Received: 31 July 2017 | Accepted: 16 February 2018
DOI: 10.1002/jcb.26793

PROSPECTS

WILEY Journal of Cellular Biochemistry

The caveolar membrane system in endothelium: From cell signaling to vascular pathology

Antonio Filippini[1] | **Gigliola Sica**[2] | **Alessio D'Alessio**[2]

[1] Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Unit of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy

[2] Istituto di Istologia ed Embriologia, Università Cattolica del Sacro Cuore, Rome, Italy

Correspondence
Alessio D'Alessio, PhD, Istituto di Istologia ed Embriologia, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1-00168 Roma, Italia.
Email: alessio.dalessio@unicatt.it

Funding information
Fondi di Ateneo Linea D.1., 2017, Università Cattolica del Sacro Cuore, Grant number: R4124500601

Abstract

Caveolae are 50- to 100-nm cholesterol and glycosphingolipid-rich flask-shaped invaginations commonly observed in many terminally differentiated cells. These organelles have been described in many cell types and are particularly abundant in endothelial cells, where they have been involved in the regulation of certain signaling pathways. Specific scaffolding proteins termed caveolins, along with the more recently discovered members of the cavin family, represent the major protein components during caveolae biogenesis. In addition, multiple studies aimed to investigate the expression and the regulation of these proteins significantly contributed to elucidate the role of caveolae and caveolins in endothelial cell physiology and disease. The aim of this review is to survey recent evidence of the involvement of the caveolar network in endothelial cell biology and endothelial cell dysfunction.

KEYWORDS

angiogenesis, cancer, caveolae, caveolin, endothelial cells, EPCs

1 | INTRODUCTION

Lipid rafts (LRs) and caveolae are distinct plasma membrane microdomains with a unique lipid composition present in a variety of different cell types. Unlike LRs, whose existence remains elusive in living cells, caveolae are 50-100 nm flask-shaped invaginations clearly detectable by electron microscope. During the last two decades both LRs and caveolae have been involved in a variety of biological processes including cell growth, proliferation, apoptosis, angiogenesis, and cancer. The shape as well as the biogenesis of caveolae depend mostly on the presence of small scaffolding proteins belonging to the caveolin gene family that includes caveolin-1, caveolin-2, and caveolin-3. These scaffolding proteins contribute to the assembly of a complex molecular platform that facilitates the integration of a variety of signaling molecules as well as the regulation of certain transmembrane signaling events. In addition, the presence of caveolin represents a useful molecular marker for the isolation of caveolae-enriched membranes from cells cultivated in vitro, that is central for the investigation of the role of these organelles in cellular functions both in physiological and pathological conditions. In this review, we mainly focus on recent findings that support the role of caveolae and caveolin proteins in the regulation of endothelial cells (EC) functions, with emphasis on potential application in angiogenic-based therapy.

2 | BIOGENESIS OF LIPID RAFTS AND CAVEOLAE

Our view of the "fluid mosaic" model of the plasma membrane illustrated more than 40 years ago, that describes

