Chinese Journal of Cancer Research 7(2):97 - 102, 1995.

THE EXPRESSION OF CATHEPSIN - D, C - *erb*B - 2 AND EGFR IN BREAST CANCER AND ITS CORRELATION TO LYMPHATIC METASTASIS

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The expression of Cathepsin - D (Cath - D), c - *erb*B - 2 and EGFR in breast cancer and its correlation to lymphatic metastases were studied in 277 cases by immunohistochemical technique. Positive staining of Cath - D was detected in 107 cases (38.62%). Among those, 89 cases (83.17%) had documented metastases in the lymph nodes. One hundred and seventy cases (61.38%) stained negative for Cath - D. Of which 64 cases (37.64%) had detectable lymphatic metastases. There is a significant difference in the rate of the lymphatic metastases between the Cath - D positive and Cath - D negative groups (x^2 = 55.05 P < 0.0001). Fifty - six out of 107 Cath - D positive cases (52.23%) were c - *erb*B - 2 positive as well. However, only 27 out of 170 Cath - D negative cases (15.88%) were c - *erb*B - 2 positive. The positive rate of c - *erb*B - 2 in Cath - D positive group was significantly different from that of Cath - D negative group (x^2 = 41.58 P < 0.0001). Among those 107 Cath - D positive cases, 49 cases (45.79%) were EGFR positive. Only 24 cases (14.12%) were EGFR positive among the 1 7 0 Cath - D negative cases. The positive rate of EGFR between these two groups was also significantly different (x^2 = 33.95 P < 0.0001). An analysis of the three mentioned markers, the lymph node metastasis and tumor size suggests that Cath - D is the most valuable indicator for tumor aggressiveness. Breast cancer cases with a positive Cath - D staining are more likely to have lymphatic metastases and a poor prognosis. Therefore, alternative therapeutic strategies and close follow ups are appropriate for these patients.

Key words: Cath - D, c - *erb*B - 2, EGFR, Breast cancer.

In breast cancer patients with negative lymph node, 30% may die of relapse or metastasis within 10 years after operation.[1] To detect the patients with high risk of recurrence and give necessary supplymentary treatments is of strategical importance in raising the total cure rate. Cath - D is a new valuable clinical prognostic index for relapse and metastasis of the breast cancer. Patients with breast cancer and positive Cath - D expression is liable to relapse and metastasize.[2] The c - *erb*B - 2 oncogene (also called HER2 or neu) is a member of the *erb*B - like oncogene family. In breast

Accepted November 19, 1994

carcinoma, 9—33% of invasive tumors overexpress the c-*erb*B-2 product,[3] and there is strong evidence that overexpression is associated with increased tumor aggressiveness.[4,5] The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein whose expression is important in the regulation of breast cancer cell growth. A growing body of studies have reported that overexpression of the EGFR was associated both with enhanced metastatic potential of some breast cancer cell lines and with high risk of early recurrence and death.[6,7]

The present work studied the expression of Cath-D and oncogenes c-*erb*B-2 and EGFR and analyzed their relation to lymph node metastases in 277 cases of breast cancer, to explore their significance in judging biological behavior of the cancer.

MATERIALS AND METHODS

Specimens

Cancer tissues from 277 cases of breast cancers (infiltrative duct carcinoma 247, medullary carcinoma 15, mucinous adenocarcinoma 7, intraductal carcinoma 5, paget's disease 3) and specimens from 70 cases of benign disease (fibroadenoma 34, lobular hyperplasia 28, mammary duct ectasia 2, breast cystis 2, breast collagenosis 2, chronic mastitis 2) were collected. All the cases were confirmed by pathological examination and received no treatment other than surgical operation. The age of patients with breast cancer were between 24 to 90 years with the median of 52. Normal breast tissues of 10 cases were used for control.

Reagents

Rabbit antihuman Cath-D was purchased from Biodesign International (USA), c-*erb*B-2 (Ab-3) and EGFR (Ab-4), from Oncogene Science Co. (USA). Biotinlabelled horse anti-mouse IgG (1:200) and ABC kit (1:100) were all purchased from Vector Laboratories (USA).

Immunohistochemical Method

Formalin fixed and wax-embeded tissue sections (4 μm) were deparaffinized with xylene, rehydrated gradually through graded alcohols, washed in Tris-buffer (TBS) $\times 2$, treated with saturated solution of mouse liver powder and washed in TBS $\times 2$. Sections were then immunostained with antibodies Cath-D (1:50), c-*erb*B-2 (1:20) and EGFR (1:20) respectively in 4℃ for 16 hrs. After washed in TBS $\times$ 2, biotinlabelled rabbit antihuman IgG was added to sections of Cath-D and EGFR and biotinlabelled house anti-mouse IgG, to sections of c-*erb*B-2. The following procedure using ABC kit and 3.3'-diamino-benzidine tetrahydrochloride was as described previously.[8] Omission of the primary antibody was taken as the negative control and HE staining was for pathological typing. The positive tiny particles of Cath-D were located in the cytoplasm. Positive staining of c-*erb*B-2 was mainly on the cell membrane; positive staining of EGFR was seen in the cytoplasm and on the cell membrane. The positive standard was referred to Dykins[9] with some modifications. The positive criteria is that the intensity of immunostain on the cell membrane and/or in the cytoplasm should be Dykins' (++) in more than 50% of the cells examined.

