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Review

An overview of caveolins and their distributions in the endocrine system

Tamaki MAEDA *Department of Nursing, Faculty of Nursing and Rehabilitation, Aino University*

Michiyo HATANAKA *Department of Medical Technology, Kobe-Tokiwa College*

Abstract

Caveolins, the proteins which were found in "little caves" named caveolae more than 50 years ago, have been revealed to work to maintain the form and functions of many kinds of cells. Therefore malfunction of caveolins is related to diseases such as tumors, atherosclerosis, muscular dystrophy, pulmonary disfunction and other diseases. The introduction of caveolin-deficient mice brought new methods to approach the mechanisms of both normal cell function and the onset of these diseases. Although we examined caveolin distribution in human tissue, it has been difficult to establish a relationship between the caveolins and hormone secretion. However, as caveolin-1 has direct functional interactions with insulin receptors, Type II diabetes mellitus has become one subject for caveolin research. In terms of tumorigenesis, there are some reports of overexpression of caveolin-1 and -2 in tumors of the thyroid and parathyroid. Caveolins are proteins which have the potential for much further research, from many points of view.

Key words : caveolin-1, caveolin-3, endocrine system

Introduction

Caveolins are membrane proteins which have three main isoforms, caveolin-1, -2 and -3, and are essential components of caveolae. Caveolae are one of the microdomains with sphingolipid and cholesterol, like rafts, but they have their own stable shape, an omega-shaped pit, on the plasma membrane. Caveolins not only drive the invagination of the membrane, but are important also for signal transduction and are associated with tumorigenesis. Moreover, these proteins relate to many kinds of diseases : not only cancer but also diabetes, atherosclerosis, muscular dystrophy and other diseases. Therefore caveolin-related topics have been reported actively from both the basic and clinical fields in the decade since their discovery. A Medline search revealed only 15 titles with caveolin (s) in 1995, but 150 in 2004. Here we will overview the history of caveolin research, introduce their distribution in endocrine cells, which is our main interest, and discuss the outlook of caveolin research especially pertaining to caveolin-3. The details of the biochemical mechanism can be found in the references.

I. Overview of Caveolins

1. Discovery of Caveolae

About 50 years ago, electron microscopic techniques revealed small invaginations of the plasma membrane. Parade (1953) described them as vesicles concentrated under the cell membranes of endothelial cells. Yamada (1955) also found similar small cave-like indentations on epithelial cells of the gall bladder, and named these 50–100 nm depressions caveolae. These structures were distinguished from clathrin-coated pits which are more electron-dense and larger coated vesicles. Additional ultrastructual studies revealed that caveolae are found in most cell types, predominantly on fibroblasts, endo-

thelial cells, adipocytes and smooth muscle cells. They have been considered to have a role in the endocytic pathway, potocytosis, transcytosis, and regulation of intracellular calcium concentration (Kurzchalia 1996). However until caveolins were recognized as biochemical markers in the 1990s, the functions of caveolae had not been sufficiently analyzed.

2. Caveolins and their isoforms

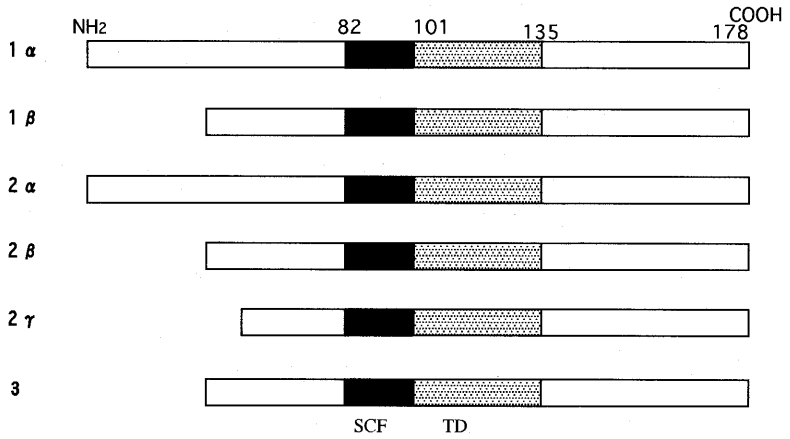
In 1989 Glenny purified 22-kDa tyrosine phosphorylated protein from Rous sarcoma virus-transformed chick embryo fibroblasts. Rothberg found that this protein is the molecular component of the caveolae membrane coat, and named it caveolin (Glenney et al. 1983), later re-termed caveolin-1 (Rothberg et al. 1992). Independently Kurzchalia et al identified VIP21 (Vesicular Integral-membrane Protein of 21 kD) from MDCK strain II cells, which are cultured epithelial cells (Kurzchalia et al. 1992). This protein in trans-Golgi-network-derived transport vesicles turned out to be the same protein as caveolin. Further studies using molecular cloning revealed that there are three main isoforms of caveolins in mammalian cells (Okamoto, 1998), and additional subtypes of caveolin-1 and 2 (Fig. 1). Two isoforms of caveolin-1 (1α and 1β) were reported to show an overlapping, but slightly different subcellular distribution in culture cells, and some results suggest that the two isoforms may have distinct physiological functions in different cell types (Kogo et al. 2004). The significance of caveolin-2β and 2γ, which are the isoforms of caveolin-2, are still under investigation.

