Breast Cancer Research and Treatment **24**: 97–102, 1992.

Report

C-erbB-2 protein in the sera of breast cancer patients

Tatsuhiko Narita, Hiroomi Funahashi, Yasuyuki Satoh and Hiroshi Takagi
From the Department of Surgery II, Nagoya University School of Medicine, Nagoya, Japan

Key words: c-erbB-2 protein, breast cancer, tumor marker, enzyme immunoassay

Summary

The c-erbB-2 protein was measured in sera of patients with breast cancer or benign breast diseases to study the significance of this protein as a tumor marker. The mean value and positive rate for this protein (assuming 20 U/ml as the cut-off value) were 11.8 U/ml (0%) in benign breast disease (n = 30), 11.8 U/ml (3.1%) in stage I/II primary breast cancer (n = 64), 38.2 U/ml (29.4%) in stage III/IV primary breast cancer (n = 17), 17.9 U/ml (33.3%) in locally recurrent breast cancer (n = 12), 298.4 U/ml (51.0%) in recurrent breast cancer with distant metastases (n = 51), and 12.9 U/ml (0%) in those with no evidence of recurrence (n = 57). Thus, the serum c-erbB-2 protein level was significantly higher in the distant metastatic group. In patients with distant metastases, there was a close association between expression of c-erbB-2 protein in the primary tumor and the serum c-erbB-2 protein level. On the basis of these results, serum c-erbB-2 protein was thought to be useful as a tumor marker for postoperative monitoring of breast cancer, especially in patients positive for expression of this protein in primary cancer tissue.

Introduction

In clinical medicine of breast cancer, especially in postoperative follow-up, tumor markers play an important role. Currently, CEA and carbohydrate antigens, such as CA15-3, are employed as tumor markers. However, new tumor markers having characteristics different from the conventional ones are desired.

The c-erbB-2 oncogene encodes a 185-kilodalton epidermal growth factor receptor-like membrane glycoprotein with tyrosine kinase activity [1]. It is believed that this oncogene is amplified at a high incidence in patients with adenocarcinoma, including breast cancer, and plays some role in the growth and metastasis of neoplasmas [2]. In addition, in breast cancer, it has been pointed out that there is a correlation between amplification of c-erbB-2 oncogene and prognosis of the disease, and an especially close correlation has been detected between c-erbB-2 protein, a product of the oncogene, and prognosis of the disease [3, 4]. Using serum samples of breast cancer patients, we quantified a c-erbB-2 oncogene product and studied the significance of this substance as a tumor marker.

Materials and methods

The subjects of this study were 231 female patients who had been treated at the Department of Surgery II, Nagoya University School of Medicine, and its affiliated institutions, and in whom histopathological diagnosis had been achieved. These patients consisted of 81 cases of primary breast cancer, 63 cases of postoperative recurrent breast cancer, 57

Address for offpints: T. Narita, the Department of Surgery II, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan

cases of postoperative non-recurrent breast cancer, and 30 cases of benign breast diseases (21 cases of mastopathy, 9 cases of fibroadenoma) (Table 1). The postoperative non-recurrent breast cancer patients were those who had been followed up for at least two years after operation and who showed no signs of recurrence for at least one year from blood sample collection in this study. Diagnosis of distant metastases was done using imaging techniques such as plain roentgenograms, CT scan, ultrasonography, bone scintigraphy, and magnetic resonance imaging.

Blood was collected in the early morning, and the serum was separated and stored at −80°C. C-erbB-2 protein in the blood sample was determined using an enzyme-immunoassay kit (Triton Diagnostics Inc., Alameda, CA), within 15 months after blood collection except for the cases with multipoint sampling. This EIA kit using polystyrene tube is improved from the microplate ELISA presented by Leitzel *et al.* [5], which includes two specific monoclonal antibodies for recognizing the external domains of c-erbB-2 protein (HRP-conjugated TAb257 and TAb259) and one monoclonal antibody for linking the sandwitched immunocomplex to the tube. Figure 1 shows the procedure.

