Am. J. Hum. Genet. 63:1578–1587, 1998

PROTEIN COMPLEXES '98
Molecular Genetics of the Caveolin Gene Family: Implications for Human Cancers, Diabetes, Alzheimer Disease, and Muscular Dystrophy

Jeffrey A. Engelman,[1] XiaoLan Zhang,[1] Ferruccio Galbiati,[1] Daniela Volonté,[1] Federica Sotgia,[1] Richard G. Pestell,[2] Carlo Minetti,[4] Philipp E. Scherer,[3] Takashi Okamoto,[5] and Michael P. Lisanti[1]

[1]Department of Molecular Pharmacology and the Albert Einstein Cancer Center, [2]Department of Developmental and Molecular Biology, Department of Medicine, and the Albert Einstein Cancer Center, and [3]Department of Cell Biology, Albert Einstein College of Medicine, Bronx; [4]Servizio Malattie-Neuromuscolari, University of Genoa, Gaslini Institute, Genoa; and [5]Department of Neurosciences, The Lerner Research Institute, Cleveland Clinic Foundation, Cleveland

Caveolae, the "little caves" first described in electron micrographs of endothelial cells, have emerged, in recent years, as the site of important dynamic and regulatory events at the plasma membrane. Both transcytosis and potocytosis occur within these cell-surface organelles, as does the uptake of atherogenic oxidized LDL particles via endothelial scavenger receptors, the uptake of cholera toxin and DNA tumor viruses, and the processing of Alzheimer disease–related protein (amyloid precursor protein [APP]) and of the scrapie prion protein (see Okamoto et al. 1998, and references cited within).

Caveolae are also implicated in signal transduction, particularly by receptor tyrosine kinases (RTKs) and G-proteins. Caveolins, a family of highly conserved integral membrane proteins, interact specifically with these signaling molecules and many of their binding partners, apparently providing a scaffold that places members of a signal-transduction pathway in close proximity with one another. In many cases, interaction with caveolins represses kinase activation, although, as we describe below, such interactions can also activate signaling. Genetic and biochemical findings have led us and others to the "caveolae signaling hypothesis," which states that caveolar localization of certain lipid-modified signaling molecules could provide a compartmental basis for organizing a subset of signal-transduction events (Sargiacomo et al. 1993; Lisanti et al. 1994*a*, 1994*b*). We argue here that caveolins may provide a selective framework that segregates one group of signaling events from the next, preventing cross-talk between functionally unrelated signaling modules while facilitating cross-talk between related signaling modules. We also review the molecular genetics of the caveolin gene family and its implications for the pathogenesis and possible prevention of four human diseases: cancer, diabetes, Alzheimer disease, and muscular dystrophy.

Received September 28, 1998; accepted for publication October 16, 1998; electronically published November 20, 1998.

Address for correspondence and reprints: Dr. Michael P. Lisanti, Department of Molecular Pharmacology and the Albert Einstein Cancer Center, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: lisanti@aecom.yu.edu

0002-9297/98/6306-0003$02.00

Structure of Caveolae and the Caveolins

Caveolae are enriched relative to the rest of the cell surface in specific lipids (glycosphingolipids, sphingomyelin, and cholesterol) and lipid-modified signaling molecules. Because of their distinctive protein and lipid profile, plasma-membrane caveolae are detergent insoluble at 4°C and can be purified away from other cellular membranes by sucrose density-gradient centrifugation. Caveolae can also be prepared by detergent-free methods (Smart et al. 1995), such as affinity purification using a recombinant form of caveolin-1 (Song et al. 1996*a*). These detergent-free caveolae preparations are dramatically enriched in lipid-modified signaling molecules (G-proteins, Src-tyrosine kinases, H-Ras, and endothelial-cell nitric oxide synthase [eNOS]) (Smart et al. 1995; Song et al. 1996*a;* Okamoto et al. 1998); however, glycosyl-phosphatidylinositol (GPI) domains are separated from caveolae by use of this detergent-free procedure, as evidenced by exclusion of the GPI-anchored protein marker—carbonic anhydrase IV (Song et al. 1996*a*).

Caveola-like vesicles can be generated by expression of caveolin-1 or -3 in insect cells, by employment of a baculovirus-based expression system, or mammalian cell lines (Li et al. 1996*b,* 1998; Engelman et al. 1997). This provides an in vivo assay for caveolin-dependent vesicle formation. In addition, caveolin-induced vesicle formation appears to be isoform specific. Expression of caveolin-2 alone under the same conditions fails to drive the formation of vesicles (Engelman et al. 1997; Li et

al. 1998), either in insect cells or in mammalian cell systems.

The mammalian caveolin family consists of caveolin-1, -2, and -3 (Parton 1996; Scherer et al. 1996; Tang et al. 1996; Okamoto et al. 1998). Caveolin-1 and -2 are coexpressed and form a hetero-oligomeric complex (Scherer et al. 1997) in many cell types, with particularly high levels in adipocytes, whereas expression of caveolin-3 is muscle specific and found in all classes of muscle cells (Song et al. 1996*b*). This pattern of expression should allow the development of drugs that target caveolar function in specific tissues.

The genomic organization of the genes encoding caveolin-1, -2, and -3 is shown schematically in figure 1. The caveolin-1 gene contains three exons, whereas both the caveolin-2 gene and the caveolin-3 gene each contain only two exons (Engelman et al. 1998*c,* 1998*d*). Interestingly, the boundary position of the last exon is essentially identical in all three caveolin genes (Engelman et al. 1998*c,* 1998*d*). Alignment of the protein sequences encoded by this segment is shown in figure 2. Note that most of the functional domains of the caveolins are contained within the last exon, including the homo-oligomerization domain (Sargiacomo et al. 1995), the scaffolding domain (Li et al. 1996*a;* Couet et al. 1997*a,* 1997*b,* 1997*c*), the membrane-spanning domain, and the C-terminal domain. This exon also encodes 10 of the 12 invariant residues that are conserved, from worms to man, in all members of the caveolin gene family (Tang et al. 1997). Given that the members of the mammalian caveolin gene family share a similar genomic organization with a virtually identical boundary for the last exon, it is likely that the caveolin gene family arose through gene duplication of this last exon (Engelman et al. 1998*c,* 1998*d*).

