Clinical significance of Caveolin-1, Caveolin-2 and HER2/neu mRNA expression in human breast cancer

Y Sagara1, K Mimori2, K Yoshinaga2, F Tanaka2, K Nishida2, S Ohno1, H Inoue2 and M Mori*2
1Department of Breast Oncology, National Kyushu Cancer Center, 3-1-1 Notame, Minami-Ku, Fukuoka 811-1395, Japan; 2Department of Surgery, Medical Institute of Bioregulation, Kyushu University, 4546 Tsurumihara, Beppu 874-0838, Japan

Caveolin-1 and -2 (CAV1, CAV2) are closely linked genes localised to the fragile region of 7q31 (FRA7G), and loss of heterozygosity involving this region has been reported in breast cancer. Several studies have suggested that CAV1 is a negative regulator of HER2/neu signal transduction in vitro. However, the clinical significance of CAV1 in breast cancer has not yet been clarified. We examined quantitatively the mRNA levels of CAV1, CAV2 and HER2/neu in 162 cases of breast cancer using real-time PCR. Caveolin-1 and -2 protein expression was also examined by Western blotting and immunohistochemistry. We then evaluated for correlations between CAV1, CAV2 and HER2/neu gene expression and clinicopathologic factors in the 162 breast cancer cases. Results showed higher HER2/neu mRNA levels and lower CAV1 and CAV2 mRNA levels in breast cancer tissues than in corresponding normal tissues (P<0.001). Caveolin-1 and -2 protein expression levels were also suppressed in cancer tissues compared to normal tissues by Western blotting. Immunohistochemistry revealed that CAV1 and CAV2 proteins were abundantly expressed in mammary gland myoepithelial cells, but only weakly in ductal epithelial cells. Reduced CAV1 mRNA level was significantly associated with increasing tumour size (P=0.041), and negative oestrogen receptor status (P=0.021). There was also a significant association between low CAV2 mRNA level and negative progesterone receptor status (P=0.013), and between high HER2/neu mRNA level and negative hormonal receptor status (ER, P=0.029; PgR, P=0.019). While there was no relationship between HER2/neu and CAV1 mRNA levels, a significant association between CAV1 and CAV2 mRNA levels was observed (P<0.001). Our results indicated that CAV1 suppression correlated closely with that of CAV2 in breast cancer, that CAV1 level was inversely correlated with tumour size, and that CAV1 and CAV2 levels were correlated with hormonal receptor status. Therefore, CAV1 and CAV2 play an important role in tumour progression in breast cancer patients.

British Journal of Cancer (2004) 91, 959–965. doi:10.1038/sj.bjc.6602029 www.bjcancer.com
Published online 10 August 2004 © 2004 Cancer Research UK

Keywords: caveolin; HER2/neu; breast cancer; trastuzumab; tumour size; hormonal receptor

Caveolae mediate molecular transport, cell adhesion and signal transduction activities in the cell (Couet et al, 1997; Zhang et al, 2000; Razani et al, 2001). Caveolin-1 (CAV1), the major coat protein of caveolae, has been reported to interact with various intracellular signalling molecules including growth factors such as epidermal growth factor receptor (EGFR) (Couet et al, 1997; Engelman et al, 1998; Kim et al, 2000) and oestrogen receptor (ER) (Razandi et al, 2002; Zschocke et al, 2002). Some studies have suggested that caveolin may function as a negative regulators of signal transduction to HER2/neu, member of the EGF family. In recent years, trastuzumab (Herceptin®) has been used as the first-line agent in the treatment of recurrent breast cancers that overexpress HER2/neu. Interestingly, some breast cancer patients with HER2/neu-overexpressing tumours do not respond well to trastuzumab treatment.

Several lines of evidence have suggested that caveolin expression in malignancies may be clinically significant. For example, CAV1 expression is increased in prostate cancer (Yang et al, 1999), oesophageal cancer (Kato et al, 2002) and ovarian cancer (Davidson et al, 2001), but reduced in colon cancer (Bender et al, 2000), lung cancer (Ho et al, 2002) and sarcoma (Wiechen et al, 2001). In the present study, we investigated the mRNA levels of CAV1, Caveolin-2 (CAV2) and HER2/neu in breast cancer tissues from 162 cases by the real-time PCR to evaluate the clinical significance of these genes.