Table 1. Clinical features of patients

	No. of patients
Primary BC	
Stage I	29
Stage II	35
Stage III	10
Stage IV	7
Recurrent BC	
Local	12
Distant metastases	
Bone (B)	19
Pulmonary (P)	11
Brain	2
Liver (L)	1
B+P	5
B+L	5
Multiple organs	8
Non-recurrent BC	57
Benign breast disease	30
Total	231

BC: breast cancer

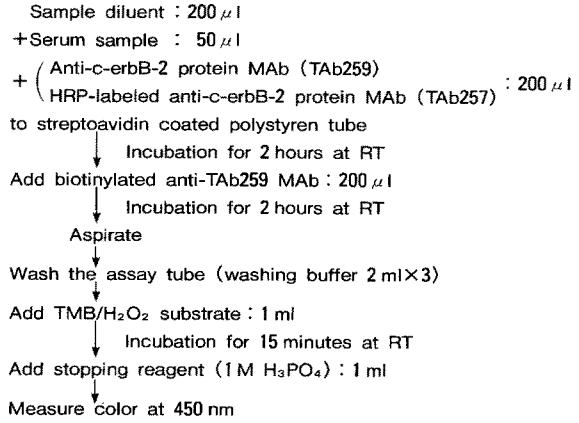

Fig. 1. Enzyme-immunoassay procedure for measurement of serum c-erbB-2 protein. TAb257: Balb-c immunized with the c-DNA transfected NIH3T3 (IgG1), Tab259: Balb-c immunized with SK-Br-3(IgG1), HRP: horseradish peroxidase, MAb: monoclonal antibody, RT: room temperature, TMB: 3,3', 5,5'-tetrametylbenzidine.

Formalin-fixed, paraffin-embedded tissue specimens collected from the 100 primary tumors and the lymph nodes with metastases of 25 cases were immunohistochemically stained by the avidin-biotin peroxidase complex (ABC) method. For greater detail, after deparaffinization and blocking of the non-specific esterase reaction with skim milk, the specimens were reacted for 60 minutes with anti-c-erbB-2 protein mouse monoclonal antibody, mAbl(IgG1, clone TA250) (Triton Diagnostics Inc., Alameda, CA) [6], as the primary antibody at room temperature in a moist chamber. This monoclonal antibody recognizes the external 185 kilodalton glycoprotein molecule of c-erbB-2 protein [6]. Next, after removing endogenous peroxidase with 0.3% hydrogen peroxide in methanol, using an ABC kit (Vector Lab. Inc., Burlingame, CA) the specimens were reacted with biotinylated horse anti-mouse immunoglobulin for 30 minutes, and after forming an avidin-biotin peroxidase complex with the ABC reagent, they were colorized with 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO). After counterstaining with hematoxylin, the specimens were examined under a microscope. The results of this immunohistochemical staining were analyzed with regard to correlation between the level of serum c-erbB-2

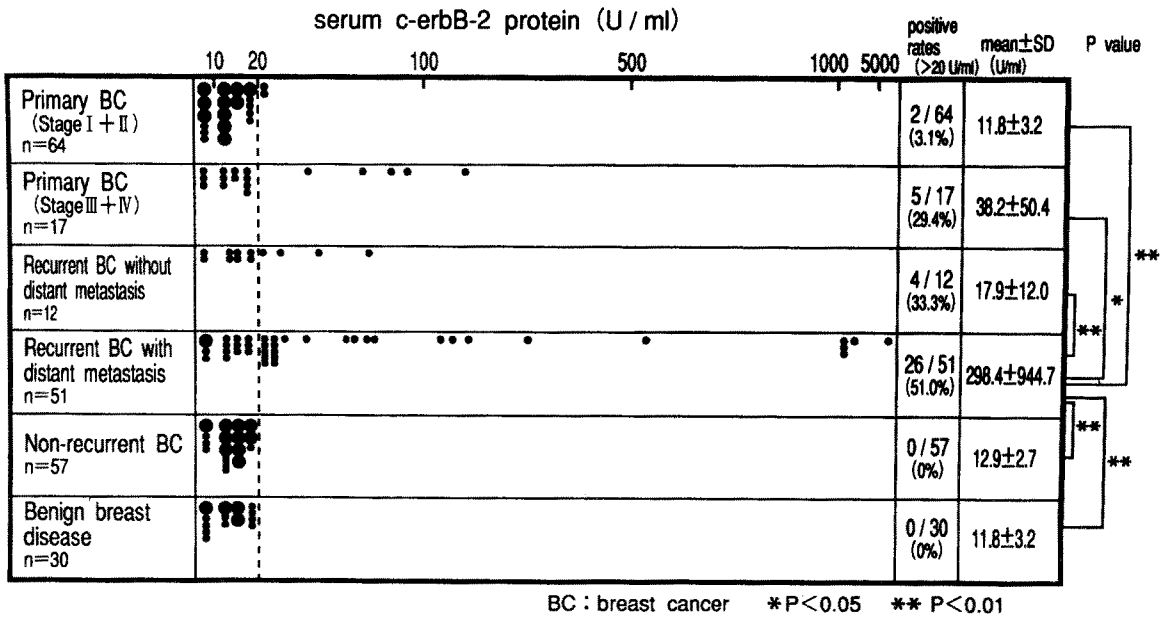

Fig. 2. C-erbB-2 protein level in the sera of patients with breast cancer and benign breast disease.

protein and its expression in the primary cancer tissue.