the cell surface as a low energy structure with randomly dispersed molecules,[1] has been deeply revised through the years. Starting from the late 80s an increasing number of evidence have revealed the existence of specific plasma membrane domains particularly enriched in glycosphingolipids, gangliosides, and cholesterol termed LRs, that have soon revealed an interesting talent to sequestrate a variety of structural and signaling molecules, leading to a fine regulation of downstream mechanisms in different cell types.[2–6] LRs are detergent-resistant non invaginated, flat, plasma membrane microdomains containing a unique combination of glycosphingolipids, and cholesterol.[7,8] Two main types of LRs are described based on the presence of the cholesterol binding protein caveolin-1.[9] LRs that contain caveolin-1 can promote the formation of clearly detectable flask-like invaginations termed caveolae. In contrast, LRs that do not contain caveolin-1 remain as presumably flat undetectable structures on the plasma membrane. However, in certain cell types such as neurons and lymphocytes, the presence of LRs along with the expression of caveolin-1 do not result in the formation of caveolae, raising the question about the function of caveolin-1 in these cell types.[10] Caveolae were initially described in the early 50s by Palade and Yamada as omega-shaped membrane invaginated "smooth" (noncoated) vesicles of 50-100 nm in size.[11,12] After their discovery, caveolae have been identified in a variety of cell types, including smooth muscle cells, EC, fibroblasts and adipocytes, challenging many investigators to explore their functional significance in cell functions. Early studies, almost exclusively based on morphological observation, reinforced the earliest hypothesis, anticipated by Palade of caveolae as structures involved almost exclusively in transcellular trafficking of molecules across the EC barrier.[13] Other well-known functions of caveolae include cholesterol transport,[14,15] potocytosis,[16] and endocytosis.[17] Structurally, caveolae display different level of organization, forming grape-like clusters, rosettes, membrane-bound or detached vesicles and tubule-like structures, clearly detectable by electron microscope in different cell types.[18] However, only with the discovery of the involvement of caveolae in the sequestration and uptake of small molecules[16] and later with the identification of caveolin-1.[19–21] as the first molecular marker used as a convenient tool for the isolation of caveolae-enriched membranes,[22–26] the research in this field has greatly boosted. Caveolin-1 was the first member of this family to be identified,[19] followed by the discovery of caveolin-2[27] and caveolin-3.[28] Caveolin-1 is expressed in most cell types such as adipocytes, epithelial cells, and fibroblasts where it is typically found co-expressed with caveolin-2.[27,29] Caveolin-3, which appears shorter but functionally and structurally similar to caveolin-1, is known to be muscle specific and expressed in skeletal, cardiac and smooth muscle cells.[28] Unlike caveolin-1 and caveolin-3, caveolin-2 appears incapable of forming caveolae and is usually found sequestered and degraded in the Golgi apparatus in the absence of caveolin-1.[27,30,31] Caveolins contain three distinct regions including a cytosolic N-terminal domain, a transmembrane domain and a hydrophilic C-terminal domain. Although the C-terminal domain has been demonstrated to be a site of palmitoylation, the significance of this modification is debated[32–34] and this modification seems not essential for proper localization of caveolins into caveolae. While the contribution of caveolin-1 is crucial for the formation of caveolae, recent studies identified a novel family of proteins termed cavins that actively participate to the biogenesis of caveolae, and specifically contribute to membrane curvature of these organelles.[35] Therefore, the persistence of caveolin and cavin oligomers is essential for the stability of plasma membrane bound caveolae. However, this association can be modulated by a variety of stimuli that destabilize caveolae and usually terminate with internalization of the caveolin proteins. Four different cavin proteins have been identified so far with different cellular distribution. Cavin 1/Polymerase I and transcript release factor (PFTR/Cav60/Cavin) appears to show an overlapping expression pattern with caveolin-1 and is a key molecule for caveolae biogenesis.[36,37] Cavin 2, encoded by the Serum Deprivation Response (SDPR) gene, cavin-3 (PRKCDBP) and the muscle-specific cavin 4 (MURC) are all also involved in the formation of caveolae.[38–40] An additional molecule, termed EHD2 belonging to the Eps15 homology domain (EHD) proteins, appears to be specifically involved in the regulation of membrane dynamics.[41,42] To this regard, it has been recently demonstrated that due to its ability to change its structural conformation in the presence of ATP, EHD2 can switch from an active membrane-bound conformation to an inactive soluble form, regulating caveolae association to the plasma membrane.[43] Hence, the caveolar network represents undoubtedly a molecular platform that has been stimulating the interest of many investigators for years in seeking of the physiological and pathological significance of these organelles in many fields of research, from membrane biogenesis to diseases.

3 | THE CONTRIBUTION OF THE CAVEOLAR NETWORK TO CELL SIGNALING

There is increasing evidence that demonstrate the involvement of the caveolar network in cell responses to incoming chemical and mechanical stimuli as well as in the regulation of the downstream signal transduction cascades. The caveolar network can function as a molecular switch that can activate, inhibit or modulate specific cellular functions in a variety of cell types depending on the precise interactions occurring

