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Expression of E-Cadherin and uPA and their Association with the Prognosis of Pancreatic Cancer

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Objective: E-cadherin (ECD) and urokinase plasminogen activator (uPA) have been noted as markers for tumor metastasis and prognosis in several tumors. We thus investigated the relationship between the expression of ECD and uPA and the clinicopathological characteristics in pancreatic cancer.
Methods: The expression of ECD and uPA was evaluated in pancreatic cancer tissues from 53 patients.
Results: Among 53 tumor tissues, those from 29 (54.7%) patients showed positive ECD expression and those from 22 (41.5%) patients showed positive expression of uPA. There were four subgroups of ECD/uPA expression: ECD-positive/uPA-negative, ECD-negative/uPA-negative, ECD-positive/uPA-positive and ECD-negative/uPA-positive. These patterns were found in 14 (26.4%), 11 (20.8%), nine (17%) and 19 (35.8%) patients, respectively. The tumor tissues with ECD-negative and uPA-positive expression were associated with larger tumor, distant metastasis and an increased clinical stage. There was a difference in the median survival time between the patients with ECD-positive/uPA-negative pancreatic tissues (median: 18.7 months) and the patients with ECD-negative/uPA-positive pancreatic tissues (median: 7.5 months, $P < 0.05$), and there was a statistically significant difference in survival curves between these two groups.
Conclusion: The combined analysis concerning uPA and E-cadherin expression may be a useful predictor of metastasis in pancreactic cancer.

Key words: pancreatic neoplasms – E-cadherins – urokinase plasminogen activator

INTRODUCTION

The incidence of carcinoma of the pancreas has increased remarkably over the past several decades, and it now ranks as the fourth leading cause of cancer death in the USA (1).

At the time when the diagnosis of pancreatic cancer becomes clinically clear, the disease makes rapid progress and metastasis has usually already occurred (2). The mechanisms whereby this cancer exhibits such an aggressive growth pattern are still unclear.

Current studies have shown that the biology of cancer is complex, with multiple different actions, reactions and molecular pathways interacting with each other to facilitate the passage of tumor cells from their primary site to a distant site (3). The essential factors in the metastatic process include changes in cellular adhesion, production of proteolytic enzymes degrading the stroma and the secretion of various cytokines that attract and activate stromal cells and endothelial cells during tumor invasion and angiogenesis (4). In epithelial tumors, the original escape of a tumor cell from its primary site requires the loss of cell–cell attachment that is mainly mediated by the molecules of the members of the cadherin family, especially E-cadherin (4). A decline of E-cadherin function in tumors results in rapid progression of the tumor, not only in metastatic carcinomas but also in relatively benign adenoma. Cells that have a germline mutation in E-cadherin have a predisposition to become diffuse, poorly differentiated cancer, and its downregulation in sporadic tumors is associated with poor clinical prognosis (5). An indispensable step in the metastatic cascade is the breach of the basement membrane and invasion to the surrounding stroma (5). Urokinase plasminogen activator (uPA) is a proteolytic enzyme that might contribute to this process. Immunohistochemical studies have demonstrated that components of this proteolytic enzyme are located at the invasive margin of cancers and also in the associated stromal cells, which suggests that complex interactions exist between the tumor and stroma during the invasive process (6). Several

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studies have reported that overexpression of uPA is strongly associated with the malignant phenotype and nodal metastasis (7). For several cancers, some investigators reported that there is close correlation between invasion and expression of E-cadherin/uPA. Yutak et al. have reported on the inter-relationship of invasion and E-cadherin/uPA for gastric cancer. However, there has been no report about metastasis and the E-cadherin/uPA inter-relationship for pancreatic cancer. In this study, we have analyzed the concomitant expression of E-cadherin and uPA in pancreatic cancer, and its correlation with the clinicopathological characteristics are discussed.

PATIENTS AND METHODS

Patients and Pathological Specimens

Fifty-three patients who underwent surgery for pancreatic cancer at the Yeungnam and Kyemyung University hospital between 1994 and 2004 were the subjects of our investigation. These patients included 24 male and 29 female patients with a mean age of 58 years (range: 33–75). No patient received radiotherapy or chemotherapy before surgery. All the tissue specimens were histologically proven cases of ductal adenocarcinoma based on WHO histological classification of tumors of the exocrine pancreas. The TNM classification system designated by the American Joint Committee on Cancer (AJCC) was employed for the clinical staging. Tissue specimens were taken at the time of operation, all of which were from the primary lesions.

