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Original Article

Significance of E-cadherin expression in pancreatic intraepithelial neoplasia and ductal adenocarcinoma

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Abstract: Pancreatic ductal adenocarcinoma (PDA) is a malignant tumor with poor prognosis. The aim of this study was to examine the expression of E-cadherin in both pancreatic intraepithelial neoplasia (PanIN) and PDA and their relationship to clinicopathologic characteristics. Eighty one cases with PDA, 25 normal pancreases, 14 PanIN-1A, 17 PanIN-1B, 16 PanIN-2, and 23 PanIN-3 were collected. Tissue microarray blocks were constructed, and immunohistochemical staining for E-cadherin was performed. All normal pancreatic ductal cells and acinar cells showed intact E-cadherin expression. Loss of E-cadherin expression was observed in 25.5% (12/47) of low-grade PanIN and 56.5% (13/23) of high-grade PanIN, respectively. High-grade PanIN showed significantly higher loss of E-cadherin expression than low-grade PanIN ($P < 0.05$). In PDAs, 61.7% (50/81) showed loss of E-cadherin expression. PDA showed significantly higher loss of E-cadherin expression than low-grade PanIN ($P < 0.01$). Loss of E-cadherin in PDAs showed significant correlation with histological grade ($P < 0.01$). No significant correlation was observed between loss of E-cadherin expression and age, sex, tumor size, lymphovascular invasion, pT classification, and stage, respectively. These findings suggest that loss of E-cadherin is usually observed in high-grade panIN-3 and PDA. Loss of E-cadherin expression may be a late event in pancreatic cancer progression.

Keywords: E-cadherin, carcinoma, pancreatic ductal, intraepithelial neoplasia

Introduction

Pancreatic ductal adenocarcinoma (PDA), more commonly known as pancreatic cancer, is the fourth leading cause of cancer deaths in the United States and has one of the highest mortality rates of any cancer [1]. Invasive pancreatic cancers are believed to arise from well-defined noninvasive precursor lesions referred to as pancreatic intraepithelial neoplasia (PanIN).

E-cadherin, a calcium-dependent adhesion molecule, plays a major role in the maintenance of intercellular adhesiveness in normal epithelial cells [2]. E-cadherin expression is lost or reduced in many types of human cancers, including lung cancer [3], cervical cancer [4], colorectal cancer [5], and nasopharyngeal cancer [6].

Studies on E-cadherin expression in PDAs have been reported over the past two decades. Loss or reduction of E-cadherin expression has shown positive correlation with histologic grade [7], lymph node metastasis [8, 9], and poor outcome [10]. Few studies examining E-cadherin expression in PanIN lesions have been reported [11, 12]. The role and mechanisms of E-cadherin expression in the progression of invasive ductal adenocarcinoma are not well known.

The aim of this study was to investigate expression of E-cadherin in PanIN and PDA and to analyze their relationship to clinicopathologic parameters.

Materials and methods

Tissue specimens

Eighty one patients with PDA who underwent surgical pancreatic resection at Yeungnam University Hospital, South Korea, between 1986 and 2015 were selected. Twenty-five normal pancreases, 14 PanIN- 1A, 17 PanIN-1B, 16 PanIN-2, and 23 PanIN-3 were collected, which were found incidentally in pancreas pa-

Table 1. Comparison of E-cadherin expression and clinicopathologic factors of pancreatic ductal adenocarcinoma

	No. Case	E-cadherin expression				*P*
		Intact	Mild loss	Moderate loss	Severe loss	
Age (y)						0.56
≤ 60	37	13	11	10	3	
> 60	44	18	21	3	2	
Sex						0.54
Male	51	20	21	6	4	
Female	30	11	11	7	1	
Tumor size (cm)						0.33
≤ 3	24	13	7	3	1	
> 3	57	18	25	10	4	
Histologic grade						< 0.0001*
Well/moderate	64	30	23	10	1	
Poor	17	1	9	3	4	
Tumor location						0.36
Head	44	17	16	9	2	
Body	24	9	8	4	3	
Tail	13	5	8	0	0	
Lymphovascular invasion						0.42
Absent	16	9	5	2	0	
Present	65	22	27	11	5	
Perineural invasion						0.47
Absent	30	9	12	7	2	
Present	51	22	20	6	3	
Resection margin						0.71
Negative	67	27	28	10	2	
Positive	14	4	4	3	3	
pT classification						0.54
pT1	0	0	0	0	0	
pT2	2	1	1	0	0	
pT3	75	29	30	12	4	
pT4	4	1	1	1	1	
Lymph node metastasis						0.93
Absent	34	14	12	6	2	
Present	47	17	20	7	3	
Distant metastasis						0.15
Absent	78	31	30	13	4	
Present	3	0	2	0	1	
Stage						0.36
I	2	1	1	0	0	
II	74	29	29	12	4	
III	2	1	0	1	0	
IV	3	0	2	0	1	