between signaling molecules within the organelle itself. With the discovery of the caveolin gene family, many evidence have been collected on the involvement of caveolae and caveolin proteins in cell signaling.[44–47] It has been demonstrated that mice carrying a single deletion of any of the caveolin genes resulted vital and fertile, whereas the double knock-out of caveolin-1 and -3 developed severe cardiomyopathy and inflammation,[48] raising interest on the pathological role of these organelles. On the other hand, the role of caveolin-2 in this scenario is less clear and the protein appears to play a regulatory role for caveolae stability.[49] Therefore, the information concerning the involvement of caveolins in signal transduction mostly come from studies carried out on caveolin-1 and have focused to investigate how the proficiency of this protein to bind and sequestrate a variety of signaling adapters can regulate downstream pathways. This scaffolding talent of caveolin-1 led to the concept of the "caveolae signaling hypothesis"[23] that predicts the existence of interactions between the 20-amino acid segment in the caveolin scaffolding domain (CSD, aa 82-101)[50] and an aromatic-rich caveolin binding motif (CBM) on associated signaling partners.[51] Binding to CSD is sufficient to inhibit the activity of many signaling molecules such as G-proteins, Src-like kinases, eNOS, H-Ras, and EGFR.[52–54] In vivo studies supported the idea that caveolins can modulate signal transduction independently of their structural role carried out during caveolae formation,[55] encouraging many groups to investigate more in deep the contribution of caveolins to specific cellular mechanisms. Different types of membrane bound and intracellular receptors involved in signal transduction, such as G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), have been found localized into caveolae or to interact with caveolins within the endothelial compartment.[56] Some GPCRs appear more dependent on the integrity of LRs whereas other members of the family have been found to interact with caveolins, suggesting that caveolar platform as well as LR microdomains contribute to their function. CCR5 is a chemokine receptor belonging to GPCRs family involved in the modulation of immune response triggered by chemokines. The contribution of functional LR microdomains to proper activation of CCR5 signaling has been demonstrated by the use of cholesterol depleting agents such as hydroxypropyl-β-cyclodextrin, in human $CD4^+$ lymphoblasts,[57] that affects CCR5 ligand ability and impairs the activation of cell signaling. Notably, even though the majority of CCR5 resides within raft microdomains, the receptor can function in non-raft domain as it happens for HIV entry into the cell.[58] Other studies have also suggested the contribution of the caveolar network during the internalization of GCPRs. For example, by using a labeled cholera toxin bound to ganglioside GM1, it has been shown the direct contribution of caveolae and caveolin-1 during the endocytosis of the adenosine receptor A1 in smooth muscle cells.[59] Based on the role of adenosine during ischemia and inflammation and the expression of adenosine receptors in EC,[60] the caveolar network may represent a potential target in the treatment of damaged tissues. In addition, other GCPRs such as the endothelin type A (ETAR) and B (ETBR) receptors were found localized into LRs/caveolae in rat peritubular smooth muscle cells.[61] To this regard, these authors demonstrated that the integrity of these plasma membrane microdomains is essential only for ETB- but not for ETA-mediated Ca^{2+} signaling, emphasizing the role of caveolae during the spatio-temporal coordination of NAADP (Nicotinic acid adenine dinucleotide phosphate)-dependent Ca^{2+} release through ETBRs. Other authors reported that, in EC, only ETBR resides within caveolae from which it is internalized upon administration of endothelin. These observations were supported by the use of specific ETBR (but not ETAR) inhibitors that prevented ET-induced budding of caveolae.[62] RTKs represent a large family of molecules also involved in signal transduction. The different types of RTKs are included into about twenty different RTK classes and many of them have been found either localized into caveolae or to interact with caveolin-1.[63–67] In addition, vascular endothelial growth factor (VEGF), angiopoietin, PDGF and Ephrin receptors, all belonging to the RTK family, have been found mostly expressed in EC where they play a central role during the angiogenic process.[68] There is no much information regarding the contribution of the caveolae network during ligand-gated ion channels signaling specifically in the endothelium. However, it has been reported the compartmentalization of $P2 \times 7R$ into caveolae and its colocalization with caveolin-1[69] in EC, even though the significance of this interaction is far from being elucidated.

4 | CAVEOLAE AND CAVEOLINS IN ENDOTHELIAL CELLS

The generation of caveolin null mice made it possible to investigate more in deep the significance and the contribution of both the caveolar system and caveolins to the animal physiology.[70–73] In cultured EC, caveolin-1 functions as a major scaffolding protein that helps to sequestrate key signaling molecules, such as TNFR1 and TNF-alpha converting enzyme (TACE), into caveolae.[64,74–76] During the past two decades, the study of caveolae and caveolin proteins has generated great attention especially because of their involvement in pathological conditions.[77,78] Caveolins, and in particular caveolin-1, have been involved in a variety of different cellular mechanisms from membrane trafficking to cell migration, angiogenesis, and cancer. Besides adipocytes, where caveolae are particularly abundant, EC show an overall high number of caveolae, mostly observed in the microvascular continuous endothelium, but excluded in