Immunohistochemical and biochemical analysis using the antibodies against caveolin-1, -2, and -3 revealed the distributions of each isoform in cultured cells and human tissues (Fig. 2) (Maeda 2000). Caveolin-1 is expressed in many cell types but abundantly in adipocytes, endothelial cells, pneumocytes, and fibroblasts (Fig. 3). Caveolin-2 mainly co-localizes with caveolin-1 in the plasma membrane and internal cellular membranes. Caveolin-3 mainly exists in muscle cells.

Caveolin-deficient mouse models showed several different aspects according to the isoforms. For example, although Cav-1/3 double knock-out mice lack caveolae in both nonmuscle and striated muscle tissues, caveolae could be observed in Cav-2 null mice (Park 2002). The characteristics of each isoform will likely be brought to light by examination using these caveolin-deficient mouse models.

3. Functions of Caveolins

Caveolins are necessary to maintain the shape of the caveolae, by binding with the actin cytoskeleton (Fig. 4). Using caveolins as markers caveolae can be isolated from other cellular components, and in this way caveolae-containing proteins have been purified. The number of proteins harvested from caveolae was unexpectedly high, and these are the important proteins related to signal transduction. Currently caveolins are thought to act as scaffolding proteins, concentrating specific signaling molecules within caveolae, so the caveolin-derived protein domain has



SCF : scaffolding domain, which binds to various proteins
TD : transmembrane domain, which forms a hairpin-like loop within the membrane

Fig. 1 Isoforms of caveolins

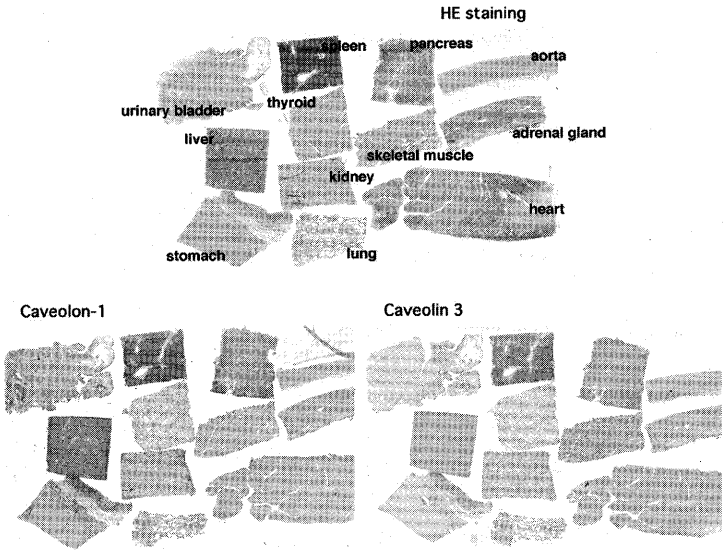


Fig. 2 Immunohistochemistry for caveolin 1 and 3 : To equalize the immunohistochemical conditions, all the tissues examined in an individual autopsy case were embedded together as 5 mm squares in a single paraffin block.