The statistical analyses were performed by Pearson's least-squares method and Student's t-test.

Results

Figure 2 shows the serum c-erbB-2 protein level in each patient group. The advanced and recurrent cancer group showed a high level of this protein in comparison with the other patient groups, and the level was especially elevated in the distant metastatic group. However, the primary cancer group did not show any significant difference in the serum c-erbB-2 protein level from the non-recurrent group or the benign disease group. The mean+2SD of the protein in 87 patients with no evidence of disease and those with benign breast disease was 18.37 U/ml. Thus, if 20 U/ml is defined as the cut-off value, the positive rate for c-erbB-2 protein was 8.6% (7/81) for the primary cancer group and 47.6% (30/63) for the postoperative recurrent group. The positive rate in patients with distant metastases was 50.9% (29/57).

Out of 100 primary tumors of breast cancer, 23 cases showed positive expression of c-erbB-2 protein in the cell membrane (Fig. 3). In 25 cases with lymph node metastases, nine cases showed positive expression in their primary tumors. Among these nine cases, eight also showed positive expression in their metastatic lymph nodes. On the other hand, the 16 cases with negative expression

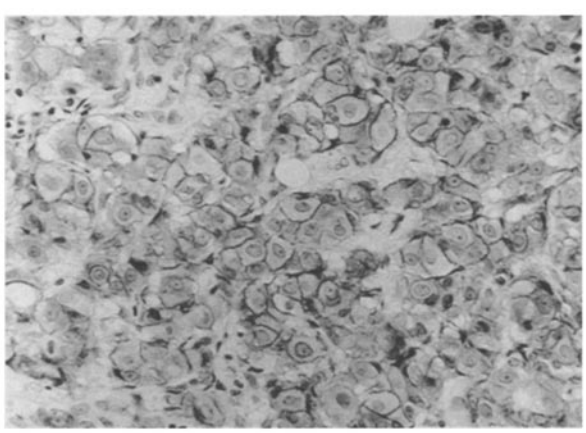

Fig. 3. Immunohistochemical staining of c-erbB-2 protein in breast cancer (×400).

Table 2. Relationship between tissue expression and serum level of c-erbB-2 protein in 55 patients with primary breast cancer

		Serum c-erbB-2 protein			
		Stage I/II (n= 42)		Stage III/IV (n= 11)	
		Mean ±SD (U/ml)	Positive rate	Mean ±SD (U/ml)	Positive rate
Tissue c-erbB-2 protein	Negative	12.3± 3.9	2/38	21.4± 23.2	1/9
	Positive	11.3± 1.6	0/4	147.3± 78.1	2/2

in primary tumors also showed negative expression in their metastatic lymph nodes.

The expression of c-erbB-2 protein in the cancer tissue was studied in 55 patients with primary breast cancer. In stage I/II patients, no correlation between tissue expression and serum level of c-erbB-2 protein was observed. In stage III/IV patients, the two cases with positive tissue expression showed an elevated serum level. These two cases had distant metastases (Table 2).

Next, the expression of c-erbB-2 protein in the primary cancer tissue was studied in 45 patients with distant metastases, and correlated with serum c-erbB-2 protein level after recurrence. The primary tumor was positive in 17 cases, and all those patients showed an elevated serum c-erbB-2 protein level on recurrence. On the other hand, although some cases with negative primary tumors had an elevated level of this protein on recurrence, this incidence was low, and the mean value of serum c-erbB-2 protein in the negative expression cases was significantly lower than in the positive expression cases. Moreover, in all cases showing a high serum level of 100 U/ml or higher, expression of the protein in the primary cancer tissue was positive (Fig. 4).

Figure 5 shows changes in the serum c-erbB-2 protein level, along with CEA and CA15-3 levels, in four recurrent breast cancer cases. In two cases positive for tumor c-erbB-2 protein expression, the serum c-erbB-2 protein level was seen to change in response to the state of disease, and these changes were thought to reflect the tumor volume more sensitively than CEA and CA15-3. On the other hand, in two cases negative for tumor c-erbB-2 protein expression, absolutely no changes were seen in the serum level of this protein.

