

Caveolae and Caveolins in the Cardiovascular System

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Abstract—Caveolae and the caveolae coat proteins, caveolins, are putatively implicated in many cellular processes, including transcytosis of macromolecules, cholesterol transport, and signal transduction. Recent insights into the physiological and pathophysiological roles of these organelles and the caveolins from genetically modified mice suggest that they may be profoundly important for postnatal cardiovascular function, including endothelial barrier function, regulation of nitric oxide synthesis, cholesterol metabolism, and cardiac function. (*Circ Res.* 2004;94:1408-1417.)

Key Words: caveolae ■ caveolins ■ signaling ■ phenotypes ■ nitric oxide

Caveolae (ie, “small caves”) are 50- to 100-nm (diameter) vesicles found throughout the various cell types of the body. These organelles, discovered >50 years ago, have been and continue to be fertile grounds for discoveries as well as interesting controversies. Many recent reviews have outlined the nature of caveolae structures, regulation of caveolae and less well-defined lipid rafts, generalized signaling mechanisms in or out of caveolae, and their roles in physiology and pathophysiology.¹⁻⁵ Therefore, the main focus of this review is to outline the role of caveolae and the caveolins in cardiovascular cells and to highlight new insights into the functions of caveolae and caveolins.

Endothelium: Paradigm Shift From Cellophane Liners to Bags Full of Caveolae?

Paradigm shifts in vascular biology, spearheaded by electron microscopy and culturing of vascular endothelial and smooth muscle cells, led to the present explosion of information regarding the dynamic nature of vascular cells. These discoveries combined with basic cellular biology identifying molecular zip codes for protein trafficking and scaffolding molecules that regionally regulate protein-protein interactions has influenced many investigators to incorporate temporally and spatially restricted signaling events into vascular physiology. At the same time, the identification of caveolins as the major coat proteins of caveolae has sparked research into the roles and function of caveolae and have stimulated the fertile ground of understanding signal transduction in cholesterol-rich microdomain platforms such as caveolae. Although there are ample data and reviews supporting this view, “not all that gleams is gold” because there are many unanswered questions regarding the nature and function of caveolae.

It is clear that the major plasmalemma vesicle structure in endothelial cells (ECs) are caveolae as opposed to clathrin-

coated vesicles. The number of caveolae is high in continuous endothelium and low in fenestrated or discontinuous endothelium. Based on electron microscopy data, caveolae can exist lumenally and ablumenally, with the largest number in perijunctional zones between endothelia.¹ This close apposition of caveolae to intercellular junctions may give rise to the conclusion that certain junctional molecules may colocalize with caveolin-1 by immunofluorescence microscopy. In addition to caveolae, there are racemose caveolin-1-positive structures in postcapillary venules termed vesiculo-vascular vesicles (VVOs) that are most likely fused caveolae.⁶ These VVOs may participate in perijunctional transcytosis of macromolecules; however, this is somewhat controversial. One issue often unappreciated is that caveolae number, quantified by electron microscopy, decreases 10- to 1000-fold in ECs in culture (0.1 to 9 per μm^2 of plasma membrane)⁷⁻¹⁰ compared with the endothelium in vivo (78 to 89 per μm^2 in continuous endothelium with less caveolae in the blood-brain barrier),^{1,11,12} although ample caveolin-1 protein expression is detected in cultured cells. Despite these caveats, recent insights into the dynamics of caveolae trafficking in cultured cells has led to the discovery of internalized caveolae or “caveosomes” as unique entities distinct from lysosomes,¹³⁻¹⁵ whereas others argue caveolae are static structures that do not move.^{16,17} Finally, because caveolae are distinct on electron microscopy but lipid rafts are not, the existence of cholesterol-rich raft domains that organize signaling systems in living cells has recently been challenged.²

Caveolae and Signal Transduction

General Considerations

There is emerging evidence supporting compartmentalization of signaling systems. As previously stated, “molecular zip