*Significant at the level of < 0.05.

renchyma adjacent to resected pancreatic specimens due to traumatic rupture or other tumors of pancreas, ampulla of Vater, or common bile duct. All specimens were fixed in 10% buffered formalin and embedded in paraffin. Tissue microarrays were constructed from representative tissue blocks for each case. Two cores with 2 mm diameter were extracted and transferred to the recipient tissue microarray blocks. According to WHO criteria, PanIN lesions are classified as PanIN-1A, panIN-1B, panIN-2, and PanIN-3 [13]. For statistical analysis, PanIN-1A, PanIN-1B, and panIN-2 were classified as low-grade PanIN, and PanIN-3 was classified as high-grade PanIN. TNM stage was classified according to AJCC cancer staging [14]. Clinicopathologic parameters including patient age, gender, tumor size, histologic grade, tumor location, lymphovascular invasion, perineural invasion, resection margin, pT classification, lymph node metastasis, distant metastasis, and stage were evaluated by review of medical charts and pathologic records. This study was approved by the institutional review board of Yeungnam University Hospital (yuh-2015-05-41).

Immunohistochemistry of E-cadherin and assessment of immunoreactivity

Tissue sections with 4 μm thickness were deparaffinized in xylene and hydrated through serial alcohol solutions. Endogenous peroxidase was blocked by incubation with 3% H_2O_2 for 10 min. Heat-induced antigen retrieval was performed using EDTA buffer (pH 8.0). E-cadherin monoclonal antibody (Clone 4A2C7, Zymed, San Francisco, CA, USA) was used at 1:100 dilution. Immunohistochemical staining

Table 2. E-cadherin expression in pancreatic intraepithelial neoplasia lesions and ductal adenocarcinomas

	No. cases	E-cadherin expression				*P*
		Intact	Mild loss	Moderate loss	Severe loss	
Normal	25	25	0	0		*
Low-grade PanIN	47	36	8	3	0	**
High-grade PanIN	23	10	6	6	1	
Adenocarcinoma	81	31	32	13	5	

*$P < 0.01$ versus low-grade PanIN, high-grade PanIN, and adenocarcinoma; **$P < 0.05$ versus high-grade PanIN, and $P < .01$ versus adenocarcinoma.

was performed on the automated immunostaining system using an I-View detection kit (Benchmark XT, Ventana Medical Systems, Tucson, AZ, USA). Immunostained slides were counterstained with hematoxylin.

Evaluation of immunohistochemical staining

In this study, the percentage of only membrane staining cells was graded in each lesion. E-cadherin expression was considered 'intact' if 100% of cells showed membrane staining. E-cadherin expression was considered 'mild loss' if 51% to 99% of cells showed membrane staining, 'moderate loss' if 5% to 50% of cells showed membrane staining, and 'severe loss' if less than 5% of cells showed membrane staining.

Statistical analysis

The X^2 test and Fisher exact test were performed to examine correlation between E-cadherin expression and each clinicopathologic variable in PDA. Overall patients' survival was defined as the time from surgical resection of PDA to the death of the patients or the date of last follow-up of the patients. Survival rates were calculated using the Kaplan-Meier method and statistical significance was evaluated using the Tarone-Ware test. Cox proportional hazards regression analyses were performed to determine the significance of prognostic factors. *P* values < 0.05 were considered significant.

