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Analysis of Cytosol CA15-3, Carcinoembryonic Antigen, Estrogen and Progesterone Receptors in Breast Cancer Tissues

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Cytosol levels of carcinoembryonic antigen (CEA), CA15-3, estrogen receptor (ER) and progesterone receptor (PgR) of 194 primary breast cancer tissues were measured. ER and PgR determined by enzyme immunoassay methods correlated inversely with Page's grades of histological atypia and mitotic rate. Cytosol CA15-3 correlated inversely with the grades of tubular and nuclear atypia and positively with the mitotic rate. CA15-3 correlated positively with ER and PgR. Cytosol CEA showed no correlation with the pathological grade or hormone receptor status. These results indicate that hormone receptor-positive breast cancers tend to have more differentiated morphology and slower growth rate than those without those receptors and that the cytosol CA15-3 level may reflect some of the intrinsic malignant potency, particularly the growth rate, of breast cancer. Cytosol CA15-3 as well as the hormone receptor status may have prognostic value for patients with breast cancer.

Key words: Breast cancer — Cytosol CA15-3 — Cytosol CEA — Estrogen receptor — Progesterone receptor

Serum carcinoembryonic antigen (CEA) has been widely used to monitor the clinical course of patients with colon, stomach, pancreas, lung, uterine cervix and breast cancer.[1)] The principal utility of the CEA measurement is, however, the monitoring of recurrence after surgical resection of the primary cancer and of the effect of systemic therapy in patients with metastatic disease.[2–4)] The sensitivity and specificity of CEA assay need to be improved before it can be employed to screen cancer patients in the early stage.[5,6)] Immunohistochemical staining of CEA of primary breast cancer tissues revealed that patients with CEA-positive tissue have a higher recurrence rate with probably poorer prognosis than those with negative tissues.[7,8)] The cytosol CEA concentration in breast cancer tissues was also measured and found not to be related to histological differentiation, but is considered to be one of the prognostic factors of breast cancer.[9–13)] In recent years, CA15-3 has come into clinical use to monitor recurrence of the disease and response to therapy in patients with advanced breast cancer.[14–16)] The antigen is determined by immunoradiometric assay with two monoclonal antibodies: DF3, which was raised against a membrane-enriched extract of human breast cancer metastasized to the liver, and 115D8 against human milk fat globules.[17–19)] Circulating CA15-3 was found to have bettre specificity for monitoring the clinical course of patients with breast cancer than CEA.[15,16)] Immunohistochemical examination revealed that expression of CA15-3 in breast cancer tissue obtained at the time of surgery correlates positively with both estrogen receptor (ER) status and histological differentiation and is thought to express the malignant potentiality of breast cancer.[20)] Another immunohistochemical study also demonstrated that positive staining for CA 15-3 is observed in the cytoplasm of breast cancer tissues but on the plasma membrane of the apical border in normal tissue or a benign proliferative breast lesion.[16–18)] Although these immunohistochemical studies indicate that tissue CA15-3 is qualitatively related to the degree of differentiation of breast cancer, no quantitative study of cytosol CA15-3 has been found in the literature.

Breast cancer patients positive for ER and progesterone receptor (PgR) are likely to have a longer disease-free period and longer survival from evidence of the first recurrence of the disease than do those patients without the receptors. It is tempting to assume that the receptor status is a reflection of the intrinsic growth rate of the cancer.

The present study was conducted to determine levels of CEA, CA15-3, ER and PgR in the cytosol of primary breast cancer tissues and to determine the relationships between these levels and pathological features.

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Table I. Characteristics of Patients

Age	54 (29–82)
Menopausal status	
Pre	83
Post	111
Clinical stage[a)]	
I	48
II	120
IIIA	12
IIIB	12
IV	2
TNM classification	
T	
1	51
2	118
3	14
4	11
N	
0	132
1	53
2	5
3	1
M	
0	192
1	2

Total 194.
a) Staging was according to the system of the Japan Mammary Cancer Society.[22)]

MATERIALS AND METHODS

Patients We included 194 female patients with primary breast carcinomas who were treated by mastectomy at the National Cancer Center Hospital in Tokyo from May 1988 to August 1989 (Table I).

