CHAPTER 9

ATHEROSCLEROSIS, CAVEOLAE AND CAVEOLIN-1

Stephanos Pavlides, Jorge L. Gutierrez-Pajares, Christiane Danilo, Michael P. Lisanti and Philippe G. Frank*

Departments of Cancer Biology and Stem Cell Biology and Regenerative Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania, USA
**Corresponding Author: Philippe G. Frank—Email: philippe.frank@jefferson.edu*

Abstract: Atherosclerosis is a disease of the blood vessel characterized by the development of an arterial occlusion containing lipid and cellular deposits. Caveolae are 50-100 nm cell surface plasma membrane invaginations that are believed to play an important role in the regulation of cellular signaling and transport of molecules among others. These organelles are enriched in sphingolipids and cholesterol and are characterized by the presence of the protein caveolin-1. Caveolin-1 and caveolae are present in most of the cells involved in the development of atherosclerosis. The current literature suggests a rather complex role for caveolin-1 in this disease, with evidence of either pro- or anti-atherogenic functions depending on the cell type examined. In the present chapter, the various roles of caveolae and caveolin-1 in the development of atherosclerosis are examined.

INTRODUCTION

The Development of Atherosclerosis: Current Understanding

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in industrialized nations. Atherosclerosis is the primary cause of CVD and is mainly characterized by the formation of plaques that develop in the arterial wall. This wall consists of three distinct cellular layers: the intima, the media and the adventitia. The intima is the innermost monolayer of the artery formed by endothelial cells (ECs) and

Caveolins and Caveolae: Roles in Signaling and Disease Mechanisms,
edited by Jean-François Jasmin, Philippe G. Frank and Michael P. Lisanti.

internal elastic lamina. The media consists of smooth muscle cells (SMCs) embedded in an extracellular matrix (ECM). The adventitia is the outer layer of the arterial wall and is made up predominantly of fibroblasts and ECM.[1]

Plaque formation is a complex multistep process that is initiated by the accumulation of lipoproteins in the arterial intima and followed by the infiltration of monocytes at lesion sites. Lipoprotein infiltration mainly involves low-density lipoprotein (LDL), which acts as a molecular suitcase for the transport and delivery of lipids to peripheral tissues. Thus, increased plasma LDL levels have been linked to increased risk of CVD.[2] Importantly, the entrapment of LDL particles and their subsequent modification (e.g., oxidation or aggregation) in the sub-endothelial space of arteries[3,4] have been demonstrated to play a major role in the initiation of atherosclerosis.[5] As a result, the transfer of LDL from the blood stream to the sub-endothelial space may be the defining initial step for the atherosclerotic process.

The presence of modified LDL particles in the sub-endothelial space induces early inflammation via the activation of ECs. This inflammatory process is initiated by the expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intracellular cell adhesion molecule-1 (ICAM-1), P-Selectin and E-Selectin.[6] Selectins play a key role in the primary interaction between monocytes and the endothelium, namely tethering and rolling of monocytes at the surface of activated endothelial cells.[7] Subsequently, monocytes differentiate into macrophages that can take-up large amounts of modified LDL and eventually become foam cells, which are enriched in cholesteryl esters (CE). The presence of T-lymphocytes, foam cells and macrophages in the intima further contribute to the inflammatory response via the secretion of chemokine and cytokine molecules, such as Monocyte Chemoattractant Protein-1 (MCP-1) and Tumor Necrosis Factor-α (TNF-α).[8]

In the early stages of this process, foam cell formation occurs via the uptake and subsequent accumulation of modified LDL in macrophages.[9] Ingested lipoprotein particles are degraded into cholesterol, amino acids and fatty acids in the lysosomes. Excess cholesterol is stored in lipid droplets as cholesteryl esters (CE).[10] These cholesterol-loaded macrophages transform into foam cells since the expression of receptors responsible for lipoprotein uptake (i.e., scavenger receptors) is not regulated by cellular cholesterol levels.[11] The scavenger receptors CD36 and Scavenger Receptor class A (SR-A) are receptors that bind modified LDL. Contrary to the LDL-receptor, mRNA levels of these receptors are not regulated by cellular cholesterol levels. Consequently, mice deficient in either of these receptors exhibit reduced atherosclerotic lesions.[12,13] This phenotype is likely due to impaired modified LDL uptake by macrophages and, consequently, reduced fatty streak formation.

During all stages of lesion progression, macrophages may undergo apoptosis[14,15] and with prolonged cholesterol loading, macrophages show characteristics of necrosis.[16] In vitro experiments have shown that free cholesterol (FC)-loading or oxidized LDL (oxLDL) treatment of macrophages leads to necrosis that is characterized by disruption of the plasma membrane and swelling of cellular organelles.[16-18] Other possible causes for macrophage death in atherosclerotic lesions include growth factor deprivation[19] and the exposure to factors such as inflammatory cytokines and nitric oxide.[20] These observations highlight the importance of the macrophage phagocytotic properties that would allow an efficient clearance of apoptotic cells. The removal of the resulting apoptotic cells by phagocytosis is carried out by infiltrating macrophages in a process known as efferocytosis.[21] This process is decreased in the more advanced stages of atherosclerosis and, as a result, increased plaque necrosis and inflammation are observed.[9,22] Besides apoptotic macrophages, lesions in the sub-endothelial space at this stage are also composed of proliferating SMCs and an ECM composed of lipid-rich cellular and necrotic debris.[1,23] The secretion of cytokines and

growth factors by macrophages and T-cells further promote the migration and proliferation of SMCs. In turn, these stimulated SMCs produce ECM proteins that can facilitate plaque rupture.[1] All of these events are believed to promote the development of an atheroma and later plaque rupture can eventually lead to blood clot and acute arterial occlusion causing a myocardial infarction or stroke depending on the location.[1,3,4,24]

A good understanding of the molecular mechanisms associated with the development of atherosclerosis has been obtained in mouse models. Mice are normally very resistant to atherosclerosis. However, under specific genetic and dietary conditions, they can develop hypercholesterolemia and extensive atherosclerotic lesions with characteristics that are similar to those observed in humans. In mice, a targeted disruption of the apolipoprotein E gene (*apoe*) is characterized by increased very low-density lipoproteins (VLDL) and LDL associated cholesterol levels in the blood stream. In addition, feeding *apoe*$^{-/-}$ mice with a western-type diet (i.e., enriched in cholesterol) leads to a further increase in plasma cholesterol levels that can reach 1500-2000 mg/dl (~ten times normal values) and therefore accelerates the appearance of lesions in the aorta.[25,26]

Caveolin-1 (Cav-1) is expressed in all of the cell types involved in the development of atherosclerosis (i.e., endothelial cells, macrophages and smooth muscle cells). Because of its role in the regulation of cellular cholesterol homeostasis and in numerous signaling pathways, it has been proposed to play an important role in atherosclerosis together with caveolae. The objective of this chapter is therefore to provide a better understanding of the role of Cav-1 and caveolae in the complex process of atherosclerosis development at the cellular and molecular levels. The study of complex diseases such as atherosclerosis is challenging because of its multi-factorial origin, most notably environmental and genetic. Based on data generated by various laboratories, including ours, we believe that the study of Cav-1 will allow the development of novel scientific approaches to study atherosclerosis by examining the different steps associated with the development of this disease. In this chapter, we present a caveolae-based approach to dissect the various steps, in particular, intimal LDL accumulation, endothelial, macrophage and SMC function. These cell types are directly involved in the development of atherosclerosis at different stages during disease progression. More specifically, we underline the multifaceted and sometimes opposing roles of Cav-1 in ECs, macrophages and SMCs. Finally, we present a working model for Cav-1 function in atherogenesis.