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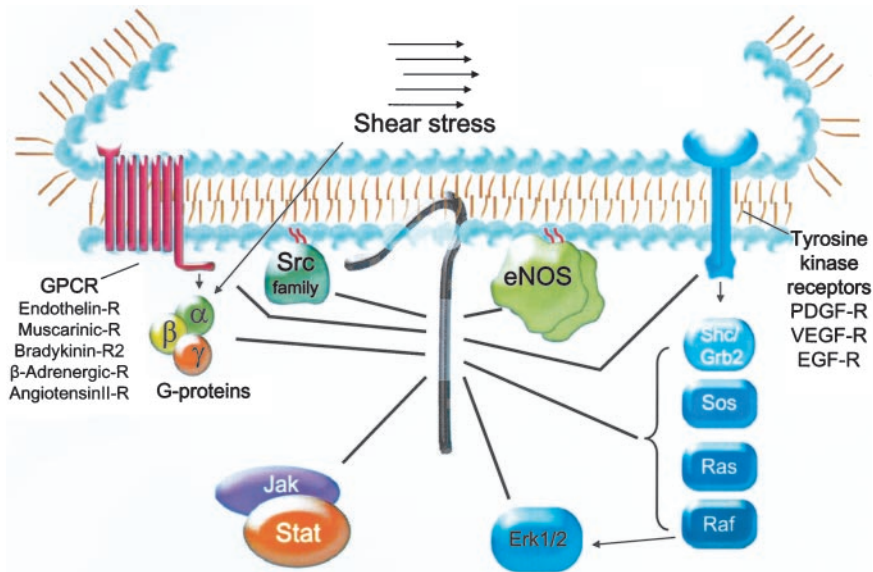
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Regulation of signaling via caveolae microdomains. G-protein-coupled receptors (GPCR), G-proteins (α and $\beta\gamma$), and receptor tyrosine kinases can reside in or translocate in and out of caveolae. Caveolin-1, the primary coat protein of caveolae (typically oligomeric in cells, but shown graphically as half hairpin) may directly regulate protein function or indirectly regulate Ras-Stat, extracellular signal-regulated kinases (Erk), and Janus kinases (Jak)-Stat signaling pathways. Lipid-modified proteins such as endothelial nitric oxide synthase (eNOS) and the Src family of kinases can target to caveolae and interact with caveolin-1.

codes" can determine the targeting of specific proteins to the nucleus, Golgi complex, mitochondria, and plasma membrane. Specific targeting coupled with the growing numbers of stimulus-dependent protein-protein interactions composed of receptors, kinases/phosphatases, scaffolding proteins, and molecular chaperones are logical mechanisms to ensure specificity of signaling, at the same time permitting similar pathways to exist in different parts of the same cell (Figure). Caveolae and lipid rafts are an attractive way to segregate or integrate certain pathways in microdomains of the plasma membrane. However, it is our opinion that it is erroneous to conclude that all signaling takes place in these organelles despite biochemical evidence in isolated caveolae/rafts, because all enrichment/purification schemes for caveolae/rafts have significant methodological concerns.^{2,18} More importantly, caveolin-1, -2, and -3 knockout mice are viable arguing against an essential role for the organelles¹⁹ and cholesterol-disrupting drugs, a standard approach to rule in or out caveolae or lipid rafts in given pathways, likely do more than just extract caveolae/raft domain cholesterol and change plasma membrane fluidity and permeability.²

However, to err on the side of caution, many of the observations of signaling pathways discovered in caveolin-1 enriched domains or rafts, may be caused by the diverse operational definitions of caveolae-dependent signaling. Several or all criteria for signaling in caveolae used by most investigators include: colocalization of a given protein with caveolin-1, electron microscopic colabeling with caveolin-1, cholesterol-sensitive signaling (ie, increase or decrease in effect when cells are exposed to cholesterol depleting agents, β -methyl cyclodextrin or oxidizing agents, cholesterol oxidase), hydrodynamic cosedimentation of proteins with caveolin-1 in sucrose gradients using detergent-free systems, detergent-insoluble membranes, sodium carbonate-resistant membranes, or isolated plasmalemma caveolae by colloidal silica.²⁰⁻²⁴ The strengths and caveats of all of these methods are hopefully obvious and may have been responsible for the explosion of molecules found in caveolin-enriched microdomains or rafts. One salient illustration supporting the problem

with an operational definition of a caveolin-enriched membrane or a raft is exemplified by recent use of proteomics to identify "lipid raft" proteins using the criterion of Triton X-100-resistant membranes in T cells or HeLa cells.^{18,25} In the latter cell type, the additional criterion of β -methyl cyclodextrin sensitivity was used to distinguish detergent-resistant proteins from cholesterol-sensitive proteins. In both circumstances, many predicted proteins were found, including flotillin and G proteins; however, many cytoskeletal proteins, heat-shock proteins, resident proteins of the endoplasmic reticulum, nucleus, or mitochondria were found in detergent- or sodium carbonate-treated membranes. In HeLa cells, it was estimated that one-third of the detergent-resistant proteins and two-thirds of the sodium carbonate-resistant proteins were "nonraft proteins," thus implying that many of the proteins "in or out" of rafts are most likely artifactually generated by these procedures. An alternative, less likely explanation is that rafts are everywhere in the cell and all these proteins are embedded in cholesterol-rich domains; however, this view is not compatible with the known facts about cholesterol synthesis and trafficking in cells. Neither study attempted to dissect proteins in caveolae versus rafts; however, with the availability of cell lines and mice deficient in caveolins/ caveolae, and the technical breakthrough of quantitative proteomics, work in the next several years will clearly delineate which proteins and signaling pathways are "in or out" of caveolae in a more definitive manner.

