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Original Article

Effect of caveolin-1 on the expression of tight junction-associated proteins in rat glioma-derived microvascular endothelial cells

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Abstract: Caveolin-1 affects the permeability of blood-tumor barrier (BTB) by regulating the expression of tight junction-associated proteins. However, the effect is still controversial. In the present work, we studied the regulative effect of caveolin-1 on the expression of tight junction-associated proteins and BTB via directly silencing and overexpressing of caveolin-1 by recombinant adenovirus transduction of glioma-derived microvascular endothelial cells in rat brain. The results show that the caveolin-1 downregulation resulted in decreased expression of tight junction-associated proteins, opening of tight junctions, and increasing the permeability of BTB, whereas the overexpression of caveolin-1 presented the opposite effects. Therefore, we conclude that caveolin-1 regulates the expression of tight junction-associated proteins in a positive manner, which further plays a role in the regulation of BTB permeability. This finding provides a novel therapeutic target for selectively opening of BTB.

Keywords: Caveolin-1, blood-tumor barrier, tight junction-associated proteins, occludin, claudin-5, ZO-1

Introduction

Blood-tumor barrier (BTB) limits the therapeutic effects of anti-tumor drugs [1]. Therefore, selectively increasing the permeability of BTB is an ideal strategy for glioma treatment with chemotherapy. Transcellular and paracellular pathways are two major strategies for BTB opening [2]. Caveolae-induced internalization belongs to transcellular pathway. Caveolin-1, as an essential structural protein for caveolae formation, regulates vesicular transport of downstream and upstream effectors by combining the caveolin-1 specific binding domain of multiple signaling molecules via its scaffolding domain [3-5]. Tight junctions, the important structure for BTB formation, consist of the integral membrane proteins (claudins and occludin) and peripheral proteins (zonula occludens, ZOs). The role of tight junctions is to mediate the paracellular transport of BTB [6, 7]. Nag et al. [8] demonstrated an increased permeability of blood-brain barrier (BBB) in rat cryogenic brain injury model, and the expression of tight junction proteins (occludin and claudin-5) were downregulated when caveolin-1 level increased; Zhong et al. [9] showed that type 1 immunodeficiency virus Tat induced the elevated expression of caveolin-1 in human brain microvascular endothelial cells, which sequentially downregulated the expression of occludin, ZO-1 and ZO-2, and then followed by the increased permeability of BBB. However, it is controversial with the study on caveolin-1 knockout mice [10]. In caveolin-1 knockout mice, the vessel function was defected and the activity of eNOS expressed by vascular endothelial cells was increased, moreover, an increased permeability of vessels was observed. In this study, we tried to modulate the caveolin-1 expression by using recombinant adenovirus-based transduction of caveolin-1 into brain microvascular endothelial cells in rat glioma. Furthermore, we studied the effect of caveolin-1 on the regulation of tight junction-associated proteins and the permeability of BTB in rat glioma model.

Materials and methods

Reagents

Recombinant adenoviral vectors of caveolin-1 were prepared and stored by our lab, amplifying and purifying by ourselves. Mice monoclonal antibody for caveolin-1 and rabbit polyclonal antibody for tight junction-associated proteins

were purchased from Abcam Ltd. Other regents, unless otherwise specified, were purchased from Sigma-Aldrich.

Cell culture

Rat glioma cell line C6 (from the Department of Neurobiology, China Medical University) were cultured in high glucose DMEM supplemented with 10% fetal bovine serum, 100 mg/ml streptomycin and 100 U/ml penicillin. Cells were placed in the 37°C with 100% humidity and 5% CO_2. Cells were collected when exponential growth phase was reached. Cell suspension was prepared in DMEM without addition of serum after centrifuge.