Results

Clinicopathologic characteristics and E-cadherin expression in PDAs

E-cadherin expression and clinicopathologic characteristics in PDAs are shown in **Table 1**. The patients' age ranged from 32 to 81 years (mean ± SD, 60.2±10.6 years). Male to female ratio was 1.7:1. The mean tumor size was 4.5±2.8 cm. In 24 cases (29.6%), tumor size was ≤ 3 cm and in 57 cases (70.4%), tumor size was > 3 cm. Lymphovascular invasion was observed in 80.2% (65/81). Perineural invasion was observed in 63.0% (51/81). In pathologic stage, 2.5% (2/81) were pT2, 92.6% (75/81) were pT3, and 4.9% (4/81) were pT4. Lymph node metastasis was observed in 58.0% (47/81), and distant metastasis was observed in 3.7% (3/81).

Of the 81 PDAs, 31 cases (38.3%) showed intact E-cadherin expression. Mild loss of E-cadherin expression was observed in 32 cases (39.5%), moderate loss in 13 cases (16.0%), and severe loss in 5 cases (6.2%). Loss of E-cadherin expression was observed in 34 cases (41.9%) of well and moderately differentiated adenocarcinoma and 16 cases (94.1%) of poorly differentiated adenocarcinoma. Significant correlation was observed between histologic grade and loss of E-cadherin expression ($P < 0.01$). No significant correlation was observed between E-cadherin expression and patient gender, age, tumor size, location, lymphovascular invasion, perineural invasion, pT classification, lymph node metastasis, distant metastasis, and stage, respectively.

E-cadherin expression in PanIN lesions

E-cadherin expression in normal pancreas, PanIN lesions, and adenocarcinomas is summarized in **Table 2**. Among normal pancreatic tissues, E-cadherin expression was intact in all normal ductal cells and acinar cells (**Figure 1A**). In low-grade PanIN lesions including PanIN-1A, 1B, and 2, mild loss of E-cadherin expression was observed in 17.0% (8/47) and moderate loss was observed in 6.4% (3/47) (**Figure 1B**, **1C**). In high-grade PanIN (PanIN-3), 26.1% (6/23) had mild loss of E-cadherin, 26.1% (6/23) had moderate loss, and 4.3% (1/23) had severe loss (**Figure 1D**). The frequency of loss of E-cadherin expression was significantly higher in high-grade PanIN than in low-grade PanIN ($P < 0.05$). Thirty-one cases (38.3%) of PDAs showed mild loss of E-cadherin expres-