MATERIALS AND METHODS

Surgical specimens

A total of 162 female cases of breast cancer were available for study and included 93 cases with both tumour and corresponding normal specimens, and 69 cases with only tumour specimens. Surgery was performed from October 1993 to October 1999 at the Medical Institute of Bioregulation, Kyushu University Beppu, and Oita Prefectural Hospital, Oita, Japan. Experimental protocols were authorised by a Bioethics Committee and informed consent was obtained from all patients in the study. The mean patient age was
55 years (range, 27–84 years), and median postoperative follow-up was 41 months (range, 1–96 months). Hormonal status was identified in 125 cases. All tumours are histopathologically diagnosed as breast cancer. To avoid degeneration, cancer tissue centres and corresponding cancer-free tissues were snap frozen in liquid nitrogen immediately after excision, handled carefully to avoid contamination with RNase, and stored at −80 °C until use. mRNA quality was assessed in several representative cases using a Bioanalyzer (Agilent Technologies, Japan) to exclude unsuitable samples. Results showed samples were appropriate for further study.

Cell culture
Human breast cancer cell lines, MCF-7, YMB-1, SKBR-3, MRKnu-1 and CRL-1500 were obtained from the Cell Resource Center at the Biochemical Research Institute of Development, Aging and Cancer (Tohoku University, Sendai, Japan). YMB-1 was maintained in DMEM and MCF-7, SKBR-3, MRKnu-1 and CRL-1500 were maintained in RPMI 1640. All cell lines were supplemented with 10% FBS and incubated at 37 °C in a 5% humidified CO2 atmosphere.

Preparation of cDNA from tissue specimens
Total RNA was extracted using the acid guanidinium thiocyanate/phenol/chloroform extraction (AGPC) method. All samples were treated with DEPC in Eppendorf tubes (Eppendorf, Germany) and handled with gloves to avoid RNase contamination. Total RNA aliquots were reverse transcription into cDNA using oligo-dT primers.

Quantitative analysis of mRNA of CAV1, 2 and HER2/neu
Reverse transcriptase reactions were performed as previously described (Mori et al., 1993). Real-time PCR was performed using the iCycler iQ detection system (Bio-Rad, Tokyo, Japan) and IQ SYBR Green Supermix. Reactions were performed in 96-well plates with Optical-Quantity 8-tube Strips (Bio-Rad). The primer sequences for HER2/neu, CAV1, 2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are detailed in Table 1. The reactions for both of them were subjected to 40 cycles for 30 s at 95 °C, 1 min at 60 °C, and 1 min at 72 °C. Increases in fluorescence were measured in real time during the extension step.

Fluorescence in situ hybridisation (FISH) of HER2/neu
Fluorescence in situ hybridisation on breast cancer cell lines was performed at Ohtsuka Assay Inc., according to the PathVysion (Vysis, Inc., Illinois, USA) protocol. Briefly, cultured breast cancer cell lines on microscope slides were hydrated with Hemo-De clearing agent (Vysis, Inc.) and a graded alcohols series. Slides were air dried, pretreated (80 °C, 30 min), and digested with protease (37 °C, 10–20 min) before hybridising with fluorescentlabelled probes for the HER-2/neu gene and chromosome 17 alpha-satellite DNA. Probes were premixed and predenatured in hybridisation buffer for ease of use. Nuclei were counterstained with intercalating fluorescent counterstain 4′,6-diamidino-2′-phenylindole (DAPI). HER2 amplification ratio was calculated as: HER2 total signal count/chromosome 17 total signal count. Presence of HER2 amplification was defined as an HER2 amplification ratio of greater than 2.00.

Western blotting and immunohistochemistry for CAV1 and CAV2
For Western blot analysis, proteins were extracted from normal and tumour tissues in five representative breast cancer cases. Proteins were subjected to SDS–PAGE in 15% acrylamide gels under reducing conditions and transferred to Immobilon-P membranes (Millipore, Bedford, MA, USA). After blocking with 5% nonfat dry milk and 0.05% Tween-20 in PBS, blots were incubated with CAV1 (BD Biosciences, CA, USA) and CAV2 (Santa Cruz, CA, USA) mAb, used as culture supernatants diluted 1:2. After several washings, blots were incubated for 1 h with goat anti-mouse IgG (1:5000) coupled to horseradish peroxidase, washed extensively, and developed using a chemiluminescence Western blot kit (ECL, Amersham, Buckinghamshire, UK).