Cytosol preparation After the breast carcinoma tissue was resected by mastectomy, approximately 0.5 g of cancerous tissue was immediately put into a liquid nitrogen tank and kept frozen until the time of cytosol preparation. The frozen tissue was pulverized in a freezer-mill (Spex Industries, Inc., Metuchen, NJ) and homogenized in 3 ml of 10 m*M* Tris; 1 m*M* EDTA buffer, pH 7.4 containing 12 mmol of thioglycerol and 10% (v/v) glycerol with a Polytron homogenizer (Kinematica GmbH, Luzerne, Switzerland) with three 15-s bursts at 45-s intervals. The homogenate was centrifuged at 105,000*g* at 4°C for 60 min to obtain a cytosol fraction which was assayed for ER, PgR, CEA, CA15-3 and protein concentration.

Hormone receptor assay The cytosol was examined for ER and PgR by using enzyme immunoassay kits from Abbott Laboratories (Dinabot Co., Tokyo) and considered positive if the values were equal to or greater than 13 and 10 fmol/mg cytosol protein, respectively.[21)]

CA15-3 and CEA assay Cytosol CEA was measured with a radioimmunoassay (RIA) kit from Roche Laboratories (Tokyo) and CA15-3 was assayed with an RIA kit from Centrocore Laboratories (Tokyo). Because these kits are designed to determine serum levels of the tumor markers, we first measured CEA and CA15-3 levels in mixtures of cytosol solutions ($n=5$) and graded amounts of the tumor markers as standards, and found that the results were always greater than 90% of the expected values in both assays. We therefore used these kits to determine the levels of the tumor markers in the cytosol without any modifications of the recommended assay methods.

Pathological evaluation Evaluation of histopathological features was carried out without any knowledge of the laboratory and clinical data by one of us (M.I.) according to the criteria of the Japan Mammary Cancer Society.[22)]

We used Page's histological grading to evaluate histopathological atypia, which is based on three categories, tubular atypia, nuclear atypia and mitotic rate. Each category is given a score of 1–3. Therefore the total points range from 3 to 9. The number of mitotic figures per 10 high-power (×400) fields was taken as mitotic rate. It has been shown that this grading system has a highly significant correlation with the outcome for patients with breast cancer.[23)]

Statistical analysis Statistical analyses were performed by the two-tailed *t* test for unequal variances and one way analysis of variance followed by Duncan's multiple range test; $P<0.05$ was considered significant. Calculations were performed with a statistical package, SPSS PC plus (SPSS Japan Inc., Tokyo) on an NEC PC98XL2 personal computer (Nippon Electric Corporation Inc., Tokyo).

RESULTS

Cytosol CEA and CA15-3 The mean cytosol CA15-3 level was 40.3 (ranged from 0 to 593) U/mg cytosol protein and the cytosol CEA level was 21.0 (ranged from 0 to 1112) ng/mg cytosol protein (Fig. 1). This divergent distribution of cytosol CEA level is in good agreement with that previously reported.[11)] Neither cytosol CA15-3 nor CEA significantly correlated with tumor size, axillary lymph-node metastasis or presence of distant metastasis (data not shown). No significant correlation between cytosol tumor markers and stage was observed (Table II).

Serum CEA and CA15-3 The mean preoperative levels of serum CEA and CA15-3 were 4.6 ± 15.9 ng/ml and 14.8 ± 11.6 U/ml, respectively. Of the 194 patients, 21

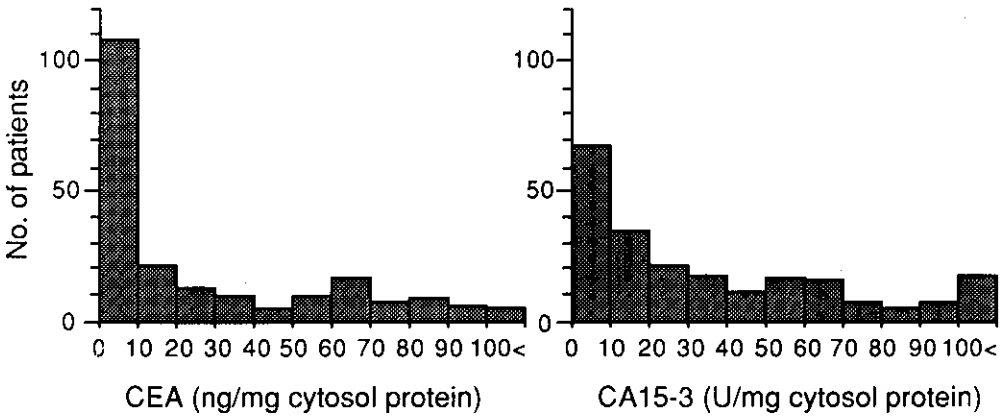

Fig. 1. Distribution of cytosol CEA and CA15-3 in 194 breast cancer tissues.

