Prognostic significance of epidermal growth factor-like domain 7 in pancreatic cancer

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BACKGROUND: **Recent studies have shown the clinical significance of epidermal growth factor-like domain 7 (EGFL7) in a variety of cancers. However, the relationship between EGFL7 and the prognosis of pancreatic cancer (PC) remains unclear. The present study was undertaken to investigate the role of EGFL7 in the prognosis of PC.**

METHODS: **The expression of EGFL7 in nine PC cell lines was first determined by Western blotting analysis. Tissue microarray-based immunohistochemical staining was performed in paired formalin-fixed paraffin-embedded tumor and non-tumor samples from 83 patients with PC. Finally, correlations between EGFL7 expression and clinicopathological variables as well as overall survival were evaluated.**

RESULTS: **EGFL7 was widely expressed in all PC cell lines tested. EGFL7 expression in tumor tissues was significantly higher than that in non-tumor tissues (P=0.040). In addition, univariate analysis revealed that high EGFL7 expression in tumor tissues was significantly associated with poor overall survival, accompanied by several conventional clinicopathological variables, such as gender, histological grade and lymph node metastasis. In a multivariate Cox regression test, EGFL7 expression was identified as an independent marker for long-term outcome of PC.**

CONCLUSION: **Our data showed that EGFL7 is extensively expressed in PC and that EGFL7 is associated with poor prognosis.**

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KEY WORDS: **epidermal growth factor-like domain 7; pancreatic cancer; prognosis; Western blotting; immunohistochemistry; tissue microarray**

Introduction

Pancreatic cancer (PC), has long been recognized as a lethal malignancy with extremely poor prognosis.[1, 2] In addition, the long-term survival of PC has not been improved within two decades, despite widely-performed surgical resections.[3] Therefore, factors predictive for poor prognosis of the disease are of interest. Among these factors, conventional clinical and pathological parameters such as lymph node metastasis, neural/perineural invasion and resection margin were previously identified as significant variables.[4-8] Recently, much attention has been paid to many tumor initiation/development-associated molecules that were associated with PC prognosis.[9, 10] However, further clues and evidences are required.

Epidermal growth factor-like domain 7 (EGFL7), a secreted protein containing two EGF-like domains, was initially identified as a modulator of smooth muscle cell migration.[11] The biological roles of EGFL7 in the vascular system have been extensively investigated.[12-15] In human cancer cells, EGFL7 accelerates migration through the focal adhesion kinase (FAK)-associated pathway,[16] and promotes tumor progression by reducing the expression of endothelial molecules that mediate immune cell infiltration.[17] A study[18] on human tissues revealed that EGFL7 is overexpressed in ten human epithelial tumor types, including hepatocellular carcinoma, lung cancer, breast cancer, prostate cancer, colorectal cancer, gastric cancer, esophageal cancer, malignant glioma, ovarian cancer and renal cancer. In addition, correlations

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between EGFL7 expression and unfavorable biological behaviors as well as poor prognosis were also suggested in many malignancies.[16, 18-21] However, controversy still remains because EGFL7 expression was associated with better prognosis and with the absence of lymph node invasion in human breast cancer.[22] So far, there is no study on the expression and prognostic role of EGFL7 in PC.

The present study was to explore the expression of EGFL7 in PC, its association with clinicopathological variables, and the role of EGFL7 in the prognosis of PC.

Methods

Cell culture

Nine human PC cell lines (AsPC-1, BxPC-3, Capan-1, Colo357, MIAPaCa-2, PANC-1, Su86.86, SW1990 and T3M4), gifts from Professor Helmut Friess, Heidelberg University, Germany, were cultured in Dulbecco's modified Eagle's medium (DMEM) or RPMI-1640 medium (Hyclone, Thermo Fisher Scientific Inc., Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS, Hyclone), as previously reported.[23]

Western blotting analysis

Western blotting detection of EGFL7 in PC cell lines was performed according to the method of Liu et al,[24] using a rabbit anti-human EGFL7 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

Patients

Eighty-three patients with PC after radical surgical resection (R0) were recruited, 53 males and 30 females, with a mean age of 61.8±10.6 years (mean±SD). The clinicopathological features of the patients are shown in Table 1. Standard lymphadenectomy (including resection of nodes of No. 12, 13, 17, 8 and 14 groups) was performed. Post-surgical gemcitabine-based adjuvant chemotherapy/chemoradiotherapy was recommended for all patients. The study was approved by the Institutional Ethics Committee.

Construction of tissue microarray (TMA)

A tissue microarray was constructed using formalin-fixed paraffin-embedded blocks of PC. Diagnosis was proven by hematoxylin-eosin staining-based routine pathologic examination. After careful review and screening of representative tumor and non-tumor regions, two cores of corresponding tissues for each patient were sampled from typical areas using a 1.5-mm punch. The TMA was constructed with a manual tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA).

Table 1. EGFL7 expression and clinicopathological features of PC

Variables	Patient number (*n*)	EGFL7 expression in TT		*P* value*
		High	Low	
Gender				0.238
Male	53	36	17	
Female	30	24	6	
Age (yr)				0.566
≥65	33	25	8	
<65	50	35	15	
Tumor location				0.328
Head	47	32	15	
Non-head	36	28	8	
Tumor size (cm)				0.655
≥4	36	25	11	
<4	46	34	12	
Histological grade				0.523
G1-2	53	40	13	
G3-4	23	15	8	
PNI				0.443
Present	34	26	8	
Absent	48	33	15	
T stage				0.470
T1-2	50	38	12	
T3	32	22	10	
N stage				0.721
N0	48	35	13	
N1	33	22	11	

*: Chi-square test. EGFL7: epidermal growth factor-like domain 7; PC: pancreatic cancer; TT: tumor tissue; G1: well differentiated; G2: moderately differentiated; G3: poorly differentiated; G4: undifferentiated; PNI: perineural invasion; T: tumor; N: lymph node.

Immunohistochemical staining

TMA-based immunohistochemical staining for EGFL7 was performed in tissues from 83 patients with PC. A rabbit anti-human EGFL7 polyclonal antibody (Santa Cruz) and a two-step staining kit (EnVision+kit, Dako, Glostrup, Denmark) were used. The tissues were cross-sectioned in 4 μm thickness and mounted, deparaffinized and rehydrated. An autoclave was used to retrieve antigen. Slides were subsequently incubated with 3% hydrogen peroxide to block the endogenous peroxidase. Then, the slides were incubated with the primary antibody (dilution: 1:20) overnight at 4 ℃. Following washing in PBS, the slides were incubated with horseradish peroxidase (HRP)-labeled secondary antibody, and after washing, the slides were stained with diaminobenzidine and then counterstained with hematoxylin. Non-immune rabbit serum at the same dilution was applied as the negative control.