Discussion

There have been few reports on serum c-erbB-2 protein in breast cancer patients [5, 7, 8], and its clinical significance remains unknown. In the present study, c-erbB-2 protein was detected in the sera of breast cancer patients, and thus this protein is thought to be a useful tumor marker. Because c-erbB-2 protein level was especially elevated in pa-

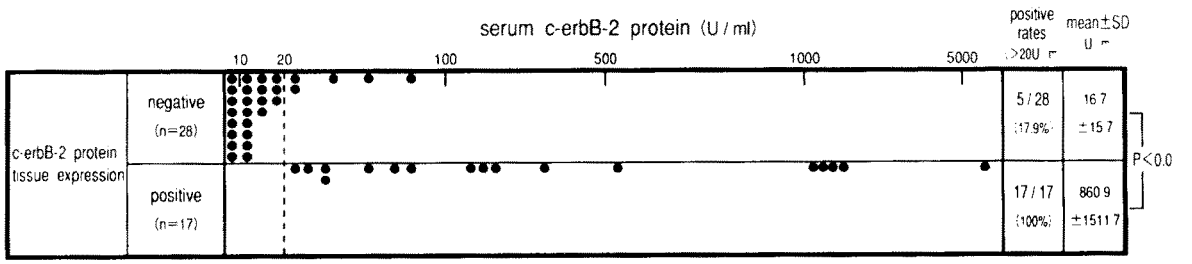

Fig. 4. Relationship between tissue expression and serum level of c-erbB-2 protein in 45 patients with distant metastases.

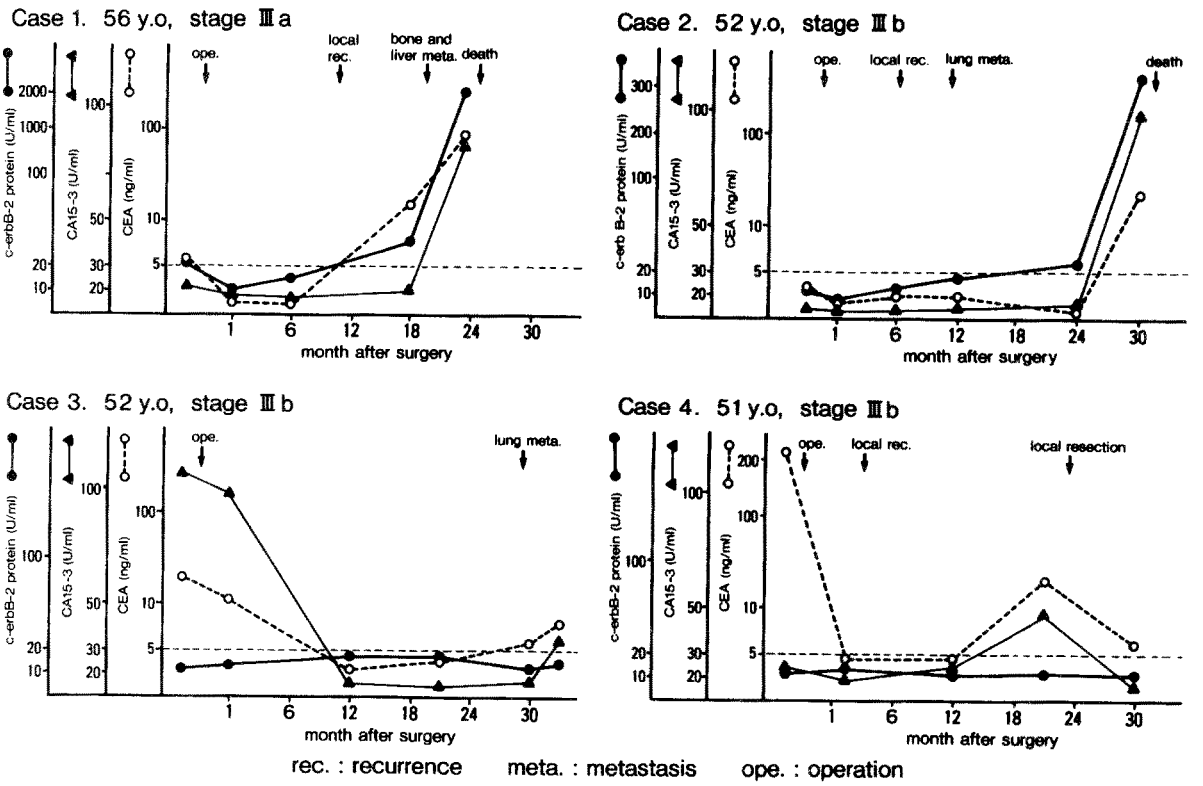

Fig. 5. C-erbB-2 protein, CEA, and CA15-3 in the sera of four recurrent breast cancer cases. Immunohistochemically, c-erbB-2 protein was positive in case 1 and 2, but negative in case 3 and 4, in the primary tumor tissues.

