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Original Article

EGFR expression in pancreatic intraepithelial neoplasia and ductal adenocarcinoma

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Abstract: Pancreatic ductal adenocarcinoma (PDA) is an aggressive malignant tumor with poor prognosis. Epidermal growth factor receptor (EGFR) is an important cell adhesion and signaling pathway mediator. The aim of this study was to evaluate the expression of EGFR in both pancreatic intraepithelial neoplasia (PanIN) and PDA and their relationship to clinicopathologic characteristics. Formalin-fixed, paraffin-embedded tissues including 81 cases with pancreatic ductal adenocarcinoma, 27 with normal pancreas, 16 with PanIN-1A, 18 with PanIN-1B, 11 with PanIN-2, and 24 with PanIN-3 were used for construction of tissue microarrays. Imunohistochemistry for EGFR was performed. Normal pancreatic ducts, PanIN-1A, and PanIN-1B did not show EGFR overexpression. EGFR overexpression was observed in 18.2% (2/9) of PanIN-2, 41.7% (10/14) of PanIN-3, and 64.2% (52/81) of PDA, respectively. Significantly higher EGFR overexpression was observed in PDAs than in PanIN lesions (P<0.05). No statistically significant correlation was observed between EGFR overexpression and patient age, sex, tumor location, size, histological grade, vascular invasion, lymph node metastasis and stage at presentation, respectively. In conclusion, EGFR expression increased from PanIN to PDA. EGFR may be involved in early stage in development of PDA.

Keywords: Epidermal growth factor receptor, pancreatic ductal adenocarcinoma, pancreatic intraepithelial neoplasia

Introduction

Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer deaths in the United States and has one of the highest mortality rates of any cancer [1]. Pancreatic intraepithelial neoplasia (PanIN) is a well-defined, non-invasive precursor lesion for PDA.

Epidermal growth factor receptor (EGFR) is a transmembrane growth factor receptor with tyrosine kinase activity. EGFR is frequently overexpressed in many tumors, including lung, breast, colorectal and vulvar cancer [2-4]. Its activation affects the signaling pathways affecting cellular growth, differentiation, and proliferation.

Although EGFR expression has been previously studied in PDAs, its expression has not been well characterized [5-10]. In PanIN lesions, knowledge regarding EGGFR expression is limited. The role of EGFR expression in carcinogenesis of PDA remains controversial.

The aim of this study was to examine the expression of EGFR in PanIN lesions and PDAs and their relationship to clinicopathologic features.

Materials and methods

Patients and specimen

Eighty-one patients with PDA were selected. All patients underwent surgical resection at Yeungnam University Hospital, South Korea, between 1986 and 2014. Twenty seven normal pancreas, 16 PanIN-1A, 18 PanIN-1B, 11 PanIN-2, and 24 PanIN-3 were collected, which were found incidentally in pancreas parenchyma adjacent to resected pancreatic specimens due to traumatic rupture, intraductal papillary mucinous neoplasm, and other tumors such as ampulla of Vater or common bile duct cancers. All tissues were fixed in 10% buffered formalin and embedded in paraffin. Representative blocks for each case were selected for construction of tissue microarrays. A pair of 2-mm-diameter tissue cores were retrieved and transferred to the recipient block. PanIN lesions and histological grade of PDA were classified according to the criteria described in the World

Table 1. Comparison of EGFR expression and clinicopathologic factors of pancreatic ductal adenocarcinoma

	No. Case (n=81)	EGFR expression		*P*
		Negative (n=29)	Positive (n=52)	
Age (y)				
≤60	39	11	28	0.169
>60	42	18	24	
Sex				
Male	52	19	33	0.853
Female	29	10	19	
Size (cm)				
≤3	25	8	17	0.633
>3	56	21	35	
Grade				
Well	16	7	9	0.265
Moderately	49	19	30	
Poorly	16	3	13	
Location				
Head	45	14	31	0.364
Body	23	11	12	
Tail	13	4	9	
VS invasion				
Absent	16	6	10	0.874
Present	65	23	42	
PN invasion				
Absent	27	6	21	0.104
Present	53	23	30	
pT stage				0.665
pT1	0	0	0	
pT2	2	1	1	
pT3	75	26	49	
pT4	4	2	2	
LN metastasis				
Absent	35	14	21	0.492
Present	46	15	31	
DT metastasis				
Absent	78	27	51	0.291
Present	3	2	1	
Stage				
I	2	1	1	0.547
II	74	26	48	
III	2	0	2	
IV	3	2	1	

VS, vascular; PN, perineural; LN, lymph node; DT, distant.

