Review

Caveolae and caveolin in transmembrane signaling:
Implications for human disease

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Received 19 September 2005; received in revised form 15 November 2005; accepted 29 November 2005
Available online 10 January 2006
Time for primary review 22 days

Abstract

The identification of various signaling molecules found within caveolae and their functional interaction with the integral membrane protein caveolin, a major structural component of caveolae, suggests that these membrane microdomains participate in transmembrane signaling. Several lines of evidence indicate that caveolin may act as a scaffolding protein by direct interaction with and modulation of the activity of multiple signaling molecules. The compartmentation of various signaling molecules in caveolae and their direct and functional interaction with caveolin provides a paradigm by which these membrane microdomains are involved in regulating signal transduction pathways. By dysregulation of these signal transduction pathways caveolins may be involved in the pathogenesis of various diseases. This review focuses on the implications as well as controversies of the contribution of caveolae and caveolins for several human diseases and the potential implications to therapeutic strategies.

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Keywords: Caveolae; Caveolin; Human disease; Atherosclerosis; Restenosis; Malignancies

1. Introduction

Signal transduction is initiated by complex protein–protein interactions for example between ligands, receptors and kinases. A recent fundamental development of molecular cell biology is the concept of membrane microdomains. Caveolae, 50–100 nm plasmalemmal vesicles, represent a subcompartment of the plasma membrane which exists most abundantly in terminally differentiated cells [1]. Caveolin, a 21–24 kDa integral membrane protein, is a major protein component of caveolae. Multiple members of the caveolin gene family have been identified (caveolin-1-α and -β, caveolin-2-α, -β, -γ, and -3) that differ in molecular structure and in tissue distribution [1]. Although caveolae play a major role in vesicular and cholesterol trafficking, the recent identification of various signaling molecules in caveolae and their functional interaction with caveolin suggest that caveolins may also participate in transmembrane signaling. Moreover, modulation of the activity of signaling molecules such as kinases by a short cytosolic domain derived from the N-terminal region of the caveolin molecule, called the caveolin scaffolding domain, could be demonstrated [2]. In effect, caveolin-1 seems to act as a scaffolding protein, able to regulate the activity of multiple signaling molecules.

Caveolae are receiving increasing attention as cellular organelles contributing to the pathogenesis of several human diseases. A large variety of in vitro and in vivo studies have implicated caveolae and caveolin in the pathogenesis of cancer, atherosclerosis and vasculoproliferative diseases, cardiac hypertrophy and heart failure, degenerative muscular dystrophies and diabetes mellitus. For the role of caveolin in different human diseases, it may be noteworthy...
to consider the distinct regional expression of the different caveolin isoforms within the different tissues and cells. Caveolin-1 is abundant in adipocytes, endothelial cells, pneumocytes and fibroblasts, whereas caveolin-3 expression is predominantly in cardiomyocytes, skeletal and smooth muscle cells [1].

It has to be noticed that at present our understanding of the relevance of caveolae and caveolins for physiology and pathophysiology is based mainly on in vitro data and experiments with caveolin knockout mice. However, an increasing number of experiments utilizing human tissues and genetic analyses support the crucial role of these membrane microdomains for the pathogenesis of several human diseases.

2. Cancer

Caveolin-1 has been shown to inhibit cellular proliferation [3–5]. This observation suggests that it may play a pivotal role in cellular transformation and tumorigenesis. Caveolin-1 was initially identified as a major substrate for tyrosine phosphorylation in Rous sarcoma virus-transformed chicken embryonic fibroblasts, suggesting its role as a target for inactivation during oncogenesis [6]. Most importantly, studies by Engelman et al. demonstrated that the human caveolin-1 gene maps to the long arm of human chromosome 7 (7q31.1) [7,8]. The (C-A)n microsatellite repeat marker D7S522 is located on human chromosome 7q31.1 and is frequently deleted in a variety of human cancers including breast, ovarian, colon, prostate and renal carcinomas [9–13]. It has been proposed that an as yet unidentified tumor suppressor gene is contained within, or located in close proximity to this locus. While no genes have been directly mapped to the D7S522 locus, the genes closest to this region encode caveolin-1 and caveolin-2 [7,8]. These data suggest that caveolin has tumor suppressor characteristics. Further support of this hypothesis arises from the observation that the caveolin-1 promoter is hypermethylated in several cancer cell lines, including those derived from the breast and prostate [8,14], suggesting that an abrogated transcription of the caveolin-1 gene might be a crucial step in carcinogenesis.

