

# Caveolin-1 upregulation in senescent neurons alters amyloid precursor protein processing

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Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Cav-1, caveolin-1; CTF, C-terminal fragment; DIG, detergent-insoluble glycolipid; GSK-3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; PKC, protein kinase C; SRE, sterol regulatory element; TGN, trans-Golgi network

## Abstract

Lipid rafts provide a platform for regulating cellular functions and participate in the pathogenesis of several diseases. However, the role of caveolin-1 in this process has not been elucidated definitely in neuron. Thus, this study was performed to examine whether caveolin-1 can regulate amyloid precursor protein (APP) processing in neuronal cells and to identify the molecular mechanisms involved in this regulation. Caveolin-1 is up-regulated in all parts of old rat brain, namely hippocampus, cerebral cortex and in elderly human cerebral cortex. Moreover, detergent-insoluble glycolipid (DIG) fractions indicated that caveolin-1 was co-localized with APP in caveolae-like structures. In DIG fractions,  $\beta$ APP secretion was up-regulated by caveolin-1 over-expression, which was modulated via protein kinase C (PKC) in neuroblastoma cells. From these results we conclude that caveolin-1 is selectively expressed in senescent neurons and that it induces the processing of APP by  $\beta$ -secretase via PKC down-

regulation.

**Keywords:** aging; Alzheimer disease; amyloid  $\beta$ -protein precursor; caveolin-1; neuron

## Introduction

Alzheimer's disease (AD) is one of the most prevalent diseases in the elderly population. Although the molecular pathogenesis of familial AD has been well proven by specific mutations, available information on age-related sporadic AD is limited. Senile plaque, composed of A $\beta$  amyloid peptide and neurofibrillary tangles differentiates the AD brain and the normal brain (Esler and Wolfe, 2001). A $\beta$  amyloid peptide can be produced from amyloid precursor protein (APP) by the proteolytic activities of  $\beta$ - and  $\gamma$ -secretases and by the dysregulation of APP metabolism in the elderly brain. Moreover, these processes are important in the pathogenesis of age-related AD.

Caveolin proteins were reported to be upregulated in senescent fibroblasts and in old rat tissues, e.g., brain and spleen, and these may attenuate responsiveness to external stimuli (Park *et al.*, 2000; Wheaton *et al.*, 2001). Although the isoforms of caveolin have not been fully elucidated in the nervous system, caveolae structures have been observed in thin-section images of neuronal cells (Galbiati *et al.*, 1998). Moreover, APP is enriched in this caveolae fraction (Bouillot *et al.*, 1996), and several reports have demonstrated the localization of A $\beta$  amyloid peptide to cholesterol-rich lipid rafts (Lee *et al.*, 1998; Simons *et al.*, 1998). Moreover, APP trafficking and metabolism occur in raft fractions, and these may determine neurotoxic A $\beta$  amyloid peptide production. Also, caveolin-3 proteins can directly regulate APP processing by promoting secretase activities in reactive astrocytes (Nishiyama *et al.*, 1999). A possible link between APP processing and caveolin-1 has been proposed, but the precise role of caveolin-1 has not been demonstrated in the pathogenesis of sporadic AD in the elderly.

Previously we demonstrated the presence of caveolin proteins in the 26-month-old rat production by Western blotting (Park *et al.*, 2000). Here, we undertook to identify cell types expressing caveolin-1 in the aged brain, and investigated the role

of caveolin-1 in neurons by over-expressing of caveolin-1 in rat neuroblastoma cells.

## Materials and Methods

### Materials, samples and rats

Human cerebral cortex of the parietal region was obtained from the autopsied brains of middle-aged or elderly donors. Anti-caveolin-1 (clone 2234 and clone 2297) and anti-flotillin (clone 18) antibodies were purchased from Transduction Laboratories (San Diego, CA). Anti-p53 (sc-126), anti- $\beta$ -actin antibody (sc-6246), and anti-PKC $\alpha$  antibody (sc-208) were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Anti-PKC $\epsilon$  antibody (539609) was from Calbiochem, antibodies against phosphorylated PKC (#9371) was from cell signaling. Anti-APP antibody 6E10 was from Senetek (CA) and 22C11 and 6687 were from Chemicon (CA). Secondary horseradish peroxidase conjugated anti-rabbit or anti-mouse antibodies were purchased from Jackson Immunochemicals (West Grove, PA). Other biochemical reagents were from Sigma (St. Louis, MO) and Gibco BRL Life Technologies (Carlsbad, CA). Aged (24 month) Sprague-Dawley rat brains were obtained from Dr. H.-Y. Chung at the Aging Tissue Bank, Pusan National University.

### Western blotting

Total cell or tissue lysates were extracted using extraction buffer (10 mM TrisHCl, pH 7.5; 1 mM EDTA; 150 mM NaCl; 1% Triton X-100; 1 mM PMSF; 2 mg/ml aprotinin; 2 mg/ml leupeptin; 50 mM NaF; 0.2 mM Na<sub>3</sub>VO<sub>4</sub>) and briefly sonicated. Lysates were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose filters (Protran, Schleicher and Schuell Inc., NH). After immunoblotting membranes with primary antibody, immune complexes were incubated with a peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody and finally visualized using an enhanced chemiluminescence detection kit (ECL kit, Amersham Biosciences, IL).

