ATVB In Focus
Endothelium: Signaling, Oxidative Stress, and Gene Expression

Caveolin, Caveolae, and Endothelial Cell Function

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Abstract—Caveolae are 50- to 100-nm cell-surface plasma membrane invaginations observed in terminally differentiated cells. They are particularly abundant in endothelial cells, where they are believed to play a major role in the regulation of endothelial vesicular trafficking and signal transduction. The use of caveolin-1–deficient mice has provided many new insights into the roles of caveolae and caveolin-1 in the regulation of endothelial cell function. These novel findings suggest an important role for caveolin-1 in the pathogenesis of cancer, atherosclerosis, and vascular disease. (*Arterioscler Thromb Vasc Biol*. 2003;23:1161-1168.)

Key Words: caveolae ■ endothelial cells ■ transcytosis ■ angiogenesis ■ atherosclerosis

Caveolae are 50- to 100-nm cell-surface invaginations that were first described ≈50 years ago.[1,2] These structures were initially believed to play an important role in the regulation of transcytosis in endothelial and epithelial cells. However, after the identification of caveolin-1 as the principal marker of caveolae,[3–5] a more complex role for caveolae has been revealed.

Several cell types express high levels of caveolin-1. They include endothelial cells, type I pneumocytes, and adipocytes.[6] It is therefore not surprising that most of the abnormalities observed in caveolin-1–deficient mice are localized within the lungs, adipose tissue, and vascular compartment.[7,8] The generation of caveolin-1–deficient mice has greatly helped to further define the role of caveolae/caveolin-1 in these organ systems. In this review, we will focus on the functional role of caveolae and caveolin-1 in the endothelium.

Caveolin-1 and Caveolae in Endothelial Cells

The endothelial cell is one of the cell types that express the highest levels of caveolin-1.[9] As a consequence, these cells contain a large number of caveolae (Figure). The classic type of caveolae (Ω-shaped cell-surface invaginations) is probably the most predominant form. However, other structures containing caveolin-1 are found in endothelial cells,[6] including detached plasmalemmal vesicles and tubular-vesicular channels.

Tubular channels might be important, because these structures link the luminal and basal plasma membranes, thereby creating a transendothelial channel. Therefore, caveolae might be involved in vesicular transport as detached vesicles.

Consistent with this observation, it is important to note that caveolae contain all of the "machinery" necessary for vesicle formation, fission, docking, and fusion with target membranes.[10] Recently, Dvorak and Feng[11] have also identified a new type of organelle present in normal venule endothelial cells, in venules associated with allergic reactions, and in capillaries observed in tumors. These structures were also shown to contain caveolin-1, and they might play an important role in the regulation of endothelium permeability.

Caveolin-1 has been shown to be involved in the regulation of numerous signaling cascades. The Table summarizes[9,33,45–47,52,57,63,75–96] some of the most important endothelial signaling molecules associated with caveolae. The role of caveolin-1 in the regulation of these signal transduction cascades will be discussed in subsequent sections.

