnoid spread of blood-derived products [6,57]. These events are followed by excitotoxicity, inflammatory and free radical reactions, microcirculatory disturbance, increased permeability of BBB, cortical spreading depolarization or depression, and others, finally leading to neuronal apoptosis [6,57]. Glial cells are considered to contribute to post-stroke brain injuries or pathophysiology in an acute phase [58,59], as well as intimately and actively control neuronal activities and synaptic neurotransmissions in brain function [60,61]. These phenomena interact each other: for example, BBB disruption causes further infiltration of toxic blood components and inflammatory cells into brain tissue and furthermore aggravates inflammatory reactions, BBB permeability, brain edema and brain injury [62–64]. Although OPNs are pleiotropic glycoproteins, OPNs have been always protective for disruption of BBB and apoptosis of neurons, which are important constituents of EBI following experimentally induced SAH [11].

4.2 BBB disruption

Post-SAH ischemia by elevated intracranial pressure, mechanical injury on cerebral tissues and extravasation of blood components as well as their degradation products disrupt BBB associated with activation of multiple inflam-

matory and other signaling pathways that are independent or interconnected, exacerbating EBI [62,65,66]. Although post-SAH BBB disruption is caused by many molecules and pathways and has crucial roles in EBI [62], BBB disruption induces only transient damage and recovery mechanisms may ensue [67]. In endovascular perforation SAH models in rats, endogenous OPN induction occurred in reactive astrocytes and brain capillary endothelial cells and peaked at 3 days after SAH, at which BBB disruption spontaneously recovered [68]. Upregulated OPN increased expression of angiopoietin-1, in addition to mitogen-activated protein kinase (MAPK) phosphatase-1 that is an endogenous MAPK inhibitor, to inactivate MAPKs such as extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinase and p38, and suppressed expressions of vascular endothelial growth factor-A through RGD-dependent integrins [68]. These bioactive molecules are all implicated in progression or regression mechanisms of BBB injuries, acting simultaneously or at different stages through the course of BBB disruption after SAH [68]. Vascular endothelial growth factor-A potently induces the disruption of BBB, while angiopoietin-1 blocks effects of vascular endothelial growth factor-A, possibly by regulating MAPK activation [69]. The blockage of endogenous OPN expression prevented the pathways and the recovery of BBB disruption [70]. Endogenous induction of OPNs is considered to serve as an important intrinsic mechanism for BBB protection or repair after SAH (Fig. 1).

OPNs have also been experimentally examined as to the possibility as therapeutic agents. In endovascular perforation SAH models in rats, r-OPN administration prevented BBB disruption, brain edema formation as well as body weight loss and improved neurological function after SAH by deactivating a nuclear factor-kappaB (NF-κB)-mediated signaling pathway, resulting in MMP-9 downregulation, tissue inhibitor of MMP-1 maintenance and the resultant preservation of substrates for MMP-9 such as brain capillary basal lamina protein laminin and the tight junction protein zona occludens (ZO)-1, both of which are important elements of BBB [28]. There is much evidence that MMP-9 degrades extracellular matrix proteins of cerebral microvessels including basal lamina proteins consisting of laminin, collagen IV and fibronectin, as well as ZO-1 belonging to endothelial tight junction-related proteins, loss of which causes disruption or increased permeability of BBB [14]. However, post-SAH increases in active interleukin-1β, which activates NF-κB signaling, were not suppressed by r-OPN, suggesting that r-OPN blocked intracellular signaling upstream of NF-κB via RGD-dependent integrins [70] (Fig. 1). In the same rat models, intranasal vitamin D3 reportedly upregulated expression of endogenous OPN isomers (OPNs-a and OPNs-c, but not OPNs-b) in astrocytic and brain capillary endothelial cells [71]. Induced OPN activated CD44 and P-glucoprotein glycosylation signals in the capillary endothelium and attenuated the disruption of BBB and cerebral edema [71]. The inhibition of complement C3 by an extract of Ephedra sinica also alleviated the disruption of BBB and cerebral edema associated with improved neurological functions, possibly via the upregulation of sonic hedgehog and OPN signaling and the consequent reduction of MMP-9 expression [45].