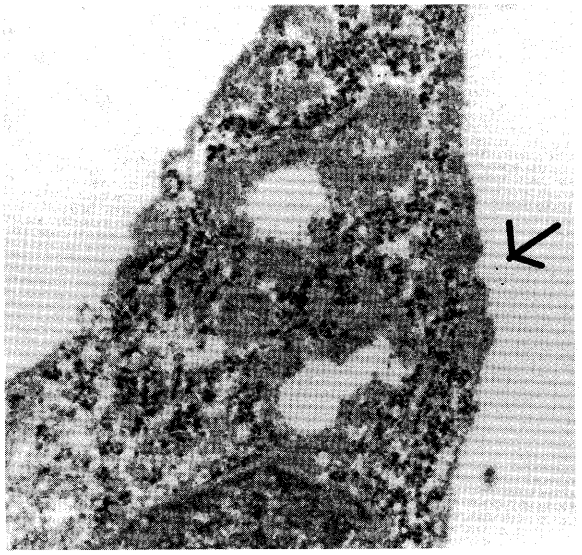


Fig. 3 The signals of caveolae on a CHO cell, one of the cultured fibroblasts, are shown by ultrastructual immunohistchemistry using colloidal gold particles.

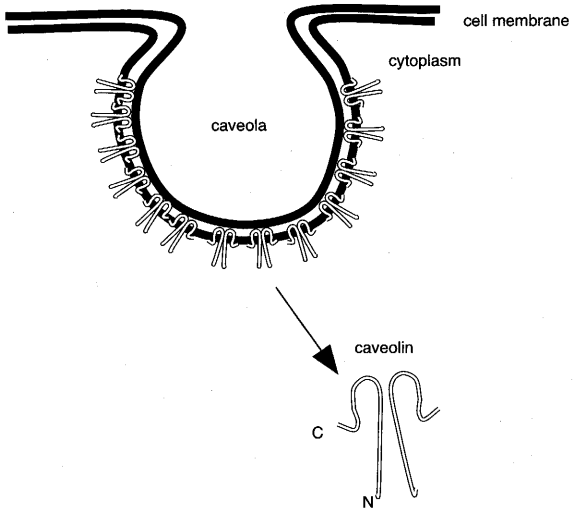


Fig. 4 Caveolae structure supported by caveolin (citation from Quest 2004)

been termed the caveolin-scaffolding domain (Couet et al. 1997).

Although the function of caveolins has not yet been clearly defined, Razani et al (2002) particularized the functional significance of caveolae/caveolins as follows (Table 1) :

A. Vesicular Transport : The structures of caveolae, which show invaginations of the plasma membrane, completely enclosed vesicles, or aggregates of several vesicles, suggested that they might be a place for the endocytosis of macromolecules (Simionescu et al., 1975). Electronmicroscopic examination using tracers has been clarifying how caveolae and caveolins mediate the selective uptake and transport of molecules, using different

Table 1 Functional significance of caveolae/caveolin

A. Vesicular Transport	1. Transcytosis 2. Endocytosis 3. Potocytosis
B. Cellular Cholesterol Homeostasis	1. Intracellular Transport of de Novo Synthesized cholesterol 2. Cholesterol Efflux from Cells
C. Signal Transduction	1. Compartmentalization of signaling as signalosomes 2. Modulators of signaling for ; G-protein α subunit eNOS, H-Ras, EGF-R, Src family tyrosine kinases, PKC isoforms etc.
D. Tumorigenesis	1. Targets of oncogenes 2. Tumor suppressors
E. Specialized function of caveolin-3 in muscle cells	Modulators of dystrophin-glycoprotein complex function
F. Emerging Functions	Possibly, portals for the uptake and transport of fatty acids to lipid droplets

processes such as transcytosis, endocytosis, and potocytosis.

B. Cellular Cholesterol Homeostasis : Caveolin is a cholesterol-binding protein, which binds at least 1 mol of cholesterol per mole of protein (Murata 1995),and is suggested to be involved in maintaining intracellular cholesterol balance by modulating its cellular influx and efflux.

C. Signal Transduction : Since Lisanti et al made the "caveolae/raft signaling hypothesis" in 1994, many caveolae-localized and caveolin-interacting molecules have been investigated, and now caveolae are thought to be signalosomes, the locations of signal transduction events and cross-talk between independent signaling pathways. The caveolin scaffolding domain also interacts with many kinds of signaling proteins. The mechanism of the inhibition of endothelial nitric-oxide synthase (eNOS) through the caveolin-1 scaffolding domain has been well studied, and a new therapeutic approach is also suggested from these experiments (Ju et al. 1997, Bucci 2000). It has also been reported that caveolin-1 and -3 inhibit many other enzymes, especially kinases.

D. Relation with Tumorigenesis : Caveolin-1 is supposed to be a tumor suppressor and a direct target of the activated oncogenes in many tumor cells. Examples will be described in the section of "Caveolins and Diseases."

