EXPERT OPINION

Caveolae: molecular insights and therapeutic targets for stroke

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Introduction: Caveolae are specialized plasma membrane micro-invaginations of most mammalian cell types. The organization and function of caveolae are carried out by their coat proteins, caveolins and adaptor proteins, cavins. Caveolae/caveolins physically interact with membrane-associated signaling molecules and function in cholesterol incorporation, signaling transduction and macromolecular transport/permeability.

Areas covered: Recent investigations have implicated a check-and-balance role of caveolae in the pathophysiology of cerebral ischemia. Caveolin knock-out mice displayed exacerbated ischemic injury, whereas caveolin peptide exerted remarkable protection against ischemia/reperfusion injury. This review attempts to provide a comprehensive synopsis of how caveolae/caveolins modulate blood–brain barrier permeability, pro-survival signaling, angiogenesis and neuroinflammation, and how this may contribute to a better understanding of the participation of caveolae in ischemic cascade. The role of caveolin in the preconditioning-induced tolerance against ischemia is also discussed.

Expert opinion: Caveolae represent a novel target for cerebral ischemia. It remains open how to manipulate caveolin expression in a practical way to recapitulate the beneficial therapeutic outcomes. Caveolin peptides and associated antagomirs may be efficacious and deserve further investigations for their potential benefits for stroke.

Keywords: caveolae, caveolin, cerebral ischemia, pro-survival signaling, stroke

Expert Opin. Ther. Targets [Early Online]

1. Introduction

Stroke is the third leading cause of death and the most frequent cause of permanent disability in adults worldwide [1]. Despite advances in the understanding of the pathways of cerebral ischemic cascade, therapeutic options for ischemic stroke remain very limited. The only accepted treatment for stroke is thrombolysis which recanalizes the occluded artery. This intervention has many defects such as narrow therapeutic window and serious side effects, thus limiting its clinical utility [2]. The alternative approach is to try to impede the ischemic cascade by targeting various components of the cascade, such as excitotoxicity, free radical-mediated injury and inflammatory mechanisms, to protect the salvageable brain tissues [3]. During the past two decades, > US$1 billion have been spent in the development of therapeutic method for stroke [4]. Over 100 Phase II/III trials of stroke protection have been completed or terminated (source: Internet Stroke Center) [5]. Unfortunately, such huge investment did not produce a safe and efficacious drug. An important reason is the inappropriate target selection which leads to the insufficient therapeutic efficacy [6]. In this context, the elucidation of the molecular mechanisms is an essential prerequisite of the drug development for stroke treatment and should deserve more attention in future studies.

Caveolae are a specialized form of lipid rafts which participate in and are the site of regulation for many cellular functions. The organization and functions of

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Article highlights.

- The elucidation of the molecular mechanisms is an essential prerequisite of the drug development for stroke treatment.
- Several lines of evidence have shed light on a check-and-balance role of caveolae and their component proteins in cerebral ischemia.
- Caveolae regulate blood–brain barrier permeability and mediate convergence of pro-survival signaling.
- Caveolin expression is both necessary and sufficient for the preconditioning-induced neuroprotection.

This box summarizes key points contained in the article.

caveolae are carried out by their coat proteins, caveolins and, adaptor proteins, cavins [7]. Several lines of evidence have implicated the beneficial role of caveolae and their component proteins in the pathophysiology of ischemic stroke injury. In this review, we focus on discussing how caveolae/caveolins modulate blood–brain barrier (BBB) permeability, pro-survival signaling, angiogenesis, neuroinflammation and preconditioning, and how this may contribute to a better understanding of caveolae as a therapeutic target in stroke.

2. Caveolae and component proteins

Caveolae are specialized micro-invaginations (50 – 100 nm) of the plasma membrane (**Figure 1**), which were first observed by Palade and Yamada independently in the 1950s [8,9]. They were originally defined as small ampullate-shaped caves. Caveolae are rich in sphingolipids, cholesterol and lipid-anchored proteins. These organelles are present in most cell types and are particularly abundant in endothelia, muscle and adipocytes. At the outset, caveolae were found to be involved in cholesterol incorporation, potocytosis and endocytosis. Later studies revealed multiple faces of caveolae, including mechanosensing, signaling transduction and macromolecular transport/permeability [10].

Owing to the discovery of the molecular composition of caveolae, the research of biochemical function about caveolae entered into a new phase. Currently, two groups of proteins have been found to be essential for the caveolae formation: caveolins and cavins.

2.1 Caveolins

Caveolins are multiply acylated 22 – 24 kDa proteins. Caveolin-1 and caveolin-2 are generally expressed in smooth muscle cells, endothelial cells, skeletal myoblasts, fibroblasts and adipocytes, whereas caveolin-3 is primarily expressed in striated (skeletal and cardiac) muscle cells [11]. Caveolin-1 was the first protein identified as an essential structure protein in caveolae [12]. Genetic depletion of caveolin-1 causes loss of caveolae in non-muscle tissues [13], and re-expression of caveolin-1 in cells devoid of caveolin-1 induces *de novo* formation of caveolae [14]. However, overexpression of caveolin-1 in endothelial cells, which already have rich caveolae, do not further increase their caveolae number [15]. Alternative splicing of mRNA generates two isoforms: the 24 kDa caveolin-1α and the 21 kDa caveolin-1β. These two isoforms share a distinct but overlapping cellular distribution and differ by 31 additional amino acids which are present in the α-isoform. The function of these two different isoforms is still unclear. However, it was indicated that they may have different potential for forming caveolae structure, with caveolin-1β hardly capable of generating caveolae in the absence of caveolin-1α [16].

Caveolin-2 is accessory to caveolin-1 and form hetero-oligomers in most cells. Its expression also needs caveolin-1. In the absence of caveolin-1, caveolin-2 degraded through proteasomal pathway [17]. In caveolin-2-deficient mice, the expression of caveolin-1 was decreased but the formation of caveolae was not affected, thus indicating that caveolin-2 is not a *sine qua non* for caveola formation [18]. However, co-expression of caveolin-2 and caveolin-1 results in deeper [16] and more abundant [19] formation of caveolae, thus suggesting that caveolin-2 presence is an enhancer for caveolae interior structure.

Caveolin-3 is generally recognized as a muscle-specific isoform of caveolin. However, it has also been detected in astrocytes, neurons and microglial cells [20-22]. Its structure and function are similar to caveolin-1. In contrast to caveolin-1 and -2, which constitute functional hetero-oligomers where they are co-expressed, caveolin-3 can form high molecular mass homo-oligomeric complexes. It has been shown that in the muscle cells of caveolin-3 knockout (KO) mice, the structure of caveolae disappeared [23]. Together with caveolin-1, caveolin-3 can drive the formation of a uniform population of vesicles (50 – 100 nm in diameter) [19], whereas in the absence of caveolin-1, caveolin-3 *per se* is capable of generating caveolae [24].