There are other possible mechanisms of OPN protection of the BBB, which have not been investigated in SAH yet. For example, administration of exogenous OPNs has reduced oxidative stress that activates NF-κB; as a result, OPNs downregulate MMP-9 expressions and upregulate a tissue inhibitor of MMP-1, leading to the maintenance of the integrity of BBB [14,28]. Furthermore, exogenous OPNs are known to promote focal adhesion kinase (FAK) phosphorylation and subsequent activation of phosphatidylinositol 3-kinase (PI3K), inducing Ras-related C3 botulinum toxin substrate 1 (Rac-1) to preserve the integrity of BBB [14]. Additionally, endogenous OPNs have been reported to induce polarization of reactive astrocytes that is pivotal to the complete coverage of neovessels by astrocytic end-feet and the BBB integrity [72].

4.3 Neuronal apoptosis

EBI finally leads to neuronal death or apoptosis, which has been demonstrated experimentally and clinically [73,74]. The PI3K–Akt pathway is related to an antiapoptotic mechanism in neurons after experimentally induced SAH [75] and the activation of the pathway has been demonstrated to exert an antiapoptotic role [76]. Activation of FAK, a cytoplasmic tyrosine kinase, which is triggered via integrin or CD44 receptors, stimulates the PI3K–Akt signaling pathways [14,77]. Phosphorylated Akt can then suppress proapoptotic proteins such as B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax) and Bcl-2-associated agonist of cell death (BAD) and upregulate antiapoptotic proteins including Bcl-2 [14]. ERK1/2 is another classic downstream protein of FAK [78,79].

Accumulating studies have demonstrated that OPNs exert direct antiapoptotic action through integrin and CD44 receptors [14]. However, the underlying mechanism for OPNs to inhibit caspase-3 activation remains unclear, although cleaved caspase-3 may be a common downstream protein to apoptosis after SAH [14]. Administration of r-OPN was reported to attenuate neuronal apoptosis in the cerebral cortex and brain edema and improved neurological status in endovascular perforation SAH models in rats, possibly through the activation of the FAK–PI3K–Akt signaling pathway that inhibits capase-3 cleavage [77]. In the same model, although SAH upregulated endogenous OPNs and autophagy (ATG)-related proteins (Beclin 1, ATG5, and microtubule-associated protein light chain 3 [LC3] II to I ratio), r-OPN administration further caused an increase in expressions of ATG-related proteins [79]. r-OPN inhibited apoptosis of neurons by ATG activation and the regulation of ATG-apoptosis interactions, accompanied by increased