### Electron microscopic analysis and immuno-electron microscopy

The parietal area of the young (6 month) and old (24 month) rat cerebral cortices were isolated and fixed with 3 % glutaraldehyde/PBS at pH 7.4. After washing with 0.2 M sodium cacodylate buffer, pH 7.4, the tissues were treated with 1% osmium tetroxide in cacodylate buffer for 1 h. Specimens

were then dehydrated in graded ethanol steps through propylene oxide and embedded in Embed812 (Electron Microscope Sciences). One-micrometer sections were cut and stained with methylene blue and azure II, and observed under the optical microscope, representative areas were then chosen for ultrathin sectioning, which were cut and stained with uranyl acetate and lead citrate. Sections were observed using a JEM 100 CX transmission electron microscope.

For immuno-electron microscopic analysis, ultrathin frozen sections were labeled with rabbit anti-caveolin-1 antibody followed by 5 nm colloidal gold-conjugated goat anti-rabbit IgG antibody (Amersham), and embedded in methylcellulose (Tokuyasu, 1986).

### Immunohistochemical staining

Twelve adult (4-6 months old) and 15 aged (24-29 months old) Sprague-Dawley rats were perfused transcardially with ice-cold 4% paraformaldehyde for 10 min at a flow rate of 50-60 ml/min. Frozen sections were cut at 40  $\mu$ m in the coronal plane, and incubated using the free-floating method for 48-72 hrs at 4°C in primary antiserum containing Triton X-100 (0.3%), bovine serum albumin (0.5 mg/ml) and normal goat serum (3 drops/10ml). Mouse anti-rat caveolin 1 antibodies were used as primary antibodies and were visualized using an ABC kit (Vectastain<sup>TM</sup>, Vector Labs, CA).

### Isolation of DIG fractions

Brain tissues from young and old rats were prepared in order to isolate the caveolin-enriched membrane fractions described previously (Song *et al.*, 1996). After two washes with ice-cold phosphate-buffered saline, brain tissue, e.g., cerebral cortex, hippocampus, cerebellum, midbrain from young and old rats or cells were scraped into 2 ml of 500 mM sodium carbonate, pH 11.0. Homogenization was carried sequentially in the following order using a loose-fitting Dounce homogenizer (10 strokes), a Polytron tissue grinder, and a sonicator (three 20-s bursts). Homogenates was then adjusted to 45% sucrose by adding 2 ml of 90% sucrose prepared in MBS (25 mM Mes, pH 6.5, 0.15 M NaCl) and then placed at the bottom of an ultracentrifuge tube. A 5-35% discontinuous sucrose gradient was formed above (4 ml of 5% sucrose/4ml of 35% sucrose; both in MBS containing 250 mM sodium carbonate) and then homogenates were centrifuged at 39,000 rpm for 16 h in an SW41 rotor (Beckman Instruments, Palo Alto, CA). A light-scattering band confined to the 5-35% sucrose interface was found to contain caveolin but to exclude the majority of other cellular

proteins. Each fraction was precipitated with TCA and then separated by SDS-PAGE for Western blot analysis.

### Plasmid DNA and transfection

We previously described the cloning of the pCav-1 construct into pcDNA3.1 plasmid vector (Invitrogen) (Park *et al.*, 2000). Here, we introduced this caveolin-1 expression vector into W4 neuroblastoma cells using Lipofectamine (Invitrogen Life Technologies).

## Results

### Up-regulation and neuronal localization of caveolin-1 in aged brain tissues

Caveolin-1 levels were found to be upregulated in each part of dissected aged rat brain (Figure 1A). Anatomically separated cerebral cortex, hippocampus, cerebellum and brain stem of old (26 month) rats were found to express caveolin-1 and p53 more than young (1.5 month) control rats. As shown in Figure 1B, caveolin-1 expression was also detected in elderly (67-90 years old) human brain.

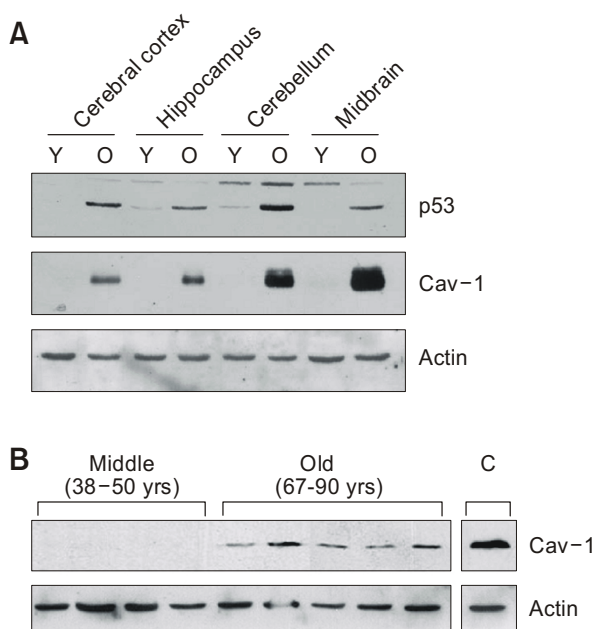
In the cerebral cortex of aged rats, caveolin-1-immunoreactivity was observed to be high in the