Animals and experimental groups

Wistar rats (provided by the Center for Experimental Animals of China Medical University) weighed between 200 g and 250 g were used as the source of microvessels. Animal studies were carried out in accordance with NIH Guide for the Care and Use of Laboratory Animals. Rats were randomly divided into 5 groups: control group (glioma modeling rats with PBS injection), caveolin-1 silencing group (named as Ad-siRNA-cav1, glioma modeling rats were injected with Ad-siRNA-cav1 in order to silence caveolin-1), caveolin-1 overexpression group (named as Ad-cav1, recombinant adenoviral vector were injected into the brain of glioma modeling rats to increase the expression of caveolin-1), and negative controls for caveolin-1 silencing group (named as Ad-siRNA-NC, no RNA was silenced) and negative controls for caveolin-1 overexpression group (named as Ad-EGFP, only expressed the protein of EGFP). 10 rats were included in each group.

Glioma modeling with C6 cells in rats

1×10^6 C6 cells in 10 μl culture medium were stereotactically (1 mm anterior and 3 mm right to the bregma, 4 mm deep from the dura after withdraw 0.5 mm) injected into the brains of rats after anesthesia with intraperitoneal injection of 10% chloral hydrate (3 ml/kg). After 10 min injection, the needle was remained in the place for another 10 min with the aim of allowing the cells to fully settle down in the caudate nucleus area. The needle was withdrawn slowly. The surgery area was disinfected with penicillin solution before stitching it.

Transduction of the recombinant adenoviral vector

On day 18 post-transplantation of C6 cells, 20 μl recombinant adenovirus was injected into the brain at the same place where C6 cells were injected. The injection procedures were performed as described above. At 48 h after injection, rats were sacrificed, and the brains were processed for further experiments.

Evans blue (EB) experiment

The permeability of BTB was evaluated based on leakage of EB. Rats were anesthetized and followed by femoral vein injection of 2 mg/kg 2% EB. Around 2 h after injection, the chest cavity was opened to expose the heart. Left ventricle was perfused with physiological saline, and the brain was extracted after clear solution was drained from the right atria. After weighing the right- and left-brain, EB dye was extracted from the brains by immersion of the brains in 1 ml/100 mg of formamide overnight at 60°C and measured spectrophotometrically at 620 nm. The formamide solution was set up as a negative control.

Transmission electron microscope

Rat decapitation was performed after anesthesia. The tumor tissues was recovered from the brains and washed with physiological saline before fixation in 2.5% glutaraldehyde for 2 h. After rinsing with PBS for 3 times (15 min for each time), the tissues were fixed in 1% osmic acid for 1 h and followed by a washing step with 0.1 M PBS. The tissues were stained with uranyl acetate for 2 h, and sequentially dehydrated for 15 min in each dilution of propanone (50%, 70%, 80%, 90%, and 100%). The tissues were final dehydrated 2 times in 100% propanone for 10min each time and followed by immersion steps (aceton:embedding medium = 1:1, placed in oven at 37°C for 2 h, aceton:embedding medium = 1:4 at 37°C overnight, and embedding medium at 45°C for 2 h). The tissues can be observed after embedding and polymerization.

Western blot

The tumor tissues from each group were homogenized at 4°C in 1 ml lysis buffer (2 mM EDTA, 10 mM EGTA, 0.4% NaF, 20 mM Tris-HCl,

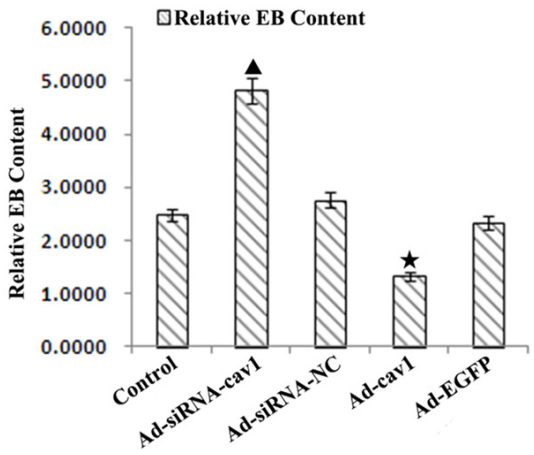

Figure 1. Altering the expression of caveolin-1 affects EB content in the brain of glioma modeling rat, the later representing the permeability of BTB. Relative EB content are shown. Values are means ± SD (n = 10, each). ▲P < 0.01 and ★P < 0.01 vs. control, Ad-siRNA-NC and Ad-EGFP group.