Immunohistochemistry

The specimens were fixed with 10% formalin and embedded in paraffin, and then they were cut into 4 μm serial sections.

The primary anti-E-cadherin monoclonal antibody (mAb) was obtained from Zymed (South San Francisco, CA). The anti-uPA mAb was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The specimens were stained immunohistochemically by the labeled streptavidin–biotin–peroxidase method for uPA and E-cadherin. Before staining, the sections were pretreated with microwaves (4 min at 900 W) in 0.1 mol/l citrate buffer for antigen retrieval. 3,3′-Diaminobenzidine (DAB) was used as the chromogen. Phosphate-buffered saline (PBS) was substituted for the primary antibodies as a negative control.

Evaluation of Immunohistochemical Staining

Two expert pathologists who had no knowledge of the patients' outcome reviewed the slides. Grading of E-cadherin expression was classified into four groups: >70% positive expression, 50–70% positive expression, 10–49% positive expression and <10% positive expression. Cancer cells that were immunostained at <70% of the cells were defined as having a reduced E-cadherin expression (E-cadherin-negative). If uPA expression could be found in >10% of the cells, the tumors were determined to be uPA positive.

Statistics

All statistical analyses were performed using SPSS for Windows (version 10.0). For the statistical analysis, frequency

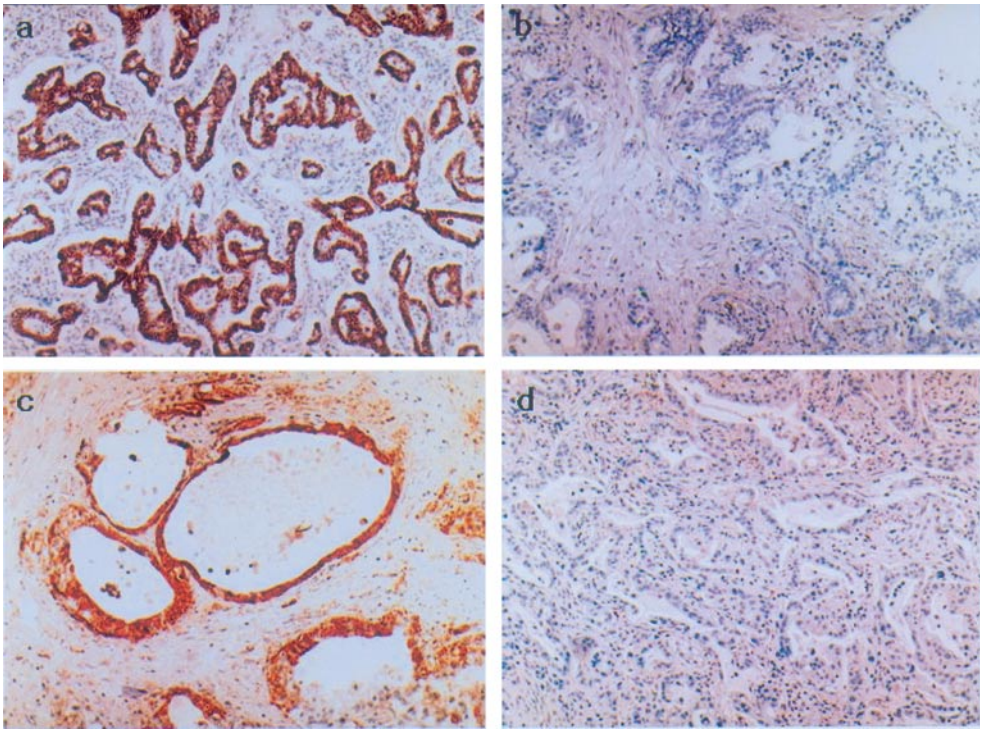

Figure 1. (a–d) Immunohistochemical staining of pancreatic cancer. Photomicrographs showing: (a) positive expression of E-cadherin; (b) negative expression of E-cadherin; (c) positive expression of uPA; (d) negative expression of uPA.

tables and the χ^2 test were used. Life-table probabilities for the overall survival were calculated by the method of Kaplan and Meier, and differences in survival between subgroups were compared with the log-rank test. To define independent risk factors for prognosis, multivariate analysis was performed with a Cox proportional hazards model. A P-value of <0.05 was considered significant.