E. Specialized Functions of Caveolin-3 : Caveolin-3 not only generates caveolae and supplies signalosomes on muscle cells like caveolin-1, but is also related to the cellular dystrophin-glycoprotein complex proteins. An experiment using anti-Cav-3 antibody showed evidence that Cav-3/caveolae have a role in T-tubule development (Parton et al. 1997).

F. Caveolins and Lipid Droplets : A participation in lipogenic processes is one of the emerging functions of caveolins. As the first known integral membrane protein components of lipid droplets, caveolins are expected to be a key to resolving the mechanism of the uptake and transport of fatty acids to lipid droplets.

4. Caveolins and Diseases

After caveolin-1 was reported to inhibit various signal pathways, it was supposed to work as a tumor suppressor, and caveolins have been investigated in many types of human tumors from the clinical point of view. Reductions in caveolin-1 protein were identified in the breast, lung, colon, and ovarian carcinomas. In an attempt to map the caveolins' loci in the human genome both genes of the human caveolin-1 and -2 are co-localized to the q31.1 region of the human chromosome 7 (Engelman et al. 1998), which is a region of high deletion frequency in many types of human epithelial tumors. The demonstration of a point mutation of the caveolin-1 gene in 16% of breast cancers (Hayashi et al. 2001) is also one of the reports supporting that caveolin-1 is a tumor suppressor. An examination using NIH 3T3 cells transformed by various oncogenes revealed the oncogenes which lead to reductions in cellular levels of caveolin-1. The oncogenes which transcriptionally suppress caveolin-1 expression are H-ras^{G12V}, v-abl, mTAg, bcr-abl, crk1, c-src, c-neu, HPV E6, and c-myc (Koleske 1995, Razani 2002).

On the other hand we identified caveolin-1 in a subline of Jurkat cells and two of five ATL cell lines (Hatanaka 1998), although neither caveolin proteins nor caveola structures are detected in peripheral blood cells or blood cell

lines including other sublines of Jurkat cells (Fig. 5). The prostate, normally with low expression of caveolin-1, also shows elevation of caveolin-1 expression in later-stage cancer cells and in metastatic lesions. These paradoxical overexpressions of caveolin-1 in tumors correlated with a worse prognosis, may reflect the diversity of caveolins' function; thus we speculate that phosphorylation of caveolin-1 might relate to tumorigenesis.

Cohen et al. (2004) suggest that caveolin knock-out mice, which are surprisingly viable and fertile, can be models of human diseases including not only cancers but also diabetes, cardiovascular disease, atherosclerosis, pulmonary fibrosis, and a variety of degenerative muscular dystrophies. Diabetes will be mentioned in the next section, and dystrophies in the final section.

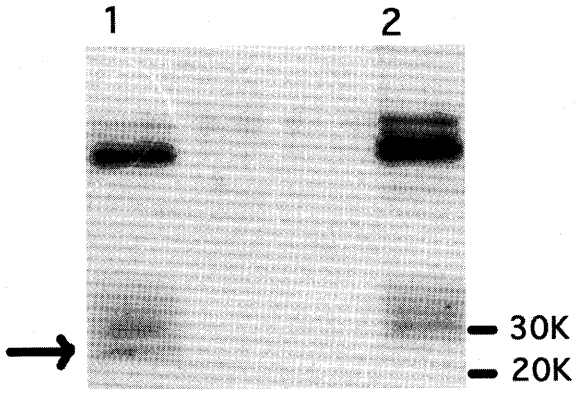


Fig. 5 Western blotting of cultured cells using caveolin-1 agarose conjugates. Line 1 : C6 shows positive signal (arrow). Line 2 : Jurkat shows no signal

II. Caveolins in the Endocrine System

We have been studying hormone secretion especially exocytosis in normal and hyperplastic pituitaries (Mori et al. 2001). From the existence of caveolins in trans-Golgi-network-derived transport vesicles, we tried to find evidence of caveolin participation in exocytosis. We investigated normal and hyperplastic endocrine cells in human tissues for caveolin-1 and -2 by immunohistochemistry using formaldehyde fixed-paraffin embedded tissues (Maeda et al. 2000). It was difficult to find evidence of a relationship between caveolins and hormone secretion, but neoplastic change in the thyroid showed the same expression as other tumors. In this section we will show some results from our experiments and introduce literature related to the endocrine system.