protease inhibitors, PH 7.5) and suspended on ice for 1 h. Samples were centrifuged at 14,000×g for 30 min at 4°C, the supernatant was collected for the cytosolic fraction (CF). The pellet was resuspended in lysis buffer containing 1% Triton X-100 and 0.1% SDS at 4°C. Samples were then centrifuged at 14,000×g for 30 min at 4°C. The supernatant was collected for the membrane fraction (MF). The protein concentrations of supernatant samples were determined using BCA™ Protein Assay Kit (Pierce). Equal amounts of MF proteins (10-20 μg) were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and processed with mice monoclonal antibody for caveolin-1 (diluted 1:250) and rabbit polyclonal antibody for occludin (diluted 1:500), claudin-5 (diluted 1:500) and ZO-1 (diluted 1:500). GAPDH (diluted 1:5000) was applied as an inner control. Immune complexes were visualized by enhanced chemiluminescence (ECL kit; Santa Cruz Biotechnology).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and post-hoc comparisons (Bonferroni test). The values were considered significantly different at $P < 0.05$. All experiments were repeated 3-5 times and data expressed as the mean ± SEM of several independent experiments.

Results

Evaluation of BTB permeability

First, the relative content of EB was calculated (EB content in the tumor side of brain/EB content in the non-tumor side of brain). Then, the relative content of EB in the different groups was compared. The results showed that the content of EB in Ad-siRNA-cav1 treated brains was significantly increased, whereas in the Ad-cav1 group, EB content was downregulated. In addition, no meaningful changes were observed in all negative control groups (**Figure 1**).

Ultrastructural changes of BTB tight junction

Tight junction of BTB opened and became broader in Ad-siRNA-cav1 treated group compared with the control groups, whereas the junction in Ad-cav1 group was tight and displayed as the narrow black belt (**Figure 2**).

Altered expression of caveolin-1 and tight junction-associated proteins

The expression of caveolin-1 was dramatically reduced, concordant with decreased expression of tight junction-associated proteins occludin, claudin-5 and ZO-1 ($P < 0.01$). However, the expression of caveolin-1 in Ad-cav1 treated group was obviously upregulated, as well as the expression of tight junction-associated proteins. There were no significant changes observed in the control groups (**Figure 3**).

Discussion

The results indicated that caveolin-1 gene silencing resulted in reduced expression of tight junction-associated proteins, broadening and opening of tight junction, and increased permeability of BTB; On the contrary, the overexpression of caveolin-1 led to the opposite effects. Therefore, we believe that caveolin-1 regulates the expression of tight junction-associated proteins in a positive manner.

Tight junction is important for the integrity and permeability of BTB. Claudins and occludin, as transmembrane molecules, compose tight junction complex. Tight junction complex combines adherence molecules of adjacent endothelial cells to form tight junction and physiolog-