In the CNS, caveolae and caveolins were initially believed to be restricted to glial and endothelial cells [25,26]. Later studies revealed existence of caveolae-like structure in the neuronal cells, and expressions of three caveolin isotypes in neurons [27-29]. Caveolin-1-deficient mice exhibit complex neurological phenotypes, including abnormal spinning, altered emotionality, gait abnormalities, spatial memory impairment and cholinergic hypofunction [30,31]. Caveolins are abundant in nerve terminals (synaptosomes), especially in postsynaptic sites. Emerging evidence has suggested the influence of caveolins in the potency and efficacy of neurotransmitter receptors [32]. First, caveolins modulate synapse function through membrane cholesterol trafficking [33]. Second, caveolins directly interact with a range of neurotransmitter receptors such as adenosine A1 receptors, muscarinic acetylcholine receptors, D1 dopamine receptors and serotonin type 2A receptors, and other membrane-associated proteins such as synaptosomal-associated protein of 25 kDa and postsynaptic density protein-95 [34]. Caveolins are prevalent

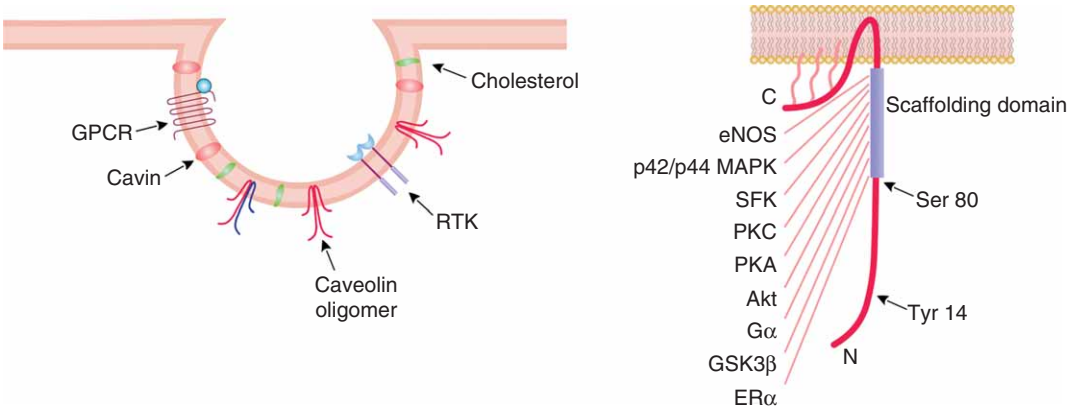

Figure 1. Schematic structures of caveolae and caveolin domains. Left panel: Caveolae are organized as oligomers of caveolin proteins, adaptor protein cavins and cholesterol in the membrane, and they serve as spatially localized signaling receptors, such as GPCR and RTK. Right panel: Caveolin monomer contains a scaffolding domain, which is the binding site for many pro-survival and pro-growth molecules, including PKA, PKC, eNOS, SFK, GSK3β, and so on.

eNOS: Endothelial nitric oxide synthase; ER: Estrogen receptor; GPCR: G-protein coupled receptor; GSK3β: Glycogen synthase kinase 3 β; MAPK: Mitogen-activated protein kinase; PKA: Protein kinase A; PKC: Protein kinase C; RTK: Receptor tyrosine kinase; SFK: Src family kinase.

at excitatory synapses [35], implying their involvement in excitotoxicity. Caveolin-1 was found to be obligatory for NMDA-mediated intracellular signaling pathways. NMDA receptor subunit NMDAR2B co-localize with caveolin-1 in rat cortical neurons. This interaction likely renders the neurotoxicity, triggered from NMDAR, because knockdown of caveolin-1 blocked the sequential phosphorylation of Src and extracellular signal-regulated kinase (ERK)1/2 on NMDA stimulation [36]. Moreover, re-expression of caveolin-1 in caveolin-1 KO neurons rescued NMDA-mediated Src and ERK1/2 activation [37]. On the other hand, AMPA receptor (AMPAR) seems to be negatively regulated by caveolin. The binding of AMPA to its receptor could be abolished by caveolin-1 peptide in hippocampal neurons. This regulation was not due to the direct interference with AMPAR, but rather the caveolin's modulation of phospholipase, which itself would augment AMPAR affinity by changing the lipid environment [38]. Thus, caveolin can divergently regulate glutamate signaling via the facilitation of NMDAR and thereby the mitigation of AMPAR signaling [34].

2.2 Cavins

Cavins are perceived as adaptor proteins cooperating with caveolins and regulating the formation of caveolae [7]. Cavins have four isoforms: cavin-1 (polymerase transcriptase release factor [PTRF]), cavin-2 (serum deprivation response protein), cavin-3 (sdr-related gene product that binds to c-kinase) and cavin-4 (muscle-restricted coiled-coil protein). Cavin-1/PTRF was first characterized as a cytoplasmic protein capable of dissociating ternary Pol I transcription complexes [39] and was later found to be a caveolar marker protein [40]. Cavin-2 and cavin-3 were identified as substrates of PKC and can bind PKC to caveolae [41]. Cavin-4, predominantly expressed in muscle, was discovered to be associated with caveolin-3 dysfunction [42]. The mutations of cavin-4 have been implicated to be a causal factor for human dilated cardiomyopathy [43], which was probably via RhoA/Rho kinase pathway [44].

Cavin family plays pivotal roles in regulating caveolar biogenesis. Cavin-1 can be recruited by caveolins to the cytosolic face of caveolar domains, thereby maintaining the level of caveolins [45]. In the absence of cavin-1, the size of caveolin-1 oligomers in the plasma membrane decreases and their lateral mobility increases, implying a pivotal role of cavin-1 in the last steps of caveolar biogenesis and stabilization of caveolin in the immobile caveolae [46]. Animals lacking cavin-1 have no morphologically distinct caveolae in all tissues examined [46]. Thus, it is not surprising to see cavin-1 null mice exhibit phenotypes parallel to caveolin-1 and -3 double-KO mice. The phenotypes involve reduced adipose tissue mass, glucose intolerance, hypertriglyceridemia and hyperinsulinemia [7,47]. Strikingly, patients with cavin-1 null mutation have been reported to present with a variety of pathologies such as generalized lipodystrophy, long-QT syndrome, bradycardia, muscular dystrophy and smooth-muscle hypertrophy [48-50]. Inconsistent with the findings in the cavin-1 KO animals, in the fibroblasts of cavin-1-deficient patients, caveolin-1 failed to localize toward the cell surface and electron microscopy revealed reduction of caveolae to < 3% [48].