Table II. Levels of Cytosol Tumor Markers and Hormone Receptors According to Stage

Stage	N	CEA (ng/mg protein)	CA15-3 (U/mg protein)	ER (fmol/mg protein)	PgR (fmol/mg protein)
I	48	18.4±3.5	38.4±6.2	90.2±14.9	116.7±27.8
II	120	23.9±5.3	42.9±7.3	119.0±32.8	89.6±15.1
IIIA	12	23.7±15.3	35.5±9.9	32.6±11.2	45.8±22.1
IIIB	12	3.7±2.3	30.4±7.5	128.2±81.4	58.3±27.4
IV	2	0.0±0	18.7±11.7	241.1±156.1	180.5±94.9

Total 194.

Table III. Levels of Cytosol Tumor Markers and Hormone Receptors According to Pathological Classification

Pathological classification	N	CEA (ng/mg protein)	CA15-3 (U/mg protein)	ER (fmol/mg protein)	PgR (fmol/mg protein)
Noninvasive carcinoma					
Noninvasive ductal	1	0	9.5	14.4	9.2
Lobular carcinoma *in situ*	1	25.4	31.7	4.3	76.1
Invasive carcinoma					
Papillotubular	12	23.6±6.1	148.0±58.5*	108.4±24.1	161.1±85.7
Solid tubular	98	25.1±11.5	28.2±3.8	77.4±11.7	76.3±13.2
Scirrhous	66	19.1±3.8	43.9±4.9	98.1±18.5	102.0±15.7
Special types					
Mucinous	6	6.2±2.2	14.9±7.0	283.6±169.0	269.8±203.0*
Medullary	5	1.8±1.4	38.0±29.4	742.5±737.1*	13.2±10.4
Others	5	1.8±1.0	11.7±6.6	44.3±29.9	10.1±5.4

Total 194. * $P<0.01$.

and 17 had positive levels of serum CEA and CA15-3, respectively. Neither serum CEA nor CA15-3 showed a correlation with its cytosol level.

Estrogen and progesterone receptors Of the 194 patients, 121 (62.4%) were ER-positive, 101 (52.1%) were PgR-positive, 94 (48.5%) were both ER- and PgR-positive, 27 (13.9%) were ER-positive and PgR-negative, 7 (3.6%) were ER-negative and PgR-positive, and 66 (34.4%) were both ER- and PgR-negetive. There was a strong correlation between ER and PgR ($P<0.001$). The recep-

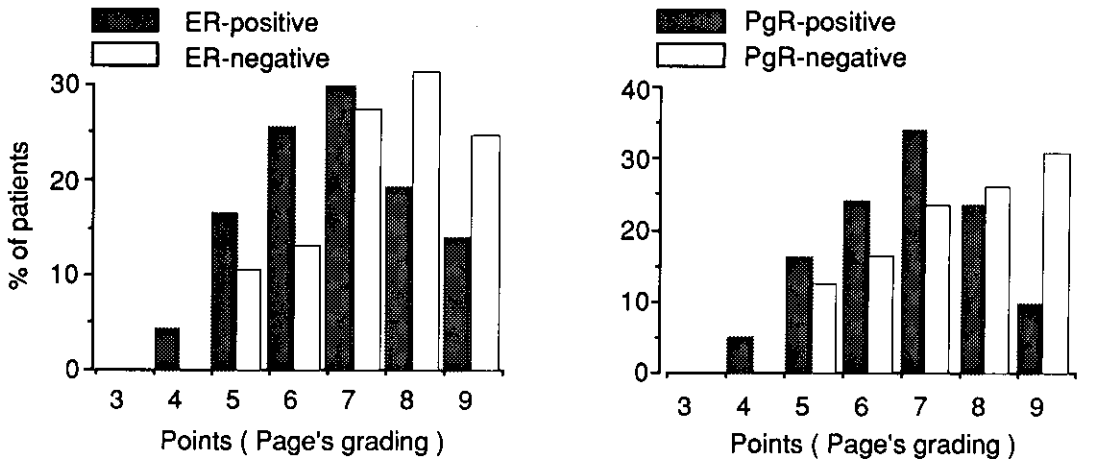

Fig. 2. Estrogen and progesterone receptor status according to the point score in Page's grading. For both hormone receptors, patients without hormone receptors were distributed at significantly higher point scores than those with receptors ($P<0.05$).