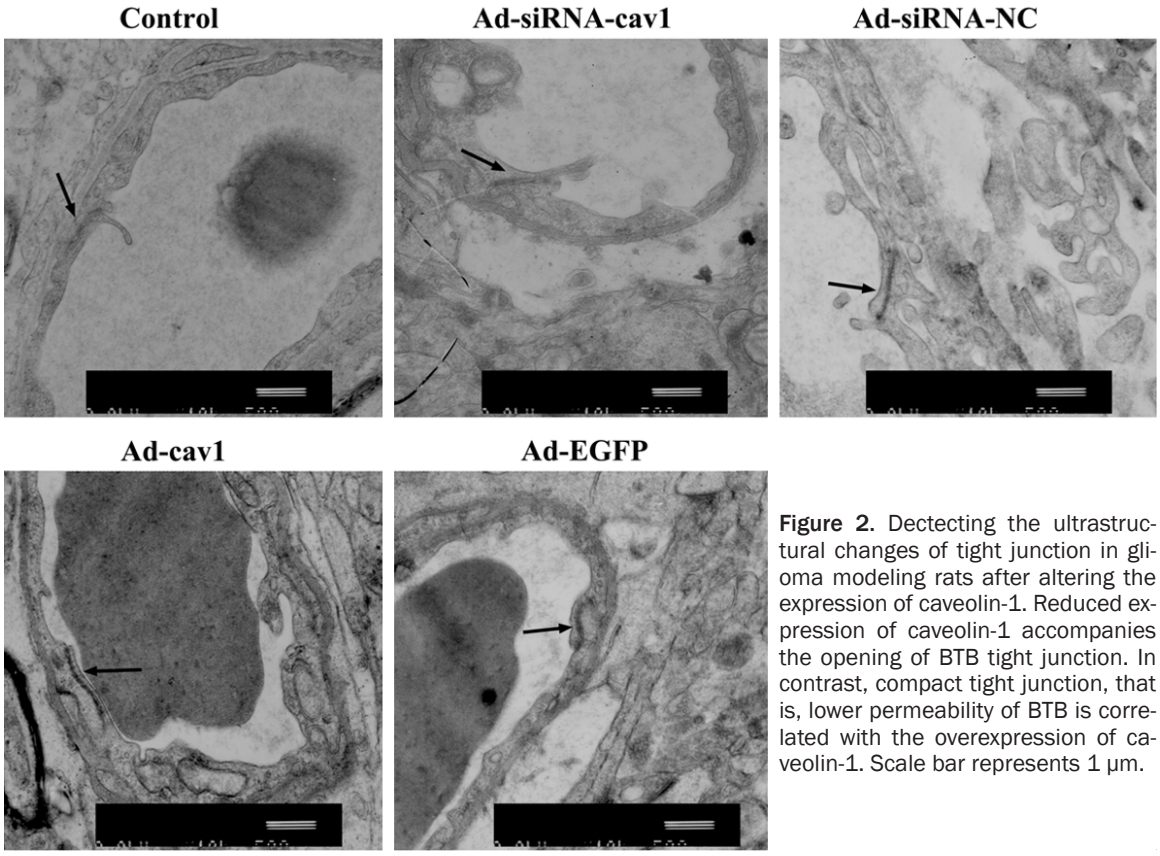

Figure 2. Dectecting the ultrastructural changes of tight junction in glioma modeling rats after altering the expression of caveolin-1. Reduced expression of caveolin-1 accompanies the opening of BTB tight junction. In contrast, compact tight junction, that is, lower permeability of BTB is correlated with the overexpression of caveolin-1. Scale bar represents 1 μm.

ical barrier for paracellular diffusion [11, 12]. Claudins is involved in the establishment of tight junction [13, 14]; occludin increases the tightness of junction and decreases the permeability of physiological barrier [15-17]. ZO-1, as a membrane cofactor protein, plays a role in the regulation of tight junction by binding together with occludin, claudins and actin cytoskeleton [18, 19]. The reduced level of tight junction-associated proteins and the alteration in their distribution cause the opening of tight junction and the increased permeability of BBB [20]. Caveolin-1 regulates the expression of tight junction-associated proteins, but the regulated effects are highly controversial. Some studies suggested the negative regulation of caveolin-1 on the expression of tight junction-associated proteins. For instance, by application of in vivo imaging system for study of occludin and ZO-1 internalization, Amanda et al. [21] reported that TNF and active MLCK (myosin light chain kinase) could stimulate the caveolin-1-dependent occludin internalization, and reduced the expression of occludin on the membrane of intestinal endothelial cells and the function of membrane barrier. Additionally, Xia et al. [22, 23] showed that caveolin-1 level increased in the microvascular endothelial cells derived from the BTB after a low-MHz ultrasonic irradiation. Meanwhile, the expression of tight junction-associated proteins ZO-1, occludin and claudin-5 decreased and the distribution of them altered. However, study from Schubert et al. [24] on caveolin-1 knockout mice demonstrated that tight junction in lung microvascular system was reduced in size and changed in shape, and the ability of paracellular transport of eosin enhanced. Miyawaki-Shimizu et al. [25] revealed that in vivo siRNA-induced caveolin-1 silencing increased the junction space between vascular endothelial cells and the permeability of the microvascular system in lung. In addition, Song et al. [26] used adenovirus delivery system of siRNA to specifically silence the caveolin-1 in cultured brain microvascular endothelial monolayer cells (BMEC). This study indicated that reduced expression of caveolin-1 accompanied the diminished expression of tight junction-associated proteins occludin and ZO-1, the heightened permeability of BMEC, and occludin exhibited dissociation from the cytoskeletal frame-

