The Prognostic Significance of a Combined Determination of Cathepsin D and Estrogen Receptors in Breast Carcinomas with Positive Axillary Lymph Nodes

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OBJECTIVE The aim of this study was to investigate the correlation between cathepsin D (Cath-D) and estrogen receptor (ER)expression in breast cancer tissue and to explore the prognostic significance of their combined determination in breast carcinoma patients with positive axillary lymph nodes.

METHODS One hundred and thirty-eight cases of breast carcinoma were examined by immunohistochemistry (IHC) and the results relating to patient follow-up analyzed.

RESULTS The overall 5-year disease-free survival rate (DFS) was 60.9% (84/138) in the series. The positive rate of Cath-D expression in the tumor cells was 55.07% and the positive ER staining was 51.4%. A definite significant negative correlation was found between the positive rates for Cath-D and ER (r=-0.294, P=0.001) The Cath-D expression for the cases in clinical Stage II, ≥10 positive-node and recurrence or distant metastasis, was higher than that those cases in clinical Stage II with fewer node-metastasis and with 5 year DFS (χ^2=13.926, P=0.000; χ^2=13.070, P=0.001; χ^2=10.545, P=0.001). However, there was no significant difference of Cath-D expression between 2 groups of patients with different ages or among the different histopathologic types of the non-specific invasive carcinoma. In the combined examination of Cath-D and ER, the cases that were ER (+) and Cath-D (-) had the highest 5-year DFS compared to other situations. In contrast, the cases that were reversed in expression , ie, ER(-) and Cath-D(+), had a lower 5-year DFS. There was a significant difference between the 2 conditions (χ^2=18.675, P=0.000).

CONCLUSION A combined determination and analysis of Cath-D and ER expression may be more useful to establish a prognosis than the biological characteristics of carcinomas with positive lymph nodes.

KEYWORS: breast carcinoma, cathepsin-D, estrogen receptor, combined determination, prognosis.

It has been reported that cathepsin-D (Cath-D), which is a proteinase, may enhance the growth of the tumor cells by degrading the basement membranes and extracellalar matrix. This action allows the cancer cells to easily invade the regional tissues and metastasize to destant sites. However, there are few reports concerning the prognostic significance of a combined determination of Cath-D and estrogen receptors. In the current study, we explored the correlation between the expression of Cath-D and ER in breast carcinomas in order to relate the correlation to the prognosis of the patients.

MATERIALS AND METHODS

Samples and groups

Tissue blocks of the breast tumors and clinical records were obtained from the Tianjin Medical University Tuomr Hospital from 138 cases during the period from June 1986 to January 1995. All of the cases met the following qualifications: 1) the range of the patients' age was from 31 to 50 years; 2) clinical Stages were II or III; 3) the pathologic diagnoses were all usual non-specific invasive breast carcinoma;[1] 4) all cases had positive axillary lymph nodes; 5) data from 5 years of follow-up were complete.

Determination of Cath-D

Reagents

Cath-D rabbit-anti-human polyclonal antibody and a immunohistochemistry (IHC) S-P kit were purchased from the ZYMED Co., USA.

Methods

Inmmunohistochemical staining was conducted on the tissue using the labelled streptavidin biotin method (S-P) with the Cath-D primary antibody (at 1:100). Positive control (breast cancer specimens known to show Cath-D positive expression) and negative controls (TBS buffer and normal serum replaced the primary antibody) were included with every determination.

Assessment of the results

The positive IHC indication for Cath-D expression was located in the cytoplasm seen by a yellow-brown staining. If the percentage of the positive cells was ≥30% (×100), the case was considered to be positive.

Determination of ER

Reagent

ER mouse-anti-human monoclonal antibody was obtained from the Zhongshan Biotechnology Co. China.

Methods

Immunohistochemical staining was conducted on the tissues using the labeled streptavidin biotin method (S-P) with the ER primary antibody (at 1:50), in a manner similar to that with Cath-D, except that the antigen was restored by microwaving for 10~20 min. Positive and negative controls were included with every assay.

Assessment of the results

IHC positive signals of ER expression were located in the nucleus as indicated by a yellow-brown staining. If the percentage of the positive cells was ≥15 % (× 100), the case was considered to be positive.

Statistical analysis

Analysis of the data was performed with the SPSS 10.0 software package. The differences among the groups were compared using the chi-square test; prognostic parameters were evaluated by Spearman rank correlation analysis; the Kaplan-Meier test was used for survival analysis. The level of significance was set at P= 0.05.

RESULTS

Consequence of follow-up: The survival of these patients was between 6 months and >60 months. The overall 5-year disease-free survival was 60.9% (84/138), 73.1% (49/67) for stage II cases, and 49.3% (35/71) for stage III cases. The difference in the survival rates is shown in the survival curve (Fig.1). The difference was statistically significant (χ^2=9.77, P= 0.018).

