

CHINESE MEDICAL SCIENCES JOURNAL

REVIEW

A Second Protein Marker of Caveolae: Caveolin-2[△]

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Key words: caveolin-2; lung; lipid metabolism; cancer

Caveolin-2, a protein about 20 kD, is a major component of the inner surface of caveolae, small invaginations of the plasma membrane. Similar with caveolin-1 and caveolin-3, it serves as a protein marker of caveolae. Caveolin-1 and -2 are located next to each other at 7q31.1 on human chromosome, the proteins encoded are co-localized and form a stable hetero-oligomeric complex, distributing similarly in tissue and cultured cells. Caveolin-3 is located on different chromosomes but confirmed to interact with caveolin-2. Caveolin-2 is similar to caveolin-1 in many respects but differs from the latter in functional domains, especially in G-protein binding domain and caveolin scaffolding domain. The mRNAs of both caveolin-1 and caveolin-2 are most abundantly expressed in white adipose tissue and are induced during differentiation of 3T3-L1 cells to adipocytes. Caveolin-2-deficient mice demonstrate clear pulmonary defects, with little or no change in caveolin-1 expression and caveolae formation, suggesting that caveolin-2 plays a selective role in lung functions. Caveolin-2 is also involved in lipid metabolism and human cancers.

Chin Med Sci J 2010; 25(2):119-124.

THE caveolin gene family in vertebrates has three members: caveolin-1 (cav-1), caveolin-2 (cav-2), and caveolin-3 (cav-3). They are integral membrane proteins weighing 20-24 kD and serve as protein markers of caveolae ("little caves"), spherical or flask-shaped invaginations of plasma membrane of strikingly regular shape and 50-100 nm in size.¹

Caveolins are found predominantly in plasma membrane, but also in Golgi bodies, endoplasmic reticulum, vesicles, and at cytosolic locations, considered to be subtypes of lipid rafts.² An ultrastructural analysis of adipocytes has shown that caveolae take up as much as 20% of the total area of plasma membrane,³ therefore greatly increase the surface area of cells, which seems to facilitate signal transduction. Cav-1 and cav-2 are expressed in most cell types, quite similar in tissue distribution,⁴ while cav-3 is found almost exclusively in muscle cells.⁵ Cav-1 is required by cav-2 for proper membrane localization.^{3,4} The genes encoding cav-1 and cav-2 are located in the same regions (7q31.1-31.2) on human chromosome, only about 19 kb apart. Unlike cav-1 and cav-3, cav-2 alone is insufficient to drive the formation of morphologically identifiable caveolae.

Received for publication May 5, 2010.

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△ Supported by National Basic Research Program of China (973 Program) (2004CB518602, 2006CB503909) and National High Technology Research and Development Program of China (863 Program) (2009AA02Z192).

In fact, in cav-1-deficient tissues, cav-2 level is also reduced by approximately 95% due to its degradation *via* the proteasome system, rather than altered transcriptional regulation.^{6,7}

Cav-2 was identified by microsequencing of adipocyte-derived caveolar membranes as a protein of about 20 kD. It is homologous with cav-1 (about 24 kD) except for differences in several residues. The identity between human cav-2 and cav-1 is about 38%, and similarity about 58%. The most conserved region is a stretch of 8 identical amino acids FEDVIAEP termed as the "caveolin signature motif", which is identical among all the three caveolin proteins. All caveolins have a central 33-amino acid-long hydrophobic domain that is thought to adopt a hairpin conformation into the membrane, leaving both N- and C-termini of the proteins facing the cytoplasm.⁸ The G-protein binding region of cav-2 is about 30% identical to cav-1, with almost half of those non-consensus representing conservative substitutions, so the peptide encoding this region of cav-2 was consequentially evaluated to generate different functions.³ The N-terminal membrane-binding domain between transmembrane domain and oligomerization domain is called the caveolin scaffolding domain (CSD). The scaffolding domain has been shown to play a dual role, acting both as an anchor holding various proteins within caveolae, and as a regulatory element capable of inhibiting or activating a given protein's signaling activity. Cav-1 appears to inhibit the downstream activation and signaling of many proteins, including c-Src, H-Ras, mitogen-activated protein kinases (MAPKs), and endothelial nitric oxide synthase (eNOS). The evidence supporting the signaling modulating function of cav-2 is less clear, perhaps partly because its CSD sequence is different from that of cav-1.⁹

The genomic organization of human cav-2 is greatly similar to cav-1. Three isoforms of cav-2 have been identified: the full-length cav-2 α , and two truncated variants termed cav-2 β and cav-2 γ . The β -isoform is thought to be an alternate splice variant, with a subcellular distribution distinct from full-length cav-2 α .^{3,10,11}