With these caveats in mind, a growing body of evidence suggests that caveolae may act as transducers or membrane-delimited sites of signal transduction. Isolation and purification of caveolin-enriched microdomains from tissues and cultured cells reveal that they contain surprisingly high levels of several intracellular signaling proteins and tyrosine phosphorylated proteins.²⁶ It is thereby plausible that targeted concentrations of preassembled signaling complexes exist or can be assembled via receptor-dependent signaling events.

Role for Caveolae in EC Signaling

Because of their high abundance in ECs, caveolae have been implicated in the regulation of many functions including

macromolecular transport in the microcirculation. Recent reviews have addressed the cell biological considerations of caveolae-type organelles in transport^{27,28} and perhaps drug delivery;²⁹ therefore, we focus on the evidence supporting the role of caveolae in signaling. An important conceptual hurdle in discussing the role of caveolae in signaling is to separate signaling regulated by the putative movement of proteins in or out of caveolae from processes regulated by caveolae-mediated endocytosis (typically interpreted through fractionation experiments and cholesterol disrupting agents), with pathways that may be regulated by direct or indirect interactions with caveolin-1 (typically determined by coprecipitation and *in vitro* binding experiments).

Caveolin-1

Caveolin-1, the major coat protein responsible for caveolae assembly, itself may positively or negatively regulate signaling via direct or indirect protein-protein interactions with resident caveolar proteins. Alternatively, because caveolin-1 is essential for formation of the caveolae, stimuli that increase or decrease caveolin levels may regulate the number of caveolae and thus signaling. As reviewed previously,^{26,30} caveolin-1 interacts with itself to oligomerize in cells and can bind to additional proteins via its caveolin scaffolding domain (CSD) (residues 82 to 101 in caveolin-1) or carboxy tail.

Recent experiments suggest that caveolin proteins in ECs are themselves regulated by intracellular signaling events. Caveolin-1 can be phosphorylated on tyrosine 14 in response to oxidative^{31,32} and shear stress,³³ likely by src family kinases.³⁴ Interestingly, this phosphorylation site is thought to serve as a phosphotyrosine binding site for the recruitment of SH2 domain-containing molecules. Caveolin-2, an additional isoform found in ECs, is not essential for caveolae formation but regulates the number of caveolae in many cell systems. Recent evidence suggests that caveolin-2 can also be phosphorylated on tyrosine 19 by c-src³⁵ and serine phosphorylated on serines 23 and 36,³⁶ likely through the action of casein kinase 2. However, there are no data supporting that caveolin-2, *per se*, can modulate signaling in a manner similar to caveolin-1.

Caveolin-1 may also modulate signal transduction through the regulation of cholesterol efflux. Caveolin-1 can bind cholesterol³⁷ and caveolin-1/caveolae may regulate the influx^{38,39} or efflux of cholesterol onto cholesterol acceptors such as low-density lipoprotein (LDL)^{40,41} and high-density lipoprotein.⁴² Plasmalemmal cholesterol, a key component of caveolae and lipid rafts, in turn, can regulate membrane fluidity and the rate of diffusion of many signaling molecules.

Endothelial Nitric Oxide Synthase

The contemporaneous identification of endothelial nitric oxide synthase (eNOS) in plasmalemmal caveolae of ECs by 2 laboratories^{43,44} has opened up insights into the capacity of caveolae-enriched molecules to participate in signal transduction. eNOS is the NOS isoform in EC that produces low levels of NO in response to mechanical forces and agonists such as acetylcholine, estrogen, bradykinin, or vascular endothelial growth factor (VEGF), and is the isoform that uniquely regulates many aspects of cardiovascular homeosta-