3. Caveolae protect against cerebral ischemia

Recently, several lines of evidence have shed light on the beneficial roles of caveolae and their component proteins in cerebral ischemia. Caveolin-1 KO mice have larger infarct size and more apoptotic cell death as compared with wild-type (WT) mice. Caveolin-2 depletion, on the other hand,

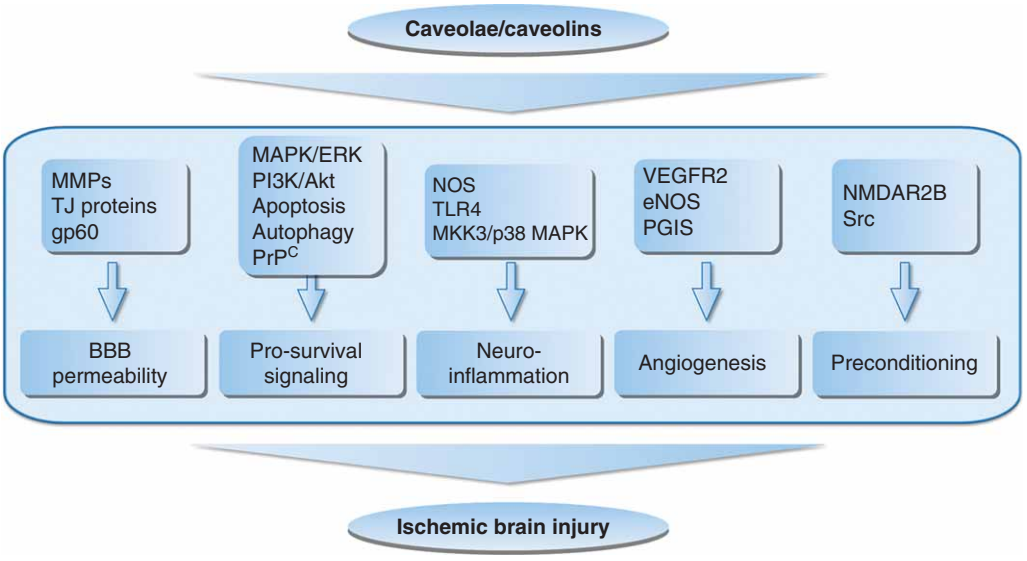

Figure 2. Schematic representation showing some of the signaling pathways coupling caveolae/caveolins with ischemic brain injury.

ERK: Extracellular signal-regulated kinase; MAPK: Mitogen activated protein kinase; MMP: Matrix metalloproteinases; NMDAR2: N-methyl-D-aspartate receptor 2; NOS: Nitric oxide synthase; PGIS: Prostacyclin synthase; PI3K: Phosphatidylinositol 3-kinase; PrP^C: Cellular prion protein; TJ: Tight junction; TLR4: Toll-like receptor 4; VEGFR2: Vascular endothelial growth factor receptor 2.

seems to have negligible influence on the histological outcomes of cerebral ischemia [51]. Mice deficient in caveolin-1 also have augmented ischemia/reperfusion injury in liver [52], kidney [53], hind limb [54] and heart [55], indicating a ubiquitous role of caveolin in the ischemic cell death. In addition, many protective strategies fail to protect caveolin-1-deficient mice from cerebral ischemia injury [36]. Caveolin, thus, represents a critical step in the ischemic cascade and may be harnessed for pharmacological manipulation. Indeed, neuronal-specific overexpression of caveolin-1 enhanced pro-survival kinase activation and dendritic growth and arborization [37]. Likewise, cardiac-specific caveolin-3 overexpression mice showed endogenous tolerance for myocardial ischemia, as exemplified by preserved ultrastructure, improved cardiac function and reduced apoptosis [56]. Supplementation with cell-permeable caveolin scaffolding domain (CSD) peptide, the functional domain of caveolin, could exert protective effects against ischemia *in vivo* [57] and *in vitro* [58]. These results demonstrated that caveolin is both necessary and sufficient to induce neuroprotection. Targeting caveolin may be a novel means to repair cerebral ischemia.

The interplay between caveolae/caveolin and cerebral ischemia is multifaceted. Following middle cerebral artery occlusion, the expression of caveolin-1 protein dramatically decreased at the core and penumbra areas of the ischemic brain. This decline continued for at least 72 h [59]. Caveolin-2 protein showed moderate downregulation at initial stages of ischemia and reperfusion and recovered afterward. Ischemia/reperfusion had no effect on the expression of caveolin-1 mRNA, thus indicating that the alterations of caveolins occurred in the post-transcriptional stage when nitric oxide (NO) might be a modulator [59]. Consistently, attenuated caveolin-1 expression was observed in cultured astrocytes subjected to oxygen glucose deprivation (OGD) [58]. Jasmin *et al.*, however, revealed marked increases in caveolin-1 and caveolin-2 protein levels, which were mainly restricted to endothelial cells [51]. This discrepancy may be due to the differences in stroke models, cell types and time intervals between ischemia and detection. Moreover, caveolin could dissociate from caveolae [60] and translocate toward cellular compartments such as mitochondria in response to ischemia [61]. The degradation and dissociation of caveolin proteins could sequentially induce a series of alterations in BBB permeability, pro-survival signaling, neuroinflammation and cerebral angiogenesis, thereby counteracting the ischemic injury. These mechanisms are discussed respectively in the following sections (**Figure 2**).

4. Caveolae regulate BBB permeability

The BBB provides a specialized interface between the blood and the brain. Historically, the diffusion restriction of BBB was mainly ascribed to the lack of fenestrations, minimal pinocytotic activity and presence of tight junctions (TJs). Recently, extracellular matrix, astrocytes, pericytes, as well as neurons and microglia, are increasingly recognized to be involved in the barrier function. Thus, BBB is currently considered as a component of neurovascular unit [62], or vascular neural network [63], the concept of which emphasizes the coordinated interaction between these various cell types.

Experimental models on both global and focal cerebral ischemia have been demonstrated to induce significant perturbation of BBB homeostasis, even though there is no major extracellular edema after global ischemia. Under thrombolysis treatment, BBB disruption also contributes to the secondary reperfusion injury, tissue plasminogen activator (tPA) neurotoxicity and hemorrhagic transformation [64]. In this context, BBB homeostasis represents an attractive therapeutic target not only for neurovascular protection but also for thrombolytic adjuvant to extend the reperfusion window and/or to prevent tPA-associated cerebral hemorrhage.

The caveolar regulation of BBB permeability is twofold. First, caveolae mediate transcytosis which transport macromolecules from the blood vessel to interstitial space [65]. This transcellular pathway involves a receptor-dependent trafficking of vesicles that detach from luminal side of the endothelial barrier and shuttle to abluminal side where they fuse and release their contents [66]. Endothelial cells are one of the cell populations that express highest level of caveolae and caveolin-1. Caveolae constitute ~ 30% of the total endothelial cell surface in capillaries [13]. Caveolin-1 is also present in other structures such as detached plasmalemmal vesicles and tubular vesicular channels [67]. Therefore, it is not surprising that the involvement of caveolae in the transcytosis has long been proposed and investigated. Numerous macromolecules, including albumin, lipoprotein and insulin [68], have been demonstrated to be transendothelially delivered by caveolae. Using a specific antibody-targeting lung endothelial caveolae, Schnitzer's group demonstrated that macromolecular transcytosis can occur via epithelial caveolae, as they found an ~ 172-fold increase in the interstitial accumulation of caveolae-specific antibodies, compared with control probes [69]. In contrast, disruption of caveolae using sterol-binding agents such as filipin or methyl-β-cyclodextrin could block the lipid raft-mediated transcytosis [70,71]. In caveolin-1 KO mice, which lack detectable caveolae in endothelial cells, the endothelial endocytosis of albumin was seriously blunted [72,73].

