

Expression of CD44v6 Protein in Human Breast Carcinoma and its Relevance for Prognosis

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Abstract Purpose To study the expression of CD44v6 protein in breast carcinoma and its prognostic significance. **Methods** 100 cases of formalin-fixed paraffin-embedded female breast invasive ductal carcinoma tissues were retrospectively analyzed using EnVision™ immunohistochemical method with the monoclonal antibody CD44v6. Statistical analysis was based on the Long-rank test and Cox analysis. **Results** Sixty-six of 100 cancer tissues expressed CD44v6. Positive staining was mainly on the cell membranes. There was significant correlation between CD44v6 immunoreactivity and lymph node metastasis and TNM stage. The 5- and 10-year survival rates were 82.76% and 78.37% of patients with CD44v6 low-expression tumors, and 64.1% and 49.88% of those with CD44v6 high-expression tumors, respectively; the difference between the two groups of patients was significant ($p < 0.05$). In multivariate analysis using the Cox regression model, CD44v6 expression did not emerge as an independent prognostic indicator. **Conclusions** The results suggest that CD44V6 plays a role in metastasis of breast carcinoma, and CD44v6 expression might be a new useful prognostic marker of breast carcinoma.

Key Words breast carcinoma; CD44v6; metastasis; prognosis

Breast carcinoma is a very common malignant tumor in China, and its incidence has increased greatly in recent years^[1], so it is important to evaluate its biological behavior. Recently, CD44, a cellular adhesion molecule, especially its variant isoforms have been linked to the tumor metastasis, but few reports have been noticed concerning CD44v and breast carcinoma, and results were confusing. We investigate the CD44v6 expression in female breast invasive ductal carcinoma by immunohistochemical method, and the relationship between CD44v6 expression status and metastasis, prognosis was discussed.

MATERIAL AND METHODS

Patients Tissue blocks of breast invasive ductal carcinoma from 100 patients were obtained from the archives of the department of pathology, Railway hospital, all the cases were followed up after the operation until Jan 1999. 65 cases of Non-recurrent group were all alive, whereas 35 cases of recurrent patients were all dead (survival range, 3.47-169.33 months), the mean follow-up period was 91.85 months. All the cases were diagnosed by different two pathologists. All the patients were female with a mean age of 54.52 years (range, 24-86 years). According to TNM staging method, 11 cases were stage I, 59 cases stage II, and 30 cases stage III. All the specimens were fixed by 4% formalin, embedded in paraffin wax.

Main reagent The rat anti-CD44v6 monoclonal antibody was purchased from the company of Bender Medsystem (dilution, 1:1:400). Envision™ were from the Dako Company.

Immunohistochemical assay 4 μ m thick sections of each specimen were deparaffinised, put in the citrated buffer (temperature 95°C, PH=6.0), antigen was repaired by microwave for 5 minutes 4 times, immunoreactivity was detected by EnVision, staining was visualized using DAB, TBS was used as a negative control instead of the specific monoclonal antibody, the normal cutaneous squamous epithelial was used as positive control.

Evaluation criteria The immunohistochemical result was evaluated by the percentage of positively stained tumor cells, negative(-), 0; weak positive(+), between 1% -10%; moderate positive(++), between 11% and 50%; strong positive(+++), >50%. Negative and weak positive group were regarded as low expression, and moderate positive and strong positive group as high expression.

Statistical analysis Microsoft SPSS8.2 was used for statistical analysis. The relationship between CD44v6 expression and clinical pathological features was evaluated using χ^2 or spearman test. Kaplan-Meier survival curve, Log-rank analysis and Cox Hazard mod-

el were used for survival analysis.

RESULTS

CD44v6 immunoreactivity in breast carcinoma CD44v6 positive staining was present at cell membrane(Fig.1). CD44v6 expression rate was 66% in breast carcinoma, among them 27% was (+), 16% was(++) and 23% (+++). All the normal breast tissues were negative.

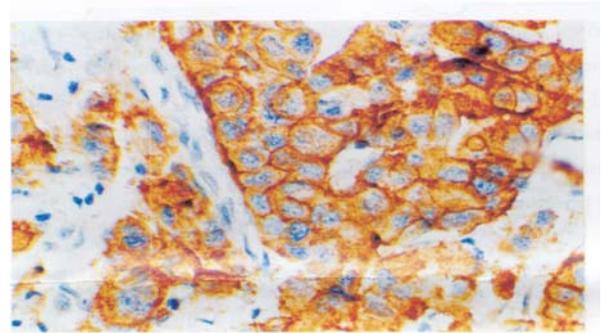


Fig. 1 Positive CD44v6 expression in breast invasive ductal carcinoma (×400)

Table 1 The relationship between CD44v6 expression and pathological features

features	case	CD44v6 expression				expression rate	x ²	P
		-	+	++	+++			
age								
≤ 50 years	40	16	9	7	8	60.0	1.561	0.668
>50 years	60	18	18	9	15	70.7		
tumor size								
≤ 3cm	52	16	19	8	9	69.2	5.535	0.137
>3cm	48	18	8	8	14	62.5		
lymph node metastasis status								
non-metastasis	39	15	12	9	3	61.5	9.226	0.026
metastasis	61	19	15	7	20	68.9		
survival status								
survival group	65	30	15	8	12	53.8	12.373	0.006
dead group	35	4	12	8	11	88.6		

x² test, *p<0.05

Table 2 The relationship between CD44v6 expression and clinical stage

clinical stage	case	CD44v6 expression				expression rate	γ	p
		-	+	++	+++			
I	11	6	4	0	1	45.5	0.082	0.004
II	59	21	17	11	10	64.4		
III	30	7	6	5	12	76.7		