RESULTS

Among the 53 tumor tissues we collected and tested, those from 29 (54.7%) patients had positive E-cadherin expression and the those from the other 24 (45.3%) showed negative E-cadherin expression. Twenty-two (41.5%) patients revealed a positive expression for uPA. Fifty-three pancreatic cancer tissues were stained with two monoclonal antibodies against E-cadherin and uPA, respectively (Fig. 1a–d).

The correlation of E-cadherin expression and the clinicopathological indices is shown in Table 1. Tumors with reduced E-cadherin expression were associated with distant metastasis. Table 2 shows the correlation between uPA expression and clinicopathological indices. There was a strong relationship between uPA expression and distant metastasis, and uPA expression correlated with an increased progression of the clinical stage. We subgrouped specimens into four categories according to the expression of E-cadherin and uPA: E-cadherin-positive/uPA-negative, E-cadherin-negative/uPA-negative, E-cadherin-positive/uPA-positive and E-cadherin-negative/uPA-positive. These patterns were found in 14 (26.4%), 11 (20.8%), nine (17%) and 19 (35.8%) of the 53 tumor tissues, respectively. According to the subgroup pattern of E-cadherin and uPA expression, the tumor tissues with E-cadherin-negative and uPA-positive expression were associated with larger tumor, distant metastasis and an increased clinical stage, as listed in Table 3.

The overall survival rate of the 53 patients with pancreatic cancer was stratified according to the E-cadherin and uPA expression, and the result is shown in Figs 2 and 3. Patients with E-cadherin-negative pancreatic tissue had a shorter survival [median 15.6 months; 95% confidence interval (CI) 6.3–24.9 months] than the patients in whom the expression of E-cadherin was positive (median 16.2 months; 95% CI 13.9–18.6 months, $P > 0.05$). Kaplan–Meier curves showed that there was no significant correlation between the

Table 1. Correlation between E-cadherin expression and clinicopathological features

Clinicopathological features	*n*	Expression of E-cadherin			
		Negative		Positive	
		n	%	*n*	%
Tumor size					
<5 cm	22	9	40.9	13	59.1
⩾5 cm	31	20	64.5	11	35.5
Lymphatics invasion					
Negative	40	23	57.5	17	42.5
Positive	13	6	46.2	7	53.8
Neural invasion					
Negative	35	19	54.3	16	45.7
Positive	18	10	55.6	8	44.4
Vascular invasion					
Negative	30	14	46.7	16	53.3
Positive	23	15	65.2	8	34.8
LN metastasis					
Negative	35	19	54.3	16	45.7
Positive	18	10	55.6	8	44.4
Distant metastasis*					
Negative	41	19	46.3	22	53.7
Positive	12	10	83.3	2	16.7
Stage					
I, II	22	9	40.9	13	59.1
III, IV	31	16	51.6	15	48.4

LN, lymph node.
*$P < 0.05$.

Table 2. Correlation between uPA expression and clinicopathological features

Clinicopathological features	*n*	Expression of uPA			
		Negative		Positive	
		n	%	*n*	%
Tumor size					
<5 cm	22	15	68.2	7	31.8
⩾5 cm	31	16	51.6	15	41.8
Lymphatics invasion					
Negative	40	23	57.5	17	42.5
Positive	13	8	61.5	5	38.5
Neural invasion					
Negative	35	21	60.0	14	42.5
Positive	18	10	55.6	8	44.4
Vascular invasion					
Negative	30	20	66.7	10	33.3
Positive	23	11	47.8	12	52.2
LN metastasis					
Negative	35	22	62.9	13	37.1
Positive	18	9	50.0	9	50.0
Distant metastasis*					
Negative	41	28	68.3	13	31.7
Positive	12	3	25.0	9	75.0
Stage*					
I, II	28	17	60.7	11	39.3
III, IV	25	14	56.0	11	44.0

LN, lymph node.
*$P < 0.05$.

E-cadherin expression and the overall survival. On the other hand, patients with uPA-positive pancreatic tissue had a shorter survival (median 9.7 months; 95% CI 4.7–14.7 months) than did the patients having pancreatic tissue with uPA-negative expression (median 18.7 months; 95% CI 6.2–31.3 months, $P < 0.01$).