A very recent study utilized two-photon imaging to investigate the relationship between caveolae and dynamic BBB opening following ischemic stroke [74]. The endothelial caveolae number increased within 6 h after transient middle cerebral artery occlusion, and reflected a greater transcytosis and early BBB hyperpermeability. As such, mice lacking caveolin-1 had reduced transcellular permeability in cortical vessels after ischemic stroke. However, caveolin-1 deficiency did not alter the delayed phase of barrier dysfunction and overall enlarged stroke area, compared to WT mice [74]. The early changes in BBB permeability after cerebral ischemia mainly involve endocytosis and transcytosis in endothelial cells, whereas the delayed phase of BBB opening is accompanied with the remodeling of multiple cell types of neurovascular unit. It cannot be ruled out that caveolin-1 depletion in other cell types (such as in neurons or astrocytes) produced antagonistic effects on BBB permeability as in endothelial cells. The signaling mechanisms of caveolae-mediated transcytosis upregulation remains much less understood. Tyrosine phosphorylation seems to be an important event regulating caveolar function [75]. The binding of some proteins to their receptors (like albumin and gp-60) induces receptor clustering in caveolae, which in turn activates phosphorylation cascades [76,77]. Src-mediated phosphorylation of caveolin-1 at Tyr14 initiates plasmalemmal vesicle fission and transendothelial vesicular transport. Inhibition of phosphatases increases caveolar internalization, whereas inhibition of kinases decreases it [78]. Under pathophysiological conditions, caveolin-1 phosphorylation can be augmented, and therefore accounts for the transcellular transport of albumin and protein-rich edema [79,80].

Second, caveolae are associated with paracellular permeability. One early study of Drab *et al.* revealed no change in the albumin concentration in cerebrospinal fluid (CSF) of caveolin-1-deficient mice and WT counterparts [13]. The authors concluded that the transcytosis of albumin from blood into CSF was either unaffected by caveolin-1 depletion, or that some alternative compensatory pathway exists. To clarify this issue, Lisanti's group later examined the microvascular permeability of caveolin-1-deficient lung capillaries and found that caveolin-1-deficient mice have a hyperpermeable microvascular system [81]. Almost all of the tissues they examined showed twofold or higher accumulation of bovine serum albumin (up to four- to eight-fold in lung, spleen, kidney and liver) in caveolin-1-deficient mice. Accordingly, caveolin-1-deficient mice represented lower levels of endogenous serum [81]. Using *in vitro* and *in vivo* vascular permeability models, knockdown of caveolin-1 by small interfering RNA (siRNA) could increase the paraendothelial permeability, as indicated by transendothelial resistance and paracellular flux [82-84]. Ultrastructural morphometry showed that the hyperpermeability was accompanied with compromised architecture of interendothelial junctions [81], which was probably due to the modulation of caveolae on the TJ- and adherens junction (AJ)-associated proteins. TJ proteins occludin, claudin-5 and zonula occluden (ZO)-1 have been shown to co-localize with caveolin-1 in lipid rafts [85,86]. Knockdown of caveolin-1 with siRNA in brain microvascular endothelial cells (BMECs) resulted in the loss and the redistribution of TJ- and AJ-associated proteins, whereas delivery of a synthesized peptide encoding caveolin-1 scaffolding domain reversed the effects of caveolin-1 siRNA on TJ- and AJ-associated proteins and its permeability to monocytes [82].

Caveolae may also preserve endothelial junctional integrity through controlling their degradation by MMPs. Aberrant MMPs, mainly gelatinases MMP-2 and MMP-9, degrade extracellular matrix and TJ proteins following stroke [87]. It has been shown that MMP-2 localizes to the caveolae [88], where its activity is greatly reduced [89]. In several tumor models, the overexpression and knockdown of caveolin-1 decreased and increased the secretion of MMP-2/9 respectively, consequently affecting the ability of tumor cell

invasion [90-92]. In the CNS, Gu *et al.* used two approaches including caveolin-1 knockdown in cultured BMECs and caveolin-1 KO mice and revealed a preservative role of caveolin-1 in the BBB. Caveolin-1's decline dramatically increased MMP-2/9 activity in BMECs. Meanwhile, following focal cerebral ischemia/reperfusion, caveolin-1-deleted mice exhibited higher gelatinase activity and BBB permeability than WT mice [93]. These findings suggest that caveolin-1 serves critical roles in regulating MMP activity and preserving BBB permeability in ischemic stroke injury [94].

It is noteworthy that there is evidence that caveolae may diminish TJ proteins and BBB integrity. In a rat cortical cold injury model, a significant increase in caveolin-1 expression at the lesion site was found to precede the disassembly of TJ proteins during BBB breakdown [95]. However, a causal link between caveolin upregulation and TJ disassembly was not proven. Using BMEC cultures, caveolin-1 knockdown could attenuate the membrane reorganization of ZO-1, occludin or claudin-5 on BBB disruption models induced by OGD [96], HIV Tat protein [97] or proinflammatory mediator chemokine (C-C motif) ligand 2 [85]. Given that TJ proteins are localized in the lipid rafts/caveolae, these results imply that caveolae may contribute to the TJ complex stability through transmembrane internalization and redistribution, not just the direct degradation processes.

5. Caveolae mediate convergence of pro-survival signaling

zCaveolins, the integral membrane proteins of caveolae, share a similar structure. They all form hairpin loops (residues 102 – 134) embedded within the cell membrane, with their C terminus and N terminus facing the cytoplasm [41]. All caveolins possess a conserved CSD (residues 82 – 101) adjacent to the intramembrane domain. CSD functions as a docking site for many intracellular signaling proteins that contain caveolin-binding motifs. This binding motif is characterized by the amino acid sequence *ΦXXXXΦXXΦ*, *ΦXΦXXXXΦ*, or *ΦXΦXXXXΦXXΦ* (*Φ* represents one of the aromatic amino acids, e.g., tyrosine, tryptophan or phenylalanine, and *X* represents other amino acids) [98,99]. Caveolins coordinate intracellular signaling via the clustering, segregation and trafficking of proteins [34]. By clustering, caveolins band a G-protein-coupled receptor (GPCR) and its G-protein together, thereby facilitating the subsequent signaling pathway. Conversely, caveolins can also isolate membrane-bound receptors from their obligatory signaling partners to attenuate signaling [100]. Other regulatory mechanisms involve trafficking, which refers to caveolae/caveolin-mediated cycling of signaling components between cell membrane and intracellular pool [101,102]. In these ways, caveolae are able to provide bidirectional regulation of signaling transduction.

A large number of signaling components have been demonstrated to be regulated by caveolae or caveolins, including upstream entities (e.g., GPCRs, receptor tyrosine kinases and steroid hormone receptors) and downstream entities (e.g., heterotrimeric and low-molecular-weight G proteins, effector enzymes and ion channels) [103]. Many of these molecules are involved in the pro-survival signaling pathways, especially in the context of ischemia and reperfusion injury. In this section, we primarily discuss the influence of caveolae on the MAPK pathway, phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway, apoptotic and autophagic pathways and cellular prion protein pathway.