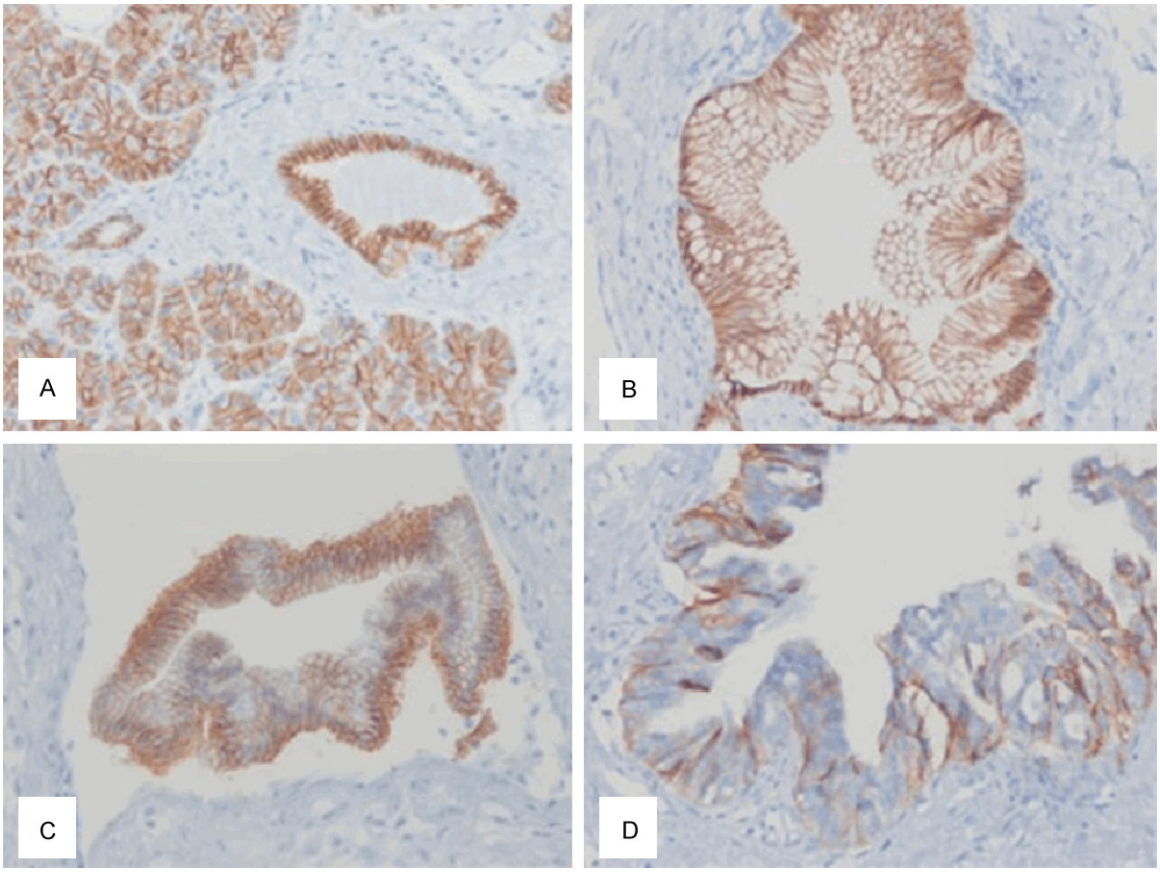

Figure 1. E-cadherin immunostaining in normal pancreas and pancreatic intraepithelial neoplasia (PanIN). A. Intact E-cadherin expression in normal ductal and acinar cells. B. Intact E-cadherin expression in low-grade PanIN-1B. C. Mild loss of E-cadherin in low-grade PanIN-2. D. Moderate loss of E-cadherin in high-grade PanIN-3.

sion, 13 cases (16.0%) showed moderate loss, and 5 cases (6.2%) showed severe loss (**Figure 2A-D**). The frequency of loss of E-cadherin expression was significantly higher in PDA than in low-grade PanIN ($P < 0.01$). No significant difference in E-cadherin expression was observed between high-grade PanIN and PDA.

Prognostic significance of E-cadherin expression

Results of univariate and multivariate analyses are shown in **Table 3**. Poor histologic grade showed correlation with unfavorable overall survival ($P < 0.05$). E-cadherin expression showed no significant correlation with overall survival in patients with PDA (**Figure 3**).

Discussion

We investigated E-cadherin expression in precursor lesions referred to as PanIN and PDA in order to evaluate the role and significance of E-cadherin expression in pancreatic cancer carcinogenesis.

In this study, E-cadherin expression was intact in all normal pancreatic ductal cells and acinar cells as previously reported. Loss or reduction of E-cadherin expression has been reported in 30.0%-61.8 % of PDAs [7-9, 15-17]. In the current study, 61.7% of PDA showed mild, moderate or severe loss of E-cadherin expression. The difference may be due to the different types of antibody used, antigen retrieval methods, different criteria for assessing positivity, and heterogeneity of the samples (type of samples, fixation). Loss of E-cadherin interferes with β-catenin signaling in the Wnt pathway, leading to decreased growth inhibition and uncontrolled proliferation and cancer development. Loss of E-cadherin expression is considered a key characteristic of PDA.