CHARACTER AND LOCALIZATION

Characters of distribution and expression

The mRNAs of both cav-1 and cav-2 are most abundantly expressed in white adipose tissue and are induced during differentiation of 3T3-L1 cells to adipocytes. The same as for cav-1, the mRNA of cav-2 is induced about 25-fold after 4 days of differentiation from 3T3-L1 fibroblasts.³ Cav-2 is also specially expressed in lung without cav-1

detected by Western blot.⁴ Another research on its expression in established cell lines and primary cultured cells revealed that cav-2 was most widely expressed among the three caveolins. Cav-2 was most abundant in endothelial cells, smooth muscle cells, skeletal myoblasts (L6 and BC3H1 cell lines), fibroblasts, and 3T3-L1 adipocytes. L6 myoblast was the only cell line expressing all three caveolins simultaneously. Cav-2 was constitutively expressed in C₂C₁₂ myoblasts and myotubes, and its level remained constant during the process of differentiation. In contrast, no cav-1 expression was detected in those cells, but cav-3 was strongly induced during the differentiation. Cav-2 and -3 were detected at low levels in immature ventricles (postnatal day 2 to day 15), compared to the much higher expression levels in 2-month-old infant ventricles. In contrast, the expression of flotillin-1 and -2 was found similar in all samples. Those two weighing 45 kD constitute another family of caveolae-associated proteins. They form stable complexes that are coimmunoprecipitated (along with cav-2) by cav-1 antibody. The interaction identified between cav-2 and cav-3 in cardiomyocytes suggested that cav-2 may participate with cav-3 in the biogenesis of caveolae in that type of cells.¹² However, functions of cav-2 have not been recognized yet. Expression of cav-2 in COS-7 cells yielded a protein product of the expected molecular mass (about 20 kD), slightly smaller than cav-1. Heat-dissociated cav-1 migrated as monomer, while undissociated cav-1 migrated predominantly as a high molecular mass oligomer of about 350 kD. In contrast, cav-2 migrated mainly as a monomer under both dissociated and undissociated conditions, though a small quantity of dimeric form (about 40 kD) was also observed. In 3T3-L1 fibroblast membranes, 90%-95% of cav-1 and -2 were co-fractionated in the same low-density fractions by equilibrium sucrose gradient centrifugation, indicating their co-localization.³ These results were consistent with the findings of immunolocalization.

Expression of cav-1 is necessary for the formation of caveolae. K562 cell is a good model for assessing the effects of cav-2, which expresses cav-2 but fails to express detectable level of cav-1. There is no caveolae in cav-1 negative K562 cells; in cav-1-expressing recombinant, newly formed caveolae vesicles attached to plasma membrane could be detected by electron microscopy. The expression of cav-1 almost quantitatively converts cav-2 from a Triton-soluble to a Triton-insoluble protein product.¹³ Further study demonstrates that cav-1 expression dramatically increases the molecular mass of cav-2-containing oligomeric complexes, the broad peak from 29-200 kD to 200-400 kD.¹⁴ Moreover, the localiza-

tion of cav-2 on plasma membrane is determined by the presence of cav-1; otherwise, cav-2 would remain trapped within the Golgi complex.¹⁵ Cav-2 is confined to the Golgi area in confluent monolayers of FRT cells over-expressing cav-2, but redistributes partially to plasma membrane when cav-1 is co-expressed after transfection. Recombinant expression of cav-1 up-regulates the endogenous expression of cav-2 in K562 cells, but only in protein level; cav-2 mRNA remains at the same level in cav-1 positive K562 cells when compared with that in cav-1 negative cells, as revealed in Northern blot analysis.¹⁴