5.1 MAPK/ERK pathway

ERK1/2, the most characterized MAPK pathway, plays a pivotal role in the signaling of cell proliferation and differentiation. In astrocytes, filipin III, which specifically disassemble caveolae, could significantly attenuate the phosphorylation of ERK, thus indicating that structural integrity of caveolae is necessary for ERK pathway [104]. In the process of Src homology 2-containing protein tyrosine phosphatase 2 (SHP-2)-induced protection against ischemic brain injury, caveolin-1 was specifically involved in astrocytes [105]. Caveolin-1 siRNA significantly reduced SHP-2 phosphorylation, which could be restored by caveolin-1 overexpression, thus suggesting that caveolin-1 may be an important regulator in SHP-2 pathway. SHP-2 has been shown to act positively in ERK activation. Accordingly, caveolin-1 would exert positive impact on SHP-2/ERK pathway [105]. The regulation of caveolin on ERK pathway was also observed in neurons. ERK signaling was involved in glial cell line-derived neurotrophic factor (GDNF)-stimulated neuronal survival and neurite outgrowth. On GDNF stimulation, caveolin and ERK expressions were upregulated in dopaminergic neurons. Increased ERK signaling was abrogated by the inhibition of caveolin and vice versa [106]. On the other hand, investigators used caveolin-1 siRNA for cultured neurons and caveolin-$1^{-/-}$ mice to acquire the further evidence of the relationship between caveolin-1 and ERK1/2 in cerebral ischemic injury [36]. In the loss-of-function experiment of caveolin-1, the increased p-ERK1/2 was almost diminished and ischemic neuronal damage was worsened. Nonetheless, re-expression of caveolin-1 in caveolin-$1^{-/-}$ mice could restore the ERK phosphorylation. Thus, caveolin-1 is essential for ERK activation following cerebral ischemia.

The caveolin regulation of ERK pathway has also been investigated in myocardial ischemia/reperfusion. Using a discontinuous sucrose density gradient to isolate caveolae/lipid rafts from ischemic heart tissue, Ballard-Croft *et al.* showed that myocardial ischemia/reperfusion was associated with a redistribution of caveolin-3, the major component of cardiocyte caveolae, from light buoyant to heavy fractions. Consistently, ERKs were dramatically activated in ischemic zone. ERK1 was activated only in the light fractions, whereas ERK2 phosphorylation was increased in both light and heavy fractions [107]. These accompanying changes in cardiomyocytes may be the results of co-localization of phosphorylated

ERKs with caveolae at the plasma membrane [108]. Because ERKs possess a putative caveolin-binding domain, signals transduced from nucleus to plasma membrane facilitated ERK localizing and activating in caveolae in adult cardiac myocytes.

5.2 PI3K/Akt pathway

PI3K/Akt is another protein kinase pathway which interacts with caveolae and promotes cell survival and growth. PI3K/Akt can be activated by growth factors or mechanical strain, both of which have been demonstrated to be dependent on caveolae as a gathering site. PI3K/Akt is required in the positive autoregulatory feedback loop of caveolin-1 with growth factors [109]. Likewise, Src-mediated phospho-caveolin-1 on Tyr14 has been confirmed to participate in downstream Akt activation [110,111]. When exposed to shear stress, cellular mRNA and protein levels of caveolin-1 were increased [112], which was accompanied by the activation of Akt-dependent signaling [113]. Disruption of caveolae and silencing of caveolin-1 blocked Akt activation *in vitro* and *in vivo* [110,114]. In contrast, upregulation of caveolin-1 can counteract Akt signaling pathway and its physiological functions such as promoting cell proliferation, migration and invasion, through the lack of ceramide synthesis, which can inhibit PI3K activity [115].

The interplay between caveolae and PI3K/Akt pathway is closely linked with caveolae-associated neuroprotection. It was reported that reduction of caveolin-1 or disruption of caveolae remarkably attenuated Akt-dependent PC12 cells survival [116]. The caveolin-dependent Akt activation sequentially attenuated ischemic cell death through the glycogen synthase kinase-3β/β-catenin/cyclin D1 pathway [117]. Akt also accounts for the caveolin-1-induced alteration of expression and distribution of TJ- and AJ-proteins. The interaction of caveolae and Akt may be due to the raft nature of caveolae. Caveolins, as a docking site for PI3K, facilitate the generation of phosphatidylinositol-3,4,5-trisphosphate, which specifically recognizes Akt and triggers the recruitment of Akt to caveolin-1 [110]. Akt undergoes phosphorylation by phosphoinositide-dependent kinases and is then activated [118].

In addition to caveolin-1, caveolin-3 has been demonstrated to enhance PI3K/Akt signaling. Caveolin-3 is crucial for myoblast differentiation and fusion. Unbalanced level of caveolin-3 leads to muscular dystrophy or hypertrophy, which is mediated by the alteration of Akt [119]. On oxidative stress, the PI3K-associated proteins PDK1 and Akt associate with caveolae where they bind to caveolin-3. The activation of this pathway promotes cell survival. Deficiency of caveolin-3 protein at the plasma membrane perturbs PI3K/Akt signaling and causes an increased susceptibility to oxidative stress [120]. In the context of ischemia, cardiac-specific overexpression of caveolin-3 *in vitro* and *in vivo* increased basal Akt phosphorylation in cardiomyocytes and generated endogenous cardiac protection against ischemia. Wortmannin, a PI3K inhibitor, blocked basal phosphorylation of Akt and cardiac protection in caveolin-3 overexpression mice, supporting a checkpoint role of PI3K/Akt pathway in the caveolin-3-induced cardiac protection [56]. There is evidence that caveolin-3 may be linked with dystrophin and form a signalosome to potentiate the Akt activity [121]. This caveolin-3-dependent Akt may switch the translocation of endothelial NO synthase/NO (eNOS/NO) from caveolae to mitochondria and causes mitochondrial protein S-nitrosylation [122], which sequentially lead to endogenous protection.

5.3 Apoptotic and autophagic pathways

In addition to the aforementioned reperfusion injury salvage kinase (RISK) pathways, caveolae/caveolins also show direct connections to both intrinsic and extrinsic apoptosis pathways. Mitochondrial dysfunction has been well established as a hallmark of intrinsic apoptosis. In response to apoptotic stimuli, mitochondria release proteins, such as cytochrome C, apoptosis-inducing factor, Smac/DIABLO and Htra2/Omi, which exert cryptic cytotoxic activity after releasing from the mitochondrial intermembrane space into the cytoplasm [123]. Morphologically, caveolae are closely apposed to mitochondria, and caveolins are co-localized within mitochondria [61]. Various caveolin-1-deficient cells have displayed compromised mitochondrial function, including increased mitochondrial reactive oxygen species (ROS) production [124], abnormal mitochondrial proliferation/aggregation [125], impaired oxidative phosphorylation and energy generation [126,127]. Likewise, caveolin-3 KO cardiac myocytes have increased generation of mitochondrial ROS, whereas caveolin-3 overexpression in mitochondria attenuated apoptotic stress and conferred remarkable tolerance to ischemic injury [61]. Nonetheless, how caveolin translocates into mitochondria, and how caveolin enhances the mitochondrial tolerance to cellular stress, remains to be answered.

As for the extrinsic apoptosis pathway, a potential caveolin-binding motif has been identified in the human death receptor Fas sequence [128]. In lung epithelial cells, short-term exposure to hyperoxia elicited the interaction between caveolin-1 and Fas. Genetic deletion of caveolin-1 disrupted Fas multimerization and death-inducing signaling complex formation [129]. Moreover, caveolin-1 could interact with Bid, a Bcl-2 interacting protein that relays apoptotic signaling from the cell surface to mitochondria [130], via the Tyr14 phosphorylation. In this way, caveolin-1 functions as a multifaceted operator of Fas receptors, which allows Fas multimerization and Bid truncation following apoptosis induction [129].