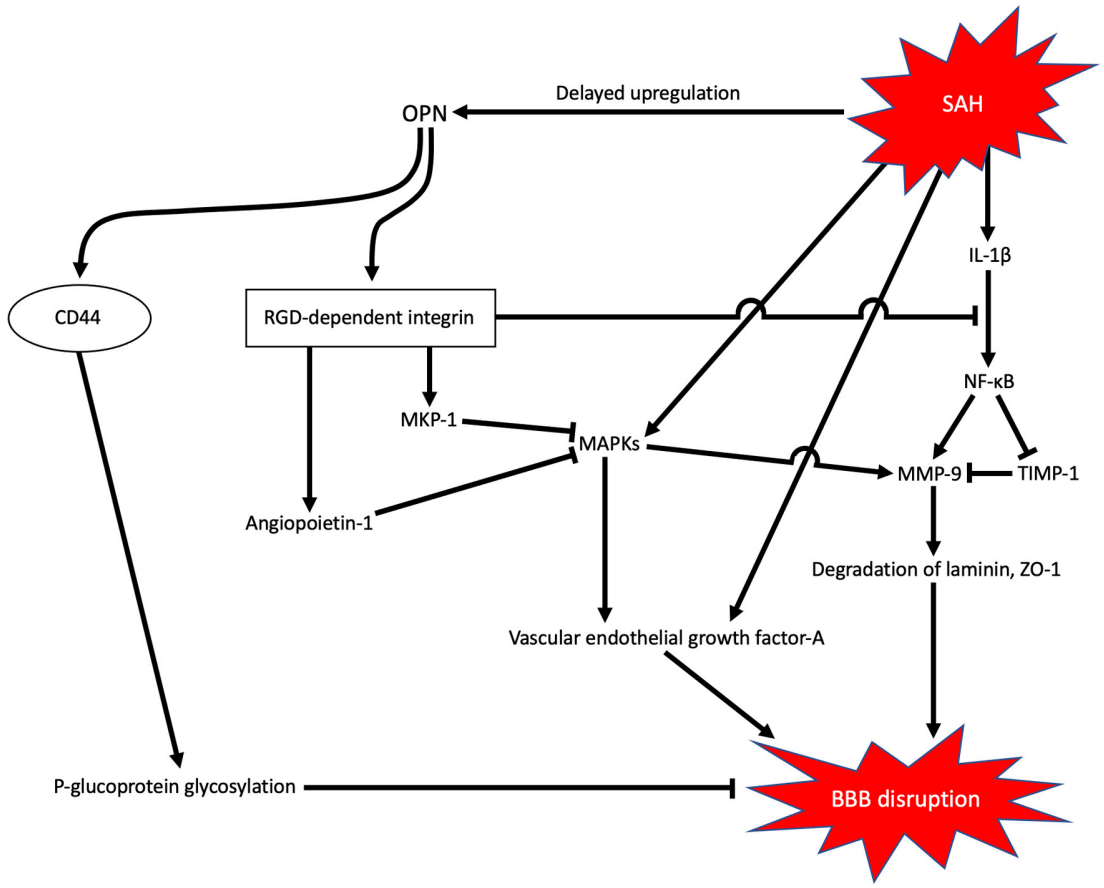

Fig. 1. Possible pathways of osteopontin (OPN) to suppress the disruption of blood-brain barrier (BBB) after subarachnoid hemorrhage (SAH). OPN induces angiopoietin-1 and mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) through Arg-Gly-Asp (RGD)-dependent integrins, then inactivates MAPKs to downregulate matrix metalloprotease (MMP)-9 as well as vascular endothelial growth factor-A. OPN also inhibits nuclear factor-kappaB (NF-κB) signaling, and downregulates MMP-9. Additionally, OPN activates CD44 and P-glucoprotein glycosylation signals. All these pathways contribute to the inhibition of BBB disruption after SAH. IL-1β, interleukin-1β; TIMP-1, tissue inhibitor of MMP-1.

expressions of an antiapoptotic protein Bcl-2 and decreased expressions of proapoptotic proteins (cleaved caspase-3 and Bax) [79]. The same group also reported that FAK–ERK1/2 signaling may be involved in r-OPN-enhanced ATG and reduced neuronal apoptosis in post-SAH EBI [78] (Fig. 2).

In a rat intracerebral hemorrhage model, intracerebroventricularly injected r-OPN increased phosphorylated Akt expression, which phosphorylated and subsequently inactivated pro-apoptotic glycogen synthase kinase 3 beta (GSK-3β), suppressing Bax-to-Bcl-2 ratios and caspase-3 activation that result in a decrease in cerebral edema and cell deaths [80]. Similar mechanisms may be implicated in r-OPN-induced antiapoptotic effects after SAH.

5. DCI, cerebral vasospasm and delayed-onset cerebral infarct

DCI develops possibly by blood breakdown products or secondary to EBI at four days or later after clinical SAH and in severe cases leads to delayed-onset non-iatrogenic cerebral infarction [57]. The pathology underlying DCI is now considered to be not only cerebral vasospasm but also microcirculatory disturbances by multiple mechanisms [57]. In the endovascular perforation or blood injection models of SAH in rats or mice that are most popularly used, however, cerebral vasospasm and microvascular dysfunction occur in the same time frame as EBI (72 hours) and therefore it is impossible to discriminate the microvascular dysfunction in DCI from that in EBI. Additionally, cerebral infarction is little observed after experimental SAH. Thus, in this section, only the potential role of OPNs in experimental cerebral vasospasm is described.

The underlying mechanisms of cerebral vasospasm are considered to be prolonged vascular smooth muscle contraction and impaired endothelium-dependent vasorelaxation and are associated with remodeling of the arterial wall [57,81] (Fig. 3). In endovascular perforation SAH models in rats, r-OPN upregulated MAPK phosphatase-1 in smooth muscle cells of the cerebral arterial walls via binding to RGD-dependent integrin receptors, followed by