fenestrated endothelium, and sinusoids.[79] The high abundance of caveolae in EC has further stimulated the investigation of these organelles in EC biology, originally started with the studies of Palade and Bruns in the vascular endothelium in the late 60s.[80,81] A variety of caveolin-1 containing vesicles are usually found in EC and, in addition to characteristic non-coated flask invaginations of the plasma membrane, endothelia appear to contain different caveolin-1 positive structures such as tubular channels or floating vesicles detached from the plasma membrane.[18] In addition to their role in trafficking of molecules across the endothelial barrier, caveolae, and caveolins can function as mechanoreceptors during EC response to extracellular hemodynamic forces such as the shear stress. Indeed, the endothelium does not exclusively function as a physical barrier between blood and the blood vessel wall but it is normally exposed to hemodynamics forces of different amplitude that can contribute to the development of vascular conditions such as inflammation and atherosclerosis. Therefore, EC have developed the ability to convert these mechanical cues into precise signal transduction mechanisms. Several molecular receptors have been proposed to play an active role during response to mechanical and hemodynamic forces in EC. These include integrins, VEGFR2, GPCRs and purinergic receptors.[82–85] It has been reported that the exposure of EC to shear stress or stretch, affects the number as well as the distribution of caveolae[86,87] indicating that these organelles may play a crucial role during EC dysfunctions.[88] Notably, the number of caveolae, but not necessarily the level of caveolin-1, decreases in primary EC, forcing researchers to employ EC lines that maintain unvaried levels of both caveolae and caveolin-1.[75] These observations are also in accordance with recent findings indicating that caveolin-1 deficient cells undergo premature senescence in fibroblasts due to mitochondrial dysfunction.[89] Since their first description, significant advances have been made in the understanding of the role of the caveolar network in EC functions and the discovery of the caveolin family of proteins has further fostered the investigation of the involvement of the caveolar network both in physiological and pathological models.

5 | CAVEOLIN AS A NEGATIVE REGULATOR OF eNOS ACTIVITY

Undoubtedly, one of the best-known function that links caveolin-1 to EC functions refers to the ability of this scaffolding protein to inhibit the activity of endothelial nitric oxide (eNOS).[90,91] eNOS was among one of the first proteins found associated to caveolae[92] where it binds to the caveolin scaffolding domain (CSD, aa 82-101) and to a lesser extent to the C-terminal region (aa 135-178)[93] of caveolin-1. Following myristoylation of its N-terminal glycine and palmitoylation of the Cys15 and Cys26 residues, eNOS is kept inactive into caveolae, associated to caveolin-1. Only in the presence of specific stimuli this interaction is lost, activating downstream signaling (Figure 1). In addition, the interaction between caveolin-1 and eNOS takes place into caveolae but not into LRs, demonstrating the distinct spatial regulation of caveolae- and rafts-dependent signaling.[94] In vivo studies confirmed the correlation between the loss of caveolin-1, the increased level of NO and the decreased arterial myogenic tone in KO mice, predicting a direct involvement of caveolin-1 in the control of blood pressure. However, whether caveolin-1 depletion is beneficial to control blood pressure is still controversial and only few studies demonstrated a decrease of systemic blood pressure in caveolin-1 KO mice.[70,95–97] These conflicting results may be explained by the activation of compensatory mechanisms in response to chronic production of NO in caveolin-1 KO mice, that are hard to investigate. However, the use of cavnoxin, a cell-permeable peptide bearing a specific mutation that disrupts the interaction between eNOS and caveolin-1,[98] both lowered blood pressure and reduced the myogenic tone in vivo. Based on the relationship that links eNOS to calmodulin activation, both caveolae and caveolin-1 have been proposed as potential candidates involved in