On the other hand, caveolins have been suggested to contribute to the crosstalk between autophagy and apoptosis. The autophagic protein microtubule-associated protein 1 light chain-3B (LC3B) positively regulated cigarette smoking-induced epithelial cell apoptosis through LC3B–caveolin-1–Fas interaction. The primary structure of LC3B contains a sequence ^{108}FLYMVYASQETF^{119}, which

served as caveolin-binding motif and mediated the connection with CSD. The knockdown of caveolin-1 resulted in higher levels of autophagy and apoptosis in the lung in response to the cigarette smoking exposure [131].

5.4 Cellular prion protein pathway

Prions exist in two major isoforms. The normal form is cellular prion protein (PrP^C), whereas its aberrant isoform, scrapie prion protein, is considered to underlie transmissible spongiform encephalopathies. PrP^C, a cell-surface glycoprotein anchored by a glycosylphosphatidylinositol moiety, is expressed throughout the brain and is particularly abundant in neurons [132]. Recently, mounting evidence indicates that PrP^C plays neuroprotective role in neuronal survival under various environmental stresses [133-135].

PrP^C was found to have a physical association with caveolin-1 in N2A neuroblastoma cells [136], PC12 neuronal-like cells [137] and GN11 hypothalamic neuronal cells [138]. Mouillet-Richard *et al.* utilized 1C11 cells to evaluate the signaling consequences of the coupling of PrP^C and caveolin-1 [139]. The 1C11 cell line behaves as neuroectodermal progenitor with an epithelial morphology that lacks neuron-associated functions. On induction, 1C11 cells develop a neural-like morphology and may undergo either serotonergic or noradrenergic differentiation. They demonstrated that caveolin forms a complex platform together with PrP^C and Fyn, a tyrosine kinase of Src family [139]. Caveolin is necessary for this coupling since immunosequestration of caveolin using antibody-coated tungsten bullets cancels Fyn activation. Noticeably, the caveolin-dependent Fyn activation was restricted to fully differentiated serotonergic or noradrenergic progenies [139,140]. Sequentially, the implementation of PrP^C-caveolin-Fyn platform could trigger the activation of the PI3K, PKCδ and nicotinamide adenine dinucleotide phosphate oxidase. The ROS generated by the latter enzyme act as second messengers and lead to downstream stimulation of ERK1/2 [139,141]. Alternatively, PrP^C–caveolin–Fyn complex may activate ERK1/2 through the classic Ras/Raf cascade. Tyrosine phosphorylation of caveolin-1, as a consequence of PrP^C stimulation, coordinates protein complexes involved in PrP^C-dependent signaling [137].

6. Caveolae counteract neuroinflammatory processes

Caveolae, together with all three caveolin isoforms, are highly expressed in astrocytes and microglia, the immunomodulatory cells in the CNS. Microglia adopt an activation state when cultured in high serum conditions but remain inactive in serum-free media. Using this model, Niesman *et al.* showed that caveolin-1 was significantly less and localized to plasmalemma and cytoplasmic vesicles of inactive microglia, whereas the active (amoeboid-shaped) microglia exhibited increased caveolin-1 expression. Conversely, caveolin-3 was highly expressed in the inactive state and localized with cellular processes and perinuclear regions [22]. The same group later employed controlled cortical impact model in caveolin-1 and caveolin-3 KO mice and investigated the role of caveolin in the neuroinflammatory response after the brain injury. Despite their differential expression in microglia, caveolin-1 and -3 KO mice have amplified proinflammatory cytokines, including IL-1β, IL-2, IL-6, IL-9, IL-10, IL-17, keratinocyte chemoattractant, monocyte chemotactic protein-1 and macrophage inflammatory protein-1α, as compared with the WT counterparts. Therefore, caveolin-1 and -3 KO mice exhibited larger brain lesions [142]. Coincidently, downregulation of caveolin-1 could attenuate leukocyte adhesion in pial venules of ovariectomized female rats [143]. In peripheral macrophages, downregulation of caveolin-1 significantly sensitized inflammatory responses to lipopolysaccharide [144,145], whereas overexpression of caveolin-1 markedly attenuated proinflammatory cytokine (TNF-α and IL-6) production and increased anti-inflammatory cytokine (IL-10) production [144]. Notably, systemic administration of CSD peptides could block carrageenan-induced acute inflammation to the same extent as glucocorticoid *in vivo* [146]. The inhibitory binding with eNOS could, at least partly, account for the anti-inflammation effects of caveolin peptide [147].

Alternatively, caveolae are associated with Toll-like receptor 4 (TLR4) in microglial cells [148], astrocytes [149] and endothelial cells [150]. TLR4 is a key receptor mediating overwhelming neuroinflammation and damage in stroke and neurodegeneration. Caveolin-1 has been shown to directly interact with TLR4 at its caveolin-binding motif (^{739}FIQSRWCIF^{747}) [99], which functionally suppresses TLR4 complex assembly with MyD88 or Toll/IL-1R domain-containing adaptor-inducing IFN-β (TRIF) [145]. The downstream NF-κB pathway and proinflammatory gene expressions were sequentially inhibited. In endothelial cells, the caveolin-dependent TLR4-MyD88-NF-κB pathway could also influence the endothelial barrier breakdown [80,151], which in turn aggravates the neuroinflammation. Taken together, these findings support the notion that caveolae play an anti-neuroinflammatory role under pathological conditions.

7. Caveolae promote angiogenesis

Angiogenesis is a well-established event which noticeably occurs in stroke-affected regions. Although excessive angiogenesis may have adverse consequences through worsening edema and hemorrhage in acute stage [152], angiogenesis is essential for ischemic brain restoration because it stimulates blood flow, collateralization and neuroplasticity in the ischemic boundary zone [153]. Various strategies to upregulate or downregulate the post-stroke angiogenesis have been shown to enhance or impair the rehabilitation, validating that angiogenesis augmentation can be harnessed as a viable therapeutic strategies for stroke recovery [154,155].

Angiogenesis is a dynamic process of endothelial proliferation, migration and differentiation. Caveolin-1 expression is

downregulated during the mitogenic stimulation of endothelial cells [156,157]. Caveolin-1 null mice develop hyperproliferative endothelium [72], thus suggesting that caveolin-1/caveolae can negatively regulate endothelial cell proliferation. Consistently, mature lung endothelial cells from caveolin-2 KO mice displayed higher proliferation rate and cell cycle progression relative to their WT counterparts [158]. It is worth to note that caveolin-2 deficiency also induced a parallel moderate reduction of caveolin-1 [18]. However, given that caveolin-1 (+/-) heterozygous mice, whose caveolin-1 level is comparable to caveolin-2-deficient mice, did not generate higher cell proliferation and the hyperproliferative phenotype observed in the caveolin-2 KO endothelial cells is unlikely due to the reduction of caveolin-1 in these cells [158]. Thus, caveolin-2 alone is capable of regulating proliferation and cell cycle progression in endothelial cells. During differentiation phase of angiogenesis, on the other hand, caveolin-1 increased in a time-dependent manner [157]. Antisense oligonucleotides-mediated ablation of caveolin-1 expression reduced endothelial differentiation, as indexed by capillary-like tubule formation in an *in vitro* Matrigel assay system [159], as well as using a three-dimensional fibrin gel assay or the chorioallantoic membrane angiogenesis system [160]. In contrast, overexpression of caveolin-1, or cell-permeable CSD peptides, clearly accelerated endothelial cell differentiation and tubule formation [159]. These findings demonstrate that caveolin-1 can promote the differentiation process and may function as a 'differentiation sensor' which readily responds to the microenvironment to coordinate proliferation and differentiation of endothelial cells during vascularization.