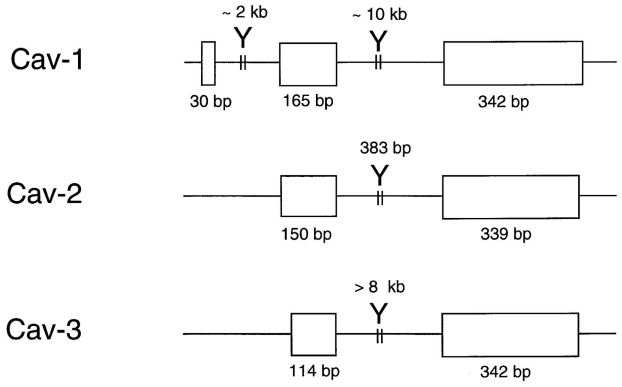

Figure 1 Genomic organization of the caveolin gene family. The overall genomic organization of the murine caveolin gene family is shown; identical intron-exon boundaries have been reported for the human caveolin gene family (Engelman et al. 1998*c,* 1998*d*).

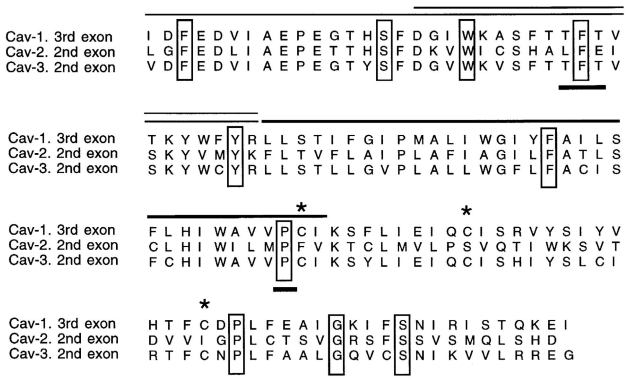

Figure 2 Alignment of the protein sequences and functional domains encoded by the last exon of murine Cav-1, -2, and -3. The positions of the oligomerization domain (*single thin overline* [Sargiacomo et al. 1995]), the caveolin-scaffolding domain (*double thin overline* [Li et al. 1996*a;* Couet et al. 19971997*a,* 1997*b,* 1997*c*]), the membrane-spanning domain (*thick overline*), and cysteines, conserved in caveolin-1 and -3, that are sites of palmitoylation (*asterisks* [*]) are shown. In addition, 10 of the 12 residues that are invariant between all known mammalian caveolins and caveolins from *C. elegans* (Tang et al. 1997) are localized to this last exon (*boxes*). Conserved residues that are deleted or mutated in human caveolin-3 and that lead to an autosomal dominant form of limb-girdle muscular dystrophy (Minetti et al. 1998) are indicated (*underlines*).

The genes encoding murine caveolin-1 and -2 are colocalized within the A2 region of mouse chromosome 6 (6-A2) (see table 1 and fig. 3). Human *CAV1* and *CAV2* map to 7q31, in a region of conserved synteny with murine 6-A2 (Engelman et al. 1998*c,* 1998*d*). Similarly, the muscle-specific *CAV3* gene is conserved, both at the level of sequence and at the level of chromosomal context, between mouse and man. The human *CAV3* gene, which underlies an autosomal dominant form of limb-girdle muscular dystrophy (Minetti et al. 1998), maps to 3p25, corresponding to the mouse region 6-E1. Caveolins are also found in the nematode *Caenorhabditis elegans,* suggesting that caveolae are an ancient and evolutionarily conserved feature of metazoan life. The *C. elegans* Cav^{ce}*-1* and Cav^{ce}*-2* genes are present on different chromosomes and appear, on the basis of sequence similarities, to correspond most closely to human *CAV1* and *CAV2* (table 2). These findings open the way toward the use of *C. elegans* as a genetic system to study caveolae and caveolins (Tang et al. 1997).

The Caveolin-Scaffolding Domain

The subcellular distribution of several signaling molecules is restricted and regulated by association with scaffolding proteins (Rubin 1994; Faux and Scott 1996). These scaffolding proteins—Ste5p, A-kinase anchor pro-

teins, and 14-3-3—simultaneously associate with distinct classes of signaling proteins to form a signaling pathway or module.

Accumulating evidence suggests that caveolins possess all the qualities of scaffolding proteins. Caveolins form multivalent homo- and hetero-oligomers, and each caveolin-interacting protein binds to the same cytosolic membrane-proximal region of caveolin (Sargiacomo et al. 1995; Li et al. 1996*a*). Domain-mapping studies have revealed that the interaction of caveolin-1 with signaling molecules is mediated via a membrane-proximal region of caveolin, termed the "caveolin-scaffolding domain" (residues 82–101). Through this domain, caveolin-1 interacts with G-protein alpha-subunits, H-Ras, Src-family tyrosine kinases, PKC isoforms, EGF-R, Neu, and eNOS (Engelman et al. 1998*b;* reviewed in Okamoto et al. 1998). In many cases, it has been shown that mutational activation of signaling molecules (G-proteins, H-Ras, or Src-family kinases) prevents regulated interaction with the caveolin-scaffolding domain. These activating mutations include H-Ras (G12V) and $G\alpha_s$ (Q227L), which are found in human cancers, suggesting that caveolin-mediated repression of signaling may suppress cell proliferation in vivo as well as in cell culture.

The caveolin-scaffolding domain recognizes a well-defined caveolin-binding motif that includes several crucial aromatic amino acid residues (Couet et al. 1997*a,* 1997*b,* 1997*c*). This motif was identified by use of the caveolin-scaffolding domain to select random peptide ligands from phage-display libraries (Couet et al. 1997*a,* 1997*b,* 1997*c*). The relevance of the motif that we identified was stringently evaluated by employment of a well-characterized caveolin-binding protein, the G-protein alpha-subunit, $G\alpha_{i2}$. Since the identification of the caveolin-scaffolding domain (Li et al. 1996*a*) and caveolin-binding sequence motifs (Couet et al. 1997*a,* 1997*b,* 1997*c*), these observations have been extended to other caveolin-interacting proteins. Functional caveolin-binding motifs have been deduced in both tyrosine and serine/threonine kinases, as well as in eNOS (reviewed in Okamoto et al. 1998). In all cases examined, the caveolin-binding motif is located within the enzymatically active catalytic domain of a given signaling molecule. For example, in the case of tyrosine and serine/threonine kinases, a kinase domain consists of 11 conserved subdomains (I–XI), and the caveolin-binding motif occurs within subdomain IX (Couet et al. 1997*a,* 1997*b,* 1997*c*). Caveolin-binding via the scaffolding domain is sufficient to inhibit the enzymatic activity of these kinases in vitro. Indeed, in many cases, a synthetic peptide corresponding to this caveolin domain is the most potent peptide inhibitor known for these enzymes. Agents that mimic the interaction with caveolins are potentially useful as general kinase inhibitors and, possibly, as antitumor drugs.

