EXPRESSION AND SIGNIFICANCE OF UROKINASE-TYPE PLASMINOGEN ACTIVATOR IN BREAST CANCER

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ABSTRACT

Objective: To study the expression and clinical significance of urokinase-type plasminogen activator (uPA) in breast cancer. Methods: Applying streptavidin-biotin complex (SABC) immunohistochemical technique, expression of uPA was studied in 100 patients with primary breast cancer. Results: There were 55 patients with high uPA expression, and 45 with lower expression. There was significant correlation between uPA expression and TNM stage, lymph node status, and the tumor size. Neither age, menopausal status, nor ER status was significantly related with level of uPA expression. The patients with high expression of uPA had significantly shorter disease-free survival (DFS) and overall survival (OS) than did those with low expression of uPA. Univariate analysis showed that uPA as a prognostic factor was of similar magnitude to lymph node status and TNM stage, but stronger than that of ER status and tumor size. UPA was an independent prognostic factor affecting disease-free survival and overall survival. Conclusion: uPA appears to be a strong and independent biologic marker for predicting prognosis of breast cancer.

Key words: Urokinase-type plasminogen activator, Breast cancer, Immunohistochemistry, Prognosis

The mortality of breast cancer is closely related to the invasive and metastatic potential of the cancer cells. Fibrinolysis mediated by urokinase-type plasminogen activator (uPA) can degrade extracellular matrix and basement membrane. Increased levels of uPA correlate with higher invasive and metastatic ability.[1] In this paper, we studied the expression of uPA in breast cancer tissue using immunohistochemical method, combining such expression with follow-up data, and exploring the relation of such expression to prognosis.

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MATERIALS AND METHODS

Patients

From 1987–1994, 100 female patients with primary breast cancer were treated in the Department of Oncology, the Second Affiliated Hospital of Hubei Medical University. Their ages ranged from 19 to 74 years, with an average value of 48. All patients underwent radical mastectomy or modified radical mastectomy, 58 had postoperative chemotherapy and 36 had radiotherapy after operation. 61 patients with positive estrogen receptor (ER) received tamoxifen treatment. One patient was lost to follow-up, the remaining 99 patients were followed by clinical visits for 48 to 120 months (median 82 months). 33 patients had recurrence or distant metastasis and 29 of them died.

Methods

Four-μm thick sections were made from the archive paraffin blocks, and stained with SABC methods, according to the technical instruction of the manufacturer's manual. The sections were pretreated by microwave in 0.01 mol/L sodium citrate solution. The uPA monoclonal antibody was from the Oncogene Science Inc. working dilution 1:400. The SABC reagent kit was purchased from Wuhan Boster Bioengineering Co. Ltd. For each assay, using positive controls of known strong uPA expression slides, and by TBS replacing the primary antibody for negative control were used to ensure inter-assay consistency.

Interpreting Criteria

The slides were viewed under microscope.

Cancer cells with yellow stained the cytoplasm were regarded as positive. Five different grades were made based on the percentage of stained tumor cells in all tumor cells: grade 0: no stained cells; grade I: 1%~24% stained cells; grade II: 25%~49% stained cells: grade III: 50%~70% stained cells; and grade IV: over 70% stained cells. <50% stained cells were designated as low uPA expression, and ≥50% as high expression.

Statistical Analysis

The clinicopathologic characteristics of the patients in relation to uPA were compared and checked with the chi-squre test. The survival rate was calculated by the Kaplan-Meier method and statistical significance was analyzed by the log-rank test. The Cox proportional hazards regression model was used for univariate and multivariate analysis, with significance defined as $P<0.05$. All the above analyses were performed on SAS Software.

RESULTS

UPA Staining Results

The uPA stain was mainly located in the cytoplasm of tumor cells. Among the 100 patients, 55(55%) had high uPA expression and 45(45%) had low uPA expression.

Relationship between uPA Expression and Other Clinicopathologic Characteristics

UPA expression had no relation with age, menopausal status or ER status. There was significant correlation between uPA expression and TNM stage, tumor size and lymph node status. High uPA expression occurred more frequently in tumors of advanced TNM stage, high lymphatic metastasis and with bigger tumor size (Table 1).

Correlation between uPA Expression and Prognosis

In the patients with high uPA expression, 33 relapsed (47.3%) and 23 died (41.8%). In those with low uPA expression, 7 relapsed (15.6%) and 6 died (13.3%). Their recurrent rate and the mortality rate were much higher in patients with high uPA expression than in those with low expression ($P<0.001$). These results showed that patients with high uPA expression had significantly shorter disease free survival (DFS) and overall survival (OS) than did those with low uPA expression.