Caveolae: Discovery and Biochemical Properties

"Caveolae", a term coined by Yamada,[27] are small, 50-100 nm, flask-shaped plasma membrane invaginations, first identified by Palade in 1953 and described as "little caves" due to their appearance by electron microscopy.[28] This type of vesicular structure is a subtype of lipid rafts, which are plasma membrane microdomains enriched in sphingolipids and cholesterol.[29] The particular lipid composition of caveolae/lipid rafts is responsible for the insolubility observed in non-ionic detergents (e.g., Triton X-100) at 4º C and a light buoyant density after sucrose gradients ultracentrifugation.[30] These properties have been instrumental for the purification and biochemical characterization of these structures.[31-35] They are involved in the regulation of signal transduction events, endocytosis, transcytosis, membrane trafficking and the regulation of cholesterol homeostasis.[33,36] They are highly sensitive to cholesterol depletion as treatment of cells with cholesterol-binding agents (e.g., cyclodextrin) flatten these structures.[37,38] Caveolae are characterized by the presence of the protein Cav-1. Caveolae and Cav-1 are abundant in terminally differentiated cells,

including fibroblasts, epithelial cells, adipocytes and ECs.[39,40] Cav-1-deficient mice lack caveolae in all of the cell types normally expressing Cav-1. These findings indicate that Cav-1 is required for the formation of caveolae.[41-43]

Caveolae and Caveolins Structure and Function

The molecular makeup of caveolae has remained mysterious for four decades after their initial morphological description. The discovery of Cav-1 as a major structural protein component of caveolae has since provided new insights into the multifaceted function of caveolae and caveolins.[38,44] Cav-1 was first identified through a screening of tyrosine-phosphorylated proteins in Rous sarcoma v-Src positive cells. This protein was detected in caveolae by immuno-electron microscopy and protein sequencing identified it as the previously characterized VIP21 protein (Vesicular integral-membrane protein of 21 kDa).[45,46] Two additional caveolin protein isoforms have also been identified by sequence identity. Together, they form the caveolin protein family, which consists of three proteins (Caveolin-1, Caveolin-2, Caveolin-3) that are well-conserved from *C. elegans* to mammals.[38]

Cav-1 has an unusual topology (Fig. 1) with the middle portion of the protein (~33 amino acids) embedded into the cytoplasmic leaflet of the lipid bilayer and its amino and carboxy termini in the cytosol, thus forming a hairpin-like structure.[44] Cav-1 and Cav-3 homo-oligomerize, while Cav-1 and Cav-2 form hetero-oligomers, via the caveolin oligomerization domain (COD, residues 61-101).[47] After synthesis in the endoplasmic reticulum (ER), Cav-1 forms high molecular oligomeric complexes with either itself or Cav-2. In skeletal muscle and cardiac myocytes, Cav-3 is the main structural component of caveolae.[48] Cav-1 oligomers organize themselves within the membrane to form a higher order umbrella-like structure (Fig. 1). As the complex traffics through the Golgi network, a higher order complex of well over 1000 subunits (Cav-1 oligomers) eventually leads to the formation of caveolae at the plasma membrane in association with cholesterol and sphingolipids. Cav-1 protein levels are highly dependent on cellular cholesterol levels.[49-52] In addition, this protein has a high affinity for cholesterol.[53-56] The initial Cav-1 oligomers allow to anchor various receptors and signaling molecules.[57,58] Besides, this structure promotes the invagination and bending of the membrane through the caveolin-induced asymmetrical conformation.

Domain mapping and deletional analysis have identified a Cav-1 scaffolding domain (CSD, residues 82-101), which allows Cav-1 to mediate protein-protein interactions and modulate signal transduction pathways. Several cytoplasmic and transmembrane proteins and downstream signaling molecules have been shown to preferentially localize to caveolae and interact with Cav-1. These molecules include Src-family tyrosine kinases, p42/44 MAPK and endothelial nitric oxide synthase (eNOS). Cav-1 can hold these signal transducing molecules in an inactive state until they are activated by the appropriate stimulus.[57,59] These properties allow caveolae and caveolins to regulate signal transduction and act as platforms for compartmentalization, engaging signaling molecules in a manner similar to lipid rafts. This function has been proposed in the "caveolin signaling hypothesis".[60]

ABSENCE OF CAVEOLIN-1 DECREASES ATHEROSCLEROSIS DEVELOPMENT

The first direct indication suggesting that Cav-1 plays a role in atherosclerosis has come from findings obtained in our laboratory. We have shown a major reduction of

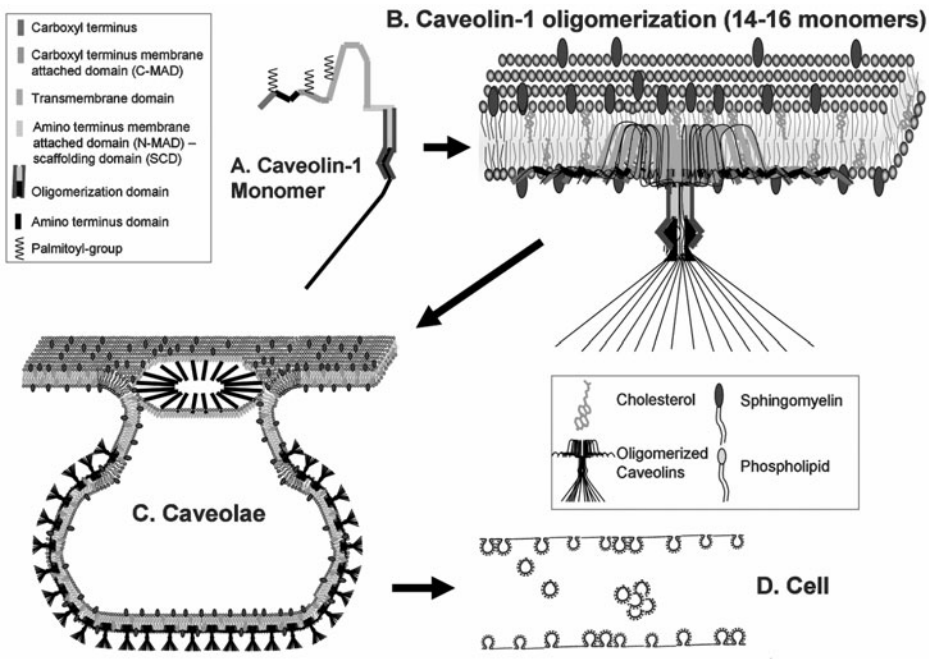

Figure 1. Caveolae Organization. A) Representation of Cav-1 hairpin-like structure with its domains, including the scaffolding domain (CSD) which allows Cav-1 to bind and regulate kinases and other downstream signaling pathways. B) Representation of Cav-1 oligomer formation (14-16 monomers) with an umbrella-like structure embedded within the plasma membrane enriched in cholesterol and sphingomyelin. C) Higher ordered complex of well over 1000 subunits eventually forms caveolae within the lipid bilayer of the plasma membrane. D) Examples of caveolae structures observed in differentiated cells. Grape-like structures plasma membrane attached caveolae are shown.

atherosclerosis in caveolin-1-deficient (*cav-1*$^{-/-}$) mice in the *apoe*$^{-/-}$ genetic background. These double knock-out mice displayed reduced aortic lesions by up to 70% compared to *apoe*$^{-/-}$ mice alone despite remarkably elevated levels of circulating plasma cholesterol.[61,62]

However, Cav-1 is expressed in all the cell types involved in the development of an atheroma. Nevertheless, its expression levels and function are different depending on the cell type. In fact, current studies suggest that Cav-1 has both a pro- and anti-atherogenic role that is context-dependent based on the cell type in which it is expressed.[61] The various roles of Cav-1 in atherosclerosis will be discussed in the following sections.

Role of Caveolin-1 in the Regulation of Endothelial Cell Function

Caveolae and the Regulation of LDL Transcytosis

Elevated plasma LDL cholesterol levels have been associated with increased risk for heart disease development. As a consequence, all mouse models used for atherosclerosis studies exhibit abnormal lipoprotein profiles. Transcytosis and retention of LDL are believed to be the initiating events that lead to downstream processes such as activation

of ECs and subsequent monocyte recruitment. The transcytosis process is defined as the transfer across ECs of a molecule (e.g., LDL) from the lumen to the subendothelial side of a blood vessel. Its occurrence may be related to the presence of Cav-1 in ECs, since it was suggested that caveolae could mediate LDL transcytosis[63] (Fig. 2). Other molecules that are known to transcytose across ECs are albumin[64] and transferrin.[65] Interestingly, ECs that lack Cav-1 display impaired transcytosis of albumin.[66,67]

Transcytosis is the first function that has been ascribed to caveolae[68] and fifteen years later it was shown that the majority of LDL transcytosis occurs via caveolae.[69] Endothelial caveolae are thought to play a role in transcytosis via receptor-mediated transfer of LDL across ECs[61,68] or fluid phase transfer of LDL across ECs. A third pathway by which LDL could cross the endothelial barrier might be via a paracellular transport, which could occur between two ECs (Fig. 2). However, the latter pathway is unlikely to occur since LDL particles may be too large (20-30 nm) to fit between the tightly apposed ECs. In fact, Simionescu et al have shown that the transfer of molecules via the paracellular pathway is limited to those in the 3-6 nm range.[70] Moreover, Vasile et al[69] have shown that LDL particles are endocytosed in small amounts in ECs by receptor-dependent and receptor-independent processes. In addition, caveolae have also been shown to be responsible for the transcytosis of LDL and HDL across ECs of the blood brain barrier.[71,72] Finally, in a recent study, we have shown that Cav-1-deficient mice present defects in the aortic uptake of LDL particles, both in vivo and in vitro.[63] In direct support of these findings, we have confirmed that downregulation of the Cav-1 protein in human umbilical vein endothelial cells leads to an over 50% reduction in LDL uptake (S Pavlides and PG Frank unpublished data). The latter studies demonstrate a critical role for caveolae-mediated transcytosis of LDL particles from the vascular lumen to the sub-endothelial space. Furthermore, they also indicate an important pro-atherogenic function for Cav-1 and caveolae in ECs. Therefore, the requirement for high cholesterol