Phosphorylation of cav-2 regulates formation and localization of caveolae

Although cav-2 protein is dispensable to formation of caveolae, it has been determined that phosphorylation of serine 23 and 36 in cav-2 modulates cav-1-dependent caveolae formation.¹⁶ Cav-2 is also phosphorylated at tyrosine 19 or 27, with the two products displaying different localization patterns. Phospho-cav-2 (pY19) is concentrated at cell edges and cell-cell contacts, whereas phospho-cav-2 (pY27) is distributed in a dot-like pattern across the cell surface and cytoplasm.⁷ Okadaic acid (serine/threonine protein phosphatase inhibitor) could affect the internalization of caveolae in HepG2 cells;¹³ and the effect is less prominent on cav-2 than on cav-1. In untreated control, cav-1 and -2 are present at the cell surface, corresponding to the typical location of caveolae; after treatment with 4 nmol/L okadaic acid, a larger amount of caveolins can be detected in the cytoplasm, suggesting the internalization of caveolae. The effect of 100 nmol/L okadaic acid is more prominent: caveolins are present as large clusters in the cytoplasm with a strict orientation. In addition, okadaic acid strongly induces tyrosine phosphorylation of cav-2 but not cav-1.¹⁷ The findings imply that protein phosphatase inhibitors could intensively induce internalization of caveolae by increasing phosphorylation of caveolins. It is known that binding of albumin to its plasma membrane receptor (gp60) can induce caveolar internalization by activating Src kinase.^{18,19} It is therefore reasonable to assume that phosphatases might stimulate internalization of caveolae by increasing the phosphorylation level of associated proteins, although the mechanism of this process is still unclear.

FUNCTIONS OF CAV-2

GAP-like activity of cav-2, corresponding to GDP dissociation inhibiting effect of cav-1

It is already mentioned that cav-1 has a G-protein binding

domain consisting of 82-101 residues, which can functionally suppress the basal GTPase activity of purified heterotrimeric G proteins, acting as a GDP dissociation inhibitor (GDI).^{20,21} The corresponding region of cav-2 is about 30% identical to cav-1, with almost half of the differences being conservative substitutions. Therefore, cav-2-derived polypeptide activates purified heterotrimeric G proteins as a GAP (GTPase activating protein). When the concentration of cav-2-derived peptide was at 10 $\mu\text{mol/L}$, the activation rate of the basal activity of G_0 protein was over 2-fold.³ Co-localization with cav-1 in a single cell yet with contrary functions demonstrates that cav-2 is not only a structural partner of cav-1 but also concerted with the latter in function. This allows a two-step mechanism for concentrating inactive G proteins within caveolae to present to activated G-protein-coupled receptor. First, cav-2 acts as a GAP to place activated G proteins in the inactive GDP-bound conformation and recruit them to the caveolae membranes. Second, cav-1 functions as a GDI to keep them in inactive conformation in the membrane.³

Roles in pulmonary functions

Lung is one of the tissues where cav-2 is most highly expressed, suggesting that cav-2 might have significant effect on lung. Razani et al²² provide a powerful and direct proof of cav-2's effect on pulmonary function by targeted disruption of cav-2 locus. Cav-2-deficient mice show clear pulmonary defects, with thickened alveolar septa, endothelial cell hyperproliferation, and exercise intolerance, but little or no change in cav-1 expression and caveolae formation.²² In addition, cav-1-deficient mice present with similar abnormalities: severe pulmonary dysfunction due to lung parenchymal hyperproliferation.⁶ Cav-2 level is dramatically reduced (by approximately 95%) due to its degradation *via* the proteasome system,^{6,7} and cav-2 but not cav-1 plays regulatory role in *Pseudomonas aeruginosa* invasion of lung epithelial cells.²³ It is hence reasonable to conclude that cav-2 is responsible for the lung disorder above-mentioned.

Complicated regulation of cav-2 in lipid metabolism, related to obesity and type 2 diabetes mellitus

In differentiated 3T3-L1 adipocytes, cholesterol depletion leads to a 2.6-fold increase in cav-2 mRNA level assessed by real-time reverse transcription-polymerase chain reaction.²⁴ While lovastatin treatment also causes cav-2 reduction to about 79% in 3T3-L1 adipocytes. Lovastatin can inhibit mevalonate formation by inhibiting the activity of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, which catalyses the committed step in cholesterol

biosynthesis.²⁵ The explanation to the two seemingly contradictory results is that cholesterol depletion reduces cholesterol uptake of cultured cells from medium, thereby activates *de novo* synthesis; and HMG-CoA reductase is increased under sterol depletion as assessed by Northern blot analysis.²⁵ In addition, cav-2 is intensely induced during differentiation of 3T3-L1 cells to adipocytes,³ and the level of HMG-CoA reductase mRNA is enhanced after cav-2 adenoviral gene transfer in liver.²⁶ It is conceivable that cav-2 expression correlates with cholesterol biosynthesis pathway.