Table 1. Relation between uPA and clinicopathologic factors

Factors	n	High uPA(%)	χ^2	*P Value*
Age (yrs)				
<50	66	40(60.6)	2.46	>0.05
≥50	34	15(44.1)		
Menopausal status				
Pre-	63	39(61.9)	3.28	>0.05
Post-	37	16(43.2)		
Tumor size(cm)				
≤2.0	18	6(33.3)		
2.1~5.0	64	35(54.7)	7.2	<0.01
>5.0	18	14(71.8)		
Nodal metastasis				
positive	56	41(73.2)	17.06	<0.005
negative	44	14(31.8)		
TNM Stage				
I	9	2(22.2)	14.74	<0.005
II	39	16(41.0)		
III	46	31(67.4)		
IV	6	6(100)		
ER status				
+	61	38(62.3)	3.36	>0.05
−	39	17(43.6)		

Univariate and Multivariate Cox Model Analysis

For DFS and OS, univariate analysis showed that uPA, just like TNM stage, lymph node status and ER, were closely related to prognosis ($P<0.01$). Moreover, the uPA risk ratio was much higher than that of ER, suggesting that variation in uPA expression had a greater impact on survival than ER. The stronger the uPA expression, the higher the risk for recurrence and death. Age, menopausal status and tumor size were not significantly correlated with prognosis ($P>0.05$, Table 2). Multivariate analysis showed that for DFS and OS, uPA was an independent prognostic factor just like TNM stage and ER, suggesting it is a risk factor. The stronger the uPA expression, the shorter the survival (Table 3).

DISCUSSION

uPA is a serine proteinase, secreted by tumor cells and other cells. It is initially secreted as a single chain zymogen form pro-uPA which binds to its specific receptors on tumor cells. Then it is activated to become double-chain active uPA, which converts plasminogen to plasmin. The plasmin degrade extracellular matrix and basement membrane components such as fribrin, fibronectin, laminin, ultimately leading to tumor cell invasion and metastasis.[1]

Table 2. Univariate Cox model analysis results

Variable	Disease-free survival			Overall survival		
	Risk ratio	χ^2	*P*	Risk ratio	χ^2	*P*
uPA	3.714	24.4547	0.0001	4.119	24.4807	0.0001
Stage	4.151	10.9955	0.0009	4.412	10.2897	0.0013
Nodal metastasis	6.348	14.3420	0.0002	7.337	13.5500	0.0002
ER	0.369	8.0859	0.0045	0.384	6.5177	0.0107
Tumor size	1.191	2.3136	0.1282	1.269	3.6140	0.0573
Age	0.995	0.0830	0.7733	0.998	0.0157	0.9004
Menopausal status	0.979	0.0035	0.9531	0.919	0.0481	0.8264

Table 3. Multivariate Cox model analysis results

Variable	Disease-free survival			Overall survival		
	RR	χ^2	*P*	RR	χ^2	*P*
Stage	3.395	16.2648	0.0001	3.822	16.5476	0.0001
uPA	2.815	5.1194	0.0237	2.836	4.4220	0.0355
ER	0.267	13.2733	0.0003	0.271	11.2174	0.008

The prognosis of breast cancer is not only related to the tumor stage and the thoroughness of resection at surgery, but also related to comprehensive therapy. Clinical practice found that even though the TNM stage, lymph node status and treatment modality is similar, patients have widely different prognosis, which might be due to the different tumor biological behaviors. Therefore, finding out biological factors affecting prognosis and studying their biological behavior are very important to making rational treatment regimen and evaluating prognosis. Researchers have found higher uPA expression in breast cancer than in breast benign tumors. And over expression of uPA in breast cancer is closely correlated with prognosis.[2,3]

Duffy, et al.[4] firstly reported that there is a relationship between uPA expression and prognosis of breast cancer in 1988. Using ELISA method, they found that patients with breast cancer containing high levels of uPA had a shorter DFS than those with low uPA. Later, they found the OS was shorter in high uPA level breast cancer patients. As an independent prognostic factor, uPA was similar to lymph node status, and its prognosis value was higher than ER, PR and tumor size.[5] These results have been confirmed by many reports.[6-8] Our study also found that uPA expression is negatively correlated with DFS and OS of the patients. Using univariate and multivariate analyses, uPA appears to be a strong marker for predicting prognosis of breast cancer and may be the strongest biological marker except for TNM stage and nodal status.

Our study found no relationship between uPA expression and age, menopausal status, ER or PR, which are in agreement with other reports.[2,6,8] What is different in our findings is the correlation between uPA expression and lymph node status, TNM stage and tumor size. High uPA expression tumors tend to be in advanced TNM stage, have a larger tumor size and a greater likelihood of lymphatic metastasis. We believe these results are more in keeping with the biological behavior which uPA promote invasion and metastasis.

Carriero, et al.[9] found that there is a positive correlation between immunohistochemical reactivity of uPA and tissue concentration of uPA as determined by ELISA on tissue extracts from the same specimens. The higher the tissue concentration of uPA was, the stronger degree of uPA staining there was. Heiss, et al.[10] found no difference in staining degree and the number of stained cells between cryostat- and paraffin-embedded sections in 30 gastric carcinomas. No difference in the immunohistochemical reactivity of uPA between cryostat- and paraffin-embedded sections was found in our experiment. All of these results support the reliability of our immunohistochemical assessment of uPA, which allows for the use of paraffin sections, thereby avoiding frozen and storage procedures of fresh tumors.

In summary, uPA is closely correlated with prognosis of breast cancer and may be an independent and strong biological marker for predicting prognosis of breast cancer. In addition, immunohistochemical assessment of uPA is a simple and readily used method, which can be easily applied to practice. The stain results can help identify patients with high risk of relapse and metastasis, for whom timely and reasonable adjuvant therapy are needed, therefore enhancing the survival of breast cancer.

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