neurons of cortical areas and hippocampus (Figure 2). Caveolin-1-positive neurons were evenly distributed in layers II-VI of the cerebral cortex (Figure 2A-E), and were composed of a heterogeneous population of small and medium-sized, multipolar and fusiform cells. The majority of caveolin-1-positive neurons were regularly oriented pyramidal cells. Under high magnification, caveolin-1-immunoreactivity was observed in apical dendrites and in the cytoplasm of pyramidal cells, not in nuclei. Caveolin-1-positive neurons were mainly observed in the frontal and parietal cortices. In hippocampi, multipolar caveolin-1-positive neurons were found in the pyramidal cell layer of CA1, whereas small-sized caveolin-1-positive cells were found in the stratum oriens and stratum radiatum (Figure 2F-J). Some dentate granule cells also showed moderate immunoreactivity. In contrast to aged rats, no caveolin-1-positive cells were observed in the cerebral cortices or hippocampi of young rats. These results suggest that caveolin-1 over-expression proceeds with aging, and that it is localized in neuron-type cells in the aged rat brain.

### Caveolae formation in the aged rat brain

To achieve functional competency, caveolin-1 should be localized to detergent-insoluble glycolipid membrane (DIG) fractions. Thus, extracts from aged brain were isolated and separated by discontinuous sucrose density gradient centrifugation. We used flotillin as a marker for DIG fractions (Bickel *et al.*, 1997) at the interface between 5% and 35% sucrose. Four parts of rat brain, namely, cerebral cortex, cerebellum, midbrain, and brain stem from aged brain revealed the co-localization of flotillin and caveolin-1 in DIG fractions (Figure 3A). Although flotillin was expressed and localized in DIG fractions in both young and aged brains, caveolin-1 was detected only in the DIG fractions of aged brain.

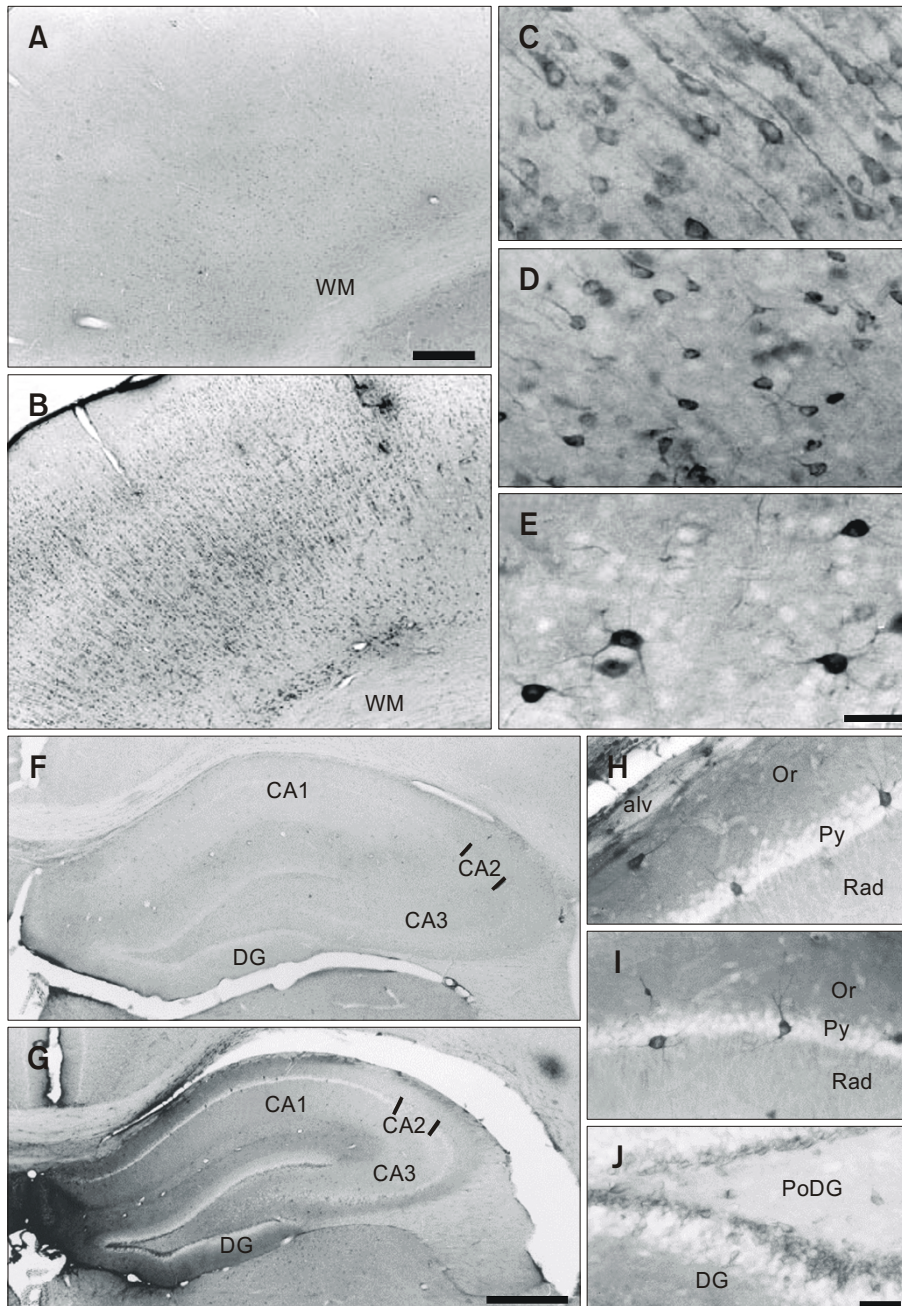
We also checked the functional competency of over-expressed caveolin-1 in aged brain by examining aged rat brain ultra-structure. By electron microscopic analysis, we found < 100 nm vesicle-like, low electron-density structures in the cell bodies of the cerebral cortices of aged rat brain (Figure 3B), and these vesicle-like structures were found to contain caveolin-1 protein by immuno-electron microscopy (Figure 3C). This result indicates that up-regulated caveolin-1 may localize to DIG fractions, but that it also forms caveolae-like structures in aged brain.