For immunohistochemistry, surgically resected specimens from the same five cases of breast cancer tissues as used above and the corresponding normal tissues were stored at −20 °C until use. Frozen sections (4 μm) were put onto silicon-coated glass slide and stained with monoclonal antibodies, as above. Staining for CAV1 and CAV2 was performed on adjacent sections. Immunohistochemical staining was performed as previously described (Mori et al., 2000) using serum specific for human monoclonal CAV1 and 2 antibodies.

Statistical analysis
mRNA levels of CAV1, CAV2 and HER2/neu were calculated with respect to GAPDH mRNA level. The 162 cases were divided into high and low mRNA groups (with 81 in each group), according to mRNA level in each breast cancer (Figure 1), and correlation between mRNA expression level and clinicopathologic factor were analysed. The predictive value of mRNA expression level for clinicopathologic variables such as age, size of tumour, lymph node metastasis, lymph vessel invasion, vascular vessel invasion, oestrogen or progesterone receptor status were univariately tested using a χ2 test. Correlation of each mRNA level was analysed using the log rank test, and the cumulative survival rate was calculated by the Kaplan–Meier method. All analyses were performed using statistical software (Statview version 5.0; SAS Institute, Inc., Cary, NC, USA), with a P-value of less than 0.05 considered statistically significant.

RESULTS
Expression levels of CAV1, CAV2 and HER2/neu in normal breast tissue, breast cancer tissue and cell lines
Low levels of CAV1 mRNA were detected in several breast cancer cell lines (Figure 2), with CAV1 and CAV2 mRNA levels significantly correlated with each other (r2 = 0.927). The SKBR-3 cell line exhibited the highest HER2/neu mRNA level of the breast cancer cell lines tested (SKBR-3, YMB-1, CRL-1500, MCF-7, MRKnu-1), and FISH detected HER2/neu overexpression only in SKBR-3 cells (Figure 3). Thus, HER2/neu mRNA levels were concordant with HER2/neu FISH results. Comparison of breast...
Cancer samples and corresponding normal breast tissues for 93 cases showed higher HER2/neu mRNA levels and lower CAV1 and CAV2 mRNA levels (P<0.001) (Figure 4).

We preliminarily confirmed the concordant expression between caveolins at the mRNA level by quantitative real-time PCR, and at protein level by Western blot analysis in five representative breast cancer samples (Figure 5). CAV1 and CAV2 protein levels were downregulated in four representative breast cancer tissues compared to corresponding normal breast tissues.

Immunohistochemical assays showed that the expression of both caveolins was more abundant in the myoepithelial cells of the...
mammary duct than ductal epithelial cell in normal breast tissues, while fewer expressions of CAV1 and CAV2 proteins were detected in cancer tissues (data not shown).

Clinicopathologic significance of HER2/neu and CAV1, 2

As shown in Table 2, HER2/neu mRNA level showed an association with hormonal receptor status (HER2/ER, P = 0.029, HER2/PgR, P = 0.019), and the high CAV1 mRNA level group tended to exhibit small tumour size (P = 0.041) and positive ER status (P = 0.021). Furthermore, there was a significant association between high CAV2 mRNA level and positive progesterone receptor status (P = 0.013). However, there were no significant differences in gene expression with respect to disease-free survival (HER2, P = 0.745; CAV1, P = 0.520; CAV2, P = 0.740). HER2/neu mRNA level was not associated with that of CAV1 or CAV2 mRNA levels in breast cancer samples (HER2/CAV1, P = 0.758; HER2/CAV2, P = 0.755) (Table 3). In contrast, CAV1 mRNA level was significantly associated with CAV2 mRNA level (P < 0.001). There were no significant associations with respect to histopathologic classification in the 162 breast cancer cases.