sis. eNOS is a peripheral membrane protein by virtue of its molecular targeting (via cotranslational N-myristoylation and posttranslational cysteine palmitoylation⁴⁵⁻⁴⁷) to the cytoplasmic aspect of the Golgi complex and to caveolae/lipid rafts.⁴⁸ eNOS can target to the Golgi and plasma membrane and isolated lipid rafts in a cyclodextrin-sensitive manner in model cell systems lacking caveolins/caveolae and is functionally active to produce basal and ionophore stimulated NO.⁴⁹ The lack of importance of caveolin-1 and caveolae for the trafficking of eNOS is further illustrated in mice deficient in caveolin-1 where Ach-stimulated relaxations, cGMP accumulation, and NO-dependent microvascular leakage are largely enhanced, not reduced,^{50,51} clearly showing that caveolae are not essential for eNOS localization and activation using these specific mechanisms of activation. However, expression of caveolin-1 to induce caveolae, reminiscent of the situation in intact ECs, does not influence eNOS trafficking in live cells or enrichment of eNOS in cholesterol-rich, caveolin-1-containing domains, but negatively regulates NO synthesis.⁴⁹ Collectively, these data in mice or cells lacking caveolae suggest that eNOS targeting to the Golgi and plasma membrane occurs in the absence of caveolins and caveolae, and caveolin-1 in caveolae is a negative regulator of eNOS function. However, because eNOS can be activated by diverse classes of stimuli (ie, shear stress, bioactive lipids, bradykinin, and VEGF), further experimentation is required to dissect the role of caveolae or caveolins, *per se*, in signal transmission. Previous studies demonstrated that localization of eNOS to caveolae was essential for eNOS activation.^{41,44,47,52} These studies were largely based on NOS activity or NO release that was enriched in "isolated caveolae," sensitive to cholesterol-disrupting drugs that cannot distinguish "rafts" from caveolae or using mutants of eNOS that do not reach the Golgi or plasma membrane. However, at that time, it was virtually impossible to discern cholesterol-rich rafts from caveolae proper, a distinction easily found in cells or mice lacking caveolins. With respect to the inhibitory function of caveolin-1, the putative CSD (amino acids 82 to 101) in caveolin-1, which potentially interacts with many proteins,⁵³ inhibits eNOS activity⁵⁴⁻⁵⁶ by reducing NADPH-dependent electron flux from the reductase to the oxygenase domain of eNOS, thereby reducing NO release from cells.⁵⁷ Interestingly, delivery of CSD to permeabilized cells⁵² or cell-permeable versions of CSD can block NO release and inhibit endothelium-dependent responses of isolated blood vessels, inflammation, vascular permeability, angiogenesis, and tumor growth *in vivo*.⁵⁸⁻⁶⁰ Although genetic data in mice deficient in caveolin-1 (see later) strongly validate the negative regulatory influence of caveolin-1 on eNOS function, a more mechanistic understanding is necessary to fully appreciate how caveolin-1 influences eNOS given the complex regulation of eNOS activity by protein phosphorylation, additional protein-protein interactions, and its cellular trafficking and activation in the Golgi complex of cells.^{61,62}

Mechanotransduction

Shear stress, the mechanical force sensed by the endothelium initiated by the laminar flow of blood, regulates EC survival versus death decisions, chemokinesis, gene expression, and

cytoskeletal remodeling in cultured EC. Because all these cellular responses are secondary to mechanotransduction, ECs are therefore capable of converting physical forces into intracellular signaling events.⁶³

The initial work by Schnitzer suggested that caveolae may act as mechanosensors or transducers.²⁴ This compelling hypothesis is based on evidence that flow-mediated tyrosyl phosphorylation of proteins is markedly enriched in proteins in isolated caveolae versus bulk plasma membrane. Indeed, eNOS activation is enhanced in caveolae isolated from lungs exposed to flow, supporting the concept that mechanosignaling can occur via proteins found in caveolae.⁶⁴ In cultured EC, shear stress increases net tyrosine phosphorylation and mitogen-activated protein kinase (MAPK) activation, effects reduced by cholesterol-disrupting drugs.⁶⁵ Caveolae numbers are drastically reduced in cultured EC as compared with *in vivo* conditions, but most importantly, caveolae density can be at least partly restored by conditioning cells under laminar flow.^{9,33} Thus, mechanotransduction may occur in caveolae and, in turn, continual shear stress may upregulate *de novo* formation of the organelle. Extrapolation of these findings to the *in vivo* setting suggests that chronic shear stress in conduit vessels may regulate caveolae turnover and adjust the number of caveolae in the plasmalemma according to the caliber of the vessel, flow rate, and perceived mean shear stress. Furthermore, wall stress and strain, especially in the vessels exhibiting myogenic tone in the microvasculature, may also initiate signaling via caveolae and regulate arteriolar remodeling. Definitive molecular approaches to dissect this concept will no doubt be forthcoming.

Tyrosine Kinases and Signaling Effectors

Tyrosine kinases are thought to be regulated by localization in caveolae or by interactions with caveolin-1 in ECs. For instance, in silica-purified caveolae from lung or cultured EC, the nonreceptor tyrosine kinases (lck, fyn, lyn, yes, and src), PI-3 kinase, and the platelet-derived growth factor receptor, some of its downstream effectors, and total tyrosyl phosphorylated proteins⁶⁶ were enriched in caveolae compared with bulk plasma membrane. The binding of albumin to one of its cognate binding partners, Gp60, triggers an increase tyrosine phosphorylation and protein-protein interactions between caveolin-1, c-src, and fyn in EC, events that presumably take place in caveolae and relate to the transcytosis of albumin.⁶⁷ More recently, the vascular endothelial growth factor receptor-2 (VEGFR-2), a key player in the induction of physiological and pathological angiogenesis, was shown to be localized in isolated caveolin-enriched membranes and interact with caveolin-1.^{34,68} Mitogen-activated protein kinases, Erk 1 and 2, also are found in caveolin-enriched domains or caveolae when cholesterol levels are depleted in fibroblasts⁶⁹ and may interact with caveolin-1.⁷⁰ This interaction also appears to attenuate Erk signaling and seems to be bidirectional, because increased Erk activity downregulates caveolin-1 mRNA and protein levels. Such a response may be stimulus-dependent, because shear stress can induce Erk activation and caveolae formation.