Mechanistically, many regulators (e.g., VEGF, VEGFR2, glypican-1, prostacyclin synthase and eNOS) involved in angiogenesis interact directly or indirectly with caveolae, thereby forming a modular unit [161]. VEGFR2 has been identified as the main receptor driving cellular angiogenic signaling cascades of VEGF in endothelial cells. Binding of VEGFs to the extracellular domains of VEGFR2 initiates receptor dimerization and phosphorylation of multiple tyrosine residues (e.g., Y951, Y1175, Y1214, Y1054 and Y1059) which, in turn stimulate activation of multiple downstream molecules [162]. VEGFR2 is localized in endothelial caveolae through physical association with caveolin-1 [163]. On VEGF stimulation, both VEGFR2 and caveolin-1 are phosphorylated and dissociated from caveolae/lipid raft [164]. Downregulation of caveolin-1 significantly reduced basal and VEGF-stimulated phosphorylation of VEGFR2 and downstream effectors such as PLCγ1, Akt and ERK1/2 [162,165]. On the other hand, caveolin-1 overexpression could inhibit VEGFR2 activity but allow VEGFR2 to undergo VEGF-dependent activation, thus suggesting that caveolin-1 confers ligand dependency to a receptor system [163]. This paradox phenomenon may arise from the nature of scaffold proteins, too much or too little of which may decrease the output of its associated signaling pathway [166]. As indicated in these studies, the level of caveolin-1 is critical for endothelial cells to sustain maximal angiogenic signaling [162,163,165], thereby supporting a check-and-balance role of caveolae/caveolin-1 in angiogenesis.

Intriguingly, a bilateral regulation of eNOS by caveolae was also found in the context of angiogenesis, during which NO is known to play pivotal roles [167]. Early reports revealed a tonic inhibitory interaction between caveolin and eNOS. The eNOS is kept inactive by caveolin or more specifically by CSD in caveolae [168,169]. Nonetheless, caveolae also exert compartmentation effect for eNOS. When palmitoylation sites were removed from eNOS, which resulted in the disappearance of eNOS from caveolae, the NO release from eNOS was diminished [170]. In caveolin-1 null endothelial cells, VEGF induced much less NO production and endothelial tube formation. The eNOS Ser1177 phosphorylation and Thr495 dephosphorylation (two hallmarks of eNOS activation) were both blunted. In such cases, re-expression of caveolin-1 to physiological level could redirect VEGFR2 in caveolar membranes and restore the eNOS activation [54]. These findings illustrate that caveolae could provide a signaling platform/compartmentation for VEGFR2/eNOS coupling [171].

8. Role of caveolae in the preconditioning

Preconditioning refers to the phenomenon that previous exposure to sublethal insults can generate robust resistance to the later lethal injuries [172]. Effective preconditioning stimuli involve ischemia, anesthetics, exercise, opioid receptor agonism and toxic agents such as NMDA, lipopolysaccharide and oxidative stress. In cardiac myocytes, several preconditioning paradigms, including opioid receptor stimulation, ischemia and isoflurane pretreatment, could increase sarcolemmal caveolar number, which was accompanied by the enrichment of phosphorylated and total caveolin-1 in caveolae-containing buoyant fractions. *In vitro* and *in vivo* depletion of caveolae fully attenuated preconditioning-induced protection [173,174], resulting in an infarct size comparable to that of ischemic group. These results suggest that the presence of caveolae is essential for the preconditioning cardiac protection from ischemia/reperfusion injury. Roth's group sequentially investigated the specific caveolin proteins in the preconditioning. Caveolin-1 KO and WT mice showed similar area at risk as a percentage of the left ventricle. Infarct size was reduced in preconditioned WT mice but not in caveolin-1 null mice [174]. In caveolin-3 KO mice, the number of myocardial caveolae decreased albeit caveolin-1 expression is normal. Nonetheless, such mice lack the ability to achieve isoflurane-induced cardiac protection from ischemia/reperfusion injury [175]. Likewise, Ma's group confirmed the role of caveolin-3 in sevoflurane preconditioning [176]. Intriguingly, delayed protection was still present in caveolin-1 null but not caveolin-3 null mice, suggesting that the caveolae formation (dependent on caveolin-3) might be a common element to both acute and delayed phases of preconditioning-induced protection [177,178]. Moreover, *in vitro* adenoviral

overexpression of caveolin-3 enhanced caveolar formation and phosphorylation of survival kinases in cardiac myocytes. Cardiac-specific caveolin-3 overexpression mice displayed preserved ultrastructure, improved cardiac function and reduced apoptosis after ischemia/reperfusion injury. The innate tolerance was comparable to that of preconditioned WT mice [56]. These findings demonstrate that caveolin-3 expression is both necessary and sufficient for preconditioning protection.

The role of caveolae in the preconditioning of the brain has likewise been investigated. Caveolin-1 co-localizes with NMDA receptor [36], a subtype of ionotropic glutamate receptors playing key roles in synaptic plasticity and excitotoxicity in ischemia. The coimmunoprecipitated complex is distributed on the cell body and along neuronal extensions resembling dendritic shafts and spines. On preconditioning stimulation, caveolin-1 [179] and phosphorylated caveolin-1 [36] were upregulated, which might facilitate the redistribution of NMDA receptor to neuronal membrane rafts and pro-survival kinase activation. Caveolin-1 KO mice exhibited compromised synaptic signaling and scaffolding proteins in hippocampal synaptosomes, and this was associated with an inability to be preconditioned against ischemia [180]. In caveolin-1-deficient neurons, pre-exposure to sublethal OGD or NMDA failed to protect cells from subsequent ischemia. In contrast, re-expression of caveolin-1 protein could restore the capacity of primary neurons to undergo the preconditioning-induced neuroprotective effects [36]. More importantly, overexpression of neuron-targeted caveolin-1 in primary neurons enhances NMDA-mediated pro-survival signaling, neuronal growth and arborization, even in the presence of inhibitory cytokines and myelin-associated glycoproteins [37]. Caveolin-1, thus, may function as a checkpoint for NMDA receptor and its downstream neuroprotection, neurotrophy and neuroplasticity. A range of molecules are involved in the preconditioning-associated pro-survival signaling, but very few of them are sufficient to be harnessed *per se* to mimic the effects. In this context, the findings on caveolin are promising and may provide a therapeutic benefit in functional recovery from brain injury.