Table 3 Prognostic analysis by Cox model

Variant	standard error	wald	degree of freedom	p	r	experimental error
Lymph node metastasis status	0.7843	7.2843	1	0.0070	0.1381	8.3042
CD44v6	0.3799	3.7495	1	0.0528	0.0794	2.0896
Age	0.3878	3.3919	1	0.0655	0.0709	2.0427
Stage	0.4087	2.6651	1	0.1026	0.0490	1.9489
Tumor size	0.4011	0.6635	1	0.4153	0.0000	1.3864

The relationship between CD44v6 expression and the clinical pathological features of breast carcinoma

The result indicated that CD44v6 expression was independent of the age or tumor size, it was associated with lymph node metastasis, clinical stage, recurrence and survival status ($P < 0.05$) (table 1,2). CD44v6 (+++) was 7.7% (3/39) in non-lymph node metastasis group, compared to 32.8% (20/61) in lymph node metastasis group; the positive rate was 45.5% (5/11) in stage I patients, whereas 76.7% (23/30) in stage III ones; in 65 cases of no-recurrent group CD44v6 positive expression was 53.8%, whereas it was 88.6% in 35 cases of recurrent group.

The relationship between CD44v6 expression and survival rate Kaplan-Meier survival curve was made, and Log-rank test was used to analyze the survival status of 100 cases of breast carcinoma, the result showed that the five-year survival rate was 82.76% and 64.10% respectively in CD44v6 low expression group and high expression group, and ten-year survival rate was 78.37% and 49.88% respectively, the difference was significant ($P = 0.0055$) (Fig.2). In addition, tumor size, lymph node status and clinical stage were associated with survival time ($P < 0.05$). the prognosis was independent of the age ($P > 0.05$).

Cox model multivariate analysis

The result showed that the lymph node metastasis status was a independent prognostic marker, but the CD44v6 was on the borderline (table 3)

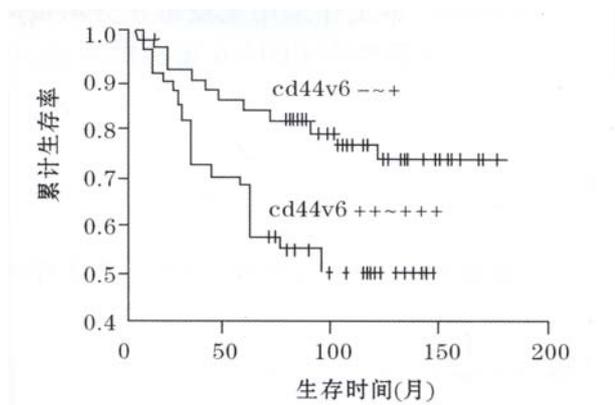


Fig.2 The relationship between CD44v6 expression and survival, prognosis

DISCUSSION

The CD44 family of cell surface adhesion molecules is expressed in a variety of normal and neoplastic tissues. This protein family has been linked to a number of functions including lymphocyte homing and activation, cell-cell, and cell-extracellular matrix interactions, and cell migration. To be able to exert such diverse effects, CD44 generates numerous isoforms through a mechanism of alternative splicing. Some of these CD44 isoforms also seem to play a role in the production of tumor metastases. Specifically, a non-metastatic cell line of a rat pancreatic adenocarcinoma has been shown to acquire metastatic potential when transfected with CD44 variants containing exon v6 (CD44v6), an effect that can be blocked by anti-v6 antibodies^[2]. Thereafter, many studies showed that CD44v was associated with metastases of many kinds of tumors^[3].

With regard to the relationship of the expression of CD44v6 with the breast carcinoma, different authors have different results. Daufman, et al^[4] investigated the CD44v expression status in breast carcinoma, and found that most tumor specimens expressed CD44v3, v5 and v6, but in normal breast tissues they were negative. CD44v6 was expressed in 84% of carcinomas in situ, 100% of breast carcinomas with lymph node metastases or local recurrences, and the prognoses were much poor in CD44v6 positive group than those in negative one. CD44v6 is a isolated prognostic marker independent of tumor size, stage or lymph node status. Sinn et al^[5] have the consistent results with the previous study. Sheen-chen et al^[6] detected the serum soluble CD44v6 in patients with breast carcinoma, study indicated that the serum soluble CD44v6 is positively correlated with distant metastases and TNM stage.

But other authors have different opinion. Some results showed that CD44v6 expression is high in breast carcinoma, which is independent of age, tumor size, histologic type, clinical stage, lymph node status or p53 expression, and it is not a prognostic marker^[7].

We used the EnVisionTM immunohistochemical assay, which has the characteristic of high sensitive, clear background and reliable results, our results indicated that CD44v6 expression has increased significantly in breast carcinoma and it is negative in normal tissues. CD44v6 expression is independent of age or tumor size, but closely associated with lymph node metastases and clinical stage, which support the idea that CD44v6 is a

metastasis-related factor. Survival analysis indicated that patients with high CD44v6 expression have poor prognoses than those with low CD44v6 expression, but in multivariate survival analysis, *p* was on the borderline, so we can not deny it as an isolated prognostic marker. We believe that CD44v6 is a new useful biological marker for the prognostic evaluation of breast carcinoma.

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