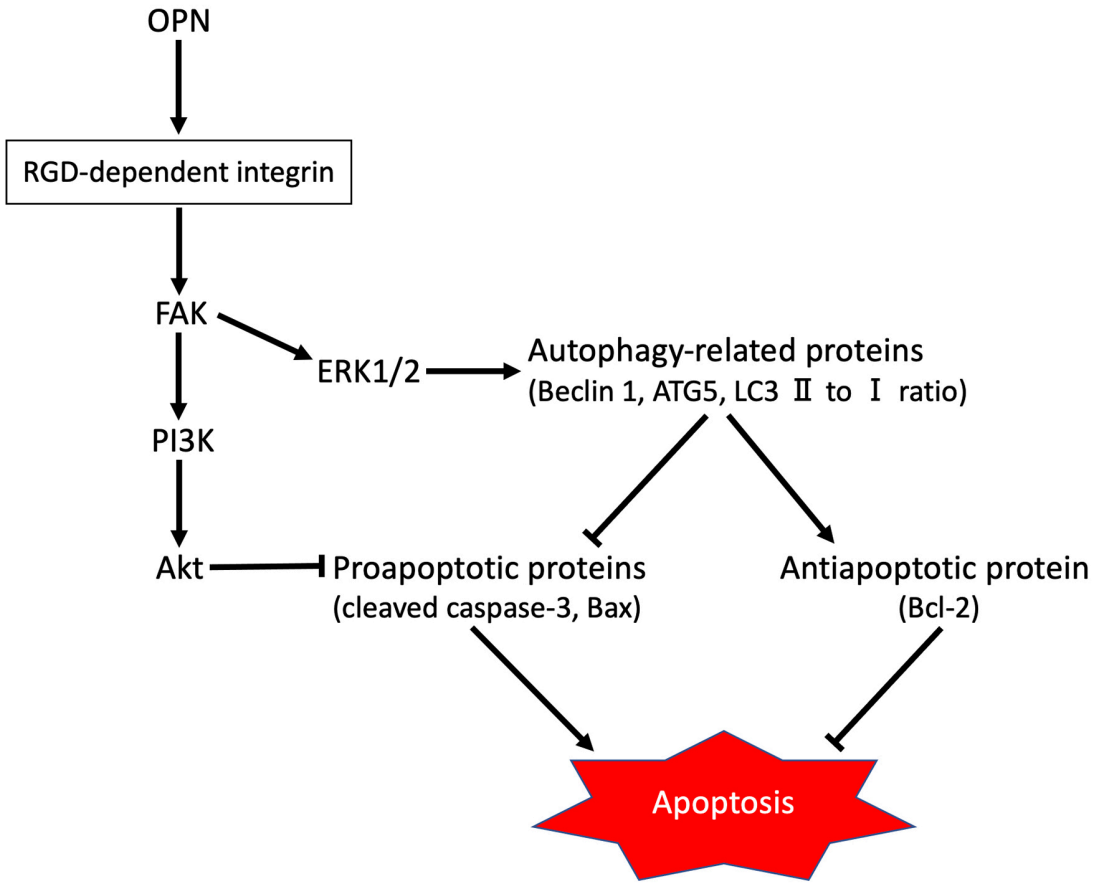

Fig. 2. Possible pathways of osteopontin (OPN) that protect against neuronal apoptosis after subarachnoid hemorrhage. OPN activates focal adhesion kinase (FAK) through Arg-Gly-Asp (RGD)-dependent integrins, subsequently activating the phosphatidylinositol 3-kinase (PI3K)-Akt signaling, which in turn inactivates proapoptotic caspase-3 and B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax). OPN-mediated FAK activation also activates extracellular signal-regulated kinase (ERK)1/2 and then upregulates autophagy (ATG)-related proteins to inhibit neuronal apoptosis. LC3, microtubule-associated protein light chain 3.

the suppressed activation of MAPKs, caldesmon and heat shock protein 27, and prevented cerebrovascular spasm after SAH [82,83]. MAPK phosphatase-1 is known to deactivate all kinds of MAPKs such as p38, c-Jun N-terminal kinase and ERK1/2, which exist in the smooth muscle cells of the cerebral artery [82]. In the same animal models, another matricellular protein tenascin-C, which exerts vasoconstrictive effects [84–86], was upregulated in the smooth muscle cells of cerebral arteries with vasospasm at day one post-SAH, but decreased at day three as vasospasm improved; in contrast, endogenous OPNs were more upregulated in the cerebral arterial walls at day three [34]. r-OPN reversed the constriction of cerebral arteries by tenascin-C, although the mechanisms were not examined [34]. r-OPN also prevented the transformation of phenotypes of arterial smooth muscle cells as well as vasospasm, possibly through the RGD-dependent integrin receptor–integrin-linked kinase–Rac-1 pathway [87]. In rat SAH models by double blood injections into the cisterna magna, r-OPN administration prevented neurological impairments and cerebral vasospasm, associated with decreased expressions of cleaved caspase-3 and Bax as well as increased expressions of p-Akt and Bcl-2, which reduced apoptosis of endothelial cells in the basilar artery after SAH [88]. In a rat model with endovascular perforation SAH, vitamin D3 treatment attenuated the remodeling of cerebral arterial walls and cerebral vasospasm by upregulating endogenous OPNs and their CD44-dependent intracellular mechanisms to activate adenosine monophosphate-activated protein kinases and endothelial nitric oxide synthases at Ser1177-Dimer in the endothelium of cerebral arteries [89].

