Pathology - Research and Practice 215 (2019) 152650

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

IL17RB expression might predict prognosis and benefit from gemcitabine in patients with resectable pancreatic cancer

Song Yao[a], Bin Ji[a], Chen-xia Jiang[b], Zhi-ming Chen[a], Ning-hua Yao[a], Naofumi Mukaida[c], Hua Huang[b,*]

[a] Department of Radiotherapy and Oncology, Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, 226362, China
[b] Department of Pathology, Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, 226362, China
[c] Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, Kanazawa, Ishikawa, Japan

ARTICLE INFO

Keywords:
Pancreatic cancer
IL17RB
Immunohistochemistry
Biomarker

ABSTRACT

Background: (Interleukin 17 Receptor Beta) IL17RB has been implicated in several malignancies. However, its role in the progression of and chemosensitivity in pancreatic cancer remains unknown. We aimed to determine the clinical significance of IL17RB expression in the prognosis of resectable pancreatic cancer and in the benefits from gemcitabine treatment.

Materials and methods: We used microarray and immunohistochemical staining techniques to evaluate IL17RB expression in 91 resectable pancreatic cancer tissues and their respective matched adjacent non-cancerous tissues. Quantitative real-time PCR and Western blotting were used to evaluate IL17RB in human pancreatic cancer cell lines after gemcitabine treatment. The correlation between IL17RB expression and overall survival and disease-free survival times were analyzed.

Results: IL17RB expression correlated with lymph node metastasis and (Vascular endothelial growth factor) VEGF expression. Cox proportional model showed that high IL17RB expression is a significant negative prognostic factor for both (overall survival) OS and (disease-free survival) DFS. Kaplan–Meier survival curves confirmed significantly reduced median overall and DFS time in high IL17RB patients as compared with low IL17RB patients. Furthermore, Cox proportional model confirmed a correlation between adjuvant treatment with gemcitabine-based chemotherapy and IL17RB expression. Kaplan–Meier survival curves showed that low IL17RB expression was associated with longer OS and DFS in patients than high IL17RB expression and gemcitabine-based adjuvant chemotherapy. In human pancreatic cancer cell lines, the messenger RNA and protein levels of IL17RB were significantly enhanced after gemcitabine treatment.

Conclusions: IL17RB predicts the prognosis and benefit from gemcitabine in patients with resectable pancreatic cancer.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a solid tumor found within the exocrine compartment of the pancreatic gland, and the 5-year survival rate for all stages has remained at less than 5% over the past few decades [1]. As PDAC has no specific symptoms, by the time the cancer is diagnosed, about 80% of patients are either in the advanced stage of this metastatic disease or have locally invasive tumors and, hence, have to be subjected to chemotherapy. The remaining 20% of the patients may be eligible for potentially curative resection of the tumor. Patients who undergo the resection surgery have an overall 5-year survival rate ranging between 10% and 25% [2,3]. However, local recurrence or distant metastasis is common even in these patients after surgery, and they often need adjuvant therapy consisting of chemotherapy and/or irradiation. Therefore, it is important to identify molecular prognostic biomarkers and new potential therapeutic targets to improve the outcomes of patients with PDAC.

Several studies have shown that the progression of PDAC is associated with members of the IL17 gene family, such as IL17A and IL17B, that induce progression by promoting the growth of tumor vasculature [4–6]. In recent times, there has been an increasing focus on the IL17 gene family (specifically on the IL17RB/IL17B axis) as a prognostic marker and new therapeutic target in several solid tumors, such as breast, gastric, thyroid, lung, and pancreatic cancers. Members of the

* Corresponding author.
E-mail address: hhua666@126.com (H. Huang).

https://doi.org/10.1016/j.prp.2019.152650
Received 5 June 2019; Received in revised form 8 September 2019; Accepted 16 September 2019

IL17 gene family are also known to be associated with chemoresistance in breast cancer [7–9]. The IL17 gene family, which comprises of six ligands (IL17A-F) and five receptors (IL17RA-E), is involved in several steps of tumor carcinogenesis and in tumor behavior [7]. Studies have shown that activation of the IL17 gene family is implicated in tumor progression, cell migration, inflammation, stem cell enrichment, vascular formation, tumor apoptosis inhibition, macrophage infiltration, and chemoresistance [7]. The main ligand- receptor combination in IL17 gene family, the IL17RB/IL17B axis, is overexpressed in tumor cells. In these cells, IL17RB/IL17B is known to enhance tumor migration and progression in PDAC and breast cancer through promoting vascular formation and inhibiting cell apoptosis [4,10,11]. IL17RB plays a vital role in the prognosis and metastasis of PDAC [6]. The reason for gemcitabine resistance is not so clear up to now. However, the role of IL17RB as a prognostic marker in resected PDAC has not been fully explored. Gemcitabine has been the standard of care drug for pancreatic cancer patients as a single-agent therapy [12]. Most patients do not respond well to gemcitabine-treatment, and those who do respond ultimately develop chemoresistance and exhibit disease progression [13]. However, no studies have explored the role of the IL17RB/IL17B axis in predicting chemo-sensitivity to gemcitabine-based adjuvant therapy.