Cross-breeding of caveolin-1 knockout (Cav-1 −/−) mice with an established transgenic mouse model of breast cancer, MMTV-PyMT (mouse mammary tumor virus-polyoma middle T antigen), was followed by onset and progression of mammary tumors and lung metastasis in female mice. Mechanistically, tumor samples derived from these mice showed a hyperactivation of the p42/44 MAP kinase cascade and cyclin D1 upregulation [15], supporting the role of caveolin-1 as a negative regulator of cell transformation and mammary tumorigenesis.

On the background of these data and the elevated proliferation of various cell lines derived from Cav-1 −/− mice [16–19] it is somewhat surprising that these animals do not show an increased incidence for carcinomas. However, it has been demonstrated that Cav-1 −/− mice subjected to the carcinogen 7,12-dimethylbenzanthracene (DMBA) develop increased rates of skin tumors [20]. Epidermal hyperplasia in these carcinogen exposed Cav-1 −/− mice was associated with a hyperactivation of the p42/44 MAP kinase pathway and an increased cyclin D1 expression [20]. Apparently, the loss of caveolin-1 alone is insufficient to induce cellular transformation and tumorigenesis in vivo, but caveolin-1 deficiency might potentiate this process when combined with a carcinogen.

The contribution of caveolin-1 to carcinogenesis has been widely investigated in many clinically oriented studies. However, the expression pattern of caveolin-1 is controversial depending on the tumor cell type. Most of the results based on ex vivo experiments are in accordance with the in vitro data, suggesting that caveolin-1 might act as an important tumor suppressor gene. For instance, a decreased caveolin-1 expression is typical for breast [21], lung [22], and ovarian carcinomas [23], as well as mesenchymal sarcomas [24]. Analysis of human breast cancer samples showed that up to 16% of these cancers harbor a dominant-negative caveolin-1 gene point mutation, with the majority of these mutations found in invasive carcinomas [21]. Using an orthotopic model of spontaneous breast cancer metastasis, Sloan and et al. demonstrated that caveolin-1 is expressed in low- and non-metastatic primary tumors, but at much lower levels in highly metastatic tumors. Exogenous expression of caveolin-1 was sufficient to suppress primary tumor growth after inoculation of cells into the mammary gland [25].

In contrast, several studies indicate possible tumor promoting effects of caveolin-1. The expression of caveolin-1 is increased consistently in prostate carcinomas and caveolin-1 expression is upregulated even further in metastatic prostate cells [26,27]. These data suggest that caveolin-1 may be a novel prognostic marker for human prostate cancer. Additionally, caveolin-1 is upregulated in human prostate cancer after androgen ablation therapy, while antisense-mediated downregulation of caveolin-1 in prostate cancer cells reduces the incidence of lymph node and lung metastasis [28,29]. Most recently, the role of caveolin-1 for prostate cancer onset and progression was investigated after cross-breeding of Cav-1 −/− mice with the well characterized TRAMP (transgenic adenocarcinoma of mouse prostate) model. Loss of caveolin-1 resulted in significant reductions in prostate tumor burden and lymph node metastasis [30]. These data indicate that caveolin-1 functions as a tumor promoter during prostate carcinogenesis rather than as a tumor suppressor.