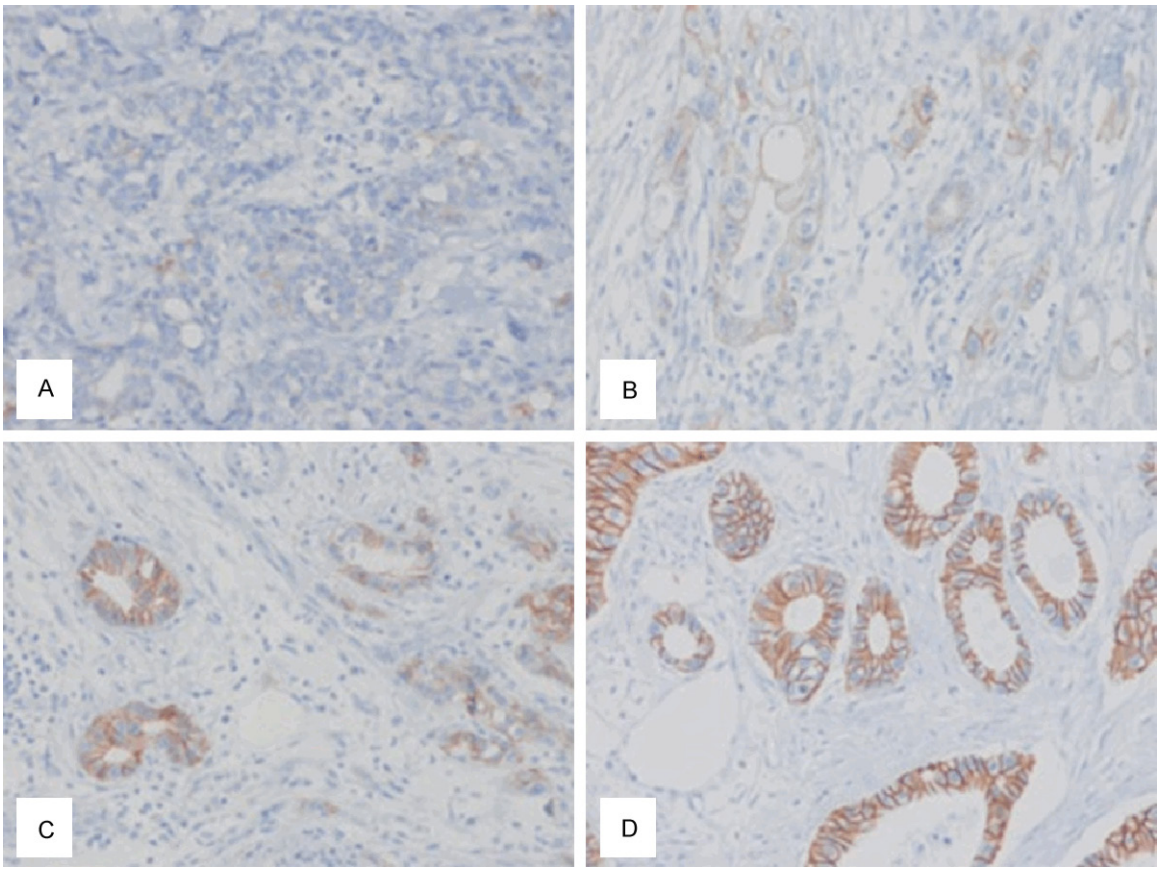

Figure 2. E-cadherin immunostaining in pancreatic ductal adenocarcinoma (PDA). A. Severe loss of E-cadherin in poorly differentiated adenocarcinoma. B. Moderate loss of E-cadherin expression in moderately differentiated adenocarcinoma. C. Mild loss of E-cadherin in moderately differentiated adenocarcinoma. D. Intact E-cadherin expression in well differentiated adenocarcinoma.

Association of loss of E-cadherin expression with histologic grade has been reported [7, 10]. In the current study, poorly differentiated PDA showed significantly higher loss of E-cadherin expression than well differentiated and moderately differentiated PDA. Our result is similar to previously reported results. In PDAs, loss of E-cadherin expression was reported to show association with lymph node metastasis [8, 9] and advanced stage [7]. In contrast, loss of E-cadherin expression has not been correlated with gender and age of patients, tumor size, invasion, lymph node involvement, distant metastasis, and survival of patients [6, 7, 16, 17]. In results of our study, loss of E-cadherin expression showed no significant correlation with gender and age of patients, tumor size, location, lymphovascular invasion, lymph node metastasis, pT classification and stage. Hong et al. reported that loss of E-cadherin expression is an independent predictor of poor prognosis in patients with PDAs [10]. In our study, loss of E-cadherin expression did not show correlation with overall survival. Further evaluation is needed to determine whether E-cadherin expression status can be used as a powerful predictor in patients with PDAs.