DISCUSSION

CAV1 has been reported to interact with various intracellular signalling pathways and is thought to suppress tumour growth in breast cancer cell lines (Lee et al, 1998; Razani et al, 2001; Fiucci et al, 2002). Prior studies have suggested that oncogenic transformation results in reduced cellular levels of caveolin (Glenney and Soppet, 1992; Sager et al, 1994; Koleske et al, 1995), and that this reduction probably contributes to a loss of caveolae (Koleske et al, 1995). Lee et al (1998) found that the CAV1 levels were inversely correlated to breast cancer progression in vitro and the overexpression of CAV1 resulted in substantial growth inhibition of breast tumour cells, which normally had no endogenous caveolin expression.

Our study confirmed that mRNA level of CAV1 and CAV2 were significantly downregulated in human breast cancer tissues compared to corresponding normal tissues (P < 0.001), and that CAV1 and CAV2 mRNA levels were significantly correlated with each other in breast cancer cell lines and tissues. Hurlstone et al reported that CAV1 was expressed in the normal breast tissue by myoepithelial cells but not by ductal epithelial cells. This is in agreement with our present study that observed much more CAV1 and CAV2 expression in myoepithelial cells than in ductal epithelial cells in normal breast tissue by immunohistochemistry. CAV1-null mice show a striking increase in the frequency and size of multifocal dysplastic lesions in mammary grans, with the nuclei and the nuclei of mammary grans showing anaplastic characteristics with increased mitotic figures (Williams et al, 2003). This suggests that CAV1-mediated interactions between myoepithelial cells and the rest of mammary grans may play an important role in oncogenesis. In addition, our study found that breast cancer patients with tumours that expressed low levels of CAV1 mRNA tended to have larger tumour sizes, which supported the hypothesis that CAV1 acts as a growth suppressor in breast tumours (Lee et al, 1998).

There was a significant correlation between high CAV1 and CAV2 mRNA level and positive hormonal receptor status. Razandi...
et al reported that E2 stimulates the synthesis of CAV1 and CAV2 proteins and activated a CAV1 promoter/luciferase reporter construct transfected into smooth muscle cells. CAV1 also stimulated ER translocation to the cell membrane in MCF-7 cells, inhibiting E2-induced ERK (MAPK) activation, required for DNA synthesis and cell survival (Razandi et al., 2002). Thus, our results support the hypothesis that CAV1 and CAV2 play an important role in the process of hormonal translocation.

Our present results also suggested that HER2/neu level and hormonal receptor expression was inversely correlated. This finding is supported by the Southwest Oncology Group Study (Elledge et al., 1998; Ferrero-Pous et al., 2000; Konecny et al., 2003). While previous studies have reported HER2/neu to be a poor prognostic factor (Slamon et al., 1989; Quenel et al., 1995), neither CAV1, CAV2, nor HER2/neu appeared to be a predictor of disease outcome in our study. This may be due to the average follow-up period in our study being only 1141 days, such that few disease recurrences were observed. Thus, future studies may require longer observation periods and higher patient numbers for analysis.

Several mechanisms could be involved in the suppression of CAV gene expression observed in our study, including loss of heterozygosity (LOH) (Tatarelli et al., 2000; Zenklusen et al., 2001; Lee et al., 2002), point mutations (Hayashi et al., 2001) and methylation (Engelman et al., 1999). As for LOH, several LOH markers are located near the CAV1 gene locus on human chromosome 7q31. Loss of heterozygosity is frequently encountered in this region in a variety of human neoplasias, indicating the presence of a tumour-suppressor gene. With regard to mutation, Hayashi et al. (2001) reported CAV1 gene mutations in approximately 16% of human breast cancers, and that these mutations may play a role in malignant progression. Although CAV1 expression is increased in prostate cancer (Yang et al., 1999), oesophageal cancer (Kato et al., 2002) and ovarian cancer (Davidson et al., 2001), future studies should include assessment

---

**Table 2** Relationship between clinicopathologic characteristics and expressions of caveolin (CAV)1, caveolin (CAV)2 and HER2