In gastrointestinal cancers the role of caveolin-1 seems to be more divergent. The caveolin-1 mRNA and protein expression is reduced in human colon carcinoma cell lines [31]. However, another study demonstrated an elevated caveolin-1 expression in the tissue of colon adenocarcinoma [32]. The expression of caveolin-1 is increased in esophageal squamous cell carcinoma as well. The overall survival rate was worse in patients with caveolin-1 positive tumors.
than in patients with caveolin-1 negative tumors. Over-expression of caveolin-1 was associated with lymph node metastasis and a worse prognosis after surgery [33].

The bulk of caveolae-related cancer research has focused on caveolin-1. Recent studies, however, implicate a possible role of caveolin-2 and -3 in human carcinogenesis as well. To identify new potential diagnostic markers for lung cancer Wikman et al. [34] analyzed the expression profiles of lung tumors using cDNA arrays. The expression of caveolin-2 correlated with shorter survival in stage I adenocarcinomas [34]. An increased expression of caveolin-2 was also observed in esophageal and bladder carcinomas [35,36]. In addition, caveolin-3 overexpression was found in germ cell tumors of the testis [37].

Taken together, various in vitro and in vivo investigations clearly support the role of caveolin-1 for cell transformation and carcinogenesis, however, cannot prove its causative role. It appears that caveolin-1, depending on the tumor cell type, might evolve functions both as a tumor promoting and as a tumor suppressing gene. Further studies will be necessary to investigate the functional importance of caveolin-2 and -3 in tumor growth and metastasis.

Another potential contribution of caveolin to growth and cancer biology, independently of any direct effects on tumor cells, is the role of caveolin in the process of angiogenesis. Pathological angiogenesis, which nourishes the growing tumor, is a hallmark of cancer [38,39]. Accordingly, various pharmaceutical approaches focus on tumor vascularization as a possible therapeutic target [38–40]. Several studies have suggested that caveolin-1 constitutes a central role in angiogenesis [41,42]. The phenotypical characterization of Cav-1 −/− mice confirmed the in vitro data obtained with antisense oligonucleotides [43], according to which angiogenesis was markedly decreased when caveolin-1 is absent from endothelial cells [44,45]. However, related to the inhibitory interaction between caveolin-1 and the endothelial nitric oxide synthase (eNOS) [46], it was previously documented that synthetic peptides derived from the caveolin scaffolding domain could block NO-dependent angiogenesis [47]. Interestingly, Brouet et al. recently demonstrated a dramatic tumor growth delay in mice transfected in vivo with caveolin-1 vs. sham-transfected animals. In these animals, recombinant caveolin-1 expression impaired NO-dependent tumor blood flow and decreased the tumor microvessel density [48].

However, it appears that a bimodal type of regulation seems to characterize the role of caveolin-1 in angiogenesis as well. Indeed, increased expression of caveolin-1 and microvessel density correlates with metastasis and poor prognosis in renal cell carcinoma [49].

### 3. Vascular proliferative disease

Cellular proliferation is also involved in the pathogenesis of vascular proliferative diseases such as primary atherosclerosis and restenosis after angioplasty [50]. Whereas early events in atherogenesis are characterized by an altered endothelial function and by the recruitment of mononuclear leukocytes to the intima, the progression of atheroma involves the proliferation of vascular smooth muscle cells (VSMC), their migration from the underlying media to the intima and their production of extracellular matrix molecules [50–52]. Thyberg et al. described the fundamental role of caveolin in the regulation of VSMC growth. Their findings indicate that caveolae and caveolin-1 are less abundant in synthetic than in contractile VSMC [53,54].