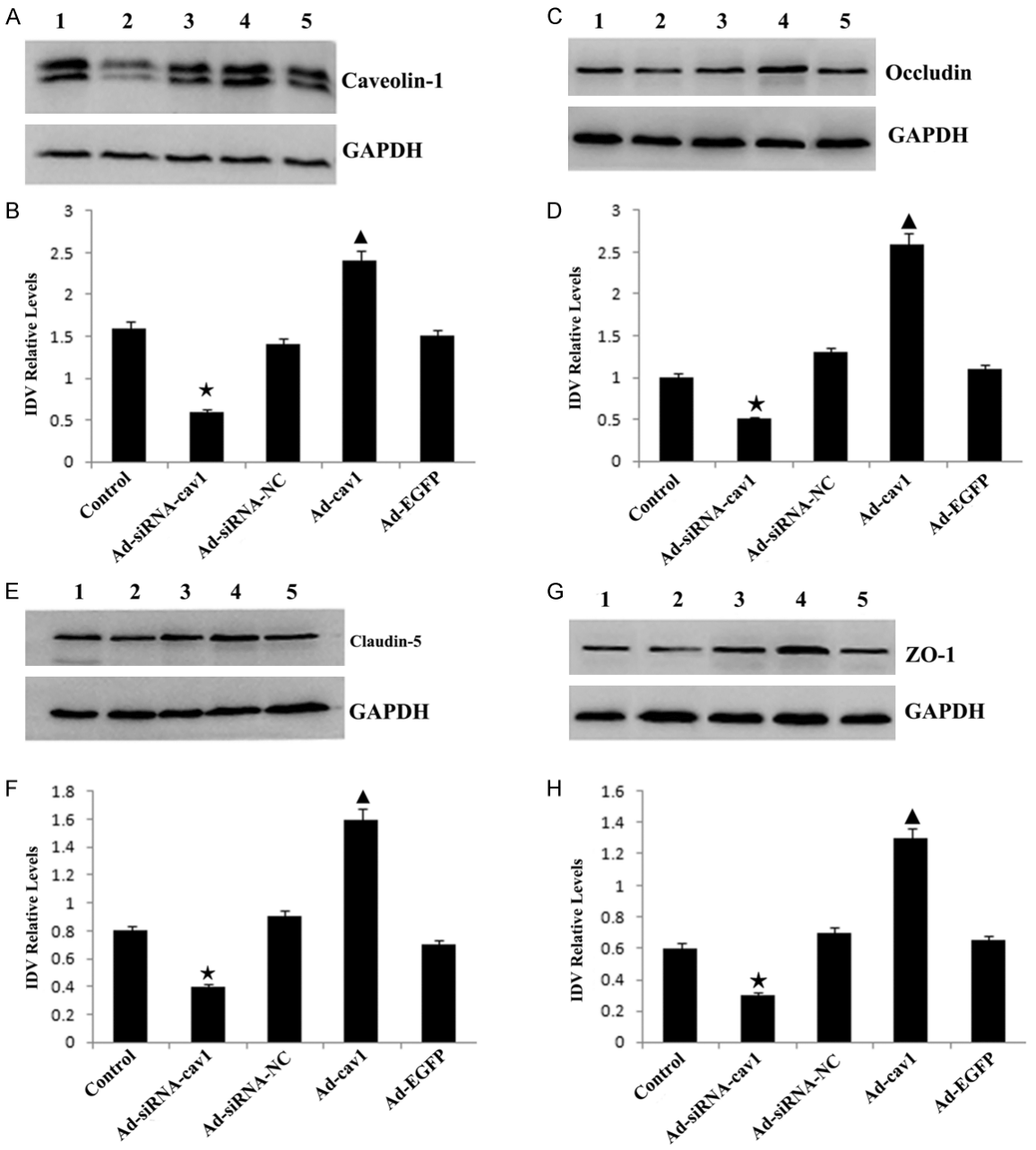

Figure 3. Caveolin-1 regulates the expression of tight junction-associated proteins. The regulation has a positive effect. (A) Expression of caveolin-1 protein; (C) Occludin; (E) Claudin-5; (G) ZO-1. Form left to right, lanes 1, 2, 3, 4, and 5 represent control, Ad-siRNA-cav1, Ad-siRNA-NC, Ad-cav1, and Ad-EGFP group, respectively. Integrated density values (IDVs) of caveolin-1 (B), occludin (D), claudin-5 (F), and ZO-1 (H) are shown. Values are means ± SD (n = 10, each). ▲$P < 0.01$ and ★$P < 0.01$ vs. control, Ad-siRNA-NC and Ad-EGFP group.

work. Studies mentioned above suggested that caveolin-1 has a positive effect on the regulation of tight junction-associated proteins, which consistent with the results described by our study. We demonstrated that, in rat glioma model, the overexpression and silence of caveolin-1 induced by recombinant adenovirus-mediated transduction to microvascular endothelial cells of BTB regulated the expression level of tight junction-associated proteins and the permeability of BTB.

The signaling pathways involved in the regulation of caveolin-1 on the permeability and integ-

rity of microvascular endothelial system following its positive regulation on the expression of tight junction-associated proteins are not well studied. With the isolation and characterization of caveolae, caveolin-1 is not only able to combine the binding domain of targeted proteins via its scaffolding domain with intention to inhibit the catalytic activity of the most signaling proteins but also has a synergic effect with caveolae on the internalization of multiple signaling proteins, such as EGF receptor, insulin receptor, Src family kinase, PKCs, eNOS, Grb2, mSOS1 and Nck [27, 28]. Caveolin-1 and its scaffolding domain regulate the activity of Src kinase in a negative manner. Several studies reported that suppressed caveolin-1 expression stimulated the Src kinase activity and resulted in the instability of the junction structures [29-32]. For