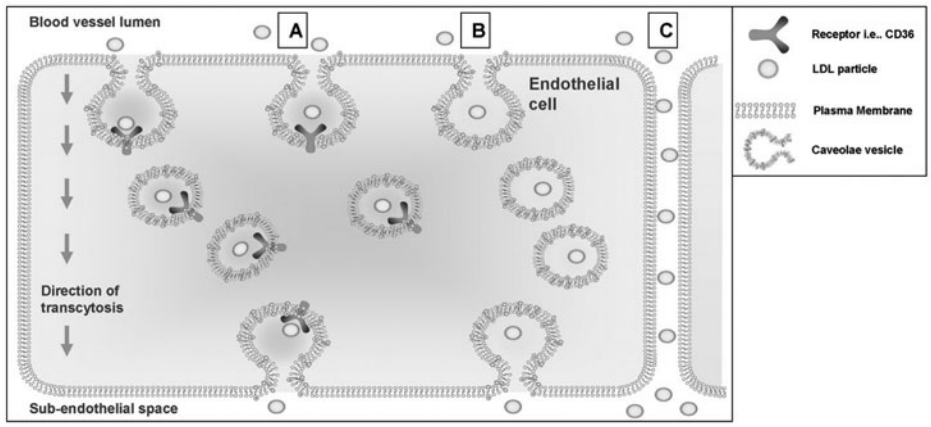

Figure 2. Transcellular model of LDL transfer across endothelial cells. LDL is transferred from the lumen to the sub-endothelial space via three possible transcellular pathways: A) Receptor-mediated transcytosis: LDL binds specific receptors that are found in caveolae (such as CD36). Caveolae vesicles endocytose and transfer LDL across the cell, where caveolae fuse with plasma membrane on the basal side to release LDL within the intima. B) Fluid phase-mediated transcytosis: LDL particles engage in caveolae vesicles in a nonspecific manner and are transferred across the cell to the intima. C) Paracellular pathway: LDL particles are transported between the space of two apposed cells in a caveolae-independent manner.

circulating levels in animal models susceptible to atherosclerosis is not sufficient but transcytosis of LDL across ECs is a prerequisite for the initiation of atherosclerotic lesion progression. Blockage of this process could prevent atherosclerotic lesion development. Further research into the factors regulating this step may lead to the development of novel drugs for the treatment of vascular diseases.

Caveolin-1: Role in the Regulation of eNOS Function and Inflammation

Endothelial Cav-1 is implicated in vascular inflammation, which is a critical element in the development of atherosclerosis. In that regard, the endothelial nitric oxide synthase (eNOS) has been demonstrated to play an important role in inflammation. eNOS is an enzyme produced by endothelial cells and it is palmitoylated and myristoylated.[73-75] These posttranslational modifications are a common feature of many signaling proteins that are targeted to caveolae.[33] Moreover, eNOS interacts with the Cav-1 scaffolding domain and is tonically inhibited by Cav-1 in vascular ECs.[42,76,77] eNOS is a complex dimeric enzyme, which activity is highly regulated. Intracellular calcium concentration rises upon agonist (e.g., acetylcholine) stimulation of ECs. This reaction leads to the binding of calmodulin to intracellular calcium and the newly-formed complex effectively displace Cav-1 from eNOS and associates with the latter. Dissociation of eNOS from caveolin allows the production of nitric oxide (NO).[78] Dysregulated eNOS activity, due to the lack of one of the major cofactors can have adverse effects by inducing superoxide production[79] (see Chapter 3 for additional details relating to the role of Cav-1 in the regulation of eNOS function).

Activation of eNOS is associated with protective effects on lesion formation via decreased expression of adhesion molecules, such as VCAM-1.[80,81] VCAM-1 is a protein that belongs to the immunoglobulin superfamily, which also includes integrins and selectins.[82] Under basal, unstimulated physiological conditions, VCAM-1 is not expressed. However, under specific pro-inflammatory conditions, such as in the presence of cytokines like Tumor Necrosis Factor (TNF)-α or Interleukin (IL)-1β, ECs are activated and quickly synthesize VCAM-1.[83] Previous studies have demonstrated the important role of VCAM-1 in the development of atherosclerosis.[84,85] Our studies using *cav-1*$^{-/-}$ *apoe*$^{-/-}$ and *apoe*$^{-/-}$ mice have shown that the absence of Cav-1 in endothelial cells could lead to a reduction in VCAM-1 production.[62] In addition, Fernandez-Hernando et al[86,127] have demonstrated the direct role of Cav-1 in the regulation of adhesion molecule expression. In this study, Fernandez-Hernando et al have shown that the re-expression of Cav-1 in endothelial cells of *cav-1*$^{-/-}$*apoe*$^{-/-}$ is sufficient to reverse the effect observed on VCAM-1 expression. In addition, these authors have also shown that the expression of other markers of inflammation (ICAM-1, E-selectin and P-selectin) is reduced in *cav-1*$^{-/-}$*apoe*$^{-/-}$ mice.[86,127] Taken together, these data suggest that endothelial Cav-1 plays an essential role in the regulation of endothelial cell activation.

Summary

The current literature strongly suggests that endothelial Cav-1 and caveolae plays critical roles in the development of atherosclerosis. Moreover, contrary to its role in macrophages and smooth muscle cells (See the following two sections), a clear pro-atherogenic role has been demonstrated for endothelial Cav-1.[62,86] It may regulate lipoprotein and cholesterol accumulation in the intima.[63] In addition, we and others have demonstrated direct and indirect effects for Cav-1 in the regulation of endothelial-mediated

inflammation.[62,86] Finally, Cav-1 may also play an important role in the regulation of endothelial cell replacement in injured blood vessels. In that case, the presence of Cav-1 may limit cellular replacement in injured blood vessels, thereby promoting lipoprotein accumulation in the intima and eventually atheroma growth.[87]

Role of Caveolin-1 in the Regulation of Macrophage Function

Macrophage Apoptosis during Atherosclerotic Development

Macrophage apoptosis occurs at all stages of atherosclerotic lesion development after foam cell formation.[14,15] Cav-1 has been implicated in the regulation of apoptosis in a number of cell types such as endothelial cells[88] and smooth muscle cells.[89] Extensive analysis shows that Cav-1 expression sensitizes certain types of cells to chemicals that induce apoptosis. For example, NIH 3T3 cells that overexpress Cav-1 are more sensitive to apoptosis mediated by the protein kinase inhibitor staurosporine. Conversely, NIH 3T3 cells that have been depleted of Cav-1 become resistant to apoptosis induced by staurosporine.[90] Similar results have been obtained with the bladder epithelial cell line T24.[90] Studies have shown that regulators of apoptosis, such as the TNF-α receptor[91] and caspase-3[88] localize to caveolae and their function may depend on the presence of caveolae. These data suggest that the localization of apoptotic regulators within caveolae is critical for apoptosis.

Several studies have now examined the role of molecules regulating macrophage apoptosis in the development of atherosclerosis. In vivo studies using bone marrow transplantation of cells lacking the pro-apoptotic gene *Bax* have revealed that decreased macrophage apoptosis leads to increased early lesion size and cellularity.[92] Similar results were obtained in bone marrow transplantation experiments using cells lacking the pro-apoptotic (or tumor-suppressor) gene *p53*.[93] Both of the above studies have used mouse models susceptible to atherosclerosis such as *apoe*$^{-/-}$ and *ldlr*$^{-/-}$ mice. However, these bone marrow-derived macrophages lacking the pro-apoptotic genes (*bax, p53*) have been shown to display increased cellular proliferation, which may further contribute to the increased lesion size.[92] On the other hand, mice that lack the anti-apoptotic factor, AIM (*a*poptosis *i*nhibitor expressed by *m*acrophages) have been shown to develop smaller early atherosclerotic lesions compared to their *ldlr*$^{-/-}$ control group.[94] The above examples are indicative of an inverse relationship between apoptosis and early atherosclerotic lesion development. Increased apoptosis leads to decreased cellularity and therefore, reduced lesion size. Conversely, decreased apoptosis leads to increased cellularity and therefore, increased lesion size.