The expression of Cath-D was located in the cytoplasm as indicated by a yellow-brown staining (Fig.2). The positive rate of Cath-D expression was 55.1%.

The expression of ER was located in the nucleus as indicated by a yellow-brown staining (Fig.3). The rate of ER positive expression was 51.4%.

Cath-D and ER expression showed a negative correlation (r=-0.294, P=0.001, Table 1).

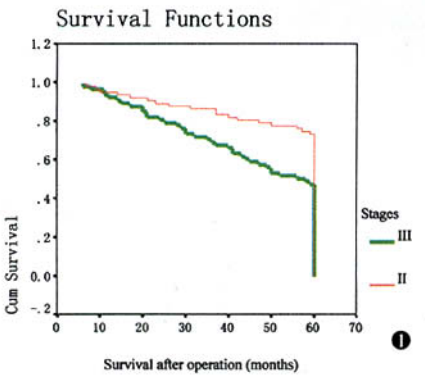
Table 1. The distribution and correlation of expression of Cath-D and ER

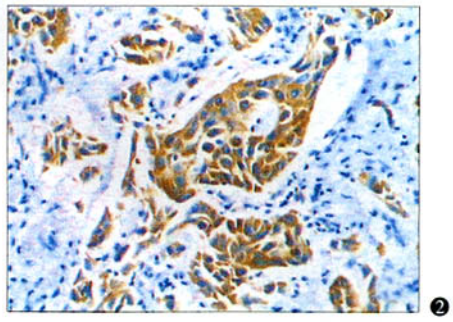
	Cases	Cases of Cath-D(+) (%)	Cases of Cath-D(-) (%)
ER(+)	71	29(38.0)	42(62.0)
ER(-)	67	47(62.0)	20(26.9)
Total	138	76(55.1)	62(44.9)

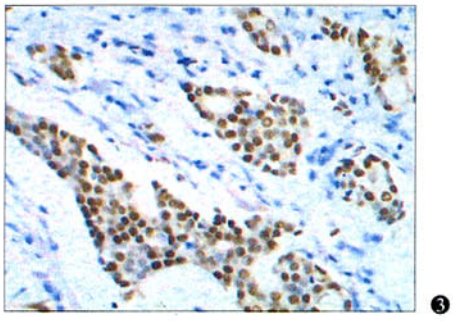
Cath-D *vs.* ER expression: r=-0.294, P=0.001.

There was a statistically significant difference in Cath-D expression between stages II and III cases (χ^2= 13.926, P=0.000). The positive rate of the cases in Stage III was higher than that in stage II.

The cases were subdivided into 3 groups based on the number of positive lymph nodes (1-3; 4-9; ≥10). The group with ≥10 positive lymphoid nodes had a







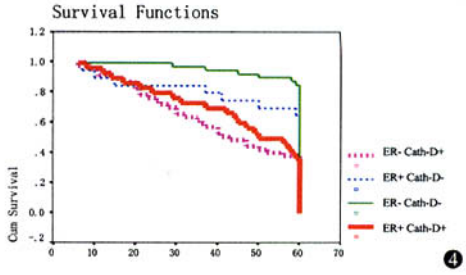


Fig.1. comparison of survival of stage II and stage III patients.
Fig.2. Positive expression of Cath-D located in the cytoplasm of breast cancer cells, IHC method(S-P)(× 200).
Fig.3. Positive expression of ER is located in the nucleus of breast cancer cells, IHC method (S-P)(× 200).
Fig.4. Correlation of 5-year-survival rate without a tumor and a combined test of Cath-D&ER.

Table 2. The relation of expression of Cath-D and patho-clinical index

Item	Cases	Positive Cases (%)	χ^2	*P*
Age			0.246	0.620
31~40 years old	68	36(52.94)		
41~50 years old	70	40(57.14)		
Clinical stages			13.926	0.000
Stage II	67	26(38.81)		
Stage III	71	50(71.43)		
Type of pathology			0.139	0.933
IDC (simple)	75	45(60.00)		
IDC	30	18(60.00)		
Other	33	21(63.64)		
Positive nodes			13.070	0.001
1~4	48	18(33.33)		
4~9	45	25(55.56)		
≥ 10	45	33(73.33)		
5 year-follow-up			10.54	0.001
Existing without cacinoma	84	37(44.05)		
Existing with cacinoma or death	54	39(72.22)		

stastically high rate of Cath-D expression (χ^2=13.070, *P*=0.001). Similarly, the Cath-D positive rate of the group of patients who died or had a recurrence was higher than the group who survived without a tumor during the 5-year follow-up (χ^2=10.545, *P*=0.001).