instance, Src family kinase inhibitor PP1 could relieve the symptoms of hydrocephalus and the elevated permeability of BBB resulted by subarachnoid hemorrhage; A phosphatase inhibitor calyculin A increased the permeability of cultured endothelial monolayer, and Src family kinase inhibitor herbimycin A could significantly improve the function of the barrier. Additionally, eNOS can be negatively regulated by caveolin-1 and its scaffolding domain [33-35]. In caveolin-1 knockout mice, the activity of eNOS and permeability of microvessels were increased and the function of vessels was defected [36]. NOS inhibitor L-NAME could mend the vessel functions [37]. Ex vivo study of the vessels derived from the caveolin-1 knockout animal revealed that continuous elevation of eNOS activity resulted in the increased permeability of vessels [38, 39]. Thus, the effect of caveolin-1 on microvascular integrity and permeability may be due to the altered expression of tight junction-associated proteins that are regulated by signaling proteins, such as, but not limited to, Src family kinase and eNOS.

To our knowledge, our findings revealed for the first time that caveolin-1 plays a positive role in the expression of tight junction-associated proteins of microvascular endothelial cells in rat glioma model. Briefly, downregulation of caveolin-1 results in the decreased expression of tight junction-associated proteins, increased permeability of BTB. This regulative function might provide novel therapeutic targets for the treatment of the selective BTB opening.

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Disclosure of conflict of interest

None.

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References

[1] Black KL, Ningaraj NS. Modulation of brain tumor capillaries for enhanced drug delivery selectively to brain tumor. Cancer Control 2004; 11: 165-173.

[2] Salama NN, Eddington ND, Fasano A. Tight junction modulation and its relationship to drug delivery. Adv Drug Deliv Rev 2006; 58: 15-28.