In mouse peritoneal macrophages (MPMs), Cav-1 expression is up regulated during simvastatin-induced apoptosis of macrophages where Cav-1 colocalizes with phosphatidylserine (PS).[49] In their study, Gargalovic and Dory have suggested that Cav-1 may be involved in the externalization of PS during early apoptosis and that increased expression of Cav-1 in MPM may serve as an early marker for apoptosis.[51] Recently, we have shown that the absence of Cav-1 in MPMs is associated with increased accumulation of CE and decreased free cholesterol (FC).[95] CE is a neutral lipid that is stored in lipid droplets within the cytoplasm. It is the accumulation of CE that leads to the formation of foam cells.[96] This event is concomitant with reduced FC synthesis and increased acyl coenzyme A: cholesterol acyltransferase (ACAT) activity.[95] Increased FC levels in the ER are toxic for macrophages and may lead to the activation of the unfolded protein response (UPR) and eventually to apoptosis.[17,48,97] Our findings[95] are in agreement with the first line of defense against cholesterol toxicity, which, in macrophages, is the esterification

of FC into CE by the enzyme ACAT.[98] Therefore, efficient conversion of FC into CE is considered a survival mechanism.[17,48,99] These results suggest that *cav-1*$^{-/-}$ macrophages may be more resistant to the toxic effects of FC and less susceptible to apoptosis than wild-type macrophages. Thus, if cell death (apoptosis) is inefficient and cholesteryl ester is stored more efficiently in macrophages lacking Cav-1, cellularity may be amplified in association with enhanced foam cell formation. This hypothesis is in agreement with our preliminary studies in which we observe an increased in early atherosclerotic lesion development in wild-type mice transplanted with bone marrow obtained from caveolin-1-deficient mice (S. Paulides and P.G. Frank, unpublished data).

Phagocytosis: Role in Lesion Development and Progression

The process of efferocytosis by macrophages infiltrating the atherosclerotic lesion is crucial for the containment of the atheroma. Many groups have suggested that apoptosis is linked to phagocytosis[100] and others have shown that at early stages of lesion development, foam cell accumulation and apoptosis are regulated by the levels of phagocytosis. In early lesion development, the process of phagocytosis is considered favorable because it helps prevent further expansion of the atheroma by decreasing cellularity and lessening the inflammatory cascade. Macrophage apoptosis is associated with diminished lesion cellularity and decreased lesion progression in early lesions, in which phagocytic clearance of apoptotic macrophages seems to be efficient.[92-94] Moreover, even if the initial response is the engulfment of foam cell apoptotic bodies by neighboring macrophages, phagocytes may become engorged with apoptotic foam cell "remnants," including abundant lipids. Eventually, the capacity of macrophages to carry out this process can be exceeded. However, in later more complex lesions, this balance is disrupted and apoptotic cell clearance is usually defective and this defect leads to advanced plaque formation.[22,101]

Data suggests that Cav-1 is involved in the modulation of macrophage inflammatory responses (e.g., to oxLDL) and in the clearance of apoptotic cells at lesion sites.[102,103] Electron microscopy studies have shown that Cav-1 is linked to the process of phagocytosis and cannibalism (an act of engulfing live cells)[104] among malignant tumor cells, through images of caveolae-like structures (caveolae-caveolae fusion) at the site of cell contact between the phagocyte and the tumor cell. Additional studies have shown that Cav-1 is present in endolysosomes of phagocytes, indicating that caveolae may contribute to the formation of the "cannibalistic vacuole".[104] Our laboratory has shown that *cav-1*$^{-/-}$ MPM have decreased phagocytic clearance ability of apoptotic thymocytes and fluorescein-labeled *E.coli* K-BioParticles.[102] To further expand on these results, Li et al,[102] have also shown that phagocytosis is impaired in wild-type MPM treated with methyl-β-cyclodextrin, which disrupts caveolae by depleting cholesterol from the plasma membrane. If Cav-1-deficient cells have reduced phagocytic capabilities, it may follow that Cav-1 is important for the proper clearance of apoptotic foam cells by macrophages in the arterial intima. In that case, macrophage Cav-1 may play an anti-atherogenic role since it allows macrophages to cleanup apoptotic foam cells and cellular debris at lesion sites.

Macrophage and Inflammation

Macrophages have also been shown to play a critical role in the regulation of vascular inflammation during atherogenesis.[1] Interestingly, Cav-1 has previously been shown to play a role in the regulation of this process.[105] Overexpression of Cav-1 in macrophages

leads to reduced secretion of TNF-α and IL-6, whereas downregulation of Cav-1 leads to increased TNF-α and IL-6 secretion.[106] In that case, signaling via the NFκB/Akt pathway is also increased. Therefore, Cav-1 is believed to play an anti-inflammatory role and prevent activation via the NFκB/Akt pathway.[106] In addition, the pro-apoptotic role of Cav-1 in macrophages may prevent a prolonged inflammatory response and may reduce the recruitment of T-cells and monocytes into atherosclerotic plaques. Taken together, these data suggest that the absence of Cav-1 in macrophage may lead to events (apoptosis-inflammation) that synergistically contribute to accelerated plaque progression in early lesions.

Summary

In general, most of the studies have provided evidence for a role of Cav-1 in the regulation of macrophage function. We propose that Cav-1 plays a role in the regulation of cellular cholesterol homeostasis, apoptosis and inflammation. In each of the function examined, we and others have shown that macrophage Cav-1 has antiatherogenic properties.

Role of Caveolin-1 in the Regulation of Vascular Smooth Muscle Cell Migration and Proliferation

Vascular tunica media is mainly composed of SMCs that can contract or relax and, as a consequence, allow the modification of blood vessel shape and blood pressure. Like the aforementioned macrophages and endothelial cells, SMCs play a critical role in atherosclerosis development. Early studies have shown that diet-induced atherosclerosis alters vascular smooth muscle morphology and/or function in rabbit,[107] swine,[108] nonhuman primate[109,110] and humans.[111] In particular, an increase in SMC proliferation leads to increased arterial wall thickness and intracellular lipid accumulation. In parallel, various research groups have realized that SMCs can present either a contractile or a synthetic phenotype.[112-115] The latter cellular state involves the acquisition of proliferative, migrating and secreting machineries, which play key roles during atherosclerosis development.[116-118]

Caveolae structures have been detected in association with the plasma membrane of brain vascular SMCs by freeze fracture ultrastructure techniques.[119] Similar to striated muscle cells, vascular SMCs express Cav-1, -2 and -3;[120-123] however, in contrast to striated muscle cells, caveolin-3 has been detected to a lesser extent in vascular SMCs.[122-124] Interestingly, while genetic deletion of Cav-3 in mice prevents caveolae formation in striated muscle cells,[125,126] Cav-1 genetic ablation is sufficient to considerably diminish the number of caveolae in vascular SMCs.[43,127-129] Moreover, while Cav-1 is expressed in all vascular SMCs, caveolin-3 expression appears to be restricted to arterial rather than venous SMCs.[130]

Regulation of SMC migration

The main role of SMCs is to aid in the distribution of blood through vascular smooth muscle contraction and relaxation. This vascular smooth muscle function is in part controlled by NO produced by eNOS in ECs,[131] although studies have also demonstrated the existence of NOS activity in SMC.[132,133] As mentioned earlier, Cav-1 regulates NO production in ECs. Therefore, a great amount of evidence in this field has shown that Cav-1 indirectly regulates SMC function. Consequently, it has been shown that aortic rings from Cav-1-deficient mice fail to contract properly when

exposed to phenylephrine and the NO-mediated relaxation effects of acetylcholine significantly increases compared to the effect observed in aortic rings obtained from wild-type mice.[42,43] Supporting the increased production of NO in *cav-1*$^{-/-}$ aortic rings, treatment with the NOS inhibitor L-NAME, causes a significantly greater contraction of aortic rings obtained from *cav-1*$^{-/-}$ mice than of those obtained from wild-type mice.[42] More recently, endothelial re-expression of Cav-1 in *cav-1*$^{-/-}$ mice has been shown to rescue low flow-mediated dilation[134] and restored SMC contractility.[135] Taken together, these data suggest that signaling mediated by NO in SMCs is regulated by endothelial Cav-1. Interestingly, several studies have now confirmed the link between defective NO-signaling and atherosclerosis. These observations have been made in human subject as well as in animal models.[136] The current literature suggests a critical role for Cav-1 in this pathway. Interestingly, endothelial Cav-1 expression has been shown to be upregulated in hypercholesterolemic subjects.[137] This fact is sufficient to explain by itself the abnormal NO-mediated vasorelaxation observed in these patients. These findings have more recently been confirmed in mice overexpressing Cav-1 in endothelial cells only.[86]

NOS activity and presence of the nNOS isoform have been demonstrated in SMC.[130,132,133] In addition, Cav-3 is expressed in SMCs[120-123] and nNOS has also been shown to interact with the scaffolding domain of Cav-3.[138] Taken together, these observations point towards the idea that Cav-3 may also regulate NO production in SMCs and may therefore play an important role in the development of CVD. However, this hypothesis has not been yet addressed. In general, the current literature suggests that Cav-1 and/or Cav-3 may play a key role in the vasorelaxation of SMCs via their ability to regulate NO production. Since NO can regulate SMC function,[139] Cav-1 and Cav-3 may therefore, indirectly regulate the phenotype of SMCs. This process may be relevant for the pathogenesis of atherosclerosis.