6. Neuroinflammation

Inflammation has crucial roles in the developing processes of EBI and DCI after experimental and clinical SAH [6,57]. Inflammatory cascades potentially exacerbate secondary brain injury in an early phase of diseases, while they favorably promote tissue remodeling and functional repairs; thus, neuroinflammatory responses secondary to EBI can be double-edged swords [14]. Interestingly, in neuroinflammation, OPN is also indicated in dual proinflammatory and anti-inflammatory roles [90]. However, OPN may be

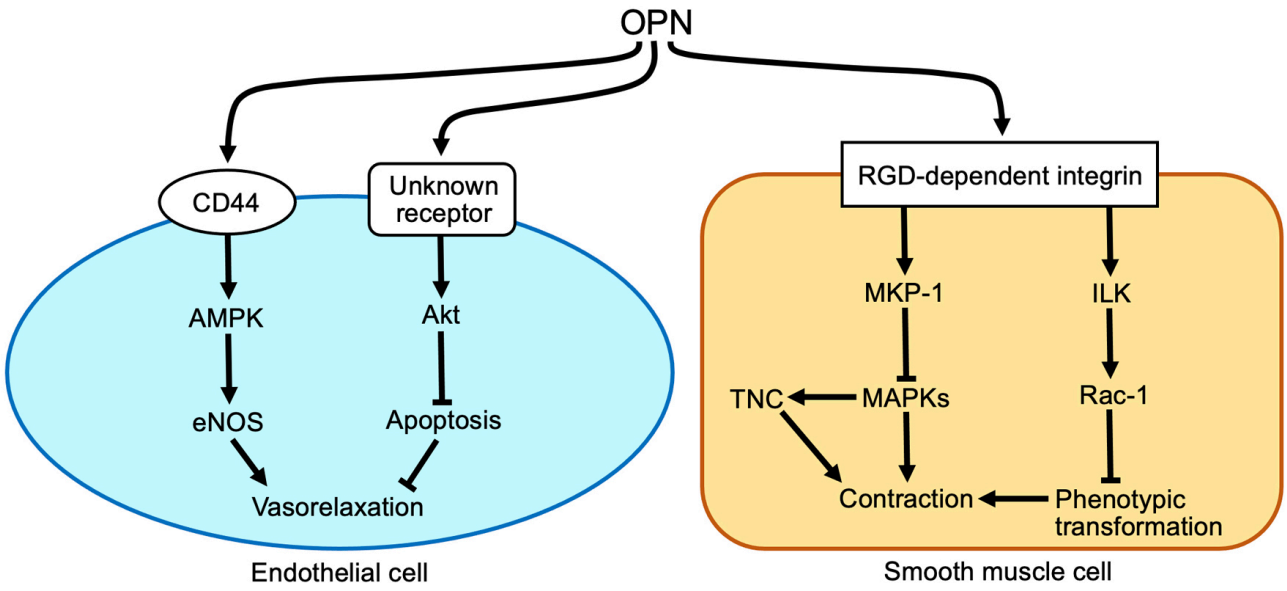

Fig. 3. Possible mechanisms of osteopontin (OPN) inhibition against vasospasm of cerebral artery after subarachnoid hemorrhage. OPN prevents phenotypic transformation of cerebral arterial smooth muscle cells through Arg-Gly-Asp (RGD)-dependent integrin receptor-mediated activation of the integrin-linked kinase (ILK)-Rac-1 pathway. OPN also activates mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) through RGD-dependent integrins, followed by inactivation of MAPKs, resulting in the attenuation of contraction of smooth muscle cells. Additionally, OPN prevents tenascin-C (TNC)-induced smooth muscle cell contraction. In arterial endothelial cells, OPN activates adenosine monophosphate-activated protein kinase (AMPK) to induce endothelial nitric oxide synthase (eNOS), as well as suppresses apoptosis via Akt activation, contributing to the preservation of endothelium-dependent vasorelaxation.