There was a difference in the median survival time between the patients with E-cadherin-positive/uPA-negative pancreatic tissue (median 18.7 months; 95% CI 5.5–31.9 months) and the patients with E-cadherin-negative/uPA-positive pancreatic tissue (median 7.5 months; 95% CI 4.5–10.5 months, $P < 0.05$), and there was a statistically significant difference in survival curves between these two groups (Fig. 4). In multivariate analysis, the stage ($P = 0.01$) and the combination between uPA and E-cadherin expression ($P = 0.011$) emerged as independent prognostic factors.

DISCUSSION

Pancreatic cancer is regarded as one of the gravest, and conventional cancer treatments have had only little impact on the disease course. Almost all of the patients who have pancreatic cancer develop metastases and die (8). The reasons for the aggressive growth behavior of pancreatic cancer cells are not well understood. An important clinical characteristic of pancreatic cancer is its early metastasis to the lymph nodes and distant organs. However, the mechanisms that contribute to the ability of pancreatic cancer cells to invade normal tissue compartments and other organs, not to mention to leave the primary tumor lesion, have not been well studied to date (9). The spread of cancer cells from the primary site to distant locations is known to follow a sequence that requires detachment of malignant cells from the original tumor mass, destruction of the subtumor basement membranes and surrounding interstitial connective tissue matrix, invasion into and then extravasations from the vascular tree, before finally migrating toward, adhering to and proliferating at a distant site to form a metastatic tumor (10). When carcinogenesis is taking place, the transformed cells have to dissociate from one another before they can invade or metastasize. Therefore, adhesion molecules are expected to play an important role in carcinogenesis and especially in metastasis (11). The coherent loss of E-cadherin expression has been reported in lobular breast cancer (12–14). Palacios et al. have reported on the correlation

Table 3. Correlation between E-cadherin/uPA expression and clinicopathological features

Clinicopathological features	*n*	Expression of uPA and ECD							
		ECD(+)/uPA(–)		ECD(–)/uPA(–)		ECD(+)/uPA(+)		ECD(–)/uPA(+)	
		n	%	*n*	%	*n*	%	*n*	%
Tumor size									
<5 cm	22	8	36.4	5	22.7	5	22.7	4	18.2
⩾5 cm	31	6	19.4	6	19.4	4	12.9	15	48.4
Lymphatics invasion									
Negative	40	11	27.5	9	22.5	5	12.5	15	37.5
Postive	13	3	23.1	2	15.4	4	30.8	4	30.8
Neural invasion									
Negative	35	11	31.4	6	17.1	4	11.4	14	40.0
Postitive	18	3	16.7	5	27.8	5	27.8	5	27.8
Vascular invasion									
Negative	30	9	30.0	6	20.0	6	20.0	9	30.0
Positive	23	5	21.7	5	21.7	3	13.0	10	43.5
LN metastasis									
Negative	35	9	25.7	9	25.7	6	17.1	11	31.4
Positive	18	5	27.8	2	11.1	3	16.7	8	44.4
Distant metastasis*									
Negative	41	14	34.1	10	24.4	8	19.5	9	22.0
Positive	12	0	0.0	1	8.3	1	8.3	10	83.3
Stage*									
I, II	28	7	25	7	25	7	25	7	25
III, IV	25	7	28	4	16	2	8	12	48

ECD, E-cadherin; LN, lymph node.
*P-value < 0.05.

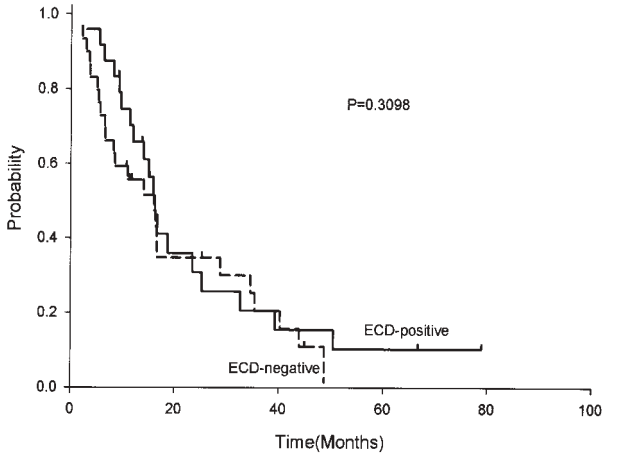

Figure 2. Survival curves of two groups subdivided according to the expression of E-cadherin.