Before proliferating, SMCs migrate into the vascular intima to form part of the occlusive mass found in atheromas.[140] SMC migration is controlled by a set of molecules including PDGF, angiotensin II, TGFβ and FGF[141] that activate tyrosine kinases.[142] Cav-1 expression has been implicated in the regulation of a number of signaling pathways that regulate SMC migration. This is possibly due to the fact that Cav-1 negatively regulates and stabilizes key players (i.e., kinases) implicated in various signaling cascades.[143-147] Moreover, Cav-1 may also modulate vascular protease activity and SMC migration.[148] Taken together, these data are consistent with those obtained in our laboratory where we have shown that aortic SMCs from *cav-1*$^{-/-}$ mice have an increased migratory potential compared to aortic SMCs obtained from wild-type mice.[149]

Role of Caveolin-1 in the Regulation of SMC Proliferation

It is well accepted that SMCs have the capacity to acquire proliferative properties (i.e., synthetic phenotype) at the initial stages of atherosclerosis.[109,150] Since Cav-1 has been shown to regulate various signaling pathways involved in the control of cellular proliferation, we can expect Cav-1 to play an important role in the regulation of vascular SMC proliferation. In agreement with this idea, several in vitro studies have demonstrated an antiproliferative function for Cav-1 in vascular SMC.[120,127] For example, Schwencke et al (2005) have demonstrated that in the absence of Cav-1, primary SMCs display increased proliferative properties.[127] Recently, vascular SMCs have been found to proliferate in response to static pressure correlating with Cav-1 downregulation and the activation of ERK1/2.[151] ERK1/2 has

been shown to be negatively regulated by Cav-1 in various studies.[146,149,152] Besides the ERK pathway, Cav-1 may regulate other signaling pathways involved in the regulation of SMC proliferation. These pathways include integrin/focal adhesion kinase[153] and tissue factor.[154]

Correlative studies have also shown that Cav-1 expression is reduced in human vascular SMC from atherosclerotic lesions[127,155] and in neointimal hyperplasia.[89] Neointima formation is a process characterized by SMC proliferation and extracellular matrix deposition in the vascular intimal layer. To evaluate the role of Cav-1 in the pathogenesis of neointimal lesions, we have used *cav-1*$^{-/-}$ mice as a model system. The right common carotid artery of wild-type and *cav-1*$^{-/-}$ mice was ligated just proximal to its bifurcation. The changes in vessel wall geometry in response to flow reduction in *cav-1*$^{-/-}$ and wild-type mice were determined by measuring the luminal, intimal and medial areas of carotid arteries after vessel ligation. Our results demonstrate that Cav-1-deficiency is associated with increased neotimal formation with the concomitant activation of the p42/44 MAP kinase cascade and upregulation of cyclin D1.[156] In support of these findings, Schwencke et al (2005) have shown that proliferation of SMCs from *cav-1*$^{-/-}$ mice is inhibited when re-expressing Cav-1.[127] Under specific conditions (i.e., cyclic strain), Cav-1 may also be involved in the activation of pro-proliferative signals.[157] However, this line of research will require further investigations.

Recent studies have shown that Cav-1 may influence vascular protease activity and potentially stabilize atherosclerotic lesions. Rodriguez-Feo et al[148] have demonstrated that low levels of SMC Cav-1 promotes plaque instability with increased lipid core size, macrophage infiltration and increased secretion of IL-6, IL-8 and matrix metalloprotease-9 activity.[148] This study implies that the absence of Cav-1 in SMCs could be directly related to impaired inflammatory responses that contribute to the formation of an atherosclerotic lesion.

Summary

In summary, these findings suggest that Cav-1 and caveolae in SMCs play an important role in the regulation of SMCs phenotype. They suggest that Cav-1 may play both antiproliferative and antimigratory roles. Therefore, we propose that SMC Cav-1 may have an anti-atherogenic role during the development of atherosclerosis.

CONCLUSION

A role for Cav-1 during atherogenesis has first been demonstrated in our laboratory. We have shown that double knockout mice *cav-1*$^{-/-}$*apoe*$^{-/-}$develop significantly less atherosclerotic lesions than the control *apoe*$^{-/-}$ mice. It is proposed that endothelial Cav-1 promotes atherogenesis through its role in the transcytosis of LDL across ECs from the blood stream into the subendothelial intima. In addition, endothelial Cav-1 appears to play an important role in the regulation of vascular inflammation. By contrast, macrophage Cav-1 may have an anti-atherogenic role. Supporting this hypothesis, we have recently shown that Cav-1 regulates intracellular cholesterol homeostasis and accumulation of CE in macrophages, but decreased FC[95] indicating a possible survival function.[16,17,99] In addition to the latter finding, we have also shown that macrophages lacking Cav-1 present impaired phagocytosis properties.[102] These findings indicate that macrophage Cav-1 can contribute to reduced atherosclerotic lesion cellularity. Another anti-atherogenic role of Cav-1 may be linked to its ability to reduce the production of cytokines by macrophages submitted to a pro-inflammatory stimulus.[106] Furthermore, Cav-1 expression in SMCs is

hypothesized to be anti-atherogenic by inhibiting migration and proliferation of this cell type during atherosclerosis progression. Finally, these findings suggest rather complex and sometimes opposing roles for Cav-1 supporting a cell-context dependent paradigm for all three types of cells that play a central role during atherosclerosis development.[61] A better understanding of the role of Cav-1 in vivo is required to better define the various functions of Cav-1. It is expected that these studies will provide us with a better rational for the treatment of patients with CAD.

REFERENCES

1. Lusis AJ. Atherosclerosis. Nature 2000; 407(6801):233-241.
2. Castelli WP, Doyle JT, Gordon T et al. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation 1977; 55(5):767-772.
3. Williams KJ, Tabas I. Lipoprotein retention—and clues for atheroma regression. Arterioscler Thromb Vasc Biol 2005; 25(8):1536-1540.
4. Ross R. Atherosclerosis is an inflammatory disease. Am Heart J 1999; 138(5 Pt 2):S419-420.
5. Gustafsson M, Boren J. Mechanism of lipoprotein retention by the extracellular matrix. Curr Opin Lipidol 2004; 15(5):505-514.
6. Hogg N, Landis RC. Adhesion molecules in cell interactions. Curr Opin Immunol 1993; 5(3):383-390.
7. Lasky LA, Singer MS, Dowbenko D et al. An endothelial ligand for L-selectin is a novel mucin-like molecule. Cell 1992; 69(6):927-938.
8. Song C, Hsu K, Yamen E et al. Serum amyloid A induction of cytokines in monocytes/macrophages and lymphocytes. Atherosclerosis 2009; 207(2):374-383.
9. Tabas I, Seimon T, Timmins J et a1. Macrophage apoptosis in advanced atherosclerosis. Ann N Y Acad Sci 2009; 1173(Suppl 1):E40-45.
10. Bhakdi S, Dorweiler B, Kirchmann R et al. On the pathogenesis of atherosclerosis: enzymatic transformation of human low density lipoprotein to an atherogenic moiety. J Exp Med 1995; 182(6):1959-1971.
11. Goldstein JL, Ho YK, Basu SK et al. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc Natl Acad Sci USA 1979; 76(1):333-337.
12. Suzuki H, Kurihara Y, Takeya M et al. The multiple roles of macrophage scavenger receptors (MSR) in vivo: resistance to atherosclerosis and susceptibility to infection in MSR knockout mice. J Atheroscler Thromb 1997; 4(1):1-11.
13. Febbraio M, Podrez EA, Smith JD et al. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. J Clin Invest 2000; 105:1049-1056.
14. Kockx MM, De Meyer GR, Muhring J et al. Apoptosis and related proteins in different stages of human atherosclerotic plaques. Circulation 1998; 97(23):2307-2315.
15. Kockx MM, Herman AG. Apoptosis in atherosclerosis: beneficial or detrimental? Cardiovasc Res 2000; 45(3):736-746.
16. Tabas I, Marathe S, Keesler GA et al. Evidence that the initial up-regulation of phosphatidylcholine biosynthesis in free cholesterol-loaded macrophages is an adaptive response that prevents cholesterol-induced cellular necrosis. Proposed role of an eventual failure of this response in foam cell necrosis in advanced atherosclerosis. J Biol Chem 1996; 271(37):22773-22781.
17. Warner GJ, Stoudt G, Bamberger M et al. Cell toxicity induced by inhibition of acyl coenzyme A: cholesterol acyltransferase and accumulation of unesterified cholesterol. J Biol Chem 1995; 270(11):5772-5778.
18. Mitchinson MJ, Hardwick SJ, Bennett MR. Cell death in atherosclerotic plaques. Curr Opin Lipidol 1996; 7(5):324-329.
19. Chin BY, Petrache I, Choi AM et al. Transforming growth factor beta1 rescues serum deprivation-induced apoptosis via the mitogen-activated protein kinase (MAPK) pathway in macrophages. J Biol Chem 1999; 274(16):11362-11368.
20. Geng YJ, Libby P. Progression of atheroma: a struggle between death and procreation. Arterioscler Thromb Vasc Biol 2002; 22(9):1370-1380.
21. Tabas I, Tall A, Accili D. The impact of macrophage insulin resistance on advanced atherosclerotic plaque progression. Circ Res 2010; 106(1):58-67.
22. Tabas I. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. Arterioscler Thromb Vasc Biol 2005; 25(11):2255-2264.