Recognition of signaling molecules by the caveolins also appears to be isoform specific. Scaffolding domains of caveolin-1 and caveolin-3 recognize a common motif, which does not interact with caveolin-2 (Couet et al. 1997*a,* 1997*b,* 1997*c*). Conversely, certain isoforms of PKC lack a defined caveolin-binding motif and, thus, are

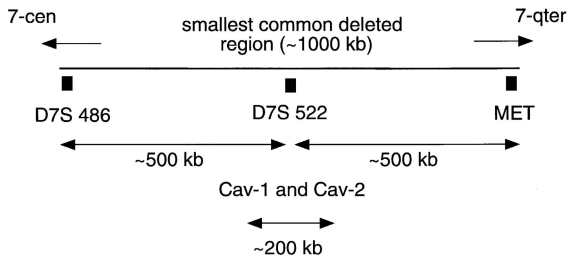

Figure 3 Human caveolin-1 and -2, located at the D7S522 locus (7q31.1), a known fragile site that is frequently deleted in human cancers. Location of the human caveolin-1 and -2 genes is shown with respect to the markers D7S486, D7S522, and the MET proto-oncogene (Engelman et al. 1998*d*). The smallest common deleted region (~1,000 kb), as previously defined by LOH analysis, is shown. Note that D7S522 is located at the center of this region. More specifically, D7S522 is ~500 kb downstream from the marker D7S486 and ~500 kb upstream from the MET proto-oncogene. Given that the average insert size of the caveolin containing bacterial-artificial-chromosome genomic clones is ~80–100 kb, the caveolin-1 and -2 genes must be located a maximum distance of ~100 kb upstream or downstream of D7S522 (Engelman et al. 1998*d*). Thus, the position of the caveolin-1 and -2 genes lies at the center of this smallest common deleted region (Engelman et al. 1998*d*).

Table 1

Molecular Genetics of the Mammalian Caveolin Gene Family

Gene	Chromosomal Localization		Exon	Closest Human Genetic Markers or Genes[a]	Associated Disease	References
	Mouse	Human				
Cav-1	6A2	7q31.1	3	D7S522, WI-5336, MET	Cancer	Engelman et al. (1998*c,* 1998*d*)
Cav-2	6A2	7q31.1	2	D7S522, WI-5336, MET	Cancer	Engelman et al. (1998*c,* 1998*d*)
Cav-3	6E1	3p25	2	ND	Muscular dystrophy	McNally et al. (1998), Minetti et al. (1998)

[a] D7S522 is a Généthon CA microsatellite-repeat marker, and WI-5336 is a Whitehead Institute sequence-tagged-site marker; the GenBank accession numbers are Z17100 and G04884, respectively. ND = not determined.

Table 2

Mammalian and *C. elegans* Caveolin Protein Products

Protein	Length (amino acids)	% Similarity (Identity) to Human Cav-1	% Similarity (Identity) to Human Cav-2	Expression Pattern	Reference(s)
Mammalian:					
Cav-1	178	100 (100)	59 (40)	Ubiquitous; Cav-1 and Cav-2 are coexpressed and are highest in adipocytes, endothelia, epithelia, smooth muscle cells, and type I pneumocytes	Scherer et al. (1996, 1997*a*)
Cav-2	162	58 (38)	100 (100)		
Cav-3	159	85 (65)	60 (39)	Muscle specific; primarily skeletal and cardiac myocytes	Parton (1996), Song et al. (1996*b*), Tang et al. (1996)
C. elegans:					
Cav^{ce}-1	235	67 (37)	65 (32)	Unknown	Tang et al. (1997)
Cav^{ce}-2	351	44 (24)	44 (24)	Unknown	Tang et al. (1997)

not inhibited by the caveolin-scaffolding domain (Oka et al. 1997). Alanine-scanning mutagenesis of the caveolin-scaffolding domain has revealed that the sequence FTVT/S is essential for proper recognition of caveolin-binding motifs; this sequence is conserved in caveolin-1 and -3 and is divergent in caveolin-2 (FEIS) (Couet et al. 1997*a*, 1997*b*, 1997*c*). Recently, we have identified a family with an autosomal dominant form of limb-girdle muscular dystrophy (Minetti et al. 1998). In this family, the FTVT/S region is deleted in caveolin-3, providing genetic evidence that this region of the caveolin-scaffolding domain is critical in vivo.

The relevance of the caveolin-binding motif within eNOS has recently been tested functionally (Garcia-Cardena et al. 1997). The caveolin-binding motif within eNOS was removed by site-directed mutagenesis by substitution of alanine in place of important aromatic residues that are required for recognition by caveolins. Removal of the eNOS caveolin-binding motif did not affect the basal enzymatic activity of eNOS, but it did block the ability of caveolin-1 to suppress eNOS activity in cotransfection experiments. This is the first demonstration that the caveolin-scaffolding domain and caveolin-binding sequence motifs are functional in vivo. These NOS-caveolin interactions have been shown to be relevant in both endothelial cells and cardiac myocytes, which express caveolin-1 and -3, respectively.

Implications for Human Genetic Diseases

Caveolins have been implicated in a variety of human diseases. We concentrate here on four such pathologies that have a strong hereditary component—cancer, diabetes mellitus, Alzheimer disease, and limb-girdle muscular dystrophy. Mutations in *CAV3* are now known to lead directly to a type of limb-girdle muscular dystrophy (fig. 4), and cell-culture or biochemical findings suggest that heritable differences in the interaction between caveolins and their partners could lead to the other conditions as well, as detailed below.