Caveolae and caveolin-1 are involved in regulating several signal transduction pathways that play a pathogenic role in atherosclerosis and restenosis after coronary angioplasty. It is noteworthy that caveolae and caveolin-1 are present in almost every cell type that has been implicated in the development of an atheroma. These include endothelial cells, macrophages, and VSMC [55–58]. Most recently, our group was able to demonstrate reduced caveolin-1 expression in proliferating VSMC of human atheroma [59], suggesting that proliferation and caveolin-1 expression is inversely correlated in human vascular disease. Indeed, overexpression of caveolin-1 in VSMC in vitro prevented growth factor-induced proliferation [59,60]. However, depending on the pathogenetic mechanisms examined, the role of caveolin-1 may either be proatherogenic or antiatherogenic. For example, Cav-1 −/− mice demonstrated increased neointimal hyperplasia in response to proximal ligation with subsequent inflammation [61], which appeared to be due to hyperactivation of the p42/44 MAP kinase cascade and upregulation of cyclin D1. While neointima formation in the above model seems to be growth factor driven, we were recently able to show that caveolin-1 also constitutes a mechanosensor. Using an in vitro and in vivo model of enhanced mechanical force, we provided evidence that caveolin-1 is critically involved in strain-induced activation of phosphoinositide-3 kinase/protein kinase B (Akt) and subsequent proliferation of VSMC [19]. But even more factors must be considered: in Cav-1 −/− mice, eNOS is activated [16,17] and eNOS gene transfer has been shown to prevent neointima formation in denudated rabbit carotid arteries [62]. Therefore, changes in nitric oxide synthesis within the plaque may additionally be responsible for modulation of plaque progression. eNOS prevents early steps that lead to the development of an atheroma such as expression of adhesion molecules and subsequent recruitment of leukocytes [63]. Accordingly, VCAM-1 was found downregulated in ApoE/Cav-1 double-knockout mice which resulted in reduced local inflammation and conferred protection against atheroma formation [64].

The studies outlined above support the hypothesis that, in the vasculature which is constantly exposed to alternating mechanical force and different growth factors, a dual role of caveolin-1 may contribute to a sensitive balance of anti- and pro-proliferative effects. Importantly, the finding of reduced caveolin-1 expression in VSMC of human plaque tissue [59]...
implies a direct pathogenetic relevance of these animal studies for human atherogenesis.

4. Cardiac hypertrophy and heart failure

Hypertrophic cardiomyopathy (HCM) is characterized by a markedly increased left ventricular mass caused by cardiomyocyte hypertrophy and prominent fibrosis [65]. With a prevalence of 0.2% in the general population, HCM might be the most common genetically transmitted cardiac disorder. Although sporadic forms are possible, HCM most frequently occurs as an autosomal dominant disease [65].

To date, several studies examining the effect of caveolin-1 and -3 gene ablation have been published. Cohen et al. demonstrated by transthoracic echocardiography (TTE) and magnetic resonance imaging (MRI) that Cav-1 --/-- mice develop progressive cardiac hypertrophy [18]. Interestingly, this cardiac phenotype was histologically characterized by interstitial fibrosis and cardiomyocyte hypertrophy. Additionally, these histological changes were accompanied by a hyperactivation of the p42/44 MAP kinase cascade in isolated cardiac fibroblasts [18]. It has been hypothesized that hyperactivation of the p42/44 MAP kinase in cardiac fibroblasts results in an elevated secretion of growth factors and a concomitant hypertrophic response of neighboring cardiomyocytes.

Since caveolin-1 is not expressed in cardiomyocytes a functional impairment of contraction may not be a direct effect on these cells. Yet dilated cardiomyopathy may develop due to the absence of caveolin-1. Zhao et al. reported that caveolin-1 knockout leads to dilated cardiomyopathy with an enlarged left ventricular diameter, wall thinning and decreased contractility [66]. A significant increase of atrial natriuretic factor (ANF) further indicated congestive heart failure in this animal model. Additionally, Cav-1 --/-- mice displayed markedly increased pulmonary artery pressure and hypertrophied right ventricles [66]. Pulmonary hypertensive could be caused by left ventricular dysfunction or might be secondary due to the pulmonary fibrosis [16,17].