23. Reddick RL, Zhang SH, Maeda N. Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression. Arterioscler Thromb 1994; 14(1):141-147.
24. Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell 2001; 104(4):503-516.
25. Smithies O, Maeda N. Gene targeting approaches to complex genetic diseases: atherosclerosis and essential hypertension. Proc Natl Acad Sci USA 1995; 92(12):5266-5272.
26. Breslow JL. Mouse models of atherosclerosis. Science 1996; 272(5262):685-688.
27. Yamada E. The fine structure of the renal glomerulus of the mouse. J Histochem Cytochem 1955; 3(4):309.
28. Palade GE. Fine structure of blood capillaries. J Appl Physics 1953; 24:1424.
29. Rietveld A, Simons K. The differential miscibility of lipids as the basis for the formation of functional membrane rafts. Biochim Biophys Acta 1998; 1376(3):467-479.
30. Waugh MG, Hsuan JJ. Preparation of membrane rafts. Methods Mol Biol 2009; 462:403-414.
31. Sargiacomo M, Sudol M, Tang Z et al. Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. J Cell Biol 1993; 122(4):789-807.
32. Lisanti MP, Tang Z, Scherer PE et al. Caveolae purification and glycosylphosphatidylinositol-linked protein sorting in polarized epithelia. Methods Enzymol 1995; 250:655-668.
33. Lisanti MP, Scherer PE, Vidugiriene J et al. Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: implications for human disease. J Cell Biol 1994; 126(1):111-126.
34. Cohen AW, Razani B, Schubert W et al. Role of caveolin-1 in the modulation of lipolysis and lipid droplet formation. Diabetes 2004; 53(5):1261-1270.
35. Chang WJ, Ying Y, Rothberg KG et al. Purification and characterization of smooth muscle cell caveolae. J Cell Biol 1994; 126:127-138.
36. Anderson RG. The caveolae membrane system. Annu Rev Biochem 1998; 67:199-225.
37. Rothberg KG, Ying Y, Kamen BA et al. Cholesterol controls the clustering of the glycophopholipid-anchored membrane receptor for 5-methyltetrahydrofolate. J Cell Biol 1990; 111:2931-2938.
38. Rothberg KG, Heuser JE, Donzell WC et al. Caveolin, a protein component of caveolae membrane coats. Cell 1992; 68(4):673-682.
39. Williams TM, Lisanti MP. The caveolin proteins. Genome Biol 2004; 5(3):214.
40. Bouras T, Lisanti MP, Pestell RG. Caveolin-1 in breast cancer. Cancer Biol Ther 2004; 3(10):931-941.
41. Razani B, Lisanti MP. Caveolins and caveolae: molecular and functional relationships. Exp Cell Res 2001; 271(1):36-44.
42. Razani B, Engelman JA, Wang XB et al. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. J Biol Chem 2001; 276(41):38121-38138.
43. Drab M, Verkade P, Elger M et al. Loss of caveolae, vascular dysfunction and pulmonary defects in caveolin-1 gene-disrupted mice. Science 2001; 293(5539):2449-2452.
44. Dupree P, Parton RG, Raposo G et al. Caveolae and sorting in the trans-Golgi network of epithelial cells. EMBO J 1993; 12(4):1597-1605.
45. Kurzchalia T, Dupree P, Parton RG et al. VIP 21, A 21-kD membrane protein is an integral component of trans-Golgi-network-derived transport vesicles. J Cell Biol 1992; 118:1003-1014.
46. Glenney JR Jr. Tyrosine phosphorylation of a 22-kDa protein is correlated with transformation by Rous sarcoma virus. J Biol Chem 1989; 264(34):20163-20166.
47. Sargiacomo M, Scherer PE, Tang Z et al. Oligomeric structure of caveolin: implications for caveolae membrane organization. Proc Natl Acad Sci USA 1995; 92(20):9407-9411.
48. Tang Z, Scherer PE, Okamoto T et al. Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. J Biol Chem 1996; 271(4):2255-2261.
49. Scherer PE, Tang Z, Chun M et al. Caveolin isoforms differ in their N-terminal protein sequence and subcellular distribution. Identification and epitope mapping of an isoform-specific monoclonal antibody probe. J Biol Chem 1995; 270(27):16395-16401.
50. Llaverias G, Vazquez-Carrera M, Sanchez RM et al. Rosiglitazone upregulates caveolin-1 expression in THP-1 cells through a PPAR-dependent mechanism. J Lipid Res 2004; 45(11):2015-2024.
51. Gargalovic P, Dory L. Cellular apoptosis is associated with increased caveolin-1 expression in macrophages. J Lipid Res 2003; 44(9):1622-1632.
52. Fielding CJ, Bist A, Fielding PE. Caveolin mRNA levels are up-regulated by free cholesterol and down-regulated by oxysterols in fibroblast monolayers. Proc Natl Acad Sci USA 1997; 94(8):3753-3758.
53. Uittenbogaard A, Ying Y, Smart EJ. Characterization of a cytosolic heat-shock protein-caveolin chaperone complex. Involvement in cholesterol trafficking. J Biol Chem 1998; 273(11):6525-6532.
54. Uittenbogaard A, Smart EJ. Palmitoylation of caveolin-1 is required for cholesterol binding, chaperone complex formation and rapid transport of cholesterol to caveolae. J Biol Chem 2000; 275(33):25595-25599.
55. Murata M, Peranen J, Schreiner R et al. VIP21/caveolin is a cholesterol-binding protein. Proc Natl Acad Sci USA 1995; 92(22):10339-10343.
56. Monier S, Dietzen DJ, Hastings WR et al. Oligomerization of VIP21-caveolin in vitro is stabilized by long chain fatty acylation or cholesterol. FEBS Lett 1996; 388(2-3):143-149.