Role of Caveolae in the Transcytosis of Macromolecules

Transcytosis is one of the first functional roles proposed for caveolae. In this process, caveolae transport macromolecules
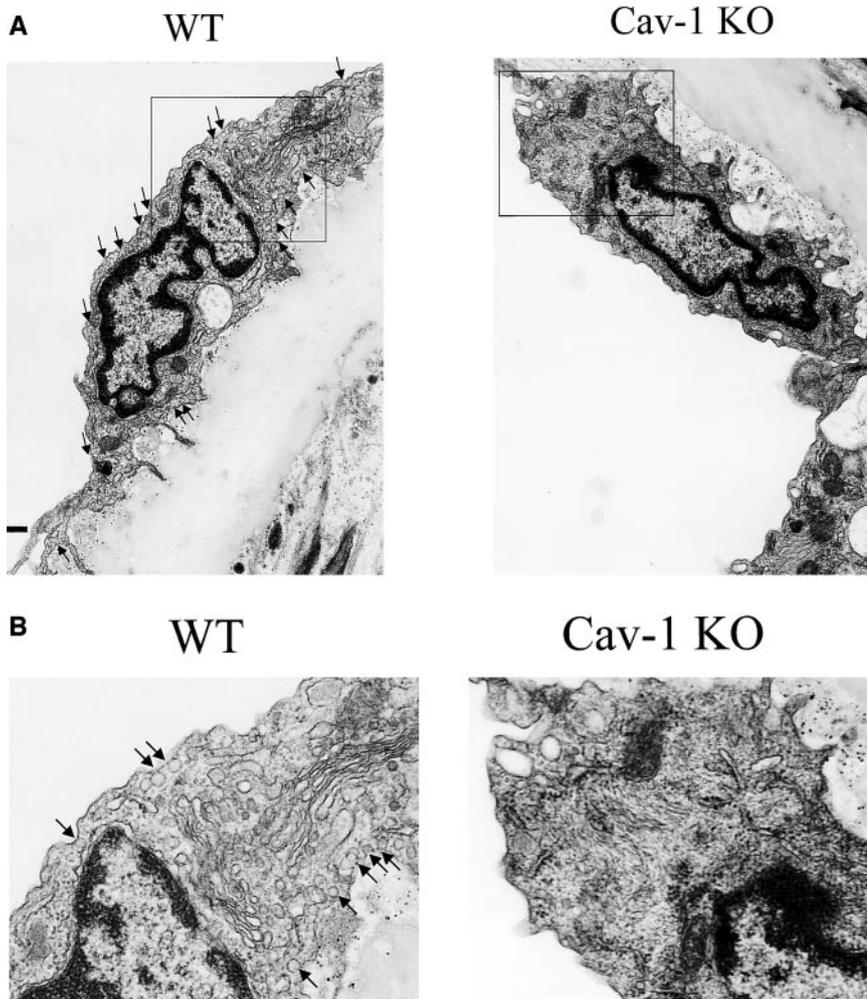
Caveolae structures in aortic endothelial cells. Electron microscopy of the aorta was performed, as described previously.[97] Electron micrographs of wild-type (WT) and caveolin-1–deficient (Cav-1 KO) aortas are compared. Wild-type endothelial cells exhibit numerous caveolae at the cell surface (arrows) in addition to detached plasmalemmal vesicles. These structures are clearly absent from caveolin-1–deficient endothelial cells. Scale bar=500 nm. B shows a ×1.5-magnified view of the boxed section presented in A.

from the luminal side of the blood vessel to the subendothelial space. Transcytosis can be both constitutive (eg, fluid-phase transport) or receptor mediated (the molecule transported requires the presence of its cognate receptor in caveolae). The transport of many macromolecules has been associated with this transcytotic pathway. The caveolar transcytosis of molecules such as albumin,[12] insulin,[13] and native LDL (as well as modified LDL)[14,15] has been clearly demonstrated.

Bendayan and Rasio[13] have shown that the transport of albumin and insulin might occur through different subsets of caveolae. This observation suggests that caveolae-mediated transcytosis is a highly regulated and specialized process. However, some of the initial observations were performed with inhibitors of caveolae formation, such as filipin or *N*-ethylmaleimide.[16] For example, Schnitzer et al[17] have demonstrated that disassembling caveolae, by using a sterol-binding agent such as filipin, could reduce the transcytosis of albumin and insulin without affecting clathrin-dependent pathways.

Following up on these findings, McIntosh et al[18] used a specific antibody targeted against lung endothelial caveolae. They showed that after injection of a gold-labeled version of this antibody, gold particles were observed at the surface of lung endothelial cells (associated with cell-surface caveolae)

within 2 to 3 minutes. After 5 minutes, gold particles were observed within internalized caveolae (plasmalemmal vesicles) inside the cells and finally, within the subendothelial space after ≈10 to 15 minutes.