**Figure 1.** Caveolin-1 expression is increased in the aged brain. Several brain regions in young (1.5 month) and old (26 month) rats were examined for p53,  $\beta$ -actin, and caveolin-1 expression. (B) The cerebral cortices of middle-aged (38-50 yrs old) and aged (67-90 yrs old) human donors were also examined for caveolin-1 expression.

### Co-localization and altered APP processing by caveolin-1

APP is a likely target of caveolin-1 in senescent neurons. Several reports have been issued on the

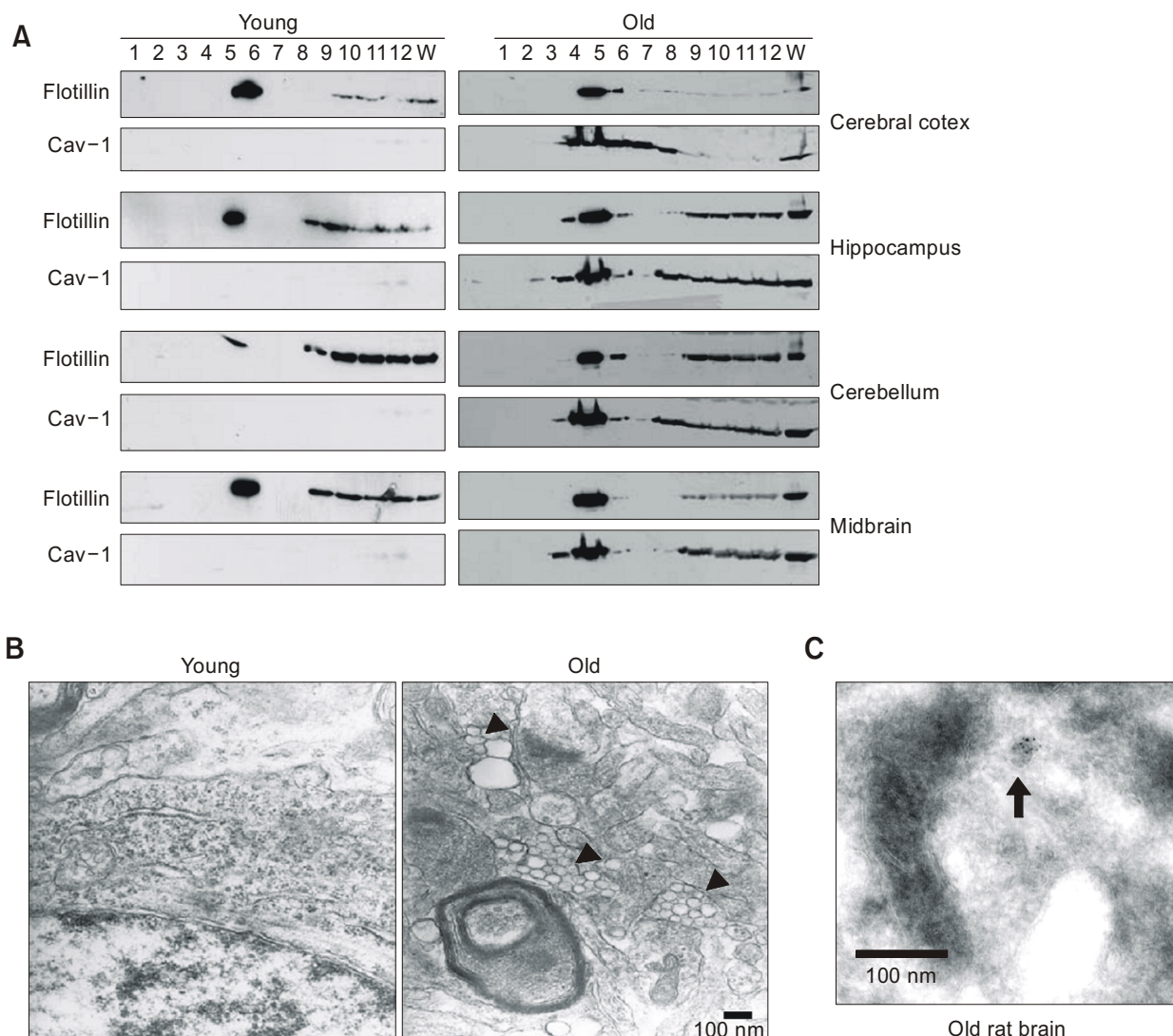


**Figure 2.** Caveolin-immunoreactive neurons in the cerebral cortices and hippocampi of adult and old rats. Caveolin-1 stained positively in the cerebral cortices (A-E) and hippocampi (F-J) of young and aged rats. alv, alveus; CA1-3, fields CA1-3 of Ammon's horn; DG, dentate gyrus; Or, stratum oriens; PoDG, polymorphic layer of dentate gyrus; Py, pyramidal cell layer; Rad, stratum radiatum; WM, subcortical white matter. Scale bar = 250  $\mu$ m (A, B), 25  $\mu$ m (C-E), 500  $\mu$ m (F, G), 25  $\mu$ m (H-J).