The aim of this study was to investigate the impact of IL17RB/IL17B axis on prognosis after curative resection of PDAC and the potential role of the IL17RB/IL17B axis in predicting the benefits from gemcitabine-based adjuvant therapy in patients.

2. Materials and methods

2.1. Cell lines and reagents

Miapaca-2 and Panc-1 cells were obtained from ATCC and were maintained in RPMI-1640 medium. The medium was supplemented with 10% fetal bovine serum. Gemcitabine were obtained from Sigma Aldrich.

2.2. RNA isolation for RT-PCR

Total RNA was isolated and was reverse transcribed to cDNA as previously described [14]. Quantitative (q)RT-PCR for IL17RB was performed using the sets of the primers, 5'-GCCCTTCCATGTCTGTGAA-3' (forward) and 5' -CAGGGGAGTGGTTGTGAAGT-3' (reverse) and mRNA amounts were normalized to the amounts of GAPDH as relative expression values as described previously [14].

2.3. Western blot analysis

Proteins were extracted from tumor cell lines. The protein concentration was measured using a BCA Assay Kit (Pierce Biotechnology, Rockford, USA). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted onto Nitrocellulose membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% skim milk for one hours at room temperature and then incubated overnight at 4 °C with the primary antibody. Primary anti-IL17RB (1:1000; TA351284, Origene, China) and β-actin (1:1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) were used to probe the target proteins. Goat anti-rabbit immunoglobulin G-horseradish peroxidase (1:5000; Santa Cruz Biotechnology Inc.) was used as a secondary antibody and incubated with the membrane for two hours at room temperature. The bands were observed with an electroche miluminescence reagent.

2.4. Patients and tissues

This study included unselected patients with primary PDAC (n = 91) who had undergone duodenopancreatectomy and distal pancreatectomy for PDAC between August 2011 and August 2014 at Nantong University Hospital and respective matched adjacent non-cancerous tissues (n = 91). Their pathologic diagnoses and classifications were made according to the UICC Classification of Malignant Tumors [15]. The exclusion criteria for the studies was either the presence of macroscopically incomplete resection (R2), administration of preoperative chemotherapy or chemoradiotherapy, presence of tumor histology other than ductal pancreatic adenocarcinoma or death within 30 days following surgery. Clinical and clinicopathological data and treatment methods were obtained from the medical records of each patient. Patient characteristics are shown in Table 1. The ages of patients ranged between 30 and 80 years with the median age being 61 years. A total of 61 patients (67%) died during the follow-up period, 68% of patients were well/moderately differentiated and 70% had received adjuvant treatment. During the treatment process, all patients were also receiving regular follow-up care with physical, blood and tomography examinations once every 2 months during the first 6 months and then once every 3 months. Survival time was calculated as the number of days from the date of surgery until either death or last follow-up. For each patient, two formalin-fixed paraffin embedded (FFPE) tissue blocks representative of each of patient's tumor were selected by a pathologist. The study protocol was approved by the Human Ethics Review Committees of Nantong University (approval no. 2018-L057).

Table 1
Patient characteristics.

Clinical characteristics	n = 91
Age(years)	
Median	60.9
range	38-80
sex	
Female	46
male	45
Surgery	
Duodenopancreatectomy	65
Distal pancreatectomy	26
Resection margins	
Negative, R0	80
Positive, R1	11
Histological grade	
Well/moderately differentiated	62
Poorly differentiated	29
Vascular emboli	
present	30
absent	61
Perineural invasion	
present	66
absent	25
tumor size(cm)	
Median	3.36
range	1.5–7
tumor stage	
I-IIA	45
IIB-III	46
Lymph node	
Absent, N0	42
Present, N1	49
Adjuvant treatment	
Yes	64
No	27
Gemcitabine treatment	
Yes	58
No	33

2.5. Tissue microarray (TMA) constitution

Tissue microarray (TMA) blocks containing a total of 182 samples (91 cancer tissue samples and 91 adjacent non-cancerous tissue samples) were constructed after two independent pathologists calculated