More recent studies from our laboratory[19] have shown that caveolae are important for the transcytosis of albumin across endothelial cells. In this study,[19] gold-labeled albumin was perfused into wild-type or caveolin-1–deficient mouse lung, which contains large numbers of endothelial cells. Whereas control endothelial cells could associate with gold-labeled bovine serum albumin, internalize, and transfer it to the subendothelial space through caveolae, no uptake or transfer was observed in caveolin-1–deficient animals. This is the first direct evidence supporting a role for caveolin-1 and caveolae in transcytosis. The function of caveolae in this process is to allow the specific transfer of molecules to the subendothelial space. Regulation of this pathway occurs at 3 levels: (1) The cell-surface availability of caveolae might be modified at any time by specific signals that alter caveolar lipid composition or caveolin-1 posttranslational modifications (eg, tyrosine phosphorylation and/or palmitoylation). (2) Caveolin-1 and/or caveolin-2 protein expression levels can be upregulated or downregulated. (3) Caveolae receptor localization or expression could be altered. The transcytotic role of caveolae is not limited to endothelial cells but might

also extend to epithelial cells.[20] During the transcytosis process, caveolin-1 tyrosine phosphorylation appears to play an important role for the release of caveolae from the plasma membrane.[21–24]

Role of Endothelial Caveolae in Vascular Permeability

When discussing the role of caveolae/caveolin in transcytosis, we must also consider their role in endothelial permeability. The existence of a transcytotic pathway suggests that vascular permeability may also be regulated by caveolae/caveolins. Indeed, studies with lipoproteins have indicated that HDL particles are transferred more efficiently than are LDL particles, whereas VLDL species are not transported.[25] The permeability of the endothelium is especially important in the case of infectious diseases, atherosclerosis, and blood-brain barrier functioning. The selective permeability of endothelial cells to a specific molecule is controlled not only by the size of caveolar vesicles but also by the presence of specific receptors within caveolae. Under certain conditions, vascular endothelial growth factor (VEGF) can induce the clustering of caveolae, therefore allowing the formation of what has been termed vesiculovacuolar organelles (VVOs[26]). Also, long-term treatment of endothelial cells with VEGF resulted in the formation of fenestrae.[27] These findings suggest a crucial role for caveolin-1 in fenestrae formation. However, more recent studies have shown that glomerular endothelial fenestrae are still formed in caveolin-1–deficient mice.[28] In addition, the ability of caveolin-1 to inhibit endothelial nitric oxide synthase (eNOS) might also contribute to the regulation of vascular permeability. In agreement with this hypothesis, eNOS deficiency in genetically engineered mice is associated with reduced VEGF-induced permeability.[29]

Studies from our laboratory have shown that although endothelium from caveolin-1–deficient mice cannot transfer gold-labeled albumin through caveolae to peripheral tissues,[19] an alternative route exists.[7,30] This route, presumably paracellular transport, becomes more important owing to the constitutive activation of eNOS in *Cav-1*–null (−/−) mice.[30] In addition, in caveolin-1–deficient mice, electron microscopic analysis revealed (1) clear defects in endothelial tight junction formation and (2) abnormalities in capillary endothelial cell adhesion to the basement membrane.[30] These results suggest that caveolin-1 plays an important role in the organization of protein interactions between cells and the extracellular matrix. In fact, caveolin-1 can also interact with integrin proteins.[31] The involvement of eNOS in this process was demonstrated with the use of *N*-nitro-L-arginine methyl ester (L-NAME), a well-established eNOS inhibitor. Schubert et al[30] showed that in caveolin-1–deficient mice injected with L-NAME, plasma albumin clearance was restored to almost normal levels.

Caveolae/Caveolin and eNOS

Endothelium-derived NO has a profound effect on vessel tonicity and permeability. The endothelial enzyme responsible for the generation of NO is known as eNOS. Within an endothelial cell, eNOS targets to lipid rafts/caveolae of the plasma membrane and the Golgi apparatus[32,33] where it is tonically inhibited by binding to caveolin-1.[34–36] Optimal eNOS activity occurs when the eNOS/caveolin-1 interaction is competitively disrupted by calcium/calmodulin binding to eNOS.