localization of APP in DIG fractions (Bouillot *et al.*, 1996). Here, we initially tested the colocalization of APP and caveolin-1 in aged rat brain. As shown in Figure 4A, caveolin-1 upregulation in aged brain was found to be co-localized with APP in caveolae fractions. Moreover, this finding was reproduced in caveolin-1-transfected W4 rat neuroblastoma cells (Figure 4B). Although the association between APP and DIG fractions was not enhanced by caveolin-1, these two proteins were found to be clustered in

caveolae structures in senescent neurons. Moreover, the C-terminal fragment of APP cleaved by  $\beta$ -secretase, namely  $\beta$ CTF, was found in the DIG fractions of caveolin-1-overexpressed neuroblastoma cells (Figure 4C).

We introduced caveolin-1 cDNA into W4 rat neuroblastoma cells expressing wild type APP to produce the aged neuronal state *in vitro*. The transient overexpression of caveolin-1 in neuronal cells reduced the amount of  $\alpha$ -secretase cleavage pro-



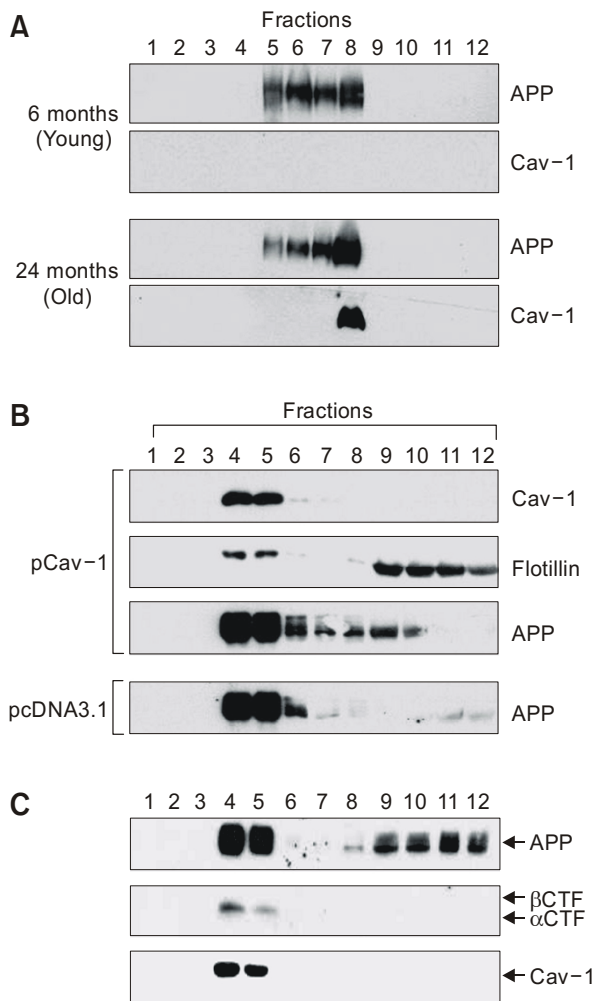
**Figure 3.** Functional analysis of caveolin-1 in the aged brain. (A) Total cell extracts from several parts of young and old rat brains were isolated and separated in a 5-30% sucrose gradient. Each fraction was analyzed for the presence of flotillin or caveolin-1. (B) Cerebral cortices from young and old rat brains were fixed in glutaraldehyde solutions and sectioned properly for EM analysis. Patches of 50-200 nm vesicle-like caveolae structures are indicated by arrows. (C) The localization of caveolin-1 protein in aged rat brain was examined by immuno-electron microscopy. Scale bar = 100 nm

duct,  $\alpha$ APP, in culture media (Figure 5A). Moreover, when the amount of caveolin-1 was gradually increased,  $\alpha$ APP secretion decreased in a dose-dependent manner. On the other hand, the secreted cleavage product of  $\beta$ -secretase was elevated by caveolin-1 over-expression. This result demonstrates that the processing of APP may be altered by the over-expression of caveolin-1 to enhance  $\beta$ -secretase cleavage product production.