In the context of vascular smooth muscle (VSM), angiotensin II signals via the angiotensin II receptor I (AT₁R) in

caveolae and may transactivate the epidermal growth factor receptor to promote tyrosine kinase signaling leading to cellular proliferation.⁷¹ Recent data suggest that caveolin-3 may act as a chaperone for the AT₁R, allowing the latter to traffic through the exocytic pathway and to localize at the cell membrane.⁷² This interaction appears to be mediated by the CSD and is required to prevent the mislocalization of AT₁R to lipid bodies or Golgi, which results in aberrant maturation and surface expression of AT₁R, effects that are not reversed by supplementing cells with cholesterol.

G-Protein-Coupled Receptors and G-Proteins

Several studies revealed that certain G-protein-coupled receptors (GPCRs) can be found in enriched caveolin-containing membranes assumed to be caveolae, such as endothelin and bradykinin receptors (bradykinin R2).^{73,74} In addition, the endocytosis of liganded GPCR may occur via caveolae and initiate or terminate signaling.⁷⁵⁻⁷⁸ An example of agonist-stimulated regulation of receptor-effector complexes in caveolin-1-enriched membranes can be illustrated by the bradykinin R2 pathway. In ECs under basal conditions, Tyk2, STAT3, and the bradykinin R2 are localized either partially or entirely in caveolin-1-enriched fractions using sucrose gradient fractionation. After bradykinin stimulation, the bradykinin R2 and STAT3 are translocated out of caveolin-1-enriched membranes, resulting in STAT3 phosphorylation, nuclear translocation, and gene expression.⁷⁹ Similar results have been shown with other GPCR agonists; however, the molecular mechanisms of ligand-induced movement out of caveolae, receptor-caveolae endocytosis, or the importance of caveolin-1 in these processes are not well-understood.

Evidence also exists for β_2 -adrenergic receptor 2 (β_2 -AR) signaling within caveolae. The β_2 -AR can target into caveolae, colocalize, coprecipitate, and coenrich with caveolin-3 in caveolin-3-enriched membrane fractions. Furthermore, dissociation of β_2 -AR from caveolin-3 increases downstream G-protein activation by 2-fold.⁸⁰ Interestingly, caveolin-3 cofractionates with several G-protein subunits such as $G_{\beta\gamma}$, G_i , $G_{2\alpha}$, and it is believed that such sequestration of G-proteins in caveolae promotes their desensitization by preventing further binding to activated receptors.^{81,82}

Two final examples illustrating agonist-mediated, caveolae-dependent signaling are noteworthy. Carbachol binds to the muscarinic acetylcholine receptors (mAChRs) and stimulates receptor clustering into isolated caveolae.^{77,83} Stimulation of myocytes with carbachol induced sequestration of mAChRs through caveolae fission. The fission of caveolae was increased by GTP and the protein dynamin and decreased by a nonhydrolyzable GTP and dominant-negative dynamin consistent with previous data showing that dynamin is critical for caveolae internalization.¹³ Functionally, carbachol-mediated mAChR sequestration and the interaction of caveolin with eNOS was stabilized by dynamin, but not dominant-negative dynamin, suggesting that caveolae fission may contribute to GPCR desensitization. However, a recent report has shown that a nonhydrolyzable GTP analog or expression of dominant-negative dynamin impairs bradykinin-stimulated NO release by preventing the internalization of eNOS-containing vesicles. These 2 studies show

Summary of Cardiovascular Phenotypes Observed in Caveolin-1 and -3 Knockout Mice

	Caveolin-1 ^{-/-}	Caveolin-3 ^{-/-}
Endothelial cells (EC)	↑ Vasorelaxation ^{50,51,72} ↑ NO production ^{50,51,72} ↑ Vascular permeability ^{93,76} ↓ Postnatal angiogenesis ^{97,80}	—
Smooth muscle cells (SMC)	↓ Myogenic tone ^{50,71}	—
Myocardium	Hypertrophy ^{118,119}	Hypertrophy ^{117,98}
	↑ MAPK signaling ¹¹⁹ (in cardiac EC and/or fibroblasts)	↑ MAPK signaling (myocytes) ^{117,98}

the importance of signaling complex recruitment into caveolae and the roles of dynamin-mediated endocytosis in attenuating or enhancing signaling.