The role of caveolin-1 as a modulator of eNOS activity and thus, vascular tone has been demonstrated in mouse models. For example, a cell-permeable peptide containing the region of caveolin-1 that binds to eNOS (ie, the caveolin-1 scaffolding domain, residues 82 to 101) was shown to inhibit acetylcholine-induced NO production and vasodilation in ex vivo mouse aorta experiments.[37] Conversely, aortas derived from *Cav-1*–null (−/−) mice showed marked enhancement of the relaxation response to acetylcholine, which is reversed by treatment with L-NAME, a specific NOS inhibitor.[7,8]

Vascular permeability is also significantly regulated by caveolin-1's effect on eNOS activity. The edematous response in mice was ameliorated by the injection of a caveolin-1 scaffolding domain–containing peptide.[37] The inverse applies as well, as loss of caveolin-1 markedly increases the vascular permeability to serum albumin by the paracellular route.[30] Importantly, this hyperpermeability is reversed by L-NAME injection, thus demonstrating the necessity of caveolin-1 for proper tonic inhibition of eNOS activity. Because both caveolin-1 and eNOS can mediate other endothelial functions, their respective roles will be described in the following sections.

Caveolin-1 and Angiogenesis

Angiogenesis is the process by which new vasculature is derived from preexisting blood vessels. Recent studies have proposed a role for caveolin-1 in the regulation of angiogenesis.[38–40] Caveolae appear to coalesce, forming larger, vesicular structures as part of the angiogenic response.[41] This observation is supported by the fact that overexpression of caveolin-1 and, thus, enhanced caveolae generation, accelerates capillary tube formation by nearly 3-fold.[40] Conversely, transfection of caveolin-1–based antisense oligonucleotides into endothelial cells results in marked reduction of capillary tube formation in a 3-dimensional fibrin gel assay, as well as in the chorioallantoic membrane system.[38] Similarly, downregulation of caveolin-1 by an antisense adenoviral approach reduces the number of capillary-like tubules formed in an in vitro Matrigel assay by >10-fold.[40] Thus, caveolin-1 expression appears to be correlated with progressive stages of the angiogenic process: caveolin-1 expression is downregulated during endothelial proliferation but is then markedly upregulated during endothelial differentiation and vessel formation.[40]

It is interesting to note that several important proteins involved in angiogenesis have been localized to caveolae (Table). Some of these macromolecules include the VEGF receptor (VEGFR), the urokinase receptor (uPAR), and eNOS. Recent studies by Labrecque et al[42] have shown that caveolin-1 tonically inhibits VEGFR-2 signaling, but interaction of VEGFR-2 with caveolin-1 appears to be required for the proper ligand-induced activation of the receptor within caveolae membranes. Brouet et al[39] found that eNOS-dependent atorvastatin stabilization of microvascular endothelial cell tube formation was associated with decreased

Proteins Associated With Caveolae in Endothelial Cells

Protein	Function	References
Membrane proteins		
PDGF-R	PDGF receptor	75
CD36	Lipoprotein receptor	57, 76
RAGE	Advanced glycated end products receptor	9
gp60	Albumin receptor	77
SR-BI	Lipoprotein receptor	76, 78
Flk-1/KDR	VEGF receptor	79
Tissue factor pathway inhibitor	Downregulates the procoagulant activity of tissue factor	80
PV-1	Component of stomatal diaphragms of caveolae and transendothelial channels	81
P-glycoprotein	ABC transporter	82
MMP-1	Matrix metalloproteinase	83
MMP-2	Matrix metalloproteinase	84
EDG-1 receptor	Endothelial differentiation gene-1 product	85
uPAR	Urokinase receptor	46
G protein–coupled receptors		
B2R	Bradikynin receptor	43
ET_A	Endothelin receptor	86
G proteins		
$G\alpha_s$	Regulates G protein–coupled receptor activity	57
$G\alpha_{i1}$	Regulates G protein–coupled receptor activity	57
$G\alpha_{i2}$	Regulates G protein–coupled receptor activity	57
$G\beta\gamma$	Regulates G protein–coupled receptor activity	57
Gq	Regulates G protein–coupled receptor activity	57, 87
Nonreceptor tyrosine kinases		
Src	Regulation of growth factor response	57, 75
Fyn	Regulation of growth factor response	57, 75
Yes	Regulation of growth factor response	57, 75
Lck	Regulation of growth factor response	57, 75
Lyn	Regulation of growth factor response	57, 75
Tyk2	Regulation of growth factor response	43
STAT3	Signal transducers and activators of transcription	43
Nonreceptor Ser/Thr kinases		
Raf	Signal transduction of mitogenic signals	52
MEK	Signal transduction of mitogenic signals	57
PI-3 kinase	Phosphorylation of phosphatidyl-inositol	57, 75
PKCα, β	Ser/Thr kinase	57, 75
Other enzymes		
eNOS	Production of NO	33, 88
PLCγ	Phospho-lipase	75
Prostacyclin synthase	Production of prostacyclin (PGI2)	45
GTPases		
Ras	GTPase	57
Rap1	GTPase	57
Rap2	GTPase	57
Cellular proteins/adaptors		
Shc	Regulates growth factor response	57
Grb2	Adaptor protein, associates growth factor receptors	57
Other proteins		
ER α and β	Estrogen receptors	63
NCX	Na^+/Ca^{2+} exchanger	89