57. Williams TM, Medina F, Badano I et al. Caveolin-1 gene disruption promotes mammary tumorigenesis and dramatically enhances lung metastasis in vivo. Role of Cav-1 in cell invasiveness and matrix metalloproteinase (MMP-2/9) secretion. J Biol Chem 2004; 279(49):51630-51646.
58. Cohen AW, Hnasko R, Schubert W et al. Role of caveolae and caveolins in health and disease. Physiol Rev 2004; 84(4):1341-1379.
59. Liu P, Ying YS, Anderson RGW. PDGF activates MAP kinase in isolated caveolae. Proc Natl Acad Sci USA 1997; 94:13666-13670.
60. Lisanti MP, Scherer P, Tang ZL et al. Caveolae, caveolin and caveolin-rich membrane domains: A signalling hypothesis. Trends Cell Biol 1994; 4:231-235.
61. Frank PG, Lisanti MP. Role of Caveolin-1 and Caveolae in Atherosclerotic Disease States: Atheroma Formation and Neointimal Hyperplasia. Curr Opin Lipidol 2004; In press.
62. Frank PG, Lee H, Park DS et al. Genetic ablation of caveolin-1 confers protection against atherosclerosis. Arterioscler Thromb Vasc Biol 2004; 24(1):98-105.
63. Frank PG, Pavlides S, Cheung MW et al. Role of caveolin-1 in the regulation of lipoprotein metabolism. Am J Physiol Cell Physiol 2008; 295(1):C242-248.
64. Ghitescu L, Fixman A, Simonescu M et al. Specific binding sites for albumin restricted to plasmalemmal vesicles of continuous capillary endothelium: Recepotor-mediated transcytosis. J Cell Biol 1986; 102:1304-1311.
65. Soda R, Tavassoli M. Transendothelial transport (transcytosis) of iron-transferrin complex in the bone marrow. J Ultrastruct Res 1984; 88(1):18-29.
66. Schubert W, Frank PG, Woodman SE et al. Microvascular hyperpermeability in caveolin-1 (–/–) knock-out mice. Treatment with a specific nitric-oxide synthase inhibitor, L-name, restores normal microvascular permeability in Cav-1 null mice. J Biol Chem 2002; 277(42):40091-40098.
67. Schubert W, Frank PG, Razani B et al. Caveolae-deficient endothelial cells show defects in the uptake and transport of albumin in vivo. J Biol Chem 2001; 276(52):48619-48622.
68. Palade GE, Bruns RR. Structural modification of plasmalemma vesicles. J Cell Biol 1968; 37:633-649.
69. Vasile E, Simionescu M, Simionescu N. Visualization of the binding, endocytosis and transcytosis of low density lipoprotein in the arterial endothelium in situ. J Cell Biol 1983; 96:1677-1689.
70. Simionescu N, Simionescu M, Palade GE. Open junctions in the endothelium of the postcapillary venules of the diaphragm. J Cell Biol 1978; 79(1):27-44.
71. Dehouck B, Fenart L, Dehouck MP et al. A new function for the LDL receptor: Transcytosis of LDL across the blood-brain barrier. J Cell Biol 1997; 138(4):877-889.
72. Balazs Z, Panzenboeck U, Hammer A et al. Uptake and transport of high-density lipoprotein (HDL) and HDL-associated alpha-tocopherol by an in vitro blood-brain barrier model. J Neurochem 2004; 89(4):939-950.
73. Yeh DC, Duncan JA, Yamashita S et al. Depalmitoylation of endothelial nitric-oxide synthase by acyl-protein thioesterase 1 is potentiated by $Ca^{(2+)}$-calmodulin. J Biol Chem 1999; 274(46):33148-33154.
74. Robinson LJ, Michel T. Mutagenesis of palmitoylation sites in endothelial nitric oxide synthase identifies a novel motif for dual acylation and subcellular targeting. Proc Natl Acad Sci USA 1995; 92(25):11776-11780.
75. Busconi L, Michel T. Endothelial nitric oxide synthase. N-terminal myristoylation determines subcellular localization. J Biol Chem1993; 268(12):8410-8413.
76. Michel JB, Feron O, Sase K et al. Caveolin versus calmodulin. Counterbalancing allosteric modulators of endothelial nitric oxide synthase. J Biol Chem 1997; 272(41):25907-25912.
77. Feron O, Belhassen L, Kobzik L et al. Endothelial nitric oxide synthase targeting to caveolae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells. J Biol Chem 1996; 271(37):22810-22814.
78. Michel T, Feron O. Nitric oxide synthases: which, where, how and why? J Clin Invest 1997; 100(9):2146-2152.
79. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 2006; 113(13):1708-1714.
80. Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. Annu Rev Physiol 2002; 64:749-774.
81. Gimbrone MA Jr, Topper JN, Nagel T et al. Endothelial dysfunction, hemodynamic forces and atherogenesis. Ann N Y Acad Sci 2000; 902:230-239.
82. Petruzzelli L, Takami M, Humes HD. Structure and function of cell adhesion molecules. Am J Med 1999; 106(4):467-476.
83. O'Brien KD, Allen MD, McDonald TO et al. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques. Implications for the mode of progression of advanced coronary atherosclerosis. J Clin Invest 1993; 92(2):945-951.
84. Ley K, Huo Y. VCAM-1 is critical in atherosclerosis. J Clin Invest 2001; 107(10):1209-1210.
85. Dansky HM, Barlow CB, Lominska C et al. Adhesion of monocytes to arterial endothelium and initiation of atherosclerosis are critically dependent on vascular cell adhesion molecule-1 gene dosage. Arterioscler Thromb Vasc Biol 2001; 21(10):1662-1667.

86. Fernandez-Hernando C, Yu J, Suarez Y et al. Genetic evidence supporting a critical role of endothelial caveolin-1 during the progression of atherosclerosis. Cell Metab 2009; 10(1):48-54.
87. Frank PG. Endothelial Caveolae and Caveolin-1 as Key Regulators of Atherosclerosis. Am J Pathol 2010.
88. Oxhorn BC, Buxton IL. Caveolar compartmentation of caspase-3 in cardiac endothelial cells. Cell Signal 2003; 15(5):489-496.
89. Peterson TE, Guicciardi ME, Gulati R et al. Caveolin-1 can regulate vascular smooth muscle cell fate by switching platelet-derived growth factor signaling from a proliferative to an apoptotic pathway. Arterioscler Thromb Vasc Biol 2003; 23(9):1521-1527.
90. Liu J, Lee P, Galbiati F et al. Caveolin-1 expression sensitizes fibroblastic and epithelial cells to apoptotic stimulation. Am J Physiol Cell Physiol 2001; 280(4):C823-835.
91. Ko YG, Lee JS, Kang YS et al. TNF-alpha-mediated apoptosis is initiated in caveolae-like domains. J Immunol 1999; 162(12):7217-7223.
92. Liu J, Thewke DP, Su YR et al. Reduced macrophage apoptosis is associated with accelerated atherosclerosis in low-density lipoprotein receptor-null mice. Arterioscler Thromb Vasc Biol 2005; 25(1):174-179.
93. Merched AJ, Williams E, Chan L. Macrophage-specific p53 expression plays a crucial role in atherosclerosis development and plaque remodeling. Arterioscler Thromb Vasc Biol 2003; 23(9):1608-1614.
94. Arai S, Shelton JM, Chen M et al. A role for the apoptosis inhibitory factor AIM/Spalpha/Api6 in atherosclerosis development. Cell Metab 2005; 1(3):201-213.
95. Frank PG, Cheung MW, Pavlides S et al. Caveolin-1 and regulation of cellular cholesterol homeostasis. Am J Physiol Heart Circ Physiol 2006; 291(2):H677-686.
96. Rader DJ. Molecular regulation of HDL metabolism and function: implications for novel therapies. J Clin Invest 2006; 116(12):3090-3100.
97. Tabas I. Apoptosis and plaque destabilization in atherosclerosis: the role of macrophage apoptosis induced by cholesterol. Cell Death Differ 2004; 11 Suppl 1:S12-16.
98. Chang TY, Chang CC, Lin S et al. Roles of acyl-coenzyme A:cholesterol acyltransferase-1 and -2. Curr Opin Lipidol 2001; 12(3):289-296.
99. Cui D, Thorp E, Li Y et al. Pivotal advance: macrophages become resistant to cholesterol-induced death after phagocytosis of apoptotic cells. J Leukoc Biol 2007; 82(5):1040-1050.
100. Savill J, Dransfield I, Gregory C et al. A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol 2002; 2(12):965-975.
101. Schrijvers DM, De Meyer GR, Herman AG et al. Phagocytosis in atherosclerosis: Molecular mechanisms and implications for plaque progression and stability. Cardiovasc Res 2007; 73(3):470-480.
102. Li J, Scherl A, Medina F et al. Impaired phagocytosis in caveolin-1 deficient macrophages. Cell Cycle 2005; 4(11):1599-1607.
103. Hegyi L, Hardwick SJ, Siow RC et al. Macrophage death and the role of apoptosis in human atherosclerosis. J Hematother Stem Cell Res 2001; 10(1):27-42.
104. Fais S. Cannibalism: a way to feed on metastatic tumors. Cancer Lett 2007; 258(2):155-164.
105. Chidlow JH Jr, Sessa WC. Caveolae, caveolins and cavins: complex control of cellular signalling and inflammation. Cardiovasc Res 2010; 86(2):219-225.
106. Wang XM, Kim HP, Song R et al. Caveolin-1 Confers Anti-Inflammatory Effects in Murine Macrophages via the MKK3/p38 MAPK Pathway. Am J Respir Cell Mol Biol 2005.
107. Imai H, Lee KT, Pastori S et al. Atherosclerosis in rabbits. Architectural and subcellular alterations of smooth muscle cells of aortas in response to hyperlipemia. Exp Mol Pathol 1966; 5(3):273-310.
108. Thomas WA, Florentin RA, Nam SC et al. Alterations in population dynamics of arterial smooth muscle cells during atherogenesis. I. Activation of interphase cells in cholesterol-fed swine prior to gross atherosclerosis demonstrated by "postpulse-salvage labeling". Exp Mol Pathol 1971; 15(2):245-267.
109. Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell: Proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. Science 1973; 180(93):1332-1339.
110. Goldfischer S, Schiller B, Wolinsky H. Lipid accumulation in smooth muscle cell lysosomes im primate atherosclerosis. Am J Pathol 1975; 78(3):497-504.
111. Aqel NM, Ball RY, Waldmann H et al. Identification of macrophages and smooth muscle cells in human atherosclerosis using monoclonal antibodies. J Pathol 1985; 146(3):197-204.
112. Thyberg J, Nilsson J, Palmberg L et al. Adult human arterial smooth muscle cells in primary culture. Modulation from contractile to synthetic phenotype. Cell Tissue Res 1985; 239(1):69-74.
113. Sjolund M, Madsen K, von der Mark K et al. Phenotype modulation in primary cultures of smooth-muscle cells from rat aorta. Synthesis of collagen and elastin. Differentiation 1986; 32(2):173-180.
114. Hedin U, Thyberg J. Plasma fibronectin promotes modulation of arterial smooth-muscle cells from contractile to synthetic phenotype. Differentiation 1987; 33(3):239-246.
115. Campbell GR, Campbell JH. Smooth muscle phenotypic changes in arterial wall homeostasis: implications for the pathogenesis of atherosclerosis. Exp Mol Pathol 1985; 42(2):139-162.