The increased concentration of GPCR in caveolae may be ligand-binding-dependent or ligand-binding-independent, and is thought to be an important initial step in the induction of the intracellular signaling events because several downstream transducers are also found in caveolin-1-enriched membranes/caveolae. Interestingly, affinity purification experiments reveal that certain G-proteins (Gq, but not Gi and Gs) are also considerably enriched in isolated ECs caveolae.⁸⁴ However, the mechanism of how or if caveolins/caveolae regulate G-protein signaling is not well-understood.^{85,86}

Physiological and Pathological Roles of Caveolins in the Vascular System

As illustrated, the roles of caveolae and caveolins in the regulation of many cellular processes in cultured cells are vast; thus, they may be considered by many investigators as essential signaling platforms or, conversely, as unsophisticated cellular glue studied with methodological naiveté. However, in the past few years, development of animal models and the usage of genetically altered mice have been instrumental in deciphering their physiological functions in vivo. Transgenic overexpression of caveolin-1 or caveolin-3 in mice^{87,88} or the targeted disruption of each of the caveolin gene locus in mice (*Cav1*, *Cav2*, and *Cav3* genes) has provided significant insights to the roles of the caveolins and caveolae (Table). The initial surprise revealed from the caveolin-1 knockout mice is that they do not exhibit any obvious developmental abnormalities or embryonic lethality;^{50,51} however, as the get older, they tend to have shorter lifespans.⁸⁹ Caveolin-1^{-/-} mice show complete ablation of the presence of the caveolae cellular organelle in the endothelia and fat. Similarly, caveolin-3^{-/-} mice lack caveolae in cells that normally express this protein such as skeletal muscle, heart, and the diaphragm.^{90,91} This demonstrated that caveolins-1 and -3 are necessary for the formation of the caveolae; however, it also indicates that this major cellular organelle is not essential for life. In contrast to caveolin-1 and -3, the loss of caveolin-2 does not prevent the formation of the caveolae, suggesting that this protein is dispensable for the formation of the membrane organelle, although these mice exhibit pulmonary fibrosis.⁹² Despite the fact that mice deficient in caveolins do not display severe abnormalities, the

careful analysis of their phenotypes has generated interesting information on the importance of these proteins in the cardiovascular system.

Caveolins and EC Functions

The first in vivo evidence of a role of caveolin-1 as a negative regulator of signaling molecules through protein-protein interactions was demonstrated based on its interaction with eNOS. As mentioned previously, in vitro experiments showed that caveolin-1 inhibits eNOS activity and NO production via a direct interaction between the CSD and eNOS. The functional evidence of this interaction in vivo was initially revealed by the use of a cell permeable peptide linked to the CSD.⁵⁸ This peptide was able to internalize into the endothelium, abrogate NO release, and endothelium-dependent vasodilation of isolated blood vessels analogous to a report in permeabilized cultured cardiac myocytes.⁵² Furthermore, administration of the cell-permeable CSD peptide inhibited NO-dependent vascular permeability, edema, and inflammation in mice.

Interestingly, the initial publications of caveolin-1 null mice also reported that the caveolin-1/eNOS interaction has functional significance.^{50,51} Isolated aortae from caveolin-1^{-/-} mice exhibited blunted responses to vasoconstrictor agents and also marked increases in vasodilatory responses to the endothelium-dependent vasodilator, acetylcholine, and both these effects were reverted by treatment of the vessels with L-NAME, a NOS inhibitor. These results suggest that the absence of caveolin-1 and caveolae in ECs leads to increased eNOS activity and thus NO release resulting in reduced vascular tone.

The enhanced eNOS activity observed in caveolin-1^{-/-} mice resulted in increased albumin extravasation, which is returned to normal after treatment of mice with the nonspecific NOS inhibitor, L-nitro arginine methyl ester.⁹³ The endothelium of caveolin-1^{-/-} mice lacked transcytotic vesicles typically important for macromolecule exchange and exhibited defects in tight junction density, both attributes that may explain the increased basal permeability. Interestingly, eNOS-derived NO also governs a significant component of the paracellular transport of plasma macromolecules across the endothelium of adult mice because eNOS^{-/-} mice show blunted increases in vascular permeability in response to VEGF.⁹⁴ In addition, inhibition of eNOS activity using a cell-permeable CSD peptide reduces vascular permeability

and edema stimulated by pro-inflammatory agents and VEGF^{58,59} as well as platelet-activating factor-mediated increases in hydraulic conductivity in isolated postcapillary venules.⁶⁰ Thus, the genetic loss of caveolin-1 increases basal NO release, which may promote albumin leakage, and blockade of eNOS reduces vascular leakage.