| CAV1 expression | CAV2 expression | HER2 expression |
|-----------------|-----------------|-----------------|
| Low            | High            | P-value | Low            | High            | P-value | Low            | High            | P-value |
| Age (years)     |                 |          |                |                 |          |                |                 |          |
| ≥ 55           | 39              | 42      | NS (0.530)     | 39              | 41      | NS (0.753)     | 35              | 45      | NS (0.116) |
| < 55           | 42              | 39      |                 | 42              | 41      |                 | 46              | 36      |          |
| Size (cm)       |                 |          |                |                 |          |                |                 |          |          |
| ≥ 2.0          | 44              | 31      | 0.041*         | 43              | 32      | NS (0.083)     | 37              | 38      | NS (0.875) |
| < 2.0          | 37              | 50      |                 | 38              | 49      |                 | 44              | 43      |          |
| Menopause      |                 |          |                |                 |          |                |                 |          |          |
| Pre            | 31              | 27      | NS (0.512)     | 27              | 30      | NS (0.743)     | 32              | 26      | NS (0.326) |
| Post           | 50              | 54      |                 | 53              | 51      |                 | 49              | 55      |          |
| n              |                 |          |                |                 |          |                |                 |          |          |
| Positive       | 43              | 40      | NS (0.753)     | 42              | 36      | NS (0.355)     | 41              | 37      | NS (0.529) |
| Negative       | 38              | 41      |                 | 39              | 45      |                 | 40              | 44      |          |
| Ly             |                 |          |                |                 |          |                |                 |          |          |
| Positive       | 37              | 37      | NS (1.000)     | 40              | 34      | NS (0.344)     | 35              | 39      | NS (0.528) |
| Negative       | 44              | 44      |                 | 41              | 47      |                 | 46              | 42      |          |
| V              |                 |          |                |                 |          |                |                 |          |          |
| Positive       | 12              | 15      | NS (0.527)     | 17              | 10      | NS (0.140)     | 13              | 14      | NS (0.833) |
| Negative       | 69              | 66      |                 | 36              | 41      |                 | 68              | 67      |          |
| ER             |                 |          |                |                 |          |                |                 |          |          |
| Positive       | 34              | 47      | 0.021*         | 35              | 46      | NS (0.053)     | 46              | 35      | 0.029*    |
| Negative       | 28              | 16      |                 | 27              | 17      |                 | 16              | 28      |          |
| PgR            |                 |          |                |                 |          |                |                 |          |          |
| Positive       | 35              | 45      | NS (0.081)     | 33              | 47      | 0.013*         | 46              | 34      | 0.019*    |
| Negative       | 27              | 18      |                 | 29              | 16      |                 | 16              | 29      |          |

*Statistical significant differences were observed between ER or PgR status and Her2 expression, between size of tumours or ER status and CAV1 expression, between PgR status and CAV2 expression. NS = not significant; n = lymph node metastasis; Ly = lymph vessel permeation; V = vascular vessel permeation.

**Table 3** Relationship among expressions of HER2, CAV1 and CAV2 in 162 cases of breast cancer

| CAV1 high | CAV1 low | Total |
|-----------|----------|-------|
| HER2 high | 42       | 39    | 81    |
| HER2 low  | 39       | 42    | 81    |
| Total     | 81       | 81    | 162   |

| CAV2 High | CAV2 low | Total |
|-----------|----------|-------|
| HER2 high | 40       | 41    | 00    |
| HER2 low  | 41       | 40    | 00    |
| Total     | 81       | 81    | 162   |

| CAV1 High | CAV1 Low | Total |
|-----------|----------|-------|
| HER2 high | 59       | 22    | 00    |
| HER2 low  | 22       | 59    | 00    |
| Total     | 81       | 81    | 162   |

*Statistical significant differences were observed between ER or PgR status and Her2 expression, between size of tumours or ER status and CAV1 expression, between PgR status and CAV2 expression. NS = not significant; n = lymph node metastasis; Ly = lymph vessel permeation; V = vascular vessel permeation.

© 2004 Cancer Research UK
of CAV1 mutation. As for methylation, Engelman et al. (1999) reported that the first and second exons of the CAV1 and CAV2 genes are embedded within CpG islands, such that it is possible that caveolin gene expression is controlled, at least in part, by methylation of these CpG regions. Zihui et al. reported that growth factor receptor stimulation activated the phosphatidylinositol 3'-kinase and β-catenin pathways in mammary epithelial cells. β-Catenin activates cyclin D1 and c-Myc transcription, and subsequently, c-Myc suppressing caveolin-1 transcription (Xie et al., 2003). Furthermore, some studies have shown that activation of HER2/neu downregulates CAV1 expression in vivo (Couet et al., 1997; Kim et al., 2000), while other studies have reported the negative regulation of expression and signal transduction between CAV1 and HER2/neu (Engelman et al., 1998; Zhang et al., 2000). However, our present study revealed no such correlations in vivo. This may be due to a discrepancy between experimental and clinical studies.