However, caveolin-3 knockout (Cav-3 --/--) mice develop cardiomyopathy characterized by hypertrophy, dilation and reduced contractility as well [67]. Histologically, the myocardium shows increased cellular infiltration with accompanying perivascular fibrosis. As demonstrated before in cardiac fibroblasts of Cav-1 --/-- mice, caveolin-3 gene ablation leads to a hyperactivation of the p42/44 MAP kinase cascade in cardiomyocytes as well [67]. Cav-1/Cav-3 double knockout mice completely lack morphologically identifiable caveolae and are deficient in all three caveolin proteins because caveolin-2 is degraded in the absence of caveolin-1. Cav-1/3 --/-- mice develop a severe cardiomyopathic phenotype with cardiac hypertrophy and decreased contractility [68].

It is intriguing to hypothesize that caveolin plays a modulating role in human myocardial hypertrophy and heart failure too. However, to date this hypothesis has not yet been proven.

5. Muscular dystrophy

The muscular dystrophies are a heterogeneous group of heritable myopathies characterized by progressive muscle degeneration and replacement with fibrous connective tissue. These disorders present a large clinical variability regarding age of onset, patterns of skeletal muscle involvement, cardiac manifestation, rate of progression and mode of inheritance. The most common and severe muscular dystrophy is Duchenne muscular dystrophy, an X-linked recessive disorder in which the genetic locus has been identified as a variety of mutations of the dystrophin gene [69]. Dystrophin is expressed predominantly in skeletal muscle, myocardium and smooth muscle cells and forms the dystrophin–glycoprotein complex (DGC) with several protein components, including dystroglycans and sarcoglycans [70]. This large multicomponent complex has both mechanical stabilizing and signaling roles by mediating interactions between the cytoskeleton, membrane, and extracellular matrix. Its absence can lead to membrane fragility, resulting in myofibril necrosis and loss of muscular fibers with fibrotic replacement. This causes the characteristic rapidly progressive skeletal muscle disease in Duchenne muscular dystrophy.

In patients suffering from Duchenne muscular dystrophy, muscle biopsies show that there is both an increase in the protein expression of caveolin-3 and an elevated number and size of caveolae at the sarcolemma [71,72]. Coimmunoprecipitation experiments and immunofluorescence microscopy demonstrated that dystrophin colocalizes with caveolin-3 and forms stable complexes in myocytes [73].

Genetic evidence for a pathogenetic role of caveolin-3 in muscular dystrophies evolves from a study by Galbiati et al. [74] who used a transgenic mouse model to overexpress caveolin-3. These animals developed a phenotype reminiscent of Duchenne muscular dystrophy with near-complete loss of skeletal muscle dystrophin, necrosis of muscle fibers and elevation of connective tissue infiltrates.

In contrast, Cav-3 --/-- mice elicit several myopathic changes consistent with those seen in patients with limb-girdle muscular dystrophy (LGMD)-1C [75,76]. LGMD is a group of hereditary myopathies, including autosomal dominant and recessive forms [69]. Several autosomal-dominant forms of LGMD have been reported, including LGMD-1A, -1B and -1C. The identification of mutations in the caveolin-3 gene, resulting in an autosomal-dominant form of LGMD-1C, suggests a pathogenetic relevance of caveolin-3 in muscular dystrophies [77–79]. Additionally, several missense mutations in the caveolin-3 gene have been reported to be responsible for the pathogenesis of rippling muscle disease, a clinically benign myopathy, characterized by stretch-induced muscle contractions [80]. To date, several
point mutations and a deletion mutation have been characterized, which are responsible for distinct but overlapping phenotypes of autosomal dominant muscular dystrophies such as LGMD-1C, rippling muscle disease, distal myopathy and hyperCKemia [81]. Appreciating their common genetic background, these inherited muscular dystrophies have recently been described as caveolinopathies.