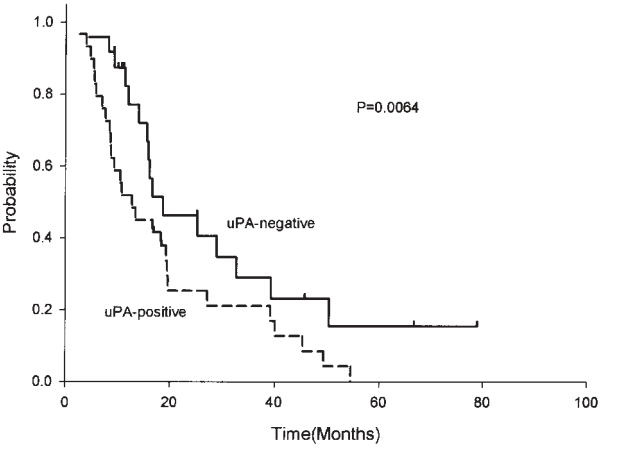

Figure 3. Survival curves of two groups subdivided according to the expression of uPA.

of E-cadherin status with poor prognostic indicators such as the grade of invasive ductal carcinoma (12). Similar correlations between the loss of E-cadherin protein and increasing malignancy have been demonstrated for carcinoma of the prostate (14), stomach (15), bladder (16), colorectum (17) and pancreas (18).

uPA changes have an important role in plasmin activation and the resultant proteolytic degradation of the extracellular

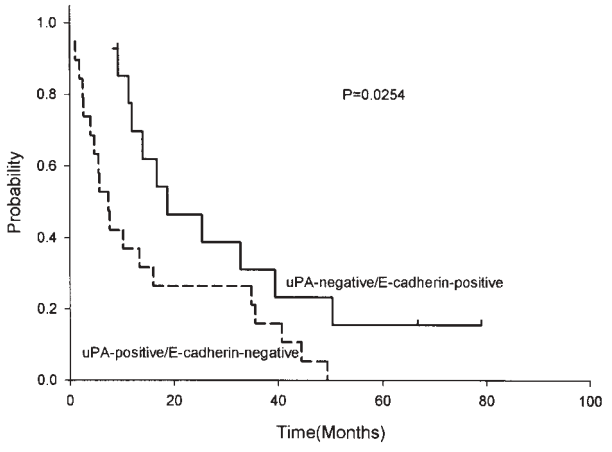

Figure 4. Survival curves of two groups subdivided according to the expression of uPA/E-cadherin tissue status.

matrix, as well as for the stimulation of tumor growth like epidermal growth factor (19). In breast cancer, higher tumor levels of uPA have been associated with a higher relapse rate, and uPA levels were found to be a better discriminator for disease-free survival than lymph node status, tumor size or estrogen receptor levels (20). Similar results have also been observed for colorectal cancer (21), gastric cancer (22), ovarian carcinoma (23), endometrial carcinoma (24), bladder cancer (25), adenocarcinoma of the lung (26) and pancreatic cancer (27). Yutak et al. have reported on the inter-relationship of invasion and E-cadherin/uPA for gastric cancer (28).

In our study, the reduced expression of E-cadherin was significantly correlated with distant metastasis. The overexpression of uPA was related to distant metastasis and stage III and IV disease. We therefore investigated the inter-relationship between the metastatic potential of pancreatic cancer and the combination of these two parameters. The results showed that E-cadherin-negative/uPA-positive tumors had a higher incidence of distant metastasis, larger tumors and a higher advanced stage (stage III and IV). The overall survival time was shorter for patients with E-cadherin-negative/uPA-positive pancreatic tissue, and there was a statistically significant difference. In multivariate analysis, stage and uPA/E-cadherin tissue status were revealed to be independent prognostic factors.

In conclusion, the combined analysis concerning uPA and E-cadherin expression may be a useful predictor of metastasis in pancreatic cancer. To prevent its metastazing potential, it may be necessary to create a novel method for blocking E-cadherin downregulation and uPA overexpression.

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