1. Caveolins in the Pituitary : Although in our studies the staining of caveolins was stable and corresponded with the other results, only pituitary cells showed a positive signal in the nucleus, and no difference was found between normal pituitaries and adenomas. Rotondo et al studied expression of caveolin-1 and caveolin-2 in 12 non-tumorous pituitaries and 97 hypophysial adenomas, but no immunoreactivity was seen in adenohypophysial and neurohypophysial cells or in the tumor cells (Rotondo et al. 2004). The possibility of caveolins' involvement in the initiation and progression of pituitary adenomas looks quite low.

2. Caveolins in the Thyroid : Ito Y et al. used a rabbit polyclonal antibody against caveolin-1 to investigate thyroid tissue and observed caveolin-1 expression in papillary carcinoma but not normal follicular cells, follicular carcinoma cells or adenoma cells. The positive signal was higher especially in microcancers, whose diameters were less than 1.0 cm, and its incidence was significantly reduced in undifferentiated (anaplastic) carcinoma cells. These results are similar to the cancers of the breast and others shown in the previous section (Aldred et al. 2003, Ito et al. 2002). We used monoclonal antibodies against caveolin-1 and -2 to stain thyroid tissues from autopsies and operations. In non-neoplastic thyroid follicles, the expressions of both caveolin isoforms were higher in flattened epithelium than in active columnar cells with absorptive vesicles. However the expressions were not stable compared to the neoplastic cells, which showed results similar to Ito's, so it is difficult to speculate upon the mechanism of these results.

3. Parathyroid : Kifor et al. (1998) demonstrated the existence of extracellular calcium concentration ($Ca^{2+}o$) sensing receptors (CaR) within caveolin-rich membrane domains in bovine parathyroid cells. They also performed immunohistochemical analysis of frozen sections of parathyroid adenomas, and reported decreased expression of caveolin-1 in adenomas (Kifor et al. 2003).

4. Adrenal Gland : There are few reports about caveolins in the adrenal gland. Our immunostaining showed positive signals in cortical cells and tumor cells of an incidental pheochromocytoma but not in cells of the

medulla.

5. Caveolins and Diabetes Mellitus: In our study pancreas islet cells didn't show a constant reaction against anti-caveolin-1 and-2 monoclonal antibodies, so it is difficult to connect caveolins to insulin secretion from the pancreas islets. However insulin receptors were found in caveolae of the adipocytes, and they were proven to interact with the scaffolding domain of caveolin-1 directly (Cohen et al. 2003a). Both Cav-1 null mice and Cav-3 null mice showed insulin resistance (Cohen et al. 2003b, Razani B et al. 2003), but this did not cause diabetes directly. In humans, mutations were found in the caveolin-binding motif of insulin receptors by investigating patients with severe insulin resistance, although the number of patients was limited. Currently type II diabetes is one target for researchers to investigate in relation to caveolins.

Ⅲ. Outlook of Caveolins

As has already been described above, caveolin-1 is being studied to clarify the mechanism and to find therapies for various diseases including endocrine diseases. In our investigation, immunohistochemical staining for caveolin-1 and -2 was not stable in endocrine cells. We speculate it maybe due to the condition of each endocrine cell secreting hormone or not.

Caveolin-2, which exists in the same cells as caveolin-1, was indicated to be necessary for normal lung formation, because of lung abnormalities in Cav-2 knock-out mice are identical to those of Cav-1 knock-out mice (Razani et al, 2002).

Caveolin-3 was reported to be limited to muscle cells, however there are some reports revealing upregulation of caveolin-3 in astroglial cells surrounding senile plaques in brain tissue sections from Alzheimer's disease patients (Nishiyama et al. 1999).

Although we already mentioned that caveolin-3 is involved in diabetes mellitus, there was no chance to discuss here the involvement of caveolin-3 in muscle diseases. It can be easily predicted because of its specific distribution in muscle cells. Indeed caveolin-3 associates with dystrophin, which is the product of the DMD gene, the X-linked recessive gene associated with Duchenne muscular dystrophy (Song et al. 1996). Fulizio L et al screened 663 patients with various phenotypes of limb-girdle muscular dystrophy (LGMD), and reported that caveolinopathies, which indicate mutations of Cav-3, are 1 % of both unclassified LGMD and other phenotypes (2005).

Since a "little cave" was first observed about 50 years ago, numerous facts pertaining to caveolae and caveolins have been accumulated using the newest methods in each period, and caveolins have become one of the most important proteins for investigations ranging from the basic mechanism of cell transportation to various human diseases.

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