Health Organization classification [11]. TNM stage was classified according to AJCC cancer staging [12]. Clinicopathologic parameters including patient age, gender, tumor size, histological grade, location, vascular invasion, perineural invasion, lymph node 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-2015-05-023).

Immunohistochemistry of EGFR and assessment of immunoreactivity

Immunohistochemical staining was performed using the Ultra View Universal DAB detection kit on a BenchMark Series automatic stainer (Ventana Medical Systems, Tuscon, AZ, USA). The primary antibody was a mouse monoclonal CONFIRM anti-EGFR (3C6) (Ventana Medical Systems). Omission of primary antibody was used as a negative control of immunohistochemical reaction and perineural fibroblasts served as a positive internal control. Semiquantative assessment of EGFR immunostaining was performed, and a 4-point scale was used for scoring as follows; Score 0: no membrane staining or incomplete membrane staining in less than 10% of cells, Score 1+: weak incomplete membrane staining in more than >10% of cells, Score 2+: moderate and complete membrane staining in more than 10% of cells, Score 3+: strong and complete membrane staining in more than 10% of cells. Score 0 and 1+ were interpreted as negative. Score 2+ and 3+ were considered positive.

Statistical analysis

Statistical analysis was performed using SPSS for window version 18.0. The x^2 test or Fisher exact test was used for determination of correlation between EGFR expression and clinicopathologic variables in PDA. Survival curves were calculated using the Kaplan-Meier method, and statistical significance between curves was tested using the Breslow test. Cox proportional hazard regression analyses were performed. A *P* value of less than 0.05 was considered statistically significant.

Results

Clinicopathologic characteristics and EGFR expression in PDAs

Clinicopathologic characteristics and EGFR expression in PDAs are shown in **Table 1**. The patient ranged in age from 32 to 81 years, with a median age of 60 years. The male-to-female