ECs are intimately involved in the regulation of the angiogenic process. The formation of new blood vessels derived from previously established vasculature involves the proliferation, migration, and recruitment of ECs and endothelial progenitors and a role for caveolin-1 in this process have been implicated. Initial experiments examining the effects of caveolin-1 overexpression on endothelial proliferation and differentiation suggested that caveolin-1 is a negative regulator of EC proliferation but promotes cellular differentiation. Adenoviral-mediated overexpression of caveolin-1 significantly enhanced ECs differentiation into tube-like structures and, conversely, downregulation of caveolin-1 protein levels using antisense oligonucleotides reduced the ability of the EC to form an organized network.⁹⁵ However, angiogenic factors such as VEGF have been shown to induce downregulation of caveolin-1, which was suggested to be important for the mitogenic effects of growth factors in ECs.⁹⁶ A more definitive role for caveolin-1 in blood vessel formation came from caveolin-1^{-/-} animals. Firstly, caveolin-1 does not seem to be a prerequisite for embryonic vasculogenesis, angiogenesis, or remodeling because these knockout animals develop a normal vasculature. However, postnatal angiogenesis seem to be reduced in caveolin-1 null mice using a growth factor-embedded matrigel assay. Similarly, tumor angiogenesis was also reduced in caveolin^{-/-} mice implanted with B16 melanoma cells.⁹⁷ These latter results suggest that EC caveolin-1/caveolae is important for the organization of a new capillary network. One must, however, bear in mind that caveolin-1 is an important regulator of eNOS signaling in ECs and that NO is also a major component postnatal angiogenesis. As mentioned previously, NO is essential for VEGF-driven angiogenesis, vascular permeability, and tumor growth.^{94,98} We recently showed that inhibition of eNOS activity in tumors using the cell-permeable CSD peptide resulted in reduce intratumor vascular leakage and tumor growth, suggesting that caveolin-1-mediated eNOS inhibition is a potential target for antitumor therapy. Moreover, tumors implanted on eNOS^{-/-} mice grew slower and exhibited reduced angiogenesis.⁵⁹ Overall, one clear phenotype that has emerged from the caveolin-1^{-/-} mice is the importance of caveolin-1 in regulating eNOS function, thus validating the initial work describing this protein-protein interaction. However, the loss of evoked angiogenesis in caveolin-1^{-/-} is more complex and cannot be rationalized via increased eNOS activation unless the loss of caveolae markedly diminishes coupling to eNOS activation.

Caveolins and VSM Function

As mentioned in the previous section, caveolin-1 and caveolin-3 are present in vascular smooth muscle cells (VSMC),⁸¹ with caveolin-1 expressed in arteries and veins and caveolin-3 expressed in arterial but not venous vasculature.⁹⁹ In addition, caveolae number, per se, and levels of

caveolin may be regulated by the phenotypic state of VSM.^{100–103} However, little is known about the physiological involvement of caveolins in VSMC function. Evidence for a role of caveolin-1 in VSMC-mediated contraction of arteries comes from caveolin-1^{-/-} mice that display reduced myogenic tone.⁵⁰ Myogenic tone, a critical autoregulatory response found in the microcirculation, is regulated by a feedback mechanism tightly linking spontaneous transient outward currents to local Ca²⁺ increases in the adjacent sarcoplasmic reticulum. These 2 events have been suggested to be dependent on the presence of caveolae.¹⁰⁴ Caveolin^{-/-} mice showed reduced spontaneous transient outward currents, a determinant of myogenic tone, indicating that caveolae integrity is necessary for efficient Ca²⁺ signaling in VSMC, an effect that may be modulated by the hyperactivation of the eNOS pathway, which may increase calcium clearing mechanisms. Recently, chemical loading of contractile VSM tissue with a synthetic caveolin-1 scaffolding domain peptide inhibited protein kinase C-dependent increases in contractility induced by a phorbol ester or an α agonist.¹⁰⁵ These results are consistent with a role for caveolin-1 in the coordination of signaling leading to the regulation of contractility of smooth muscle. However, to date, there is no evidence for altered hemodynamics in caveolin-1^{-/-} mice to support these *in vitro* findings.

Caveolin-1 and Atherosclerosis

Caveolin-1 and caveolae have the propensity to influence atherogenesis in many ways. Caveolin-1 is a cholesterol-binding protein that can transport cholesterol from the endoplasmic reticulum to the plasma membrane,³⁷ and major receptors for high-density lipoprotein, SR-B1, and a scavenger receptor for modified forms of LDL, CD36, can reside in and signal in caveolae-type microdomains.¹⁰⁶ In addition, oxidized LDL can extract caveolae cholesterol, mislocalize eNOS, and impair NO release.⁴¹ Conversely, blockade of HMG CoA reductase with statin-based drugs reduces caveolin levels and promote eNOS activation.^{107,108} This concept has been validated in apolipoprotein E-deficient (ApoE^{-/-}) mice when statin treatment decreases caveolin-1 expression and promotes NOS function *in vivo*.¹⁰⁹ However, to date, there are no data showing changes in caveolin-1 levels in atherosclerotic lesions from humans.