How do preconditioning stimuli manipulate the caveolar turnover? Current evidence has indicated a cascade which initiates via GPCRs and involves downstream Src as a main mediator. Src is a nonreceptor protein tyrosine kinase. Its membrane association is critical for Src signaling and activity. Various stress stimuli could induce tyrosine phosphorylation of caveolin-1 and this phosphorylation is dependent on activation of Src [150,181,182]. In agreement with the phosphorylation of caveolin-1 following preconditioning, Src undergoes an autophosphorylation at tyrosine 416 [36,174,183]. Because this residue is present in the activation loop domain, its phosphorylation promotes kinase activity. Both phosphorylated and total Src are enriched in caveolae-containing membrane/raft fractions. If Src-specific inhibitor PP2 was administered shortly before preconditioning (NMDA or sublethal OGD), phosphorylation of Src (Y416) and caveolin-1 (Y14) was significantly attenuated, which eventually led to the abolishment of preconditioning-induced neuronal protection [36]. Caveolin in turn regulates Src dynamics. Caveolin-1 selectively enhances the membrane association of activated Src through SH2 domain and the phosphorylation of caveolin-1 (Y14) by Src [182]. Isoflurane-pretreated caveolin-1 null mice showed decreased recruitment of C-terminal Src kinase (Csk), a negative regulator of Src, and deactivation of Src (Y527 phosphorylation), when compared to WT mice [174]. Consistently, phosphor-caveolin has been shown to be a transient nexus for Csk to target Src in membranes to phosphorylate Y527 and inactivate Src in response to oxidative stress [184]. Given that Ras/Raf and PI3K are well-defined downstream substrates of Src, the crosstalk between caveolin and Src may provide important convergence of preconditioning-induced pro-survival RISK signaling.

9. Expert opinion

Protective strategies geared toward cerebral ischemia injury are challenging and are of great interest. The collective experience of past failed trials should not be misconstrued as evidence that neuroprotection in patients is unattainable [5]. Above all, numerous studies have characterized the pathophysiology of ischemic brain injury and have provided scientifically irrefutable proof-of-principle that ischemic neuroprotection, in fact, is feasible with a variety of agents [185-188]. Many reasons have been suggested for the unsuccessful translation of neuroprotective strategy. An important reason is that inappropriate target selection leads to the insufficient therapeutic efficacy. For example, those therapies aiming at tackling the event occurred at the very early beginning of ischemia may be unlikely to work within the clinical window of treatment [4]. With hindsight, the failures of Cerestat (noncompetitive NMDA antagonism) and clomethiazole (GABA agonism) can be clued from their relatively short therapeutic window revealed in preclinical studies [189]. Also, pathophysiological mechanisms in ischemic phase may partially differ from those in reperfusion phase, as exemplified by ROS which plays a less important role in the permanent ischemia model [190]. In this context, neuroprotection for stroke should not perish with the past failures but instead deserves continued assessment with more carefully selected molecular targets.

Perhaps blocking a single deleterious event is not enough to ameliorate the outcomes of a multifactorial condition such as ischemic stroke. Simultaneous inhibition of various steps in the ischemic cascade may protect the brain tissue more efficiently [191]. Caveolae and caveolins undoubtedly regulate various aspects of pathophysiological mechanisms in ischemic stroke, making them a potential target for prophylactic neuroprotection, adjuvant for thrombolysis and neurorestoration. Thus, caveolae may be ideal for producing the so-called *dirty* drugs with amplified therapeutic efficacy and extended therapeutic window. A series of signaling pathways couple caveolae

with ischemia (**Figure 2**), thereby suggesting caveolae as a novel pathologically activated therapeutic target [192]. Nonetheless, many of the reports here have utilized non-stroke scenario to generate testable hypotheses about signaling pathways associated with cerebral ischemia. Future studies are urged to delineate the role of caveolae in the *in vitro* and *in vivo* models of stroke. It is noteworthy that caveolae may play a check-and-balance role in stroke. Uncareful augmentation of caveolin expression may generate double-edged effects, thereby missing the maximal benefits. On the one hand, caveolin deficiency worsens cerebral ischemia [51], whereas overexpression of caveolins unequivocally regains tolerance from ischemia injury [56-58]. On the other hand, there is evidence that caveolin deficiency betters intracerebral hemorrhage [193], and overexpression of caveolin-1 impairs post-ischemic recovery [15]. Such controversy may not be simply explained by the different ischemia models. The complex nature of caveolae/caveolin as a scaffold dock [166] must also be considered. In this context, the following issues are critical for the translation of caveolae as therapeutic target in stroke.

First, caveolins may present differential roles in different cell types in the CNS, especially in the neurovascular unit. For BBB homeostasis, caveolae surely facilitate endothelial vesicle trafficking in the endothelial cells [72-74], which justifies caveolae as a vesicle carrier to transport agents across BBB [69]. However, transcytosis is paralleled with early BBB breakdown. Caveolin-1 KO attenuated macromolecular extravasation during early phase following ischemic stroke. Surprisingly, such BBB improvement seemed not to reduce the infarction size [74]. This discrepancy might be explained by disconnection of early transcytosis and overall histopathological outcome, or by other compensatory mechanisms of caveolin downregulation during later phase. Indeed, caveolae have been demonstrated to fasten MMP activity, thereby preserving paracellular permeability following ischemic stroke [59,93,94]. As such, endothelium- or astrocyte-targeted manipulation of caveolin is awaited to clarify the cell-specific role of caveolae in the BBB disruption of stroke.

Second, caveolae may present dynamic roles during the rehabilitation of stroke. For angiogenesis, caveolin-1 seems capable of slowing down cell proliferation and speeding up endothelial differentiation and tube formation. Accordingly, caveolin-1 is downregulated in the proliferation phase but gradually upregulates when endothelial cells go into differentiation. Ischemic stroke stimulates angiogenesis and vasculogenesis [155]. Persistent downregulation of caveolin-1 reduces endothelial cell number and collateral perfusion after ischemia [51,54,194]. It is unclear whether caveolin-1 could improve stroke outcomes through angiogenesis regulation, but the double-edged effects of caveolin on angiogenesis and timing of manipulation are worthy of attention in the future investigations.

Third, it remains open how to manipulate caveolin expression in a practical way to recapitulate the beneficial therapeutic outcomes. The common strategy for gene therapy is to develop a gene delivery vector that is safe and efficacious for patients. However, such method is still in its infancy. One alternative approach is to directly deliver CSD peptides as a supplementation of caveolin protein. CSD peptides function as molecular partners, which is independent of caveolae formation. It has been shown to increase astrocyte survival after OGD [58] and to exert bioactive effects via systemic administration [57]. Most, if not all, caveolins are located in caveolae of plasma membranes. Therefore, it is not necessary for CSD peptide to gain access to the cytoplasm to bind its protein partners. Yet, its brain distribution and efficacies in animal stroke model and in clinical settings still await investigation. Another approach is to employ post-transcriptional regulators such as microRNAs to modulate caveolin expression. MicroRNAs are small noncoding RNA molecules which bind to mRNA and suppress the translation of mRNA, thus influencing the post-transcriptional gene expression. Its small size allows its easy incorporation into cellular compartments. It has been reported that antagomirs of caveolin-associated microRNAs (miR-103/107) could upregulate caveolin-1 protein expression and enhance insulin-stimulated glucose uptake [195]. Further investigations are encouraged to examine the potential efficacy of miR-103/107 antagomirs in cerebral ischemia or to design novel caveolin microRNA antagomirs with potential pharmaceutical implications.

Collectively, caveolae have a beneficial role in stroke. More sophisticated work in this field has great potential to lead to the development of novel therapies for cerebral ischemia.

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L Xu and R Guo have equally contributed to this work.

Declaration of interest

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