6. Insulin signaling and diabetes

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia [82]. However, the prognosis of individuals suffering from diabetes is determined by the frequent development of macro- and microvascular diseases. Most diabetes die of cardiovascular diseases such as coronary artery disease, cerebral and peripheral vascular disease. There is a two- to four-fold increased risk of a macrovascular event in patients with diabetes mellitus, compared with those patients without diabetes. Haffner et al. noted that the risk of a cardiovascular complication in a patient with diabetes was similar to that of a patient without diabetes who survived a myocardial infarction [83]. Microvascular disease causes, for instance, diabetic retinopathy and diabetic renal disease.

Type 2 diabetes is preceded by insulin resistance, in which the action of insulin is impaired, largely in adipose tissue and skeletal muscles [82]. Insulin binding to insulin receptors evokes a cascade of phosphorylation events, beginning with the autophosphorylation of the insulin receptor, followed by the phosphorylation of downstream signaling molecules, including the insulin receptor substrate (IRS) family [84]. Initial evidence suggesting that caveolae and caveolins may play a role in insulin receptor signaling came from experiments demonstrating that the insulin receptor contains the characteristic caveolin-binding motif [85]. In the first functional study of the caveolin–insulin receptor interaction, Yamamoto et al. showed that overexpression of caveolin-3 in HEK-293T cells augments insulin-dependent phosphorylation of IRS-1 [86]. Furthermore, it has been demonstrated that the scaffolding domain of caveolin-1 and -3 directly interacts with the insulin receptor. Peptides derived from the caveolin-1 and -3 scaffolding domain potently stimulated insulin receptor kinase activity [86]. In addition to these findings, cholesterol depletion of adipocytes caused, besides a disappearance of caveolae, a significantly reduced response to insulin stimulation [87]. Replenishment of plasma-membrane cholesterol resulted in morphologically identifiable caveolae and a reversal of this phenotype [87]. Taken together, these data indicate the compartmentation of the insulin receptor signaling cascade in caveolae and that caveolin serves to augment the insulin receptor kinase as well.

Although Cav-1−/− mice are not overtly diabetic, they are resistant to diet-induced obesity and develop adipose tissue atrophy [88]. Metabolically, Cav-1−/− mice are characterized by elevated free fatty acid and triglyceride levels, but no changes in plasma insulin and glucose levels [88]. However, Cav-1−/− mice showed insulin resistance. When placed on a high-fat diet for 9 months, Cav-1−/− mice develop postprandial hyperinsulinemia [89]. Furthermore, these mice revealed a significantly decreased protein expression of insulin receptors in adipose tissue. In line with these data, Cav-3−/− mice develop insulin resistance as well, as exemplified by decreased glucose uptake in skeletal muscles, impaired glucose tolerance test performance, and increases in serum lipids. Insulin-stimulated activation of insulin receptors and downstream molecules, such as IRS-1, was attenuated in skeletal muscles of Cav-3−/− mice [90].

Despite the fact that loss of caveolin-1 and -3 is not sufficient to induce overt diabetes, it is obvious that caveolin plays a central role in insulin signaling and the pathophysiology of insulin resistance. This background, the previous identification of mutations located in the caveolin binding motif of the insulin receptor [91–95] retrospectively supports the clinical importance of the caveolin–insulin receptor interaction in the pathogenesis of insulin resistance in humans. Reaven previously proposed that insulin resistance is the link between hyperglycemia, dyslipidaemia, hypertension, and macrovascular disease [96]. Hence, further work is needed to confirm the relevance of caveolae and caveolins for the development of insulin resistance and diabetes.

7. Conclusion and future directions

Experimental in vitro studies and numerous animal models clearly suggest that signaling through caveolin and caveolae is involved in the pathogenesis of several human diseases. Indeed, studies of human tissue samples and genetic analyses are able to point towards a significant role of caveolins and caveolae in human pathology. However, as in cancer and atherosclerosis, fertile controversies still exist. It seems that the impact of caveolin on cellular signaling depends upon the setting a particular cell finds itself in. Future research will undoubtedly be necessary to link experimental research to human diseases and to provide a base for clinically applicable therapeutic strategies.

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