Cancer: CAV1 *as a Tumor-Suppressor Gene and a Negative Regulator of the Ras-p42/44 MAP Kinase Cascade*

Modification and/or inactivation of caveolin-1 expression appears to be a common feature of the transformed phenotype. Historically, caveolin was first identified as a major v-Src substrate in Rous sarcoma virus–transformed cells (Glenney 1992). Caveolin-1 mRNA and protein expression are reduced or absent in NIH 3T3 cells transformed by a variety of activated oncogenes (v-Abl, Bcr-Abl, and H-Ras [G12V]), and caveolae are also missing from these transformed cells (Koleske et al. 1995); caveolin-2 protein is not down-regulated in response to oncogenic transformation (Scherer et al. 1997*a*). In addition, caveolin-1–expression levels correlated inversely with the ability of these cells to grow in soft agar. Thus, cells expressing the smallest amount of caveolin-1 and lacking detectable caveolae formed the largest colonies in soft agar, as would be predicted if caveolin represents a critical suppressor of cell transformation.

Down-regulation of caveolin-1 is a direct consequence of the oncogenic stimulus, since it can be reversed by employing a temperature sensitive form of v-Abl or by treating Ras-transformed 3T3 cells with an inhibitor of the p42/44 MAP kinase pathway. Reintroduction of caveolin-1 under the control of an inducible-expression system is sufficient to block the anchorage-independent growth of these transformed cells in soft agar and to restore the formation of morphologically detectable caveolae (Engelman et al. 1997). Consistent with its antagonism to Ras-mediated cell transformation, caveolin-1 expression dramatically inhibited both Ras/MAPK-mediated and basal transcriptional activation of a mitogen-

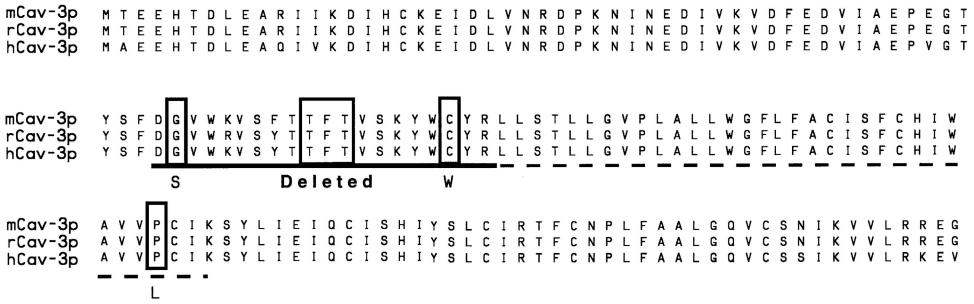

Figure 4 Caveolin-3 and muscular dystrophy. Residues that are deleted or mutated in human caveolin-3 and that lead to a form of limb-girdle muscular dystrophy are indicated (*boxes*) [McNally et al. 1998; Minetti et al. 1998]). Note that three of the four mutations thus far identified cluster within the caveolin-scaffolding domain, a 20-amino-acid membrane proximal region of caveolin-3 (*unbroken underline* [McNally et al. 1998; Minetti et al. 1998]). One mutation occurs within the transmembrane domain (*dashed underline* [Minetti et al. 1998]). Prefixes to "Cav" are as follows": m = mouse; r = rat; h = human.

sensitive promoter (Engelman et al. 1997). Taken together, these results indicate that down-regulation of caveolin-1 expression and caveolae may be critical to maintenance of the transformed phenotype in certain cell populations (Engelman et al. 1997).

Human tumor cytogenetic data are consistent with this proposal. Loss of heterozygosity (LOH) analysis implicates 7q31.1 in the pathogenesis of multiple types of cancer, including breast, ovarian, prostate, and colorectal carcinomas, as well as uterine sarcomas and leiomyomas. The locus of the presumed 7q31.1 tumor-suppressor gene has been narrowed to an ~1-Mb region that includes the highly polymorphic marker D7S522. Zenklusen and colleagues have shown that the D7S522 locus is the most commonly deleted marker in primary breast cancers, and they note that LOH at this site is strongly associated with systemic progression and death in prostate cancers (see references cited in Engelman et al. 1998*c,* 1998*d*). D7S522 also spans the aphidicolin-induced fragile site FRA7G, at 7q31. Given the utility of 7q31.1 and D7S522 LOH as markers for carcinogenesis, many laboratories are currently searching this chromosomal region for a novel tumor-suppressor gene. Recently, we have shown that *CAV1* and *CAV2* map to <100 kb from D7S522, in the middle of the critical region for the presumed tumor-suppressor gene (fig. 3; also see Engelman et al. 1998*c,* 1998*d*). Evidence that caveolin-1 can suppress cell transformation in murine fibroblasts and human breast cancer cell lines provides independent support for the model that *CAV1* is the missing tumor-suppressor gene (Engelman et al. 1997; Lee et al. 1998*b*).

Interestingly, Yang et al. (1998*b*) recently reported that elevated caveolin-1 levels may be associated with prostate-cancer lymph-node metastases, raising the possibility that *CAV1* could also act as an oncogene. However, because the closest known gene to *CAV1* is the *MET* proto-oncogene (Engelman et al. 1998*d*), this finding may simply reflect coamplification of *CAV1* along with *MET. MET* was first identified and cloned as a metastasis-associated gene (Giordano et al. 1989); mutational activation or amplification of *MET* is known to be associated with invasive cell growth and metastasis, including prostate-cancer progression (Pisters et al. 1995). Alternatively, caveolin-1 expression may be up-regulated in response to drug resistance, as has recently been documented for the lung carcinoma–derived cell line, A549 (Yang et al. 1998*a*). Finally, caveolin-1 may be mutated in these metastatic cancer cells, a possibility that deserves direct examination.

These findings may also have relevance for other human cancers. Using differential display and subtractive hybridization techniques, Sager et al. (1994) have identified a number of candidate tumor-suppressor genes, whose mRNAs are down-regulated in human mammary carcinomas. In this screening approach, caveolin-1 was independently identified as 1 of 26 gene products down-regulated during mammary tumorigenesis. In addition, caveolin-1 expression was absent in several transformed cell lines derived from human mammary carcinomas, including MT-1, MCF-7, ZR-75-1, T47D, MDA-MB-361, and MDA-MB-474 (Sager et al. 1994). In contrast, caveolin-1 mRNA was abundantly expressed in normal mammary epithelium. However, it remains unknown whether loss or reductions in caveolin-1 protein expression are sufficient to mediate cell transformation and/or tumorigenicity.