#### Down-regulation of PKC activity by caveolin-1

We next investigated the signaling molecules poten-

tially involved in the differential processing of APP,  $\alpha$ APP reduction and the  $s\beta$ APP increase following the introduction of caveolin-1. PKC is known to participate in the processing of amyloid precursor protein (APP) (Gandy and Greengard, 1994). In addition, the scaffolding domain peptides of caveolin-1 and -3 have been reported to interact with protein kinase C and to inhibit the kinase activity and autophosphorylations of protein kinase C- $\alpha$  and - $\zeta$ , but not protein kinase C- $\epsilon$  (Oka *et al.*, 1997). Thus, we examined the phosphorylation of PKC in the absence or presence of caveolin-1. The introduction of caveolin-1 blocked the PKC (pan)



**Figure 4.** Colocalization of caveolin-1 with APP *in vivo* and *in vitro*. (A) Young (6 months old) and old (26 months old) rat brain extracts were prepared in DIG buffer with 2% sodium carbonate and separated in a 5–45% sucrose gradient. Each fraction was analyzed for APP or caveolin-1 by Western blotting. (B) After W4 rat neuroblastoma cells had been transiently transfected with caveolin-1 cDNA, the distribution and localization of APP and caveolin-1 were examined in DIG fractions. (C) The cleavage product of  $\beta$ -secretase,  $\beta$ CTF, was localized by membrane fractionation. Extracts of caveolin-1-transfected W4 cells were separated in a 5–45% sucrose gradient and fractions were analyzed for the localization of  $\beta$ CTF, caveolin-1, and APP.

phosphorylation (Figure 5B).

## Discussion

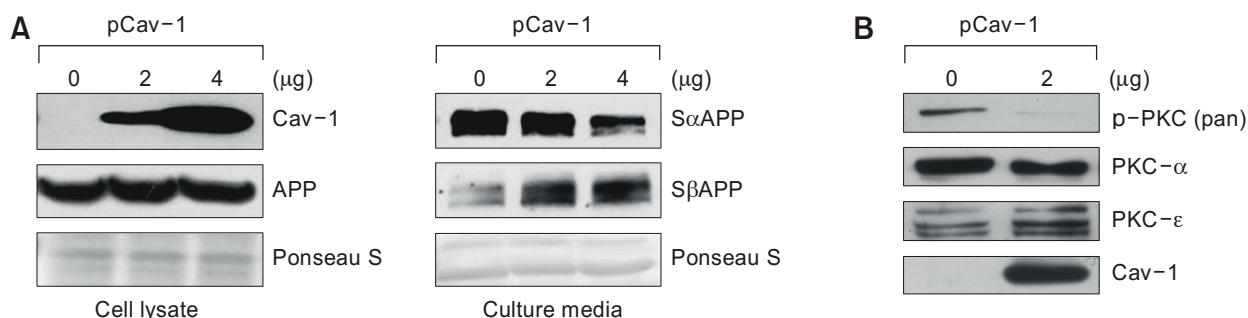
This study shows that caveolin-1 expression increases with aging in human and rat brains. Interestingly, caveolin-1 up-regulation was confined to neuron-type cells in the aged brain (Figure 2). In senescent neurons, up-regulated caveolin-1 was found to be co-localized to caveolae fractions with

APP. Moreover, the  $\beta$ -secretase cleavage product of APP was found to be increased by caveolin-1 over-expression in senescent neurons. Thus, we suggest that the pathogenesis of sporadic Alzheimer's disease in the elderly might be due to the up-regulation of caveolin-1 in neurons.

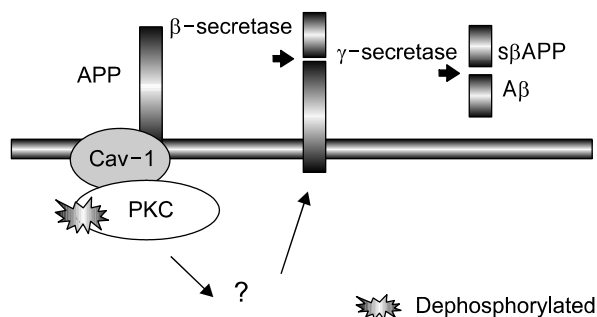
The presence of caveolae structures in neuronal cells like PC-12 or DRG neurons has been reported (Galbiati *et al.*, 1998), but no evidence has been provided concerning the presence of caveolae *in vivo*. In this study, we found both an increase in caveolin levels and in caveolae structures in cortical neurons *in vivo*. In addition, we report for the first time based on immunohistochemical and ultrastructural analysis, that caveolin-1 is expressed and localized in neuronal cells (Figure 2, 3). Because caveolin protein recruits sets of proteins like APP, prion proteins, and various signaling molecules to caveolae, the appearance of caveolin may result in a series of changes in senescent neurons.

Biochemically and ultrastructurally, caveolin-1 was found to form caveolae in senescent neuronal cells (Figure 3), which were localized to axon hillocks or the cell body of various neurons by immunochemical staining and EM analysis. In contrast to a previous report which found that DRG neurons and PC-12 cells show rather different caveolae localizations at plasma membranes (Galbiati *et al.*, 1998), our data show that caveolae conglomerated to form large structures in the cytosol by EM analysis. These structures resembled the type of caveolae previously reported in baculovirus-mediated caveolin overexpression (Li *et al.*, 1996). In addition, it has been reported that caveolin may be found in cytosolic fractions, especially those containing trans-Golgi network (TGN) or somewhat distinct structures, like caveosomes (Dupree *et al.*, 1993; Li *et al.*, 2001). In particular, TGN might confer an environment for APP processing (Lee *et al.*, 1998). Therefore, the formation of caveolae in the cytosol might affect APP processing.