To directly test if caveolin-1 influenced lesion progression in mice, the Lisanti group¹¹⁰ bred mice deficient in caveolin-1^{-/-} mice to ApoE^{-/-} mice that develop atheromas. Interestingly, the loss of caveolin-1 in the ApoE^{-/-} background resulted in a proatherogenic lipid profile, similar to that seen in CD36^{-/-} mice bred to an ApoE background.^{111,112} Surprisingly, despite a pro-atherogenic lipid profile, the loss of caveolin-1 reduced lesion burden by 80%, suggesting caveolin-1 regulated LDL-mediated vascular dysfunction, inflammation, and lesion progression. The authors suggested this may be caused by a decrease in stability of the scavenger receptor for oxidized or modified LDL, CD36 in macrophages, and an increase in endothelium-derived NO production, which would reduce vascular inflammation. These remarkable findings unequivocally support the importance of caveolin-1/caveolae in the pathogenesis of atherosclerosis in

mice and stimulate many questions regarding the roles of caveolin-1 in lipid homeostasis.

Caveolins and Myocardial Function

Caveolin-3 expression, in contrast to caveolin-1 and -2, is mostly confined to striated (cardiac and skeletal) and smooth muscle. Caveolin-3 has been shown in muscle cells to be responsible for organelle biogenesis in these tissues and caveolin-3^{-/-} mice lack the presence of caveolae only in these tissues.^{90,91} Caveolin-3 is expressed during muscle differentiation and is localized to the sarcolemma, where it indirectly interacts with dystrophin and dystrophin-associated proteins. An alteration in the assembly of the caveolin-3/dystrophin complex either by reduced or increased caveolin-3 expression results in dystrophic condition of the skeletal muscle.⁸⁷ Also, caveolin-3 is endogenously expressed in cardiac myocytes, where it was suggested to negatively regulate NOS activity.^{52,113,114} Transgenic overexpression of caveolin-3 results in cardiomyopathy characterized by myocyte degeneration and disorganization. These animals exhibit increased cellular infiltrates, inflammation, and cardiac fibrosis. Cardiac function in these animals is characterized by increased QRS duration but normal heart rate and corrected QT intervals. No evidences of cardiac hypertrophy were observed.⁸⁸ Interestingly, cardiac NOS activity was significantly reduced without any change in eNOS or nNOS protein levels, suggesting that increased expression of caveolin-3 resulted in tonic inhibition of both isoforms through increased caveolin-3-eNOS/nNOS interaction in the transgenic animals. Because previous evidence suggested a modulatory role of NO in cardiac function,^{52,115,116} it is tempting to speculate that reduction in NO levels in the caveolin-3 transgenic mice may be responsible, at least in part, for the impaired cardiac function.

In contrast to caveolin-3 overexpression in mice, which does not lead to cardiac hypertrophy, genetic ablation of the Cav-3 gene results in cardiomyopathy but is characterized by significant hypertrophy, dilatation, and reduced cardiac shortening.¹¹⁷ This increase in cardiac mass has been proposed to be a consequence of the hyperactivation of the MAPK cascade, which drives myocyte hypertrophy, suggesting that caveolin-3 is a negative regulator of the p42/44 MAPK and that loss of caveolin-3 expression is sufficient to induce cardiomyopathy. Loss of caveolin-1 expression also results in cardiomyopathy and hypertrophy;^{118,119} however, because caveolin-1 is not normally expressed in myocytes, the deficiencies of this protein in cardiac fibroblasts¹¹⁹ or perhaps ECs¹¹⁸ may contribute to this phenotype in mice. Again, the hyperactivation of the MAPK cascade was associated with observed cardiac hypertrophy. As expected, the genetic loss of both caveolin-1 and -3 also results in severe cardiomyopathy and displays dramatic increases in left ventricular wall thickness, as compared with caveolin-1^{-/-}, caveolin-3^{-/-}, and wild-type mice. As predicted, biochemical markers of hypertrophy such as atrial natriuretic peptide levels were markedly upregulated.¹²⁰ However, in all these studies, it is still not clear if the alteration in cardiac function caused by modulation of caveolin levels is because of the role of caveolins as a negative regulator of proliferation, hypertrophy, or remodeling by modulating signal transduction (such as β -adrenergic

signaling, NO, and the MAPK cascade), or the result of intrinsic defects within cardiac myocytes caused by structural derangements of the cardiac T-tubule system or impaired calcium homeostasis.^{121–123}

Conclusions

Caveolae and caveolins are undoubtedly influencing various aspects of the cardiovascular system. Clearly, the loss of caveolin-1 is having a profound effect on the eNOS pathway, indicating the prominence of this important interaction, whereas the loss of caveolin-3 impacts NOS as well as MAPK activation. Considering the caveats associated with caveolae isolation and their biochemistry, and the growing number of potential targets that may exist in isolated caveolae, much more work is required in vivo to dissect which signaling pathways are “in or out” of caveolae in a more meaningful way.

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