Table IV. Levels of Cytosol Tumor Markers and Hormone Receptors According to the Grade of Tubular Atypia

Tubular atypia	N	CEA (ng/mg protein)	CA15-3 (U/mg protein)		ER (fmol/mg protein)	PgR (fmol/mg protein)	
1+2	65	13.4±2.0	64.8±13.0	**	106.4±21.2	138.9±27.9	*
3	129	25.2±9.0	28.3±3.0		108.4±30.5	68.6±11.0	

Total 194. * $P<0.05$. ** $P<0.01$.

tor status showed no correlation with stage (Table II), or age or menopausal status of the patients.

Levels of cytosol CEA, CA15-3 and hormone receptors according to pathological classification The cytosol CA15-3 level was significantly higher in the papillotubular type than in the other pathological types (Table III). In contrast, cytosol CEA was not significantly different among the pathological types. ER and PgR levels were significantly high in the medullary and mucinous types, respectively. No difference in hormone receptor levels was found among the three major types of invasive carcinomas.

Hormone receptor status according to Page's grade of histological atypia The mean levels of PgR in grades defined by Page's system were 157.1±44.5 fmol/mg cytosol protein in grade 1, 118.4±19.9 in grade 2 and 40.2±9.4 in grade 3. The difference was significant ($P<0.01$). The level of ER was 117.9±23.0 in grade 1, 117.7±19.9 in grade 2 and 94.1±48.7 in grade 3. The distribution of the number of patients according to the total points of Page's method is shown in Fig. 2. For both ER and PgR, tumors without the hormone receptor had significantly higher points than those with the receptors ($P<0.05$). These results show that hormone receptor-negative tissues have a higher grade of histological atypia. Similar analyses were made for cytosol CEA and CA15-3 and no significant correlations were observed.

Levels of cytosol CEA, CA15-3 and hormone receptors according to each of Page's categories Because there were only eight patients with grade 1 tubular atypia, we combined tissues of grades 1 and 2 into one group (Table IV). The level of cytosol CA15-3 was significantly higher in tissues with grade 1+2 than in grade 3. The cytosol CEA level tended to be high in higher grade of tubular atypia, but without a significant difference. ER was present at almost the same level in both grades. Levels of PgR were 133.9±27.9 fmol/mg cytosol protein in grade 1+2 and 68.6±11.0 in grade 3 ($P<0.05$).

When nuclear atypia grades 1 and 2 were combined, there was a tendency for CA15-3 to be higher in grade 1+2 (48.4±10.7 U/mg cytosol protein) than in grade 3 (34.9±4.0 U/mg cytosol protein) ($P=0.095$). No sig-

Table V. Levels of Cytosol Tumor Markers and Hormone Receptors According to Mitotic Rate

Type	CEA (ng/mg protein)		CA15-3 (U/mg protein)		ER (fmol/mg protein)		PgR (fmol/mg protein)	
	<10	≥10	<10	≥10	<10	≥10	<10	≥10
Papillotubular	20.7±9.4	27.6±7.5	131.7±72.0	171.0±107.0	116.5±40.9	97.1±16.1	216.7±146.2	83.3±31.4
Solid tubular	20.1±4.9	27.6±17.2	19.5±2.9	*-32.6±5.4	96.6±21.5	67.7±13.8	102.1±27.7	*-63.2±14.3
Scirrhous	18.3±5.5	20.3±5.5	26.9±5.4	*-57.2±7.0	131.8±41.0	69.9±14.1	127.0±27.7	79.9±18.1
Others	5.7±1.7	3.6±2.5	17.0±6.0	24.4±14.8	230.2±129.4	381.3±367.8	180.9±156.2	37.3±21.2

<10: mitotic rate less than 10 per 10 fields (×400). ≥10: mitotic rate equal to or more than 10 per 10 fields (×400). * $P<0.05$.

Table VI. Levels of Cytosol Tumor Markers According to Hormone Receptor Status

		N	CEA (ng/mg protein)	CA15-3 (U/mg protein)
ER	positive	116	18.2±2.7	47.4±6.2
	negative	78	25.2±14.3	29.8±7.5
PgR	positive	110	18.6±2.9	48.7±6.5]*
	negative	84	24.3±13.2	29.3±7.0]

ER (positive) > 13 fmol/mg protein. PgR (positive) > 10 fmol/mg protein. * $P<0.05$.

nificant correlations were observed between the cytosol levels of CEA, ER and PgR with nuclear atypia.

Because the proportion of cancer cells to connective tissues in a microscopical field was different in each pathological type, analysis of the correlation between mitotic rate and cytosol values was performed separately for each major type and "others" (Table V). In every pathological type, CA15-3 levels were higher in the group whose mitotic rate was equal to or more than 10 compared to the group with a rate of less than 10. The difference was significant for solid tubular and scirrhous carcinomas ($P<0.05$). In contrast, the PgR was higher in tumors with fewer mitotic figures. The difference was observed in all histopathological types. ER also had a tendency to be high in tumors with less mitosis in the three major types. No apparent difference was observed in CEA levels according to the mitotic rate.