overall neuroprotective in SAH and the other stroke types [11]. After brain injuries by ischemic and hemorrhagic strokes, microglia/macrophage and astrocyte induced OPN, by which additional microglia/macrophage and astrocyte were inversely recruited, activated and polarized in the lesions and the perilesional areas via $\alpha v\beta 3$ integrins and/or CD44 receptor [14]. These integrin and CD44 receptors are also known to be induced after brain injuries [14]. These post-stroke reactions are followed by succedent secretion of cytokines, removal of necrotic tissues and remodeling of injured tissues including gliosis, formation of extracellular matrices and new blood vessels [14].

Inducible nitric oxide synthases (iNOSs) are also involved in neuroinflammation, BBB disruption and cell death [63]. Induced OPN was reported to dose-dependently suppress or downregulate iNOS expression associated with increased expressions of integrin-$\beta 1$ and the subsequent inhibition of the Janus kinase/signal transducers and activators of the transcription 1 pathways in an intracerebral hemorrhage model [91,92]. In rat models of permanent focal cerebral ischemia, induced OPN shifted the phenotypes of microglia towards an M2, reducing iNOS-expressing M1 microglia [93]. OPN also downregulated the expression of MMP-9 by inhibiting the interleukin-1β/NF-κB pathway and suppressed BBB disruption after experimental SAH [28].

7. Chronic hydrocephalus requiring shunt surgery

Chronic shunt-dependent hydrocephalus is a most frequently encountered sequela following aneurysmal SAH in a clinical setting and its incidence is reported to be 8.9–36.9% [94]. Although a CSF shunting procedure is the established treatment of chronic hydrocephalus after SAH, it cannot completely reverse neurological impairments and cognitive deficits and chronic hydrocephalus patients are frequently associated with poor outcomes even after shunt surgery [95]. Although the exact pathogenic mechanism of chronic shunt-dependent hydrocephalus developing after SAH remains unclear, the most prevalent theory is that post-SAH cell proliferation and fibrosis of leptomeninges and arachnoid granules cause either an impairment of CSF circulation and absorption or CSF outflow from the subarachnoid space, leading to shunt-dependent hydrocephalus [96]. Severe leptomeningeal fibroses along the CSF circulation pathway may also interfere with brain functions [97]. Leptomeningeal fibrosis may be caused by SAH-induced inflammatory cytokine and growth factor in the CSF, which may also induce a variety of pathological conditions in the subependymal parenchyma of the brain and the interstitial spaces to impair neurological and cognitive functions and to contribute to ventriculomegaly, likely explaining why neurological and cognitive deficits cannot completely recover even after shunt surgery in chronic hydrocephalus [97,98].

As to relationship between OPNs and post-SAH chronic hydrocephalus, thus far, to the knowledge of the authors, only one clinical study has been published [16]. In that study, plasma OPN-a levels in patients with the subsequent chronic hydrocephalus development requiring shunt surgery increased from days one to three to days four to six and high levels were kept until at least days 10–12, while those in the other SAH patients peaked at days four to six, then decreased thereafter [16]. Higher plasma OPN-a is considered to reflect more severe preceding injuries of brain tissues and the consequent neuroinflammation, not systemic inflammatory reactions [30,31], which are known to be a predisposing factor for the occurrence of chronic hydrocephalus requiring shunt surgery [99,100]. Although the effect of sustained high levels of OPNs has never been studied in an experimental SAH model, expressions of OPNs are enhanced by inflammatory or oxidative reactions and excessive OPNs are reported to facilitate excessive fibroses and wound healing in diverse organs [101,102]. OPN is considered to induce fibrosis or exert its wound repairing effects through direct binding to or interacting with collagen [103] and via signal activation mediating transforming growth factor-β [104] and the other pathways [105]. Increased OPN may be initially implicated in early intrinsic repair-promoting mechanisms against tissue injuries, but markedly and prolongedly increased OPN expression may cause excessive remodeling, leading to functional or anatomical disarrangement and in SAH cases the development of chronic shunt-dependent hydrocephalus [101,102].