However there was no statistically significant differences between 2 aged groups (31~40 years; 41~50 years) (χ^2=0.246, *P*=0.620) or among distinct types of pathology (types of invasive non-specific carcinoma) (χ^2=0.139, *P*=0.933, Table 2).

The results of the combined determination of Cath-D and ER expression are presented in Table 3. The comparison of the survival rate among groups is presented in Fig.4. Five year disease-free survival rates among the 4 groups showed a stastistically significant difference (χ^2=25.99, *P*=0.0000). The combined determination of Cath-D and ER showed that cases with positive ER expression and negative Cath-Dexpression had the highest 5-year disease-free survival rate. However cases with negative ER expression and positive Cath-D expression had the poorest prognosis. These 2 survival curves were stastically different (χ^2=18.675,

P=0.000) but the 2 survival curves with ER(+)Cath-D (+) versus ER(-)Cath-D(-) showed no statistical difference (χ^2=0.044, P=0.834). However the survival curve of cases with ER (+) and Cath-D (-) expression approached the curve with ER(-) and Cath-D(+) expression at 60 months (Fig.4).

Table 3. The prognostic significance of the combined detection of ER and Cath-D

Expression of ER&Cath-D	Cases	Cases of 5-year-living without tumour (%)
ER(+)Cath-D(+)	29	18(62.1)
ER(+)Cath-D(-)	42	35(83.4)
ER(-)Cath-D(+)	47	18(38.3)
ER(-)Cath-D(-)	20	13(65.0)

χ^2=19.112 P=0.000 compared among 4 groups
χ^2_1=4.099 P1=0.000 compared with group of ER+ Cath-D+ &ER+ Cath-D-
χ^2_2=4.065 P2=0.044 compared with group of ER+ Cath-D+ &ER- Cath-D+
χ^2_3=0.044 P3=0.834 compared with group of ER+ Cath-D+ &ER- Cath-D-
χ^2_4=18.675 P4=0.000 compared with group of ER+ Cath-D- &ER- Cath-D+
χ^2_5=2.605 P5=0.107 compared with group of ER+ Cath-D- &ER- Cath-D-
χ^2_6=4.024 P6=0.045 compared with group of ER- Cath-D+ &ER- Cath-D-

DISCUSSION

Cath-D is an estrogen-inducible aspartyl protease, a 52kD glycoprotein, shown to be secreted into the medium by MCF-7 cells.[2] Studies have demonstrated that the protein has 2 biological activities at an acidic pH: a mitogenic and a proteolytic activity, both of which suggest that Cath-D may have prognostic significance for breast cancer. It was reported that reported that Cath-D is the most active proteinase in breast carcinomas. Cath-D can play an important role in the progress of tumor metastasis by degrading the basal lamina and the extracellular matrix.[4,5] So most scholars believe that tissues from cases with a high level of Cath-D expression would have a poor prognosis. In the 138 breast carcinoma cases with positive lymph nodes, those with a high level of Cath-D expression had the most positive nodes, and suffered a recurrence or death, or were in stage III. The expression of Cath-D can reflect the prognosis of patients.

In a general sense, cases with positive ER expression would have low a recurrence rate, but some also had a poor prognosis perhaps because of many interacting factors.

More studies have confirmed[7,8] that the secretion of Cath-D in breast cancers is induced by estradiol. As the level of estrogen increases, the expression of Cath-D increases. It is believed that Cath-D is an estrogen-dependent protease.[9] But the results of the 94 cases in that study with ER(+) and Cath-D overexpression suggested that these patients had a long life span. These results are not in accord with the theory that Cath-D expression is correlated to a poor prognosis.

The cases in our study were subdivided and analyzed by many aspects, such as age and clinical stages. The purpose was to remove different interfering factors as far as possible. The results showed that the cases which were ER (+) and Cath-D (+) would have a poorer prognosis compared to those which was ER(+) and Cath-D (-), probably because with ER increasing, the secretion of Cath-D in the tumor increases, thus promoting invasion of malignant cells and advancement of metastasis. These results support our belief that Cath-D is an estrogen-dependent protein relating to poor prognosis. The reason for the poorest prognosis of cases that are Cath-D(+) and ER(-) may be that there were subgroups of breast cancer cells. The Cath-D secretion of these kinds of subgroups may relate to other autocrine growth factors, but the dependence of Cath-D secretion on ER falls, and fails to be influenced by the hormone regulation, that leads to ER negative expression, Cath-D(+) and ER(-) leading to poor prognosis.

The result of our study showed that: a combined determination and analysis of Cath-D and ER expression may provide a more accurate diagnosis than the biological characteristics of breast carcinomas with positive lymph nodes. This method of evaluation may be useful to improve the prognostic accuracy for breast carcinoma patients.

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