tients with distant metastases, the level of this protein is surmised to reflect the volume of tumor. Furthermore, there was a correlation between expression of the protein in cancer tissue and the serum level of the protein. Therefore, as the mechanism of elevation of the serum c-erbB-2 protein level, it is speculated that this protein is produced in cancer cells and liberated to blood.

In immunohistochemical staining, the rate of expression of c-erbB-2 protein in breast cancer tissue has been reported to be 20~40% [3, 4]. In the present study, however, elevation of the serum level of the protein was seen in 50.9% of patients with distant metastases. This finding suggests that the serum c-erbB-2 protein can be a tumor marker even for patients who are judged to be negative for primary tumor tissue expression by immunohistochemical staining. However, it should be noted that there was a difference in the degree of elevation of the serum level of this protein between cases positive for tissue expression and cases negative for tissue expression. From the immunohistochemical study in 25 patients with lymph nodes metastases, it is considered that the mutation of c-erbB-2 protein with metastasis is rare. Therefore, it is inferred that even breast cancer cells which are negative for immunohistochemical staining produce a small amount of c-erbB-2 protein, and this protein becomes detectable in the serum in associaton with the increase in the tumor volume.

It appeared that elevated serum c-erbB-2 protein levels were seldom detected in early stage cancer even though the primary cancer tissue was c-erbB-2 protein positive. This finding suggests that serum c-erbB-2 protein measurement is not useful in the preoperative diagnostic setting. On the other hand, as described earlier, breast cancer positive for expression of c-erbB-2 protein in cancer tissue is likely to undergo distant metastasis, its prognosis is poor, and thus it requires careful postoper-

ative follow-up. Serum c-erbB-2 protein is thought to be especially useful as a tumor marker for post-operative monitoring of breast cancer which is positive for expression of the protein in the cancer tissue.

Acknowledgements

The authors would like to express their special thanks to Torey-Fuji Bionics Inc. (Tokyo, Japan) for their kind support in this investigation.

References

1. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T: The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. Science 232: 1644–1646, 1986
2. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science, 235: 177–182, 1987
3. Tsuda H, Hirohashi S, Shimosato Y, Tanaka Y, Hirota T, Tsugane S, Shiraishi M, Toyoshima K, Yamamoto T, Terada M, Sugimura T: Immunohistochemical study on overexpression of c-erbB-2 protein in human breast cancer: its correlation with gene amplification and long-term survival of patients. Jpn J Cancer Res 81: 327–332, 1990
4. Kallioniemi OP, Holli K, Visakorpi T, Koivula T, Helin HH, Isola JJ: Association of c-erbB-2 protein over-expression with high rate of cell proliferation, increased risk of visceral metastasis and poor long-term survival in breast cancer. Int J Cancer 49: 650–655, 1991
5. Leitzel K, Teramoto Y, Sampson E, Mauceri J, Langston BC, Demers L, Podezasky E, Harvey H, Shambough S, Volas G, Weaver S, Lipton A: Elevated soluble c-erbB-2 antigen levels in the sera and effusions of a proportion of breast cancer patients. J Clin Oncol 10: 1436–1443, 1992
6. Longton BC, Crenshaw MC, Chao LA, Stuart SG, Akita RW: An antigen immunologically related to the external domain of gp185 is shed from nude mouse tumors overexpressing the c-erbB-2(HER-2/neu) oncogene. Cancer Res 51: 2593–2598, 1991
7. Mori S, Mori Y, Mukaiyama T, Yamada Y, Sonobe Y, Matsushita H, Sakamoto G, Akiyama T, Ogawa M, Shiraishi M, Toyoshima K, Yamamoto T: In vitro and *in vivo* release of soluble erbB-2 protein from human carcinoma cells. Jpn J Cancer Res 81: 489–494, 1990
8. McKenzie SJ: Diagnostic utility of oncogenes and their products in human cancer. Biochem Biophys Acta 1072: 193–214, 1991