Statistical Analysis of Data

The x^2 test was used for statistical analysis, a level of $P < 0.05$ was taken as significant.

RESULTS

Expression of Cath-D, C-*erb*B-2 and EGFR

In 277 cases of breast cancer, 107 were Cath-D positive (Figure 1), 83, c-*erb*B-2 positive (Figure 2), and 73, EGFR positive

(Figure 3), the positive rate being 38.62%, 29.96% and 26.35% respectively (Table 1). None was positive in Cath - D, c - *erb*B - 2 and EGFR in all the 70 cases of benign breast disease and 10 cases of normal breast tissue.

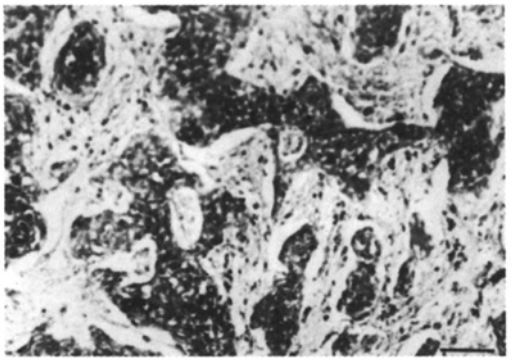

Fig. 1. Infiltrative duct carcinoma, Cath-D positive, brown granules were located in cytoplasm, ×400.

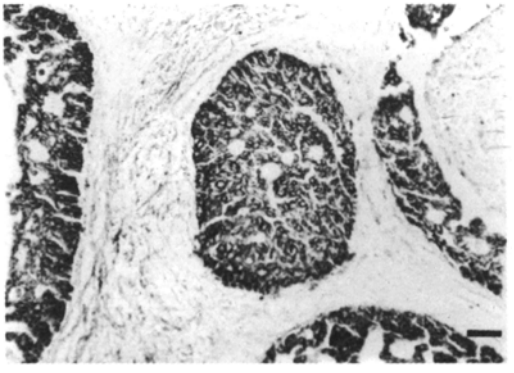

Fig. 2. Intraductal carcinoma, c - *erb*B - 2 positive, strong staining was mainly on cell membrane, ×200.

In the 107 cases of Cath - D positive breast cancer, 56 were c - *erb*B - 2 positive (52.34%) and in the 170 Cath - D negative cases, 27 were positive (15.88%). The positive rate of c - *erb*B - 2 in Cath - D positive cases was significantly higher than that in the Cath - D negative cases (x^2

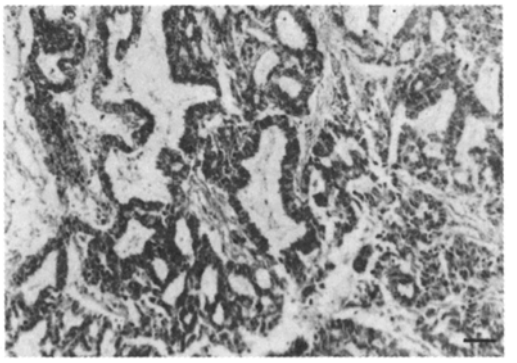

Fig. 3. Infiltrative duct cacinoma, positive staining of EGFR was both on cell membrane and in cytoplasm, ×200.

$= 41.58$ $P < 0.0001$). EGFR was positive in 49 (45.79%) of the 107 Cath - D positive cases and 24 (14.12%) of the 170 Cath - D negative cases. The positive rate of EGFR in the Cath - D positive cases was also significantly higher than that in the Cath - D negative cases ($x^2 = 33.95$ $P < 0.0001$).

Relationship between Cath - D, c - *erb*B - 2 and EGFR Expression and Lymph Node Metastases

One hundred and fifty-three of 277 cases were lymph node positive. Among them, 89 were from 107 Cath - D positive cases, and 64, from 170 Cath - D negative cases. Thus, lymph node metastatic rate in Cath - D positive cases was 83.17% significantly higher than that 37.64% of the Cath - D negative cases ($x^2 = 55.05$ $P < 0.0001$). No significant difference in metastatic rate was found either between c-*erb*B-2 positive and negative or EGFR positive and negative cases (Table 2).

Relationship between Cath - D Expression and Tumor Size

Two hundred and fourty-five cases had systematic clinical records of tumor size. In the Cath - D positive cases, number of tumors with diameter 2—3.5cm or > 3.5 cm was significantly

higher than that of tumors with diameter less than 2 cm ($x^2 = 25.82$ $P < 0.0001$; $x^2 = 16.06$ $P < 0.0001$) (Table 3).