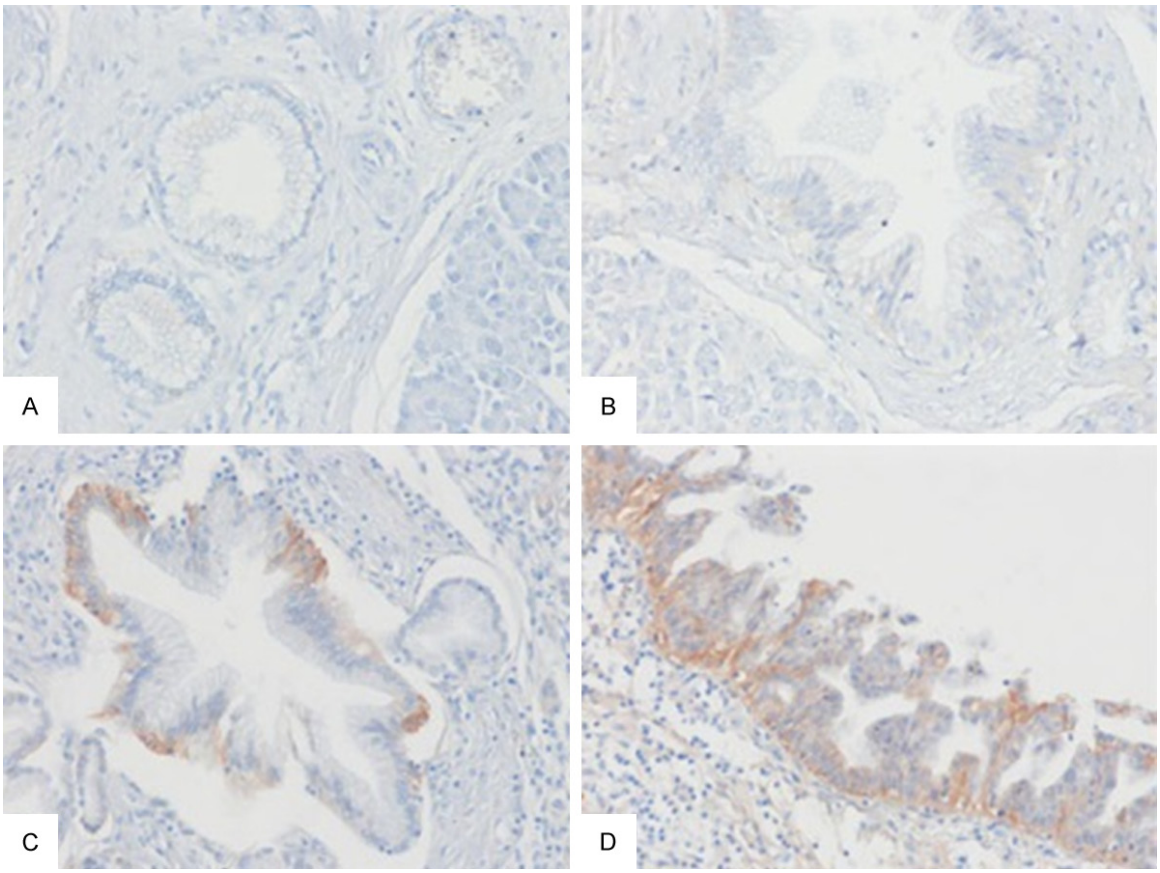

Figure 1. EGFR immunostaining in pancreatic intraepithelial neoplasia (PanIN). A. No EGFR expression in PanIN-1A. B. No EGFR expression in PanIN-1B. C. Score 2+ in PanIN-2. D. Score 2+ in PanIN-3.

Table 2. EGFR expression in pancreatic intraepithelial neoplasia and ductal adenocarcinoma

	No. Case	EGFR expression		*P*
		Negative	Positive	
Normal	27	27 (100)	0 (0)	
PanIN-1A	16	16 (100)	0 (0)	
PanIN-1B	18	18 (100)	0 (0)	
PanIN-2	11	9 (81.8)	2 (18.2)	
PanIN-3	24	14 (58.3)	10 (41.7)*	
Adenocarcinoma	81	29 (36.8)	52 (64.2)**	

*P<0.01 versus normal, PanIN-1A, and PanIN-1B, **P<0.01 versus normal, PanIN-1A, PanIN-1B, PanIN-2, and P<0.05 versus PanIN-3.

ratio was 1.7:1. Forty-five cases (55.6%) of PDAs arose in the head of the pancreas, and the remainder in the body (28.4%) and tail (16.0%). Expression of EGFR was observed in 64.2% (52 of 81) of PDAs (**Figure 1**). EGFR expression was observed in 56.3% (9/16) of well differentiated PDAs, 61.2% (30/49) of moderately differentiated PDAs, and 81.3% (13/16) of poorly differentiated PDAs. No significant correlation was observed between EGFR expression and patient age, sex, tumor size, histological grade, location, vascular invasion, perineural invasion, lymph node metastasis, and stage.

EGFR expression in PanIN lesions

EGFR expression in PanIN lesions is summarized in **Table 2**. Normal pancreatic ducts, PanIN-1A, and PanIN-1B did not show EGFR expression. Expression of EGFR was detected in 18.2% (2/11) of PanIN-2 and in 41.7% (10/24) of PanIN-3 (**Figure 2**), respectively. Significantly higher EGFR expression was observed in PanIN-3 than in normal, PanIN-1A and PanIN-1B (P<0.01). Significantly higher frequency of EGFR expression was observed in PDA than in PanIN-1A, panIN-1B, and PanIN-2 (P<0.01). No significant difference in EGFR expression was observed between PanIN-3 and PDA.