A $\beta$  amyloid peptide is the principal component of senile plaque in the Alzheimer's disease brain. The production of A $\beta$  amyloid peptide is derived from a full-length precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretases (Esler *et al.*, 2001). APP can be alternatively processed by  $\alpha$ -secretase, which cleaves APP in the middle of its A $\beta$  sequence. Moreover, the proteolytic cleavage product of  $\alpha$ -secretase can be secreted into media as sAPP, which precludes the formation of A $\beta$  amyloid peptide. We monitored the levels of APP, sAPP, and of s $\beta$ APP after over-expressing caveolin-1 in rat neuroblastoma cells, and found that caveolin-1 accelerated the processing of APP by  $\beta$ -secretase in neuronal cells (Figure 5A). It is well known that the activation of PKC has positive



**Figure 5.** Increased secretion of the  $\beta$ -secretase cleavage product of APP due to caveolin-1 over-expression in W4 neuroblastoma cells. Caveolin-1 cDNA was introduced into W4 rat neuroblastoma cells at 0, 2, 4  $\mu$ g using Lipofectamine. (A) Culture media were collected after 4 hours and loaded onto SDS-PAGE to check APP quantities and its cleavage products by Western blotting using anti-APP antibody (6E10 for  $s\alpha$ APP or APP, and 22C11 for  $s\beta$ APP). (B) Cell extracts were separated by 12% SDS-PAGE, and proteins were transferred to nitrocellulose paper. Blots were incubated with antibodies directed against phosphorylated PKC, PKC $\alpha$ , PKC $\epsilon$ , or caveolin-1, and then with a peroxidase-labeled goat anti-rabbit or anti-mouse IgG antibody. Proteins were visualized by incubating blots with an enhanced chemiluminescence substrate mixture and x-ray film exposure.



**Figure 6.** A suggested model for the caveolin-1-induced  $s\beta$ APP release.

effects, as reflected by an increase in the non-pathogenic  $s\alpha$ APP or a decrease in  $\beta$ -amyloid (Fuller *et al.*, 1995; Leblanc *et al.*, 1998; Savage *et al.*, 1998). Moreover, PKC as a component of caveolae, interacts with caveolin-1 through the caveolin interacting motif (Couet *et al.*, 1997), which results in inhibition of its activity (Couet *et al.*, 1997; Oka *et al.*, 1997). We observed that PKC phosphorylation decreased as caveolin-1 expression increased (Figure 5C). Although we were not able to classify which subtype of PKC phosphorylation is associated with decreased  $s\alpha$ APP, PKC $\epsilon$  is likely the responsible subtype. Moreover, because PKC $\epsilon$  activation favors the  $\alpha$ -secretase mediated processing of APP (Yeon *et al.*, 2001), caveolin-1 might inhibit the activity of PKC $\epsilon$ , which blocks the  $\alpha$ -secretase-mediated processing of APP, and conversely increases the  $\beta$ -secretase-mediated processing of APP. Based on previous reports, it seems that caveolin-1 acts on PKC to reduce its phosphorylation. As a result, we propose that  $s\alpha$ APP is de-

creased and  $s\beta$ APP is increased via down-regulation of PKC activity by caveolin-1 (Figure 6).

In summary, this study shows that caveolin-1 is associated with APP protein in senescent neurons, and that the over-expression of caveolin-1 induces the processing of APP by  $\beta$ -secretase. The study also shows that  $s\beta$ APP up-regulation by caveolin-1 occurs via a PKC down-regulation (Figure 6). Furthermore, our results indicate that it is possible that the rate of Alzheimer's disease progression can be determined by measuring caveolin-1 expression in the aged.

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### References

- Bickel PE, Scherer PE, Schnitzer JE, Oh P, Lisanti MP, Lodish HF. Flotillin and epidermal surface antigen define a new family of caveolae-associated integral membrane proteins. *J Biol Chem* 1997;272:13793-802
- Bouillot C, Prochiantz A, Rougon G, Allinquant B. Axonal amyloid precursor protein expressed by neurons in vitro is present in a membrane fraction with caveolae-like properties. *J Biol Chem* 1996;271:7640-4
- Couet J, Li S, Okamoto T, Ikezu T, Lisanti MP. Identification of peptide and protein ligands for the caveolin-scaffolding domain. Implications for the interaction of caveolin with caveolae-associated proteins. *J Biol Chem* 1997;272:6525-33
- Couet J, Sargiacomo M, Lisanti MP. Interaction of a receptor ty-