High-fat feeding was found to affect cav-2 expression in rat white adipose tissue and skeletal muscle.²⁷ Cav-2 is decreased or increased by high-fat diet depending on the length of feeding, but always in the opposite way of cav-1. Cav-2 mRNA was downregulated 3.8-fold²⁸ with cav-1 upregulated about 2.21-fold in rats fed with high-fat cafeteria diet for 11 days, assessed by Northern blot analysis;²⁷ while cav-2 mRNA was upregulated 5.37-fold and cav-1 downregulated to 0.41 of the level in control group when the high-fat feeding period was prolonged to 65 days without other conditions changed, assessed by the same method.²⁹ These findings suggest that caveolins have a role in lipid metabolism and storage, pointing them as candidate genes involved in diet-induced obesity.²⁸ The data in the short-term feeding study may imply an initial trend to improve insulin sensitivity and facilitate lipid usage and storage. However, after being exposed to a continuous high-fat supply, rats are becoming obese, possibly due to the associated insulin resistance. The inverse correlation between cav-1 and cav-2 expression may indicate a compensatory mechanism, by which cav-2 is less necessary when the functionally more relevant cav-1 is at a high level, but induced by long period of high-fat diet (65 days) to compensate the reduction of cav-1 expression.²⁹ Obesity arises as a consequence of prolonged imbalance between calorie intake and consumption.³⁰ This nutritional disorder is directly related to many metabolic alterations such as hyperinsulinaemia, dislipidaemia, and type 2 diabetes mellitus, and also associated with several health threats such as atherosclerosis, hypertension, immune impairment, and a low-grade, systemic inflammatory condition.³¹ Although in cav-2 null mice, adipose tissue remains normal and the protein levels of insulin receptor- β are not altered,^{22,31} the regulatory function of cav-2 in cell cycle regulation by insulin was investigated in human insulin receptor-overexpressed rat 1 fibroblast (Hirc-B) cells, in which insulin was found to increase induction of cav-2 gene in a time-dependent manner.³² The phosphotyrosine cav-2 (pY19 and pY27) is a novel regulator for transcriptional

activation of signal transducer and activator of transcription 3 (STAT3) in response to insulin.³³ And pY27-cav-2 maintains a long-term tyrosine kinase activation of insulin receptor.³⁴

Relationship between cav-2 and human cancers

Genes encoding human cav-1 and cav-2 are co-localized to the (CA)_n microsatellite repeat marker D7S522 locus (7q31.1-31.2), a known fragile site (FRA7G) that is frequently deleted in human cancers, including squamous cell carcinomas of the head and neck, prostate cancers, renal cell carcinomas, ovarian adenocarcinomas, colon carcinomas, and breast cancers.³ The mRNA and protein levels of cav-1 and -2 are found to be suppressed in breast cancer tissues.³⁵ Cav-2 expression is closely associated with basal-like immunophenotype and proved by univariate analysis to be a prognostic factor of breast cancer.^{36,37}

Other functions

Cav-2 is involved in intercellular chlamydial inclusions independently of cav-1, indicating that cav-2 may affect the developmental cycle of chlamydia, which is yet to be elucidated.³⁸ In addition, decreased expression of cav-2 significantly impairs the ability of *Pseudomonas aeruginosa* to invade MLE-12 cells, and the lipid raft-dependent tyrosine phosphorylation of cav-2 appears to be a critical regulator of *Pseudomonas aeruginosa* invasion.³⁸ New evidence has recently shown that cav-2 contributes to intercellular cell signaling and intracellular trafficking. For example, it regulates gap junctional intercellular communication as cav-1 does by co-localizing and interacting with connexin 43 in keratinocytes.³⁹ Cav-2 is also considered as an integral component of trafficking network in intestinal cells of *Caenorhabditis elegans* for apical uptake of lipid.⁴⁰

CONCLUSIONS AND FRONTIERS

Cav-2 is strikingly homologous to cav-1 but different in some functional regions, including the G-protein binding region and coding sequence (CDS). It has functions independent of cav-1, as shown by the dramatic reduction by about 95% of its level in cav-2-deficient mice. Together with cav-1, they may drive a compensatory two-step mechanism for concentrating inactive G proteins within caveolae for presentation to activated G-protein-coupled receptors. Cav-2 also exerts some functions in lungs, since cav-2-deficient mice display severe pulmonary dysfunction without disruption of caveolae and cav-1. In the process of lipid metabolism, the role of cav-1 has been clarified, but the functions of cav-2 remain less clear. Hepatic over-

expression of cav-1 and/or cav-2 increase bile flow and bile salt-dependent biliary lipid secretion in mice, and the mRNA level of cav-2 changes after high-fat feeding in rats, suggesting that cav-2 may have a role in lipid metabolism and storage as cav-1 does. The nutritional disorder of obesity is directly related to many metabolic diseases, such as hyperinsulinaemia, dislipidaemia, and type 2 diabetes. Further studies will therefore be necessary to dissect the molecular pathways through which cav-2 regulates lipid metabolism.

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