Recently, we have employed an antisense approach to derive stable NIH 3T3 cell lines that contain normal amounts of caveolin-2 but express dramatically reduced levels of caveolin-1 (Galbiati et al. 1998*a*). NIH 3T3 cells harboring antisense caveolin-1 spontaneously formed foci, exhibited anchorage-independent growth in soft agar, formed tumors in immunodeficient mice, and

appeared morphologically transformed as seen by scanning electron microscopy (Galbiati et al. 1998*a*). Biochemically, these cells also showed increased levels of activated MEK and ERK (Galbiati et al. 1998*a*). However, the SAPK/JNK pathway, the p38 MAPK pathway, and CREB were not activated in these cells. Taken together, these results suggest that down-regulation of caveolin-1 expression is sufficient to drive oncogenic transformation and to constitutively activate the p42/44 MAP kinase cascade (Galbiati et al. 1998*a*). Importantly, cell transformation induced by targeted down-regulation of caveolin-1 expression was completely reversed when caveolin-1 protein levels were restored to normal by loss of the caveolin-1 antisense vector (Galbiati et al. 1998*a*).

Several laboratories have now shown, by both in vitro biochemical methods and in vivo immunolabeling techniques, that components of the p42/44 MAP kinase cascade, MEK and ERK, are concentrated within caveolar membranes (Song et al. 1996*a;* Liu et al. 1997*a,* 1997*b*). For example, morphological studies have directly shown that ERK-1/2 is concentrated in plasma-membrane caveolae (Liu et al. 1997*b*).

How might the down-regulation of caveolin-1 protein expression activate the p42/44 MAP kinase cascade? Recently, Anderson and colleagues have shown that the pool of ERK-1 that localizes to caveolae is initially inactive and can be activated by regulated stimulation with growth-factor ligands, such as PDGF (Liu et al. 1997*b*). After such stimulation, ERK-1 is activated and translocates from the caveolar membrane to the cytosol, suggesting that ERK-1 is activated as it leaves the caveolar membrane (Liu et al. 1997*b*). These results are consistent with our current findings that down-regulation of caveolin-1 expression constitutively activates signaling from MEK and ERK in vivo, perhaps by prematurely releasing activated ERK-1/2 and other components of the p42/44 MAP kinase cascade into the cytosol. In support of this hypothesis, we have shown that transient coexpression of caveolin-1 with activated H-Ras (G12V) blocks its ability to transcriptionally activate a mitogen-sensitive promoter in vivo (Engelman et al. 1997). Because the G12V mutation of H-Ras prevents or destabilizes its interaction with caveolin-1 (Song et al. 1996*a*), overexpression of caveolin-1 is unlikely to influence Ras directly in this assay; rather, the activity of one or more of the downstream targets of H-Ras, such as Raf-1, MEK-1/2, or ERK-1/2, is probably inhibited.

In accordance with this idea, we have recently shown that (i) transient coexpression with caveolin-1 dramatically inhibits signaling from Raf-1, MEK-1, and ERK-2 to the nuclear-transcription factor Elk in vivo and (ii) peptides derived from caveolin-1 inhibit the in vitro kinase activity of purified MEK-1 and ERK-2 (Engelman et al. 1998*a*). Thus, overexpression of caveolin-1 can inhibit signal transduction from the p42/44 MAP kinase cascade, via a direct interaction of caveolin-1 with MEK and ERK (Engelman et al. 1998*a*), whereas targeted down-regulation of caveolin-1 is sufficient to activate the p42/44 MAP kinase cascade constitutively (Galbiati et al. 1998*a*).

On the basis of our current and previous observations, we suggest that a novel form of negative reciprocal regulation exists between p42/44 MAP kinase activation and caveolin-1 protein expression. The evidence is as follows:

1. *up-regulation of caveolin-1 protein expression down-regulates p42/44 MAP kinase activity* (Engelman et al. 1998*a*)—that is, transient coexpression of caveolin-1 with MEK or ERK inhibits their ability to transcriptionally activate an in vivo Elk-luciferase reporter system in CHO cells;
2. *down-regulation of caveolin-1 protein expression up-regulates p42/44 MAP kinase activity* (Galbiati et al. 1998*a*)—that is, expression of caveolin-1 antisense down-regulates caveolin-1 protein expression in NIH 3T3 cells and leads to constitutive activation of MEK and ERK;
3. *up-regulation p42/44 MAP kinase activity down-regulates caveolin-1 mRNA and protein expression* (Koleske et al. 1995; Engelman et al. 1997)—that is, expression of activated Ras (G12V) in NIH 3T3 cells leads to a loss of caveolin-1 mRNA and protein expression;
4. *down-regulation of p42/44 MAP kinase activity up-regulates caveolin-1 protein expression* (Engelman et al. 1997)—that is, treatment of Ras-transformed NIH 3T3 cells with a MEK inhibitor restores the expression of caveolin-1 protein to normal levels observed in non-transformed NIH 3T3 cells.

Importantly, these four independent observations are consistent with the changes–in response to cell density, growth-factor deprivation, and growth-factor stimulation—that we observed in caveolin-1 protein levels in normal NIH 3T3 cells (Galbiati et al. 1998*a*).

Diabetes: Caveolae, Caveolins, and Insulin Signaling

Signal transduction by the insulin receptor—and by its downstream interacting partners such as IRS-1—is important for understanding the basic mechanism of glucose uptake in muscle and adipose tissue, as well as the pathogenesis of human metabolic diseases, most notably diabetes. In adipocytes, the cell type that expresses the highest level of CAV1, caveolae represent as much as 20% of the total plasma-membrane surface area. Both caveolae and caveolin-1 are induced 10–25 fold during the differentiation of 3T3-L1 preadipocytes into mature adipocytes (Scherer et al. 1994). Previous studies have localized the ligand-bound insulin receptor to plasma-membrane caveolae by electron microscopy (see refer-

ences cited in Scherer et al. 1994). This finding raises the question of whether caveolin-1 binding inhibits the activity of insulin RTKs, as it inhibits other RTKs, such as EGF-R and Neu (Couet et al. 1997*c;* Engelman et al. 1998*b*).