**REFERENCES**

Bender FC, Reymond MA, Bron C, Quest AF (2000) Caveolin-1 levels are down-regulated in human colon tumors, and ectopic expression of caveolin-1 in colon carcinoma cell lines reduces cell tumorigenicity. *Cancer Res* 60: 5870–5878

Couet J, Sargiacoamento M, Lisanti MP (1997) 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

Davison B, Neuland JM, Goldberg I, Polovich J, Gotlieb HW, Bryne M, Ben-Baruch G, Berner A, Reich R (2001) Caveolin-1 expression in advanced-stage ovarian carcinoma – a clinicopathologic study. *Gynecol Oncol* 81: 166–171

Elledge RM, Green S, Ciocca D, Pugh R, Allred DC, Clark GM, Hall J, Ravdin P, O'Sullivan J, Martino S, Osborne CK (1998) HER-2 expression and response to tamoxifen in estrogen receptor-positive breast cancer: a Southwest Oncology Group Study. *Clin Cancer Res* 4: 7–12

Engelman JA, Lee RJ, Karnezis A, Bearss DJ, Webster M, Siegel P, Muller WJ, Windle JJ, Jessup M, Lisanti MP (1998) 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: 20419–20455

Engelman JA, Zhang XL, Lisanti MP (1999) Sequence and detailed organization of the human caveolin-1 and -2 genes located near the D7S522 locus (7q31.1). Methylation of a CpG island in the 5' region of the caveolin-1 gene in human breast cancer cell lines. *FEBS Lett* 448: 221–230

Ferrero-Pous M, Hacene K, Bouchet C, Le Doussal V, Tubiana-Hulin M, Spyratos F (2000) Relationship between c-erbB-2 and other tumor characteristics in breast cancer prognosis. *Clin Cancer Res* 6: 4745–4754

Fiucci G, Ravid D, Reich R, Liscovitch M (2002) Caveolin-1 inhibits anchorage-independent growth, anoikis and invasiveness in MCF-7 human breast cancer cells. *OncoGene* 21: 2365–2375

Glenney Jr JR, Soppe D (1992) Sequence and expression of caveolin, a protein component of caveole plasma membrane domains phosphorylated on tyrosine in Rous sarcoma virus-transformed fibroblasts. *Proc Natl Acad Sci USA* 89: 10517–10521

Hayashi K, Matsuda S, Machida K, Yamamoto T, Fukuda Y, Nimura Y, Hayakawa T, Hamaguchi M (2001) Invasion activating caveolin-1 in advanced-stage tumor-prone transgenic mice. *Am J Pathol* 161: 1647–1656

Kato K, Hida Y, Miyamoto M, Hashida H, Shinohara T, Itoh T, Okushiba S, Kondo S, Katoh H (2002) Overexpression of caveolin-1 in esophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. *Cancer* 94: 929–933

Kim YN, Wiep JG, Guadarrama AG, Bertsic PJ (2000) Epidermal growth factor-stimulated tyrosine phosphorylation of caveolin-1. Enhanced caveolin-1 tyrosine phosphorylation following aberrant epidermal growth factor receptor status. *J Biol Chem* 275: 7481–7491

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

Konecny G, Puletti G, Pegram M, Unctch D, Danekar S, Aguilar Z, Wilson C, Rong HM, Bauerfeind I, Felber M, Wang H, Berit M, Seshadri R, Hepp H, Slamon DJ (2003) Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. *J Natl Cancer Inst* 95: 142–153

Lee H, Park DS, Razani B, Russell RG, Pesta RG, Lisanti MP (2002) Caveolin-1 mutations (P123L and null) and the pathogenesis of breast cancer: caveolin-1 (P123L) behaves in a dominant-negative manner and caveolin-1 (−/−) null mice show mammary epithelial cell hyperplasia. *Am J Pathol* 161: 1357–1369

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

Mori M, Barnard GF, Saniouan JS, Jessup M, Steele Jr GD, Chen LB (1993) Prothymosin-alpha mRNA expression correlates with that of c-myc in human colon cancer. *Oncogene* 8: 2821–2826