Despite significant advances in our understanding of the pathogenesis of PDA in recent decades, studies for E-cadherin expression in PanIN lesions are limited. To the best of our knowledge, few studies addressing this issue have been reported [11, 12]. Al-Aynati et al. reported that the loss of E-cadherin membranous staining was significantly more common in adenocarcinoma than in normal ductal epithelium and PanIN lesions [12]. In our study, comparing low-grade PanIN, high-grade PanIN, and PDA with E-cadherin expression, significantly higher loss of E-cadherin expression was observed in high-grade PanIN rather than in

Table 3. Univariate and multivariate analyses on the overall survival of pancreatic ductal adenocarcinomas

	Univariate			Multivariate		
	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
Age > 60 y	0.68	0.34-1.39	0.29			
Tumor size > 3 cm	0.39	0.15-1.04	0.06			
Histologic grade	3.02	1.03-8.81	0.04*	2.00	0.66-6.12	0.22
Size > 3 cm	0.96	0.46-2.00	0.92			
Location	1.02	0.48-2.19	0.95			
Lymphovascular invasion	3.16	0.96-10.41	0.06			
Perineural invasion	1.99	0.89-4.44	0.09			
Resection margin	1.17	0.45-3.05	0.75			
Lymph node metastasis	1.88	0.92-3.92	0.09			
Distant metastasis	0.48	0.00-2.30	0.58			
Stage	1.72	0.40-7.30	0.46			
E-cadherin expression	1.26	0.63-2.53	0.52			

*Significant at the level of < 0.05.

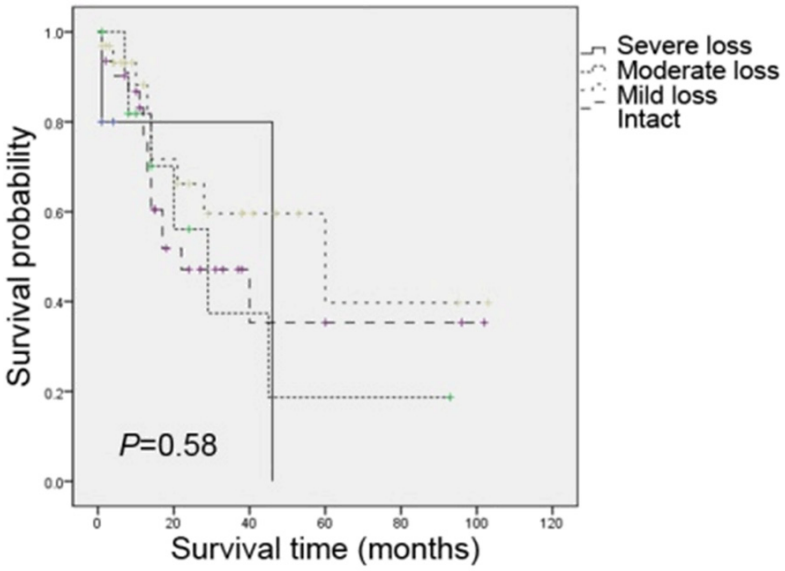

Figure 3. Kaplan-Meier curves in pancreatic ductal adenocarcinoma according to E-cadherin expression. There was no significant survival difference among four groups ($P = 0.58$).

low-grade PanIN ($P < 0.05$). Loss of E-cadherin expression increased from low-grade PanIN to high-grade PanIN to PDA. Similar to PanIN lesions and PDAs, down-regulation of E-cadherin increased from normal epithelium to carcinoma in situ and microinvasive tumors in the cervical cancer. Progressive accumulation of molecular alterations that accompany the histological progression from low-grade PanIN-1A to high-grade PanIN-3 lesions has been demonstrated [18]. We found that loss of E-cadherin expression was usually seen in high-grade panIN and PDA, which is consistent with loss of E-cadherin being a late event in pancreatic cancer progression.

Mutations in the E-cadherin gene have been identified in cancer cells, making them more susceptible to epithelial-mesenchymal transition and metastasis [19]. Little is known about the molecular events of the E-cadherin gene in the development of PDA. Hypermethylation of E-cadherin gene was observed in 3% of pancreatic cancers [20]. Winter et al. reported that promoter methylation of the E-cadherin gene is a possible mechanism of E-cadherin gene silencing in non-cohesive pancreatic cancer [21]. The prevalence of CDH1 inactivation by mutation or hypermethylation is less common in PDA [9, 20]. Future studies should include molecular analyses of the E-cadherin gene and study of E-cadherin protein expression in a larger series of PanIN lesions and PDAs to further clarify the relationship between alteration of E-cadherin gene and protein expression in pancreatic cancer carcinogenesis.

In conclusion, loss of E-cadherin expression is usually seen in high-grade panIN and PDA. Loss of E-cadherin may be associated with tumor progression to invasive cancer and a late event in pancreatic cancer carcinogenesis.

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Disclosure of conflict of interest

None.

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