TABLE Continued

Protein	Function	References
Ca^{2+}-ATPase	Calcium pump	90, 91
IP3 receptor-like protein	Involved in calcium influx	91, 92
Sprouty-1 and -2	Inhibitor of development-associated RTK signaling	47
Cationic arginine transporter-1	Arginine transporter	93
Structural proteins		
Actin	Involved in cell motility	57
Annexin II and VI	Promotes membrane fusion and is involved in exocytosis	57, 10
Dynamin	Involved in vesicular trafficking	94, 95
NSF	Involved in vesicle fusion	10
SNAP	Involved in vesicular transport	10
SNARE	Involved in vesicular transport	10
VAMP-2	Involved in the targeting/fusion of transported vesicles to their target membrane	10

caveolin-1 expression, as well as other modifications that enhance eNOS activity. On stimulation of cells with bradykinin, the G protein–coupled bradykinin B2 receptor (B2R) and downstream effectors (Tyk2 and STAT3) are translocated outside the caveolae.[43] This finding also has repercussions for eNOS regulation, because B2R can also interact with eNOS and inhibit its activity in a ligand- and calcium-dependent manner.[44]

Prostacyclin synthase has also been associated with caveolae.[45] The regulation of this enzyme might have an important role in angiogenesis. However, further studies on the subject are required. Wei et al[46] have shown that uPAR associates with and stabilizes caveolin/integrin complexes. This interaction may promote more efficient signal transduction and could affect the migration of endothelial cells. In addition, two known negative regulators of angiogenesis, Sprouty-1 and -2, have been localized within caveolae.[47] Taken together, these findings suggest a complex role for caveolin-1 in the regulation of angiogenesis.

Finally, in support of a role for caveolin-1 in the regulation of angiogenesis in vivo, we have recently shown, using Matrigel plugs supplemented with basic fibroblast growth factor, that angiogenesis in *Cav-1*–null (−/−) mice is markedly reduced.[48] Similar observations were made regarding tumor angiogenesis that was induced by injecting the B16 melanoma cell line into *Cav-1*–deficient (−/−) and wild-type animals. In addition, ultrastructural analysis of newly formed capillaries within the exogenous tumors revealed disorganized and incomplete capillary formation in *Cav-1*–null mice.

Caveolin-1 and Shear Stress

Endothelial cells are normally exposed to mechanical forces that affect their function.[49] Shear stress is important in the pathogenesis of coronary artery disease and atherosclerosis, as atheromatous lesions tend to develop at areas of high shear stress.[50] It is believed that laminar and disturbed flows regulate endothelial function differently. Studies by Rizzo et al[51] have shown that upon exposure of endothelial cells to

shear stress, NO is rapidly released and this release is due to the dissociation of eNOS from caveolin-1.