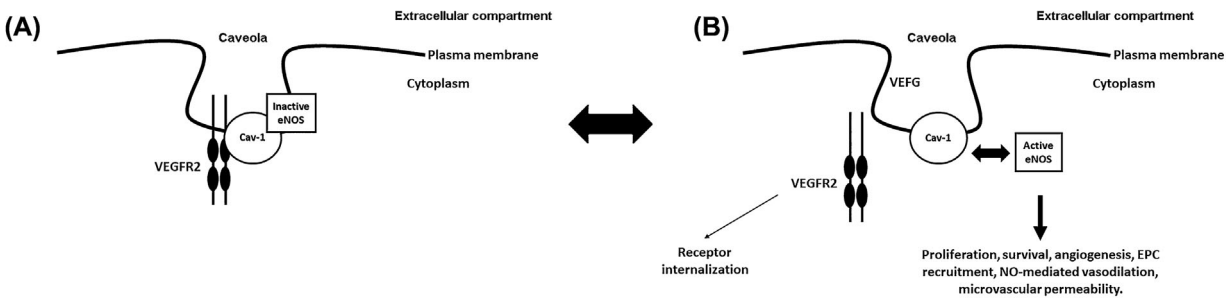

FIGURE 1 Contribution of caveolin-1 to eNOS and VEGFR2 activity. A, in non-stimulated cells, binding of caveolin-1 to eNOS into caveolae, keeps the enzyme in its inactive form. In the same compartment caveolin-1 binds to and sequestrate VEGFR2. B, In the presence of specific stimuli (eg VEGF) binding between caveolin-1 with eNOS or VEGFR2 is lost, favoring downstream signaling

calcium mobilization, both from the extracellular compartment and from internal stores. Notably, calmodulin activates eNOS by displacing its interaction with caveolin-1. Although caveolae have been proposed to be involved in the regulation of intracellular calcium concentration and calcium dependent signal transduction,[99] and caveolin-1 has been found determinant for calcium entry into the cell,[100,101] the precise involvement of the caveolar platform in these mechanisms in endothelium will require further studies.

6 | CAVEOLINS AND ENDOTHELIAL PROGENITORS CELLS (EPCs)

Blood vessel development in the embryo takes place through two specific mechanisms, namely vasculogenesis or the differentiation and growth of blood vessels from mesodermal derived hemangioblasts and angiogenesis, which ensures further development and remodeling of the primitive vascular network. On the other hand, it is now widely accepted that new vessel formation can arise from differentiating EPCs, residing in the bone marrow.[102] Since their first appearance in the literature,[103] EPCs contributed to revise the original concept of vasculogenesis as a mechanism that exclusively takes place in the embryo. Differentiation of EPCs can, indeed, occur also in the adult and may represent a key mechanism during reendothelialization and neovascularization after injury as well as adult vasculogenesis.[104–107] EPCs have been described as cells expressing VEGFR2, CD34 and CD133, although there is still considerable debate regarding their nature. Many groups have focused their attention on the role of EPCs and their potential contribution to therapeutic angiogenesis.[108–111] Among known molecules involved in postnatal vasculogenesis, Stromal cell-Derived Factor-1 (SDF-1) and VEGF represent crucial molecules during the recruitment of EPCs.[112] Although highly debated, some evidence suggesting a role of the caveolar network during EPCs recruitment in the bone marrow, have stimulated the investigation of caveolae/caveolins during postnatal vasculogenesis.[113,114] The recruitment of EPCs from bone marrow to the peripheral blood appears strictly dependent on the expression of VEGFR2, which resides into caveolae, SDF-1, and VEGF itself.[115] The contribution of caveolin-1 to EPCs recruitment has been recently reported in caveolin-deficient mice ($Cav^{-/-}$). This study demonstrated that defect in caveolin-1 expression impairs internalization of CXCR4 receptor induced by SDF-1 therefore affecting the recruitment of EPCs from bone marrow.[116] Therefore, understanding the involvement of caveolae/caveolin-1 during the recruitment of EPCs can be central for the development of potential therapeutic applications aimed to induce postnatal vasculogenesis. In addition, recent findings have suggested that NAADP-induced calcium mobilization from intracellular stores promotes EPC proliferation,[117] suggesting a role of NAADP-induced signaling during neovascularization.[118] Interestingly, this mechanism is strictly linked to the caveolar network and increasing interest has developed concerning the role of caveolae as potential sites for Ca^{2+} entry.[99,119] In addition, studies in the literature suggest the existence of two different EPC populations, named early EPCs (eEPCs) and Outgrowth endothelial cells (OECs) that show a different behavior when cultured in vitro. Interestingly, among a number of different markers evaluated, OECs but not eEPCs express high level of caveolin-1 in addition to VE-cadherin and Von Willebrand Factor.[106] However, whether the high expression of caveolin-1 in OECs has a biological significance is not known. Nevertheless, since OECs, rather than eEPCs, look more similar to EC and possess intrinsic angiogenic features, they are potential candidates for developing specific therapies for vascular based diseases. Notably, the inhibitory function of caveolin-1 seems to correlate with an increasing expression of stem cell markers in different organs of caveolin-1 KO mice, suggesting a role of caveolin-1 in stem cell differentiation.[120]