8. Conclusions and future prospects

In this review, potential functional role of OPN in post-SAH pathologies has been discussed. OPN acts in a neuroprotective manner and could be a molecular therapeutic target in direct and indirect ways for post-SAH EBI and vasospasm of cerebral artery as well as possibly for DCI that is caused by non-vasospasm pathogenic events. However, the timing and amount of OPN exclusively required to exert neuroprotective effects are unknown; moreover, delayed and prolonged upregulation of OPNs could be toxic and risky for the occurrence of chronic hydrocephalus that requires shunt surgery. Other potential and unexamined possibilities are that different isoforms of OPN are produced, or OPN undergoes different post-translational modifications between acute and delayed or chronic phases of SAH, so that the biological function of OPN is different between the phases. In this regard, no data are available, and further study is required.

A further problem, as noted elsewhere [106], concerns bench-to-bedside applications. As mentioned, many pathways have been revealed experimentally and the values of findings are paramount. Physiological differences between humans and rodents make the definition of EBI or DCI complicated. For example, EBI in humans is defined as brain damage within 72 hours of the ictus and before the "so-called" vasospasm phase, but it may not be intrinsically so long for rodents [5]. Cerebral vasospasm is another complicated pathophysiology. In humans, delayed vasospasm, which generally occurs at days 4–14 or later, because the etiology is different, should be distinguished from immediate or early vasospasm that is diagnosed before or at acute (within three days of the ictus) treatment for a ruptured intracranial aneurysm [57]. In most experimental research, however, cerebral vasospasm of rodents is evaluated at 24 or 72 hours after the ictus, and vasospasm at 24 and 72 hours post-SAH in rodents is assumed to be equal to delayed vasospasm in humans [5]. The rigorous definition of "early" or "delayed" pathologies in rodents and humans should be rethought, as well as whether the underlying mechanisms between them are similar.

Recently, organ-on-a-chip technologies including a perfused human BBB on-a-chip, which consists of human cell lines of brain capillary endothelial cells, pericytes and astrocytes in a 2- or 3-lane microfluidic platform, have been developed for high-throughput evaluation of BBB functions [107,108]. The perfused human BBB on-a-chip models show sufficient barrier function, are available for the purpose of drug screening and amenable for advanced imaging including transmission electron microscopy, 3-dimensional live fluorescence imaging using traditional spinning disk confocal or advanced lattice light-sheet microscopies [107, 108]. This enables real-time monitoring of BBB penetration and permeability [107,108]. Additionally, recently generated 3-dimensional cerebral organoids from human pluripotent stem cells overcome the limitation of stem cell-based transplantation therapies to a certain extent and show an advantage in a variety of types of cerebral cells (including, but not limited to, neural progenitor, neural stem, mature and immature neuronal and glial cells), rich sources of cells, considerable numbers of cells, controllable degrees of cellular differentiation and certain volumes of tissues with neural connectivity and brain functionality [109]. These advanced techniques may enhance OPN research in post-SAH pathologies and the development of new therapies utilizing only the good side of the molecule. In short, OPN is a promising neuroprotective molecule for SAH pathologies. It is expected that further research will overcome the issues and introduce beneficial aspects of OPN into clinical practice, and that new therapies using OPN as a molecular target will be developed for the improvement of outcomes in a patient with aneurysmal SAH.

Abbreviations

ATG, autophagy; BAD, B-cell lymphoma 2-associated agonist of cell death; Bax, B-cell lymphoma 2-associated X protein; BBB, blood-brain barrier; Bcl-2, B-cell lymphoma 2; CSF, cerebrospinal fluid; DCI, delayed cerebral ischemia; EBI, early brain injury; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GSK-3β, glycogen synthase kinase 3 beta; iNOS, in-

ducible nitric oxide synthase; LC3, microtubule-associated protein light chain 3; MAPK, mitogen-activated protein kinase; MMP, matrix metalloprotease; NF-κB, nuclear factor-kappaB; OPN, osteopontin; OPN-N, N-terminal osteopontin fragment; OPN-C, C-terminal osteopontin fragment; PI3K, phosphatidylinositol 3-kinase; Rac-1, Ras-related C3 botulinum toxin substrate 1; RGD, Arg-Gly-Asp; r-OPN, recombinant osteopontin; SAH, subarachnoid hemorrhage; ZO, zona occludens.

Author contributions

RA and HS are co-authors, and both conceived and designed the study, and wrote the paper.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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