Correlation between cytosol tumor markers and hormone receptors The cytosol CA15-3 level was higher in ER-positive tissues than in negative ones ($P=0.07$) and significantly higher in PgR-positive tissues than in negative ones (Table VI). These results showed a positive correlation between cytosol CA15-3 and the hormone receptor status. In contrast, cytosol CEA did not show any apparent difference depending upon hormone receptor status.

The number of axillary lymph node metastases and the patient's age did not correlate with the level of cytosol ER, PgR, CEA, or CA15-3 (data not shown).

DISCUSSION

A number of investigators have reported the status of CEA in breast cancer tissues. Schwartz *et al.*,[24] Walker[25] and Wahren *et al.*[10] found no relationship between cytosol CEA and histological type, degree of differentiation and axillary lymph node status. Jakes *et al.*[26] and Duffy *et al.*,[9] using cytosol of breast cancer, found no correlation between CEA and stage. In our study, the status of cytosol CEA is compatible with that in those previous studies. Although the prognostic value of tissue CEA still remains controversial, Shousha *et al.*,[13] Mansour *et al.*[7] and Majima *et al.*,[8] who used the immunohistochemical method, found that patients with CEA-positive tissue have a poorer prognosis than those with CEA-negative tissues.

Immunochemical studies by Hilkens *et al.*,[17] Kufe *et al.*[18] and Lundy *et al.*[27] demonstrated that tissue CA15-3 correlated positively with the degree of tumor differentiation. These reports also showed that CA15-3 is located in the epithelial brush borders of benign proliferative lesions and normal breast tissues, whereas malignant cells show CA15-3 staining not only in the epithelial brush borders but also characteristically in their cytoplasm. We therefore thought it useful to determine the cytosol level of CA15-3.

In the present study, we measured the level of cytosol CA15-3 with a radioimmunometric assay kit which had been developed to determine the tumor marker in serum, because no significant interference was observed in the cytosol preparation. In relation to histopathological atypia, we found that the CA15-3 level is higher in the low grade group of tubular and nuclear atypia. This finding is comparable with those of the previous reports. On the other hand, mitotic rates correlate positively with the levels of cytosol CA15-3. This may imply that the

higher the cytosol CA15-3 level, the higher is the tumor growth rate.

We evaluated cytosol levels of CEA and CA15-3 in relation to hormone receptor status. CEA had no correlation with either ER or PgR, as reported by other authors.[7, 28, 29] On the other hand, the cytosol CA15-3 level was higher in hormone receptor-positive tissues than in tissues without the receptors. A report that serum level of CA15-3 correlates with the ER status may support our observation.[30] In our study however, no conclusion was drawn concerning the relationship between serum level of CA15-3 and hormone receptor status, partly because of the small number of patients with increased CA15-3 in the sera at the time of operation. It seems to be of interest to elucidate the functional linkage, if any, between estrogenous stimulation and the mechanisms of production or release of CA15-3.

Cytosol CA15-3 has quite a different character from cytosol CEA in its relation to pathological or clinical features. Cytosol CA15-3 correlated with the grade of tumor atypia, mitotic rate and hormone receptor status. Our observation showed that breast cancer tissues with a high CA15-3 content have both functionally and morphologically differentiated features. One observation that does not go well with the above-mentioned characteristics of cytosol CA15-3 is that CA15-3 correlated with mitotic rate. At this moment, we have no explanation for this apparent contradiction. It is therefore necessary to follow the clinical course and to analyze the relative significance of contradictory factors in predicting the prognosis of this patient population.

Positive ER and PgR are well known to be better prognostic factors of patients with breast cancer.[31] A correlation between ER and histological differentiation of breast cancer has also been reported.[32, 33] Our observation showed that a tumor with a positive hormone receptor status had a low grade of histological atypia, in particular the mitotic rate. These data support the hypothesis that the better outcome for ER- and/or PgR-positive patients is due to the low growth rate or low malignant potency of the tumors.

In summary, we demonstrated a correlation between the cytosol components of breast cancer tissue and its pathological features. Cytosol CA15-3 has a very different character from CEA and substantially reflects the malignant potentiality of the breast cancer. CA15-3 and hormone receptors correlate with histophathological atypia, and in particular the mitotic rate. Follow-up study of these 194 patients to evaluate disease-free survival is necessary to confirm the prognostic value of cytosol CA15-3.

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