[3] Sotgia F, Lee JK, Das K, Bedford M, Petrucci TC, Macioce P, Sargiacomo M, Bricarelli FD, Minetti C, Sudol M, Lisanti MP. Caveolin-3 directly interacts with the C-terminal tail of beta -dystroglycan. Identification of a central WW-like domain within caveolin family members. J Biol Chem 2000; 275: 38048-58.

[4] Bender F, Montoya M, Monardes V, Leyton L, Quest AF. Caveolae and caveolae-like membrane domains in cellular signaling and disease: identification of downstream targets for the tumor suppressor protein Caveolin-1. Biol Res 2002; 35: 151-67.

[5] Minshall RD, Malik AB. Transport across the endothelium: regulation of endothelial permeability. Handb Exp Pharmacol 2006; 107-144.

[6] Brown RC, Davis TP. Hypoxia/aglycemia alters expression of occludin and actin in brain endothelial cells. Biochem Biophys Res Commun 2005; 327: 1114-1123.

[7] Harhaj NS, Antonetti DA. Regulation of tight junctions and loss of barrier function in pathophysiology. Int J Biochem Cell Biol 2004; 36: 1206-1237.

[8] Nag S, Venugopalan R, Stewart DJ. Increased caveolin-1 expression precedes decreased ex-

pression of occludin and claudin-5 during blood-brain barrier breakdown. Acta Neuropathol 2007; 114: 459-469.
[9] Zhong Y, Smart EJ, Weksler B, Couraud PO, Hennig B, Toborek M. Caveolin-1 regulates human immunodeficiency virus-1 Tat-induced alterations of tight junction protein expression via modulation of the Ras signaling. J Neurosci 2008; 28: 7788-7796.
[10] Fleming I. Molecular mechanisms underlying the activation of eNOS. Pflugers Arch 2010; 459: 793-806.
[11] Tsukamoto T, Nigam SK. Role of tyrosine phosphorylation in the reassembly of occluding and other tight junction proteins. Am J Physiol 1999; 276: F737-750.
[12] Tsukita S, Furuse M. Occludin and claudins in tightjunction strands: leading or supporting players? Trends Cell Biol 1999; 9: 268-273.
[13] Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, Furuse M, Tsukita S. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. J Cell Biol 2003; 161: 653-660.
[14] Liebner S, Fischmann A, Rascher G, Duffner F, Grote EH, Kalbacher H, Wolburg H. Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. Acta Neuropathol 2000; 100: 323-331.
[15] Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S. Occluding: a novel integral membrane peotein localizing at tight junctions. J Cell Biol 1993; 123: 1777-1788.
[16] Furuse M, Itoh M, Hirase T, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S. Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. J Cell Biol 1994; 127: 1617-1626.
[17] Hirase T, Staddon JM, Saitou M, Ando-Akatsuka Y, Itoh M, Furuse M, Fujimoto K, Tsukita S, Rubin LL. Occluding as a possible determinant of tight junction permeability in endothelial cells. J Cell Sci 1997; 110: 1603-1613.
[18] Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. J Biol Chem 1998; 273: 29745-29753.
[19] Itoh M, Furuse M, Morita K, Kubota K, Saitou M, Tsukita S. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2 and ZO-3, with the COOH termini of claudins. J Cell Biol 1999; 147: 1351-1363.
[20] Zheng K, Trivedi M, Siahaan TJ. Structure and function of the intercellular junctions: barrier of paracellular drug delivery. Curr Pharm Des 2006; 12: 2813-2824.
[21] Marchiando AM, Shen L, Graham WV, Weber CR, Schwarz BT, Austin JR 2nd, Raleigh DR, Guan Y, Watson AJ, Montrose MH, Turner JR. Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. J Cell Biol 2009; 189: 111-126.
[22] Zhang Z, Xia C, Xue Y, Liu Y. The synergistic effect of low-frequency ultrasound and bradykinin in increasing permeability of the blood-tumor barrier by opening tight junction. J Neurosci Res 2009; 87: 2282-9.
[23] Xia CY, Zhang Z, Xue YX, Wang P, Liu YH. Mechanisms of the increase in the permeability of the blood-tumor barrier obtained by combining low-frequency ultrasound irradiation with small-dose bradykinin. J Neurooncol 2009; 94: 41-50.
[24] Schubert W, Frank PG, Woodman SE, Hyogo H, Cohen DE, Chow CW, Lisanti MP. Microvascular hyperpermeability in caveolin-1 (-/-) knock-out mice: treatment with a specific nitricoxide synthase inhibitor, L-name, restores normal microvascular permeability in Cav-1 null mice. J Biol Chem 2002; 277: 40091-40098.
[25] Miyawaki-Shimizu K, Predescu D, Shimizu J, Broman M, Predescu S, Malik AB. siRNA-induced caveolin-1 knockdown in mice increases lung vascular permeability via the junctional pathway. Am J Physiol Lung Cell Mol Physiol 2005; 290: L405-L413.
[26] Song L, Ge S, Pachter JS. Caveolin-1 regulates expression of junction-associated proteins in brain microvascular endothelial cells. Blood 2007; 109: 1515-1523.
[27] Smart EJ, Graf GA, McNiven MA, Sessa WC, Engelman JA, Scherer PE, Okamoto T, Lisanti MP. Caveolins, liquid-ordered domains, and signal transduction. Mol Cell Biol 1999; 19: 7289-304.
[28] Chidlow JH Jr, Sessa WC. Caveolae, caveolins, and cavins: complex control of cellular signalling and inflammation. Cardiovasc Res 2010; 86: 219-25.
[29] Li S, Couet J, Lisanti MP. Src tyrosine kinases, G alpha subunits and H-Ras share a common membrane-anchored scaffolding protein, caveolin: caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. J Biol Chem 1996; 271: 29182-29190.
[30] Kusaka G, Ishikawa M, Nanda A, Granger DN, Zhang JH. Signaling pathways for early brain injury after subarachnoid hemorrhage. J Cereb Blood Flow Metab 2004; 24: 916-925.
[31] Meyer TN, Hunt J, Schwesinger C, Denker BM. Galpha12 regulates epithelial cell junctions through Src kinases. Am J Physiol Cell Physiol 2003; 285: C1281-C1293.
[32] Mucha DR, Myers CL, Schaeffer RC. Endothelial contraction and monolayer hyperpermeabilty are regulated by Src kinase. Am J Physiol Heart Circ Physiol 2003; 284: H994-H1002.