The caveolae-signaling hypothesis predicts that caveolin-1 will not necessarily inhibit all signaling but, rather, that it will confer differential activation and repression of distinct signaling cascades. In collaboration with Ishikawa and colleagues, we have recently found that cotransfection of caveolin-1 with the insulin receptor can stimulate the phosphorylation of IRS-1 in vivo (Yamamoto et al. 1998). In addition, peptides encoding the scaffolding domain of caveolin-1 dramatically stimulate the ability of the insulin-receptor kinase to phosphorylate IRS-1 in vitro (Yamamoto et al. 1998). The latter experiments employed only purified recombinant insulin-receptor kinase and its substrate, purified recombinant IRS-1, suggesting that stimulation occurs through a direct interaction with caveolin-1. Similar stimulatory effects were observed with caveolin-3 that is expressed predominantly in muscle cells. Thus, caveolins may demonstrate either inhibitory or stimulatory functions, in the context of different signal-transduction cascades.

This observation may provide the key to understanding how the proliferative effects of the insulin receptor are squelched in adipocytes; for example, caveolin-1 expression in adipocytes would promote insulin signaling, and, at the same time, it would, by inhibiting the p42/44 MAP kinase cascade, down-regulate mitogenic signaling. This is consistent with the finding that caveolins are most abundantly expressed in insulin-sensitive tissues: fat (caveolin-1 and -2) and skeletal muscle (caveolin-3) (Scherer et al. 1994, 1997*a;* Tang et al. 1996).

Alzheimer Disease: Caveolae, Caveolins, and APP degradation

APP is a source of Aβ amyloid peptide, the principal protein component of the senile plaques found in the brains of patients with Alzheimer disease. The Aβ amyloid peptide is generated by the processing of APP with β- and γ-secretases. Alternatively, APP is processed by α-secretase, which cleaves APP within the Aβ sequence, thereby precluding the formation of Aβ. The competition between these alternative proteolytic pathways is thus crucial to the etiology of Alzheimer disease.

Our recent evidence shows the enrichment of APP in caveolae, where caveolin provides a direct means for APP to be concentrated (Ikezu et al. 1998*a*). Interestingly, the α-secretase cleavage event also occurred in caveolae, and overexpression of recombinant caveolin-1–promoted α-secretase–mediated proteolysis of APP. Conversely, α-secretase–mediated cleavage of APP was abolished by caveolin-1–based antisense oligonucleotides that effectively block caveolin-1 expression (Ikezu et al. 1998*a*). In the brain, caveolae-like microdomains of the plasma membrane that have a similar lipid composition have been termed "detergent- insoluble glycolipid membrane complexes" (DIGs), or "caveolae-related domains" (CRDs [Okamoto et al. 1998]). CRDs isolated from whole brain contain not only APP but also the Aβ amyloid peptide (Lee et al. 1998*a*); therefore, it has been suggested that brain CRDs represent sites of amyloid biogenesis or transport. In support of this notion, cholesterol depletion, which leads to the loss of CRD integrity, efficiently inhibits Aβ amyloid–peptide secretion in cultured hippocampal neurons (Simons et al. 1998). Since recent studies have provided direct evidence that caveolins are also expressed within cells of the nervous system, including astrocytes (Ikezu et al. 1998*b*) and neurons (Galbiati et al. 1998*b*), it is possible that caveolins play a key role in APP processing in brain.

Muscular Dystrophy: Caveolin-3 and the Dystrophin Complex

The expression of caveolin-3 is induced during the differentiation of skeletal myoblasts, and caveolin-3 is localized to the sarcolemma, where it forms a complex with dystrophin and its associated glycoproteins (Song et al. 1996*b*). PFK represents the rate-limiting enzyme in glycolysis, and its muscle-specific isoform, PFK-M, is a prominent caveolin-3–binding protein. This interaction is modulated by the availability of extracellular glucose and by intracellular allosteric effectors of PFK (Scherer and Lisanti 1997), suggesting that regulation of glycolysis by caveolin-3 could be important in pathogenic states of skeletal muscle, such as muscular dystrophies.

In support of this notion, we have recently identified an autosomal dominant form of limb-girdle muscular dystrophy (i.e., type LGMD1C) in two Italian families that is due to a deficiency in caveolin-3 expression. Analysis of their genomic DNA reveals two distinct mutations in *CAV3*: (i) a 9-bp microdeletion that removes the sequence TFT from the caveolin-scaffolding domain and (2) a missense mutation that changes a proline to a leucine (P→L) in the transmembrane domain (Minetti et al. 1998) (fig. 4). Both mutations lead to a loss of >95% of caveolin-3 protein expression.

The evident loss, in cells of heterozygous individuals, of both wild-type and mutant caveolin-3 may reflect the targeting of mixed caveolin-3 oligomers to a degradative pathway, but the mechanism of this process has not been explored. However, the identity of the *CAV3* lesions in these LGMD1C families is instructive. Of the 12 amino acid residues that are shared among all three human caveolins as well as between the two *C. elegans* homologues, two are affected by the mutations identified in

these two LGMD1C families. One of the invariant prolines is changed to leucine in family A, whereas one of the invariant phenylalanines is deleted in family B (Minetti et al. 1998). These results suggest that mutation of these evolutionarily conserved residues may have dire consequences for the structure or function of the other caveolin-family members as well. Indeed, alanine-scanning mutagenesis of a peptide encoding the caveolin-scaffolding domain reveals that the FTVT/S sequence in caveolin-1 and -3 is important for the correct recognition of caveolin-binding signaling molecules (Couet et al. 1997*a*), and the FT residues in this sequence are deleted in family B. Two other mutations within the coding sequence of *CAV3* have been described that are associated with a proximal form of muscular dystrophy (McNally et al. 1998). One of these mutations was homozygous, suggesting a possible autosomal recessive inheritance (McNally et al. 1998). Interestingly, these two additional mutations map to the caveolin-3–scaffolding domain. Thus, the mutations in three of the four known *CAV3* disease alleles cluster within the 20-amino-acid caveolin-scaffolding domain.

Future Prospects

Caveolins share with other scaffolding factors the ability to bind multiple components of a signaling pathway. The existence of such factors clearly affords the cell tighter control of the activation and repression of signaling than would be possible if all players diffused freely throughout the cytoplasm. Scaffolds also allow for integration of signal-transduction pathways into discrete modules, so that they reduce the likelihood of indiscriminate cross-talk among distinct pathways. The significance of interactions between caveolins and their partners is now well established in cell-culture models, and their physiological importance is beginning to emerge from the study of various human diseases. Heritable defects in *CAV3* are now implicated in at least one form of muscular dystrophy, and other lesions in the genes for either caveolins or other scaffolds will probably be found in cancer and in other human genetic disorders. A novel class of disease mutations may now come to light, in which the root cause of the disorder is the failure of a regulatory protein to interact properly with scaffolding factors.