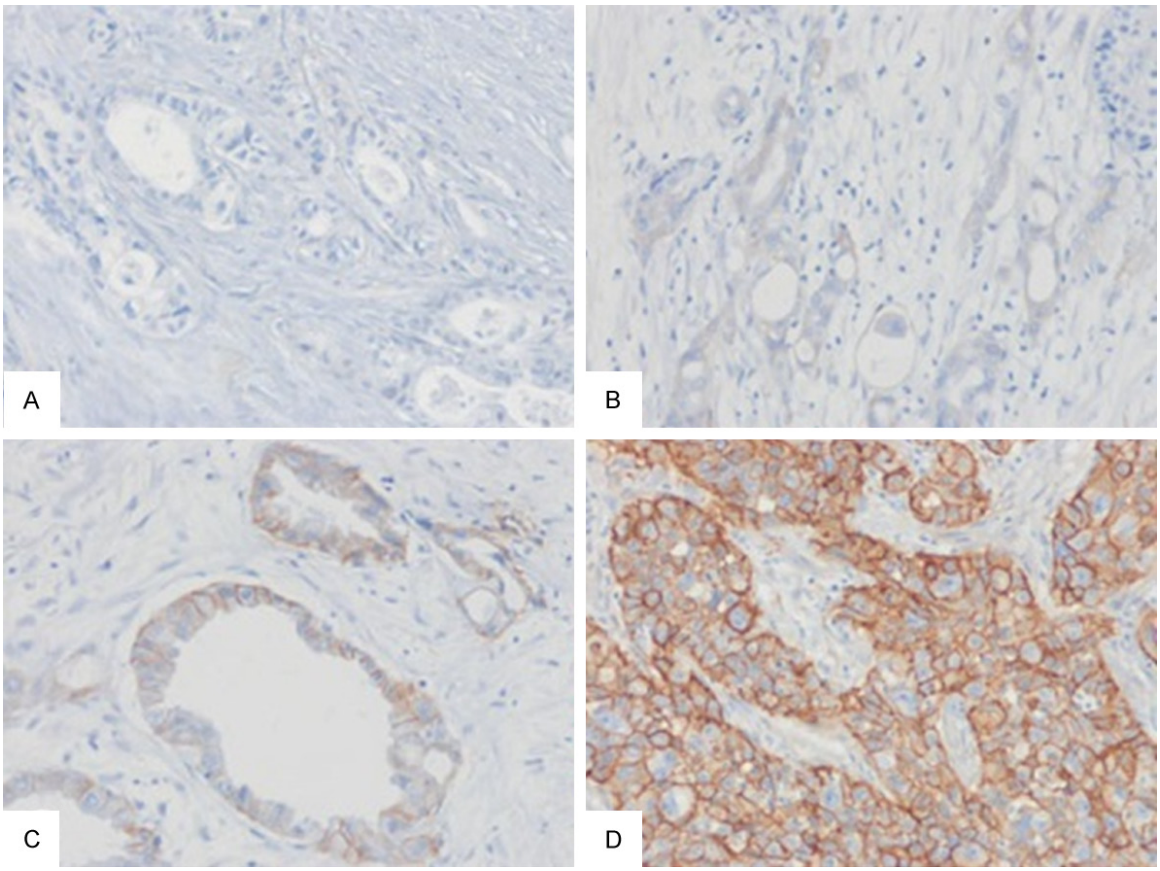

Figure 2. EGFR immunostaining in pancreatic ductal adenocarcinoma (PDA). A. Score 0 in moderately differentiated PDA. B. Score 1+ in poorly differentiated PDA. C. Score 2+ in well differentiated PAD. D. Score 3+ in poorly differentiated PDA.

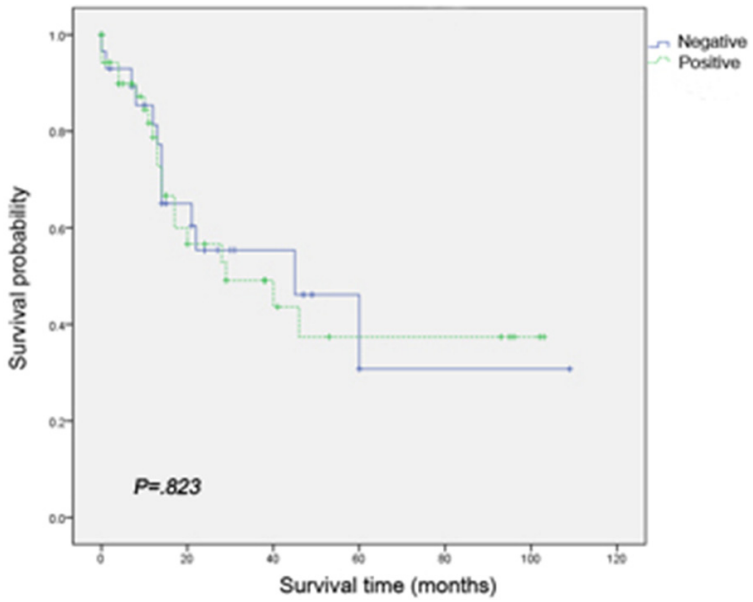

Figure 3. Kaplan-Meier curve demonstrates that 5-year survival is not significantly worse in cases with EGFR expression compared with EGFR-negative cases.

Prognostic significance of EGFR expression

EGFR expression had no significant correlation with overall survival in patients with PDA (**Figure 3**). Results of univariate and multivariate analyses are shown in **Table 3**. Vascular invasion and lymph node metastasis were associated with unfavorable overall survival ($P<0.05$).