7 | THE CAVEOLAR NETWORK IN ANGIOGENESIS

The formation of new capillary blood vessels, termed angiogenesis, is regulated by a precise balance of growth, and inhibitory factors in healthy tissues. Early during embryo development, mesoderm-derived stem cells give rise to hemangioblasts that differentiate into hematopoietic stem cells (the precursors of blood cells), and angioblasts (the precursors of the blood vessels).[121] The proliferation and differentiation of angioblasts give rise to EC and endothelial tubes during vasculogenesis.[122,123] VEGF is among the key factors involved in the differentiation of angioblasts. To this regard, it has been demonstrated that the lack of a single VEGF allele is sufficient to induce severe defects in the development of the cardiovascular system leading to death before birth. A similar scenario was observed following the disruption of the VEGF receptor genes.[124,125] The subsequent expansion of this primitive vessel network is carried on during angiogenesis occurring as the result of endothelial sprouting and microvascular growth. Recent studies focused on the development of the retinal vasculature have demonstrated that the expression of plexin-D1 mRNA is rapidly downregulated in the central vascular plexus, where sprouting starts, but remains higher in the actively sprouting vessels. These data suggest a dynamic expression as well as an active role of plexin-D1 in the discrimination between tip and stalk cells during vessel growth in response to VEGF.[126] However, in the adult the scenario changes because post-natal EC are relatively quiescent during adulthood, when physiological

angiogenesis is mainly limited to few processes such as wound healing, the uterine cycle (menstruation) and during the development of the placenta throughout the course of pregnancy. Therefore, abnormal development of new blood vessels in the postnatal life is often associated to an imbalanced expression between stimulators and inhibitors of vessel growth that can result in an excessive (switched on) or insufficient (switched off) angiogenesis. Many pathological conditions, including ocular diseases, inflammatory disorders, cancer and metastasis are well known examples of switched on angiogenesis, whereas in other conditions such as ischemic heart disease, the angiogenic switch is inadequate, resulting in EC dysfunction.[127] Angiogenesis typically takes place under hypoxic condition or in the presence of pro-angiogenic factors such as VEGF[128–130] which acts by increasing vascular permeability and promotes EC migration by weakening inter-EC contacts, destabilizing the pre-existing vessels. In the presence of angiogenic stimuli, EC secrete and activate many proteolytic factors, such as matrix metalloproteases (MMPs), that induce the release of growth factors normally trapped in the extracellular matrix (ECM), facilitating EC migration, and their further development into new blood vessels during sprouting angiogenesis. The growth of new capillaries requires the presence of a specialized endothelial "tip cell" that drives the outgrowing vessels by extending motile filopodia toward gradients of angiogenic factors.[131] A second cell type termed "stalk cell," endowed with a high proliferative aptitude, follows the tip cell, and guarantees the elongation as well as the lumenization of the new vessel. The notch signaling mainly contributes to determine which cells will become tip or stalk cells. Basically, VEGF-induced Delta-like-4 (Dll-4) expression by tip cell activates the notch signaling and dampens VEGFR2 expression in stalk cells, reducing their migratory activity, contrary to what happens to tip cells.[132,133] The final step in the formation of new blood vessels requires their stabilization through the recruitment of periendothelial pericytes and smooth muscle cells.[134]