Acknowledgments

Because of space limitations, we were unable to cite many important primary references. This work was supported by National Institutes of Health FIRST Award GM-50443 (to M.P.L.) and by grants from the Charles E. Culpeper Foundation (to M.P.L.), the G. Harold and Leila Y. Mathers Charitable Foundation (to M.P.L and P.E.S.), and the Sidney Kimmel Foundation for Cancer Research (to M.P.L.). P.E.S. was supported by a grant from the Pfizer Corporation, a pilot grant from the Diabetes Research and Research Training Center of the Albert Einstein College of Medicine, and a research grant from the American Diabetes Association. T.O. is supported by National Institutes of Health FIRST Award MH-56036 and the Prentiss Foundation. J.A.E. was supported by National Institutes of Health Medical Scientist Training Program grant T32-GM07288. C.M. was supported by Italian Telethon grant 1111. R.G.P. was supported in part by National Institutes of Health grants R29CA70897, RO1 CA75503, and P50-HL56399 and is a recipient of the Ira T. Hirschl award and an award from the Susan G. Komen Breast Cancer Foundation.

Electronic-Database Information

Accession numbers and URL for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for D7S522 [accession number Z17100] and WI-5336 [accession number G04884])

References

Couet J, Li S, Okamoto T, Ikezu T, Lisanti MP (1997*a*) Identification of peptide and protein ligands for the caveolin-scaffolding domain: implications for the interaction of caveolin with caveolae-associated proteins. J Biol Chem 272: 6525–6533

Couet J, Li S, Okamoto T, Scherer PS, Lisanti MP (1997*b*) Molecular and cellular biology of caveolae: paradoxes and plasticities. Trends Cardiovasc Med 7:103–110

Couet J, Sargiacomo M, Lisanti MP (1997*c*) Interaction of a receptor tyrosine kinase, EGF-R, with caveolins: caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. J Biol Chem 272:30429–30438

Engelman JA, Chu C, Lin A, Jo H, Ikezu T, Okamoto T, Kohtz DS, et al (1998*a*) Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo: a role for the caveolin-scaffolding domain. FEBS Lett 428:205–211

Engelman JA, Lee RJ, Karnezis A, Bearss DJ, Webster M, Siegel P, Muller WJ, et al (1998*b*) Reciprocal regulation of Neu tyrosine kinase activity and caveolin-1 protein expression in vitro and in vivo: implications for human breast cancer. J Biol Chem 273:20448–20455

Engelman JA, Wycoff CC, Yasuhara S, Song KS, Okamoto T, Lisanti MP (1997) Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. J Biol Chem 272:16374–16381

Engelman JA, Zhang XL, Galbiati F, Lisanti MP (1998*c*) Chromosomal localization, genomic organization, and developmental expression of the murine caveolin gene family (Cav-1, -2, and -3): Cav-1 and Cav-2 genes map to a known tumor suppressor locus (6-A2/7q31). FEBS Lett 429:330–336

Engelman JA, Zhang XL, Lisanti MP (1998*d*) Genes encoding human caveolin-1 and -2 are co-localized to the D7S522 locus (7q31.1), a known fragile site (FRA7G) that is frequently deleted in human cancers. FEBS Lett 436:403–410

Faux M, Scott J (1996) Molecular glue: kinase anchoring and scaffold proteins. Cell 85:9–12

Galbiati F, Volonte D, Engelman JA, Watanabe G, Burk R, Pestell R, Lisanti MP (1998*a*) Targeted down-regulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44 MAP kinase cascade. EMBO J 17: 6633–6648

Galbiati F, Volonté D, Gil O, Zanazzi G, Salzer JL, Sargiacomo M, Scherer PE, et al (1998*b*) Expression of caveolin-1 and -2 in differentiating PC12 cells and dorsal root ganglion neurons: caveolin-2 is upregulated in response to cell injury. Proc Natl Acad Sci USA 95:10257–10262

Garcia-Cardena G, Martasek P, Siler-Masters BS, Skidd PM, Couet JC, Li S, Lisanti MP, et al (1997) Dissecting the interaction between nitric oxide synthase (NOS) and caveolin: functional significance of the NOS caveolin binding domain in vivo. J Biol Chem 272:25437–25440

Giordano S, Ponzetto C, Di Renzo MF, Cooper CS, Comoglio PM (1989) Tyrosine kinase receptor indistinguishable from the c-met protein. Nature 339:155–156

Glenney JR (1992) The sequence of human caveolin reveals identity with VIP21, a component of transport vesicles. FEBS Lett 314:45–48

Ikezu T, Trapp BD, Song KS, Schegel A, Lisanti MP, Okamoto T (1998) Caveolae, plasma membrane micro-domains for α-secretase-mediated processing of the amyloid precursor protein (APP). J Biol Chem 273:10485–10495

Ikezu T, Ueda H, Trapp BD, Nishiyama K, Sha JF, Volonté D, Galbiati F, et al (1998) Affinity-purification and characterization of caveolins from the brain. Differential expression of caveolin-1, -2 and -3 in brain endothelial and astroglial cell types. Brain Res 804:177–192

Koleske AJ, Baltimore D, Lisanti MP (1995) Reduction of caveolin and caveolae in oncogenically transformed cells. Proc Natl Acad Sci USA 92:1381–1385

Lee SJ, Liyanage U, Bickel PE, Xia W, Lansbury PT, Kosik KS (1998*a*) A detergent-insoluble membrane compartment contains A-beta in vivo. Nat Med 4:730–734

Lee SW, Reimer CL, Oh P, Campbell DB, Schnitzer JE (1998*b*) Tumor cell growth inhibition by caveolin re-expression in human breast cancer cells. Oncogene 16:1391–1397

Li S, Couet J, Lisanti MP (1996*a*) Src tyrosine kinases, G alpha subunits and H-Ras share a common membrane-anchored scaffolding protein, caveolin: caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. J Biol Chem 271:29182–29190