Mori M, Mimori K, Shiraiishi T, Alder H, Inoue H, Tanaka Y, Sugimachi K, Huebner K, Croce CM (2000) Altered expression of Fhit in carcinoma and precarcinomatous lesions of the esophagus. *Cancer Res* 60: 1177–1182

Quenel N, Waflart J, Bonichon F, de Mascarel I, Trajani M, Durand M, Avril A, Coindre JM (1995) The prognostic value of c-erbB2 in primary breast carcinomas: a study on 942 cases. *Breast Cancer Res Treat* 35: 283–291

Razandi M, Oh P, Pedram A, Schnitzer J, Levin ER (2002) ERs associate with and regulate the production of caveolin: implications for signaling and cellular actions. *Mol Endocrinol* 16: 100–115

Razani B, Schlegel A, Liu J, Lisanti MP (2001) Caveolin-1, a putative tumour suppressor gene. *Biochem Soc Trans* 29: 494–499

Sager R, Sheng S, Anisowicz A, Sotirioupolou G, Zou Z, Stenman G, Swisshelm K, Chen Z, Hendrix MJ, Pemberton P (1994) RNA genetics of breast cancer: maspin as paradigm. *Cold Spring Harb Symp Quant Biol* 59: 537–546

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Avril A, Coindre JM (1995) The prognostic value of c-erbB2 in primary breast carcinomas: a study on 942 cases. *Science* 244: 707–712

Tatarella C, Linnenbach A, Mimori K, Croce CM (2000) Characterization of the human TESTIN gene localized in the FRA7G region at 7q31.2. *Genomics* 68: 1–12

Wiechen K, Sers C, Agoudik A, Arlt K, Dietel M, Schlag PM, Schneider U (2001) Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. *Am J Pathol* 158: 833–839

Williams TM, Cheung MW, Park DS, Razani B, Cohen AW, Muller WJ, Di Vizio D, Chopra NG, Pesta RG, Lisanti MP (2003) Loss of caveolin-1 gene expression accelerates the development of dysplastic mammary lesions in tumor-prone transgenic mice. *Mol Biol Cell* 14: 1027–1042

In summery, our results revealed that CAV1 suppression correlated closely with that of CAV2 in breast cancer, that CAV1 level was inversely correlated with tumour size, and that CAV1 and CAV2 levels were correlated with hormonal receptor status. Therefore, CAV1 and CAV2 play an important role in tumour progression in breast cancer patients.

**ACKNOWLEDGEMENTS**

We thank Drs Ueo and Takeuchi, Oita Prefectural Hospital, Oita for providing breast cancer samples, Haraguichi N for technical advice, Ms Yasunami H and Ms Kayashima M for wetern blotting, Ms Oda M for Real-time PCR and Ms Ando N for Immunohistochemistry. This work was supported by a Health and Labour Sciences Research grant, 2004 Kakenhi-B (2), from the Ministry of Health, Labour, and Welfare of Japan.
Xie Z, Zeng X, Waldman T, Glazer RI (2003) Transformation of mammary epithelial cells by 3-phosphoinositide-dependent protein kinase-1 activates beta-catenin and c-Myc, and down-regulates caveolin-1. Cancer Res 63: 5370–5375

Yang G, Truong LD, Wheeler TM, Thompson TC (1999) Caveolin-1 expression in clinically confined human prostate cancer: a novel prognostic marker. Cancer Res 59: 5719–5723

Zenklusen JC, Conti CJ, Green ED (2001) Mutational and functional analyses reveal that ST7 is a highly conserved tumor-suppressor gene on human chromosome 7q31. Nat Genet 27: 392–398

Zhang W, Razani B, Altschuler Y, Bouzahzah B, Mostov KE, Pestell RG, Lisanti MP (2000) Caveolin-1 inhibits epidermal growth factor-stimulated lamellipod extension and cell migration in metastatic mammary adenocarcinoma cells (MTLn3). Transformation suppressor effects of adenovirus-mediated gene delivery of caveolin-1. J Biol Chem 275: 20717–20725

Zschocke J, Manthey D, Bayatti N, van der Burg B, Goodenough S (2002) Estrogen receptor alpha-mediated silencing of caveolin gene expression in neuronal cells. J Biol Chem 277: 38772–38780