Discussion

The aim of the current study was to examine the expression of EGFR in pancreatic intraepithelial neoplasia (PanIN) and pancreatic ductal adenocarcinoma (PDA) and their relationship to clinicopathologic features.

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

	Univariate			Multivariate		
	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
Age >60 y	0.712	0.354-1.434	0.342			
Tumor size >3 cm	1.281	0.304-5.395	0.736			
Histologic grade						
Well vs moderate	2.300	0.874-6.050	0.091			
Well vs poor	0.857	0.204-3.597	0.832			
Location						
Head vs body	1.081	0.508-2.300	0.840			
Head vs tail	0.524	0.122-2.252	0.385			
Vascular invasion	3.646	1.108-11.991	0.033	2.350	0.680-8.119	0.177
PN invasion	2.295	0.992-5.308	0.052			
LN metastasis	2.164	1.043-4.489	0.038	2.094	0.959-4.573	0.064
EGFR expression	1.059	0.526-2.133	0.872			

CI, confidence interval; PN, perineural; LN, lymph node.

EGFR expression from 30.4% to 61.8% in PDAs has been reported [5-10]. In the current study, EGFR expression in PDAs was observed in 64.2% of PDAs when more than score 2(+) was considered positive. This 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) [3]. The standardization of techniques to determine EGFR overexpression should be considered a priority [3].

In PDA, EGFR expression has been reported to show association with increased invasiveness and poor prognosis [6, 9, 10]. In contrast, meta-analysis of immunohistochemical prognostic markers in resected pancreatic cancers showed no significant overall association between EGFR expression and survival [13]. Data regarding the prognostic role of EGFR expression is inconsistent. In our study, EGFR overexpression was not associated with a poorer prognosis. Univariate and multivariate analyses revealed that vascular invasion and lymph node metastasis were statistically significant factors for poorer prognosis.

EGFR expression has been reported to show association with tumor dedifferentiation, mitotic activity, and pleomorphism [10]. In our study, although no significant correlation was observed between EGFR expression and histological grade, EGFR expression tended to be higher in poorly differentiated PDA than in well differentiated and moderately differentiated PDA. No relationship was observed between EGFR expression and other clinicopathologic parameters. Accurate and conclusive evaluation of the clinical significance of EGFR expression is important in pancreatic cancer for selection of appropriate future molecular targets [8].

There is a progression in the pancreas from intraductal proliferation to invasive proliferation to invasive ductal carcinoma. This progression is associated with increasing degrees of cytological and architectural atypia, with accumulation of genetic alterations in cancer-associated genes [14]. To the best of our knowledge, no study on the interrelationship of EGFR expression in normal pancreas, PanIN, and PDA has been reported. In an attempt to examine interrelationship of EGFR expression by which pancreatic cancers progress, we compared normal pancreas, PanIN lesions and PDA with EGFR expression. In our study, neither normal pancreas, Pan-1A nor PanIN-1B showed EGFR expression. Significantly higher EGFR expression was observed in high-grade PanIN-3 rather than in low-grade PanIN-1A and PanIN-1B, and EGFR expression increased from PanIN to PDA. These results suggest that EGFR expression may be related to early event in carcinogenesis of PDA. Similar to PanIN lesions in pancreas, EGFR expression rate increased

from normal epithelium to carcinoma in situ and microinvasive tumors in the lung [15]. EGFR overexpression is more common in PDAs, and therefore may prove to be a useful marker to aid in differentiating reactive atypia from a well differentiated adenocarcinoma. EGFR signaling inhibition may prevent development of PDA [16].

EGFR mutation rate of 1.5% to 3.6% in PDA has been reported [17-19]. The correlation between EGFR mutation and EGFR protein overexpression by immunohistochemical staining was not linear [3]. PDA cases with EGFR overexpression by immunohistochemistry failed in EGFR gene amplification by FISH [20]. Although EGFR alterations were not examined at the genetic level, further study is mandatory in order to understand their role in multistep carcinogenesis of PDA. Conduct of more studies on relationship between EGFR gene alterations and EGFR protein expression is needed.

In conclusion, EGFR expression increased from PanINs to PDAs. EGFR expression may be an early event in carcinogenesis of PDA and associated with tumor progression to invasive cancer. In the future, understanding the molecular control of progression of PanIN into PDA will be extremely critical for prevention and treatment of PDA.

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Disclosure of conflict of interest

None.

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