[33] Garcia-Cardena G, Martasek P, Siler-Masters BS, Skidd PM, Couet J, Li S, Lisanti MP, Sessa WC. Dissecting the interaction between nitric oxide synthase and caveolin: functional significance of the NOS caveolin binding domain in vivo. J Biol Chem 1997; 272: 25437-25440.

[34] Ju H, Zou R, Venema VJ, Venema RC. Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. J Biol Chem 1997; 272: 18522-18525.

[35] Michel JB, Feron O, Sacks D, Michel T. Reciprocal regulation of endothelial nitric-oxide synthase by Ca^2-calmodulin and caveolin. J Biol Chem 1997; 272: 15583-15586.

[36] Fleming I. Molecular mechanisms underlying the activation of eNOS. Pflugers Arch 2010; 459: 793-806.

[37] Gratton JP, Lin MI, Yu J, Weiss ED, Jiang ZL, Fairchild TA, Iwakiri Y, Groszmann R, Claffey KP, Cheng YC, Sessa WC. Selective inhibition of tumor microvascular permeability by cavtratin blocks tumor progression in mice. Cancer Cell 2003; 4: 31-39.

[38] Bauer PM, Yu J, Chen Y, Hickey R, Bernatchez PN, Looft-Wilson R, Huang Y, Giordano F, Stan RV, Sessa WC. Endothelial-specific expression of caveolin-1 impairs microvascular permeability and angiogenesis. Proc Natl Acad Sci U S A 2005; 102: 204-209.

[39] Bucci M, Gratton JP, Rudic RD, Acevedo L, Roviezzo F, Cirino G, Sessa WC. In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. Nat Med 2000; 6: 1362-1367.