In addition, Rizzo et al[52] have shown that exposure of endothelial cells to shear stress is associated with tyrosine phosphorylation of proteins localized to caveolae. Moreover, the translocation of several signaling molecules into caveolae has been observed and results in activation of the Ras-p42/44 mitogen-activated protein (MAP) kinase cascade. In fact, recent studies have shown that upon exposure of endothelial cells to laminar flow, caveolin-1 undergoes translocation to the upstream side of the cell.[53] Thus, the 3-dimensional reorganization of caveolae in endothelial cells might contribute to the adaptive response observed in cells exposed to shear stress.

Caveolae, Caveolin-1, and Atherosclerosis

Several lines of evidence now suggest that caveolin-1 might play a proatherogenic role. In endothelial cells, caveolin-1 is upregulated on LDL exposure.[54] Moreover, downregulation of caveolin-1 is associated with reduced uptake of oxidized LDL by endothelial cells.[55] This finding is especially important, because caveolae are proposed to play a major role in the transcytosis of native and modified LDL.

Caveolin-1 translocation to the plasma membrane is also enhanced on incubation of endothelial cells with LDL. This movement is accompanied by increased association of Ras with caveolae and results in the activation of Ras, an important upstream activator of the p42/44 MAP kinase pathway.[54] Blair et al[56] have also shown that oxidized LDL can modify the distribution of both caveolin-1 and eNOS. This redistribution is accompanied by a reduction in eNOS activation by acetylcholine. This observation might be the result of disruption of the signal transduction complex containing eNOS, caveolin-1, and other molecules required for eNOS activation. Recent work by Kincer et al[58] has shown that CD36, a class B scavenger receptor associated with caveolae, was probably responsible for this effect.[58] This observation is important in view of the fact that in hypercholesterolemic patients or animal models, impairment of endothelium-derived relaxation is observed.[59–61] In agreement

with this finding, Feron et al[62] have shown that exposure of endothelial cells to serum from hypercholesterolemic patients promotes an increase in the caveolin-1–eNOS interaction.

The estrogen receptor is also localized within caveolae, and its interaction with estrogen appears to play an important role in the modulation of the eNOS–caveolin-1 association.[63] This observation might in part explain the antiatherogenic effects of short-term 17-β-estradiol injection observed in healthy postmenopausal women.[64] In agreement with this finding, long-term estrogen depletion after ovariectomy is accompanied by an upregulation of (cerebral) endothelial caveolin-1 expression in vivo.[65,66]

Ion Channel Regulation

Several ion channels have been shown to be associated with caveolae (Table). These channels play a very important role in the regulation of endothelial barrier functioning, because they are involved in the regulation of endothelial cell permeability, transcytosis, angiogenesis, and the response to shear stress.[67] Preliminary studies have suggested an important role for intact caveolae in the regulation of proper signaling after the activation of channels, such as the volume-regulated anion channel.[68]

It is also important to note that several calcium channels are localized to caveolae and, as a consequence, they might play a very important role in the regulation of eNOS activation.[69] In fact, recent work by Anderson and collaborators (Isshiki et al[70,71]) has shown that Ca^{2+} waves originate in caveolin-rich regions of endothelial cells and can move with caveolae to the trailing edge of migrating endothelial cells.

Conclusions

Much of the literature reviewed in this article suggests that caveolin-1 and caveolae are required for the proper organization of signaling pathways within endothelial cells. As previously proposed,[72] it suggests that preformed signaling complexes exist within caveolae, allowing for a rapid, efficient, and regulated response to extracellular stimuli. In addition to its regulatory role, caveolin-1 also functions in the proper targeting of certain proteins, such as caveolin-2, glycosylphosphatidyl inositol–linked proteins, and c-Src.[73] Mistargeting of proteins secondary to caveolin-1 deficiency might result in the elimination or alteration of their functional roles. It is therefore important to examine the subcellular localization of certain affected proteins in caveolin-1–deficient mice.

In addition, it is interesting to note that caveolin-1 can be tyrosine phosphorylated under certain conditions, such as oxidative stress.[23,74] However, the role of this posttranslational modification in endothelial cell function remains to be established.

Acknowledgments

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