Li S, Galbiati F, Volonté D, Sargiacomo M, Engelman JA, Das K, Scherer PE, et al (1998) Mutational analysis of caveolin-induced vesicle formation: expression of caveolin-1 recruits caveolin-2 to caveolae membranes. FEBS Lett 434:127–134

Li S, Song KS, Koh SS, Kikuchi A, Lisanti MP (1996*b*) Baculovirus-based expression of mammalian caveolin in Sf21 insect cells: a model system for the biochemical and morphological study of caveolar biogenesis. J Biol Chem 271: 28647–28654

Lisanti MP, Scherer P, Tang ZL, Sargiacomo M (1994*a*) Caveolae, caveolin and caveolin-rich membrane domains: a signalling hypothesis. Trends Cell Biol 4:231–235

Lisanti MP, Scherer PE, Vidugiriene J, Tang ZL, Hermanoski-Vosatka A, Tu YH, Cook RF, et al (1994*b*) Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: implications for human disease. J Cell Biol 126:111–126

Liu J, Oh P, Horner T, Rogers RA, Schnitzer JE (1997*a*) Organized endothelial cell signal transduction in caveolae. J Biol Chem 272:7211–7222

Liu P, Ying YS, Anderson RGW (1997*b*) PDGF activates MAP kinase in isolated caveolae. Proc Natl Acad Sci USA 94: 13666–13670

McNally EM, de Sá Moreira E, Duggan DJ, Bönnemann CG, Lisanti MP, Lidov HGW, Vainzof M, et al (1998) Caveolin-3 in muscular dystrophy. Hum Mol Genet 7:871–877

Minetti C, Sotgia F, Bruno C, Scartezzini P, Broda P, Bado M, Masetti E, et al (1998) Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. Nat Genet 18:365–368

Oka N, Yamamoto M, Schwencke C, Kawabe J, Ebina T, Couet J, Lisanti MP, et al (1997) Caveolin interaction with protein kinase C: isoenzyme-dependent regulation of kinase activity by the caveolin-scaffolding domain peptide. J Biol Chem 272:33416–33421

Okamoto T, Schlegel A, Scherer PE, Lisanti MP (1998) Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. J Biol Chem 273:5419–5422

Parton RG (1996) Caveolae and caveolins. Curr Opin Cell Biol 8:542–548

Pisters LL, Troncoso P, Zhau HE, Li W, von Eschenbach AC, Chung LW (1995) c-met proto-oncogene expression in benign and malignant human prostate tissues. J Urol 154: 293–298

Rubin CS (1994) A kinase anchor proteins and the intracellular targeting of signals carried by cyclic AMP. Biochim Biophys Acta 1224:467–479

Sager R, Sheng S, Anisowicz A, Sotiropoulou G, Zou Z, Stenman G, Swisshelm K, et al (1994) RNA Genetics of breast cancer: maspin as a paradigm. Cold Spring Harb Symp Quant Biol 59:537–546

Sargiacomo M, Scherer PE, Tang ZL, Kubler E, Song KS, Sanders MC, Lisanti MP (1995) Oligomeric structure of caveolin: implications for caveolae membrane organization. Proc Natl Acad Sci USA 92:9407–9411

Sargiacomo M, Sudol M, Tang ZL, Lisanti MP (1993) Signal transducing molecules and GPI-linked proteins form a caveolin-rich insoluble complex in MDCK cells. J Cell Biol 122: 789–807

Scherer PE, Lewis RY, Volonté D, Engelman JA, Galbiati F, Couet J, Kohtz DS, et al (1997*a*) Cell-type and tissue-specific expression of caveolin-2: caveolins 1 and 2 co-localize and form a stable hetero-oligomeric complex in vivo. J Biol Chem 272:29337–29346

Scherer PE, Lisanti MP (1997*b*) Association of phosphofructokinase-M with caveolin-3 in differentiated skeletal myotubes: dynamic regulation by extra-cellular glucose and intracellular metabolites. J Biol Chem 272:20698–20705

Scherer P, Lisanti MP, Baldini G, Sargiacomo M, Lodish HF (1994) Induction of caveolin during adipogenesis and as-

sociation of GLUT4 with caveolin-rich vesicles. J Cell Biol 127:1233–1243

Scherer PE, Okamoto T, Chun M, Nishimoto I, Lodish HF, Lisanti MP (1996) Identification, sequence and expression of caveolin-2 defines a caveolin gene family. Proc Natl Acad Sci USA 93:131–135

Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K (1998) Cholesterol depletion inhibits the generation of beta-amyloid in hippo-campal neurons. Proc Natl Acad Sci USA 95:6460–6464

Smart EJ, Ying YS, Mineo C, Anderson RGW (1995) A detergent-free method for purifying caveolae membrane from tissue culture cells. Proc Natl Acad Sci USA 92: 10104–10108

Song KS, Li S, Okamoto T, Quilliam LA, Sargiacomo M, Lisanti MP (1996*a*) Copurification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains: detergent free purification of caveolae microdomains. J Biol Chem 271:9690–9697

Song KS, Scherer PE, Tang Z, Okamoto T, Li S, Chafel M, Chu C, et al (1996*b*) Expression of caveolin-3 in skeletal, cardiac and smooth muscle cells: caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. J Biol Chem 271: 15160–15165

Tang ZL, Okamoto T, Boontrakulpoontawee P, Katada T, Otsuka AJ, Lisanti MP (1997) Identification, sequence, and expression of an invertebrate caveolin gene family from the nematode *Caenorhabditis elegans:* implications for the molecular evolution of mammalian caveolin genes. J Biol Chem 272:2437–2445

Tang ZL, Scherer PE, Okamoto T, Song K, Chu C, Kohtz DS, Nishimoto I, et al (1996) Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. J Biol Chem 271:2255–2261

Yamamoto M, Toya Y, Schwencke C, Lisanti MP, Myers M, Ishikawa Y (1998) Caveolin is an activator of insulin receptor signaling. J Biol Chem 273:26962- 26968

Yang CP, Galbiati F, Volonté D, Horwitz SB, Lisanti MP (1998*a*) Upregulation of caveolin-1 and caveolae organelles in Taxol-resistant A549 cells. FEBS Lett 439:368–372

Yang G, Truong LD, Timme TL, Ren C, Wheeler TM, Park SH, NasuY, et al (1998*b*) Elevated expression of caveolin is associated with prostate and breast cancer. Clin Cancer Res 4:1873–1880