- rosine kinase, EGF-R, with caveolins: caveolin-binding negatively regulates tyrosine and serine/threonine kinase activities. *J Biol Chem* 1997;272:30429-38
- Dupree P, Parton RG, Raposo G, Kurzchalia TV, Simons K. Caveolae and sorting in the trans Golgi network of epithelial cells. *EMBO J* 1993;12:1597-605
- Ehehalt R, Keller P, Haass C, Thiele C, Simons K. Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *J Cell Biol* 2003;160:113-23
- Esler WP, Wolfe MS. A portrait of Alzheimer secretases—new features and familiar faces. *Science* 2001;293:1449-54
- Fuller SJ, Storey E, Li QX, Smith AI, Beyreuther K, Masters CL. Intracellular production of beta A4 amyloid of Alzheimer's disease: modulation by phosphoramidon and lack of coupling to the secretion of the amyloid precursor protein. *Biochemistry* 1995;34:8091-8
- Galbati F, Volonte D, Gil O, Zanazzi G, Salzer JL, Sargiacomo M, Scherer PE, Engelman JA, Schlegel A, Parenti M, Okamoto T, Lisanti MP. Expression of caveolin-1 and -2 in differentiating PC12 cells and dorsal root ganglion neurons: caveolin-2 is up-regulated in response to cell injury. *Proc Natl Acad Sci USA* 1998;95:10257-62
- Gandy S, Greengard P. Processing of Alzheimer A beta-amyloid precursor protein: cell biology, regulation, and role in Alzheimer disease. *Int Rev Neurobiol* 1994;36:29-50
- Howland DS, Trusko SP, Savage MJ, Reaume AG, Lang DM, Hirsch JD, Maeda N, Siman R, Greenberg BD, Scott RW, Flood DG. Modulation of secreted beta-amyloid precursor protein and amyloid beta-peptide in brain by cholesterol. *J Biol Chem* 1998;273:16576-82
- Kaunitz H. Adaptive changes in aging and arteriosclerosis—role of cholesterol. *Mech Ageing Dev* 1988;44:35-43
- LeBlanc AC, Koutroumanis M, Goodyer CG. Protein kinase C activation increases release of secreted amyloid precursor protein without decreasing A beta production in human primary neuron cultures. *J Neurosci* 1998;18:2907-13
- Lee SJ, Liyanage U, Bickel PE, Xia W, Lansbury PT Jr, Kosik KS. A detergent-insoluble membrane compartment contains A beta *in vivo*. *Nat Med* 1998;4:730-4
- Li S, Song KS, Koh SS, Kikuchi A, Lisanti MP. Baculovirus-based expression of mammalian caveolin in Sf21 insect cells. A model system for the biochemical and morphological study of caveolae biogenesis. *J Biol Chem* 1996;271:28647-54
- Li WP, Liu P, Pilcher BK, Anderson RG. Cell-specific targeting of caveolin-1 to caveolae, secretory vesicles, cytoplasm or mitochondria. *J Cell Sci* 2001;114:1397-408
- Mizuno T, Nakata M, Naiki H, Michikawa M, Wang R, Haass C, Yanagisawa K. Cholesterol dependent generation of a seeding amyloid beta-protein in cell culture. *J Biol Chem* 1999;274:15110-4
- Nishiyama K, Trapp BD, Ikezu T, Ransohoff RM, Tomita T, Iwatsubo T, Kanazawa I, Hsiao KK, Lisanti MP, Okamoto T. Caveolin-3 upregulation activates beta-secretase-mediated cleavage of the amyloid precursor protein in Alzheimer's disease. *J Neurosci* 1999;19:6538-48
- Oka N, Yamamoto M, Schwencke C, Kawabe J, Ebina T, Ohno S, Couet J, Lisanti MP, Ishikawa Y. Caveolin interaction with protein kinase C. Isoenzyme-dependent regulation of kinase activity by the caveolin scaffolding domain peptide. *J Biol Chem* 1997;272:33416-21
- Okamoto T, Schlegel A, Scherer PE, Lisanti MP. Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. *J Biol Chem* 1998;273:5419-22
- Park WY, Park JS, Cho KA, Kim DI, Ko YG, Seo JS, Park SC. Up-regulation of caveolin attenuates epidermal growth factor signaling in senescent cells. *J Biol Chem* 2000;275: 20847-52
- Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurti K, Duff K, Pappolla MA. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis* 2000;7:321-31
- Refolo LM, Pappolla MA, LaFrancois J, Malester B, Schmidt SD, Thomas-Bryant T, Tint GS, Wang R, Mercken M, Petanceska SS, Duff KE. A cholesterol-lowering drug reduces betaamyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis* 2001;8:890-9
- Savage MJ, Trusko SP, Howland DS, Pinsker LP, Mistretta S, Reaume AG, Greenberg BD, Siman R, Scott RW. Turnover of amyloid beta-protein in mouse brain and acute reduction of its level by phorbol ester. *J Neurosci* 1998;8:1743-52
- Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K. Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci USA* 1998;95:6460-4
- Song KS, Li S, Okamoto T, Quilliam LA, Sargiacomo M, Lisanti MP. Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caveolae microdomains. *J Biol Chem* 1996;271:9690-7
- Stephens DJ, Walters CE, Davies H, Austen BM. The role of cholesterol in the biosynthesis of beta-amyloid. *Neuroreport* 1999;10:1699-705
- Tokuyasu KT. Application of cryoultramicrotomy to immunocytochemistry. *J Microsc* 1986;143:139-49
- Wheaton K, Sampsel K, Boisvert FM, Davy A, Robbins S, Riabowol K. Loss of functional caveolae during senescence of human fibroblasts. *J Cell Physiol* 2001;187:226-35
- Yeon SW, Jung MW, Ha MJ, Kim SU, Huh K, Savage MJ, Masliah E, Mook-Jung I. Blockade of PKC epsilon activation attenuates phorbol ester-induced increase of alpha-secretase-derived secreted form of amyloid precursor protein. *Biochem Biophys Res Commun* 2001;280:782-7