7.1 | The caveolar network and the angiogenic machinery

Among the VEGF receptors, VEGFR2, the major player of VEGF-induced angiogenesis, and VEGFR3 have been found localized into caveolae where they interact with caveolin-1.[135–138] Interestingly, in EA.hy926 EC line, the silencing of caveolin-1 by RNAi has been demonstrated to affect cell migration, MMPs activity and VEGF-induced angiogenesis in vitro.[139] In addition, a variety of proteins that participate in VEGF signal have been shown localized into caveolae,[75,140,141] and other studies demonstrated that both stimulators and inhibitors of angiogenesis affect the expression of caveolins.[45,142] Angiogenesis activators, such as VEGF, basic fibroblast growth factor (bFGF), and HGF have been reported to reduce caveolin-1 expression. By contrast, this effect is reversed in the presence of angiogenesis inhibitors such as angiostatin, thalidomide and others.[142] In addition, in vivo studies have shown that bFGF-induced angiogenesis was strongly compromised in caveolin-1 KO mice, compared to the wild-type mice.[143] These data support the idea that angiogenic stimuli require the displacement of adaptor molecules from their inhibited status within the caveolar network. The presence of a functional caveolar network has been found paramount during VEGF-induced ERK activation as well as for ensuring endothelial cell migration following the dissociation of VEGFR2 from the inhibitory effect of caveolin-1.[64] More recently, it has been suggested that in EC caveolin-1 contributes to the assembly of a complex IL1β-induced proangiogenic molecular platform, consisting of Tumor Necrosis Factor Receptor 6 (TRAF6), p38-MAPK, and MAPK-activated protein kinase 2. Notably, most of these molecules had been already associated with the caveolae network in EC.[75,144] Unusual angiogenic process takes place during abnormal blood vessel growth in tumors in response to an unconventional concentration of angiogenic factors in the presence of a hypoxic environment.[145] Tumor vessels commonly show abnormalities such as tortuosity, fragility, lack of pericytes, tendency to bleeding, exudation and elevated expression of VEGF and VEGFR2.[146] Non-physiological concentration of oxygen can contribute to the rearrangement of the plasma membrane proteome and of its endocytic activity via caveolin-1 dependent mechanisms.[147] It is well known, indeed, that the finely regulated endocytic mechanism as well as the expression of endocytosis associated proteins are frequently affected during malignant transformation and can be regulated by oxygen level.[148] Therefore, caveolin-1 may play a key role in the regulation of endocytosis within the hypoxic environment, making this protein a potential therapeutic target during malignant transformation.

7.2 | Pro- and anti-angiogenesis function of caveolin

Because of the compartmentalization of VEGFR2 into caveolae and its association with caveolin-1, the presence of a functional caveolar network appears to be crucial for VEGF-induced signaling, both under physiological and pathological conditions. Supporting this scenario, it is worth to mention that VEGF functions by disrupting the interaction between caveolin-1, and VEGFR2 within caveolae,[64] leading to the activation of downstream signaling. This endogenous inhibitory function of caveolin-1 results also evident in its ability to regulate cell polarity, directional movement and cell migration of primary EC, a key step during blood vessel growth in

response to VEGF. These data demonstrate that not only the expression but even the precise distribution of caveolin-1 within the cellular compartment contributes to determine cell polarity and influences cell functions.[149] Early evidence regarding the pro-angiogenic role of caveolin-1 date back to the early 2000s, when it was demonstrated that antisense oligodeoxynucleotides against caveolin-1 reduced the number of detectable caveolae and suppressed capillary-like tube formation.[150] Afterwards, Liu et al. demonstrated that the expression of caveolin-1 increased during EC differentiation, reaching its maximum level just before to the formation of capillary-like tubules. Interestingly, when caveolin-1 was overexpressed, tube formation resulted dramatically accelerated. On the contrary, when caveolin-1 was downregulated, the number of capillary-like tubules was reduced by 10- folds and over.[151] These findings were further proved in vivo, where the neovascularization of caveolin-1 KO mice injected with a matrigel plug supplemented with bFGF was strongly reduced.[143] More recently Madaro et al[139] investigated the role of caveolin-1 at morphological and functional level in EC and demonstrated that silencing of caveolin-1 by RNAi not only induced morphological changes of EC but also reduced cell migration and tubulogenesis in response to VEGF. This talent of caveolin-1 to regulate angiogenesis might be associated to the inhibition of eNOS, which is commonly found associated to caveolin-1 in EC. Indeed, studies performed in both caveolin-1 and eNOS KO mice demonstrated the impairment of cell migration, cell sprouting from aortic rings, tube formation and tumor growth.[152] Nevertheless, in addition to its pro-angiogenic function in EC, both in vitro and in vivo studies support the antiangiogenic role of caveolin-1. For example, studies from Bauer and collaborators demonstrated that VEGF-mediated angiogenesis is reduced by 40% in transgenic mice overexpressing caveolin-1 in endothelium compared with control littermates.[55] Another study performed using a caveolin-based plasmid delivery corroborates the antiangiogenic effect of caveolin-1 in microvascular density and tumor growth.[153] These authors demonstrated that VEGF-induced EC migration and tube formation were strongly reduced in cells overexpressing caveolin-1 and similar results were obtained with the inhibitor of eNOS, confirming the key importance of caveolin-1/eNOS interaction in the regulation of EC functions. Additional findings supporting the inhibitory activity of caveolin during EC functions, come from an in vitro study showing that overexpression of caveolin-1 in HUVEC reduced VEGF-induced activation of p42/44 MAP kinase.[154] This controversial function of caveolin-1 may be due to different extracellular environments regulating specific cell functions. Therefore, distinct responses to the same cue may activate different signaling pathways, depending on the specific location and distribution of surface receptors (eg inside or outside caveolae). To this regard, it shouldn't be overlooked the fascinating role of caveolae as mechanosensing structures that cells can employ to adapt to particular extracellular conditions.[155]