116. Yerneni KK, Bai W, Khan BV et al. Hyperglycemia-induced activation of nuclear transcription factor kappaB in vascular smooth muscle cells. Diabetes 1999; 48(4):855-864.
117. Srivastava AK. High glucose-induced activation of protein kinase signaling pathways in vascular smooth muscle cells: a potential role in the pathogenesis of vascular dysfunction in diabetes (review). Int J Mol Med 2002; 9(1):85-89.
118. Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of atherosclerosis. Lab Invest 2005; 85(1):9-23.
119. Tani E, Yamagata S, Ito Y. Cell membrane structure of vascular smooth muscle of circle of Willis. Cell Tissue Res 1977; 179(1):131-142.
120. Peterson TE, Kleppe LS, Caplice NM et al. The regulation of caveolin expression and localization by serum and heparin in vascular smooth muscle cells. Biochem Biophys Res Commun 1999; 265(3):722-727.
121. Thyberg J. Differences in caveolae dynamics in vascular smooth muscle cells of different phenotypes. Lab Invest 2000; 80(6):915-929.
122. Song KS, Scherer PE, Tang Z et al. Expression of caveolin-3 in skeletal, cardiac and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and cofractionates with dystrophin and dystrophin-associated glycoproteins. J Biol Chem 1996; 271(25):15160-15165.
123. Doyle DD, Upshaw-Earley J, Bell E et al. Expression of caveolin-3 in rat aortic vascular smooth muscle cells is determined by developmental state. Biochem Biophys Res Commun 2003; 304(1):22-25.
124. Ishizaka N, Griendling KK, Lassegue B et al. Angiotensin II type 1 receptor: relationship with caveolae and caveolin after initial agonist stimulation. Hypertension 1998; 32(3):459-466.
125. Galbiati F, Engelman JA, Volonte D et al. Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin-glycoprotein complex and t-tubule abnormalities. J Biol Chem 2001; 276(24):21425-21433.
126. Park DS, Woodman SE, Schubert W et al. Caveolin-1/3 double-knockout mice are viable, but lack both muscle and nonmuscle caveolae and develop a severe cardiomyopathic phenotype. Am J Pathol 2002; 160(6):2207-2217.
127. Schwencke C, Schmeisser A, Walter C et al. Decreased caveolin-1 in atheroma: loss of antiproliferative control of vascular smooth muscle cells in atherosclerosis. Cardiovasc Res 2005; 68(1):128-135.
128. Cho WJ, Daniel EE. Colocalization between caveolin isoforms in the intestinal smooth muscle and interstitial cells of Cajal of the Cav1(+/+) and Cav1 (–/–) mouse. Histochem Cell Biol 2006; 126(1):9-16.
129. Cheng X, Jaggar JH. Genetic ablation of caveolin-1 modifies Ca^{2+} spark coupling in murine arterial smooth muscle cells. Am J Physiol Heart Circ Physiol 2006; 290(6):H2309-2319.
130. Segal SS, Brett SE, Sessa WC. Codistribution of NOS and caveolin throughout peripheral vasculature and skeletal muscle of hamsters. Am J Physiol 1999; 277(3 Pt 2):H1167-1177.
131. Arnal JF, Dinh-Xuan AT, Pueyo M et al. Endothelium-derived nitric oxide and vascular physiology and pathology. Cell Mol Life Sci 1999; 55(8-9):1078-1087.
132. Pandolfi A, Grilli A, Cilli C et al. Phenotype modulation in cultures of vascular smooth muscle cells from diabetic rats: association with increased nitric oxide synthase expression and superoxide anion generation. J Cell Physiol 2003; 196(2):378-385.
133. Cheah LS, Gwee M, Das R et al. Evidence for the existence of a constitutive nitric oxide synthase in vascular smooth muscle. Clin Exp Pharmacol Physiol 2002; 29(8):725-727.
134. Yu J, Bergaya S, Murata T et al. Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. J Clin Invest 2006; 116(5):1284-1291.
135. Murata T, Lin MI, Huang Y et al. Reexpression of caveolin-1 in endothelium rescues the vascular, cardiac and pulmonary defects in global caveolin-1 knockout mice. J Exp Med 2007; 204(10):2373-2382.
136. Shaul PW. Endothelial nitric oxide synthase, caveolae and the development of atherosclerosis. J Physiol 2003; 547(Pt 1):21-33.
137. Feron O, Dessy C, Moniotte S et al. Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. J Clin Invest 1999; 103(6):897-905.
138. Venema VJ, Ju H, Zou R et al. Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/inhibitory domain. J Biol Chem 1997; 272(45):28187-28190.
139. Dubey RK, Jackson EK, Luscher TF. Nitric oxide inhibits angiotensin II-induced migration of rat aortic smooth muscle cell. Role of cyclic-nucleotides and angiotensin1 receptors. J Clin Invest 1995; 96(1):141-149.
140. Newby AC, George SJ. Proliferation, migration, matrix turnover and death of smooth muscle cells in native coronary and vein graft atherosclerosis. Curr Opin Cardiol 1996; 11(6):574-582.
141. Schwartz SM. Smooth muscle migration in atherosclerosis and restenosis. J Clin Invest 1997; 100(11 Suppl):S87-89.
142. Shimokado K, Yokota T, Kosaka C et al. Protein tyrosine kinase inhibitors inhibit both proliferation and chemotaxis of vascular smooth muscle cells. Ann N Y Acad Sci 1995; 748:171-175; discussion 175-176.

143. Shaul PW, Anderson RG. Role of plasmalemmal caveolae in signal transduction. Am J Physiol 1998; 275(5 Pt 1):L843-851.
144. Okamoto T, Schlegel A, Scherer PE et al. Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. J Biol Chem 1998; 273(10):5419-5422.
145. Hulit J, Bash T, Fu M et al. The cyclin D1 gene is transcriptionally repressed by caveolin-1. J Biol Chem 2000; 275(28):21203-21209.
146. Engelman JA, Chu C, Lin A et al. Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo. A role for the caveolin-scaffolding domain. FEBS Lett 1998; 428(3):205-211.
147. Capozza F, Williams TM, Schubert W et al. Absence of caveolin-1 sensitizes mouse skin to carcinogen-induced epidermal hyperplasia and tumor formation. Am J Pathol 2003; 162(6):2029-2039.
148. Rodriguez-Feo JA, Hellings WE, Moll FL et al. Caveolin-1 influences vascular protease activity and is a potential stabilizing factor in human atherosclerotic disease. PLoS One 2008; 3(7):e2612.
149. Hassan GS, Williams TM, Frank PG et al. Caveolin-1-deficient aortic smooth muscle cells show cell autonomous abnormalities in proliferation, migration and endothelin-based signal transduction. Am J Physiol Heart Circ Physiol 2006; 290(6):H2393-2401.
150. Lavezzi AM, Ottaviani G, Matturri L. Biology of the smooth muscle cells in human atherosclerosis. Apmis 2005; 113(2):112-121.
151. Luo DX, Cheng J, Xiong Y et al. Static pressure drives proliferation of vascular smooth muscle cells via caveolin-1/ERK1/2 pathway. Biochem Biophys Res Commun 2010; 391(4):1693-1697.
152. Galbiati F, Volonte D, Engelman JA et al. Targeted down-regulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44 MAP kinase cascade. EMBO Journal 1998; 17:6633-6648.
153. Morla AO, Mogford JE. Control of smooth muscle cell proliferation and phenotype by integrin signaling through focal adhesion kinase. Biochem Biophys Res Commun 2000; 272(1):298-302.
154. Ghrib F, Brisset AC, Dupouy D et al. The expression of tissue factor and tissue factor pathway inhibitor in aortic smooth muscle cells is up-regulated in synthetic compared to contractile phenotype. Thromb Haemost 2002; 87(6):1051-1056.
155. Batetta B, Mulas MF, Petruzzo P et al. Opposite pattern of MDR1 and caveolin-1 gene expression in human atherosclerotic lesions and proliferating human smooth muscle cells. Cell Mol Life Sci 2001; 58(8):1113-1120.
156. Kumar A, Lindner V. Remodeling with neointima formation in the mouse carotid artery after cessation of blood flow. Arterioscler Thromb Vasc Biol 1997; 17(10):2238-2244.
157. Sedding DG, Braun-Dullaeus RC. Caveolin-1: dual role for proliferation of vascular smooth muscle cells. Trends Cardiovasc Med 2006; 16(2):50-55.