Table 1. Expression of Cath - D, c - erbB - 2 and EGFR in breast cancer

Tissues	Cases	Cath - D				c - *erb*B - 2				EGFR			
		+	%	-	%	+	%	-	%	+	%	-	%
Infiltrative duct carcinoma	247	98	39.68	149	60.32	78	31.57	169	68.42	64	25.91	183	74.08
Medullary carcinoma	15	5	33.33	10	66.67	4	26.66	11	73.33	4	26.66	11	73.33
Mucinous adenocarcinoma	7	1	14.28	6	85.71	0	0	7	100	2	28.57	5	71.42
Intraductal carcinoma	5	1	20.00	4	80.00	1	20.00	4	80.00	2	40.00	3	60.00
Paget's disease	3	2	66.67	1	33.33	0	0	3	100	1	33.33	2	66.67
Total	277	107	38.62	170	61.37	83	29.96	194	70.03	73	26.35	204	73.64

Table 2. Relationship between expression of Cath - D, c - erbB - 2, EGFR and lymph node metastases

		Cases	Lymph node			
			+	%	-	%
Cath - D	+	107	89	83.17	18	16.83
	-	170	64	37.64	106	62.36
c - *erb*B - 2	+	83	48	57.83	35	42.16
	-	194	105	54.12	89	45.87
EGFR	+	73	44	60.27	29	39.72
	-	204	109	53.43	95	46.56

Table 3. Relationship between expression of Cath - D and tumor size

Tumor size (cm)	< 2	2 - 3.5	> 3.5	Total
Cath - D +	17(17.34)	59(60.20)	22(22.45)	98
Cath - D -	75(51.02)	52(35.37)	20(13.60)	147

Number in parenthesis: Percentage of cases

DISCUSSION

Breast cancer kills the patients via its metastatic potential and removal of a primary tumor is not always sufficient to cure a patient who may develop distant metastasis. Tumor size and node invasiveness are the most potent classical prognostic markers for predicting tumor aggressiveness. However, in 30% of node-negative patients, breast cancer will relapse and new predictive markers are required to help in making treatment decisions.

Cath-D, a lysosomal acidic protease,[10] possibly degrades extra-cellular matrix[11] when autoactivated. Thus, it may facilitate the dissemination of tumors.[12] In clinical studies, high cystosolic Cath-D concentration in primary breast cancer is correlated with a higher frequency of relapse and metastasis.[13] Compared with histopathological factors, Cath-D is an independent marker for prognosis, expecially in node-negative breast cancer.[14] McGuire et al. investigated the patients with lymph node negative and found that 5 years relapse rate may reach 50% in those with higher concentration of Cath - D.[15] Tandon et al.[16] reported the results of examining 199 cases of breast cancer, and demonstrated that 5 years relapse rate was 60% in patients with high concentration of Cath - D, but only 29% in those with low concentration. However, the prognostic value of Cath - D was obtained from immunoassays performed in cytosol or total cell extracts which requires a large amount of tissue. Due to the increasing progress in early detection of breast cancer, prognostic markers should be quantified in small tumors. Immunohistochemistry analysis might be able to fulfil this objective if its validity compared to the cytosol assay can be demonstrated. A good correlation between these two techniques was obtained by several authors denoting immunohistochemical technique is reliable for detection.[17,18]

The prognostic value of Cath-D has not been reported in breast cancer of Chinese women. We analyzed Cath-D in 277 cases of breast cancer by immunohistochemical method. Our results showed that the lymph node metastases rate in Cath-D positive cases was significantly higher than that in the Cath-D negative ones. In addition, most tumors in Cath-D positive cases were of larger size. Thus, Cath-D is also valuable as a predictor for breast tumor aggressiveness in Chinese women.

It was reported that overexpression or amplification of c - *erb*B - 2 in breast cancer indicated a poor prognosis.[19,20] We reported previously the investigation of c - *erb*B - 2 in 109 cases of breast cancer, and found that over - expression of it was closely correlated with the malignant grades.[21] In the present work, we found that positive rate of c - *erb*B - 2 in the Cath - D positive cases was significantly higher than that in the Cath - D negative. However, no significant difference was found in its positive rate between lymph node positive and negative cases.

Costa et al.[22] measured the concentration of EGFR in biopsies of 376 cases of patients with operable breast cancer, and found that one year relapse - free survival rate of patients with EGFR positive was lower than that of EGFR negative. We found that positive rate of EGFR in the Cath - D positive cases was significantly high than that in the Cath - D negative, but no significant difference was found in its positive rate between lymph node positive and negative cases.

The analysis of the results from the co - examination of Cath - D, c - *erb*B - 2 and EGFR, the lymph node metastasis and the tumor size revealed that among the three markers, Cath - D is the most valuable predictor for prognosis, it thus should be in a necessary examination program. In Cath - D positive cases, special treatment scheme and close follow ups should be suggested.

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