8 | FUTURE PERSPECTIVES

Since their discovery more than 50 years ago, caveolae have been linked to many cellular processes from vesicular transport and cholesterol homeostasis to the onset of pathological conditions. The identification of the caveolin gene family in the late 90s provided researchers an innovative molecular tool to isolate caveolin enriched membranes thus opening new approaches to investigate the role of the caveolar network in cellular functions. This explains why the investigation in this field has greatly boosted over the years. Among the three caveolin proteins, caveolin-1, the main coat protein of caveolae, appears to be the major player involved in a variety of cellular processes mostly via its CSD through which the protein regulates the activity of key signaling molecules. Although the contribution of the caveolar network has been undoubtedly involved in some pathological conditions (Figure 2), the contribution of caveolins to the onset of cancer and tumor angiogenesis remains still controversial. This is mostly due to the great variability among the different kinds of tumors and to the high heterogeneity of tumor microenvironments. Undoubtedly, tumor cells show altered caveolae as well as changes or loss of caveolin expression, a condition that generates atypical cell signaling. In addition to the structural role, endothelial caveolin-1 and caveolae have been shown to play an important role in angiogenesis by regulating the activity of key molecules as well as downstream signaling events. Despite the ever-growing reports of the involvement of

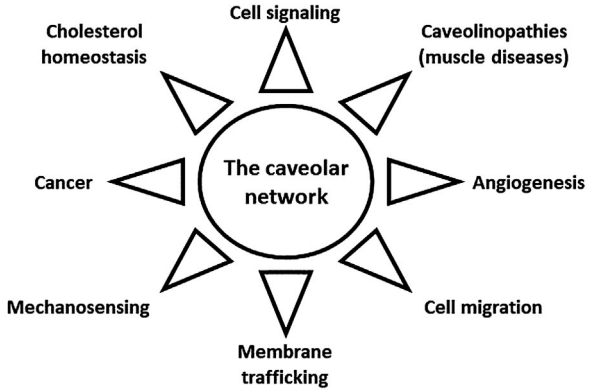

FIGURE 2 Summary of the most common processes regulated by the presence of the caveolar network

caveolae and caveolins in endothelial cell functions, many aspects concerning their contribution to angiogenesis and angiogenesis based diseases remain open. The generation of caveolin-1 null mice has greatly improved our knowledge of the involvement of the caveolar network in cell functions. However, in most cases the molecular details of disrupted signaling and the intracellular fate of signaling partners in the absence of caveolin-1 remain unclear. Notably, a bioactive secreted form of caveolin-1 has been also reported to regulate cell survival and tumor progression in prostate cancer.[149] However, the specific role of the soluble form of caveolin-1 in other cell functions, of its trafficking into the extracellular space and its possible interactions with other signaling molecules, represent a potential interest in the field of vascular pathology and tumor angiogenesis. Finally, since the involvement of caveolin-1 in the regulation of the Notch signaling, it is worth investigating more in detail the possible contribution of this protein to the early steps of the angiogenic process that determines, for example, the specification of tip and stalk cells.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Alessio D'Alessio http://orcid.org/0000-0002-3340-2634

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How to cite this article: Filippini A, Sica G, D'Alessio A. The caveolar membrane system in endothelium: From cell signaling to vascular pathology. *J Cell Biochem*. 2018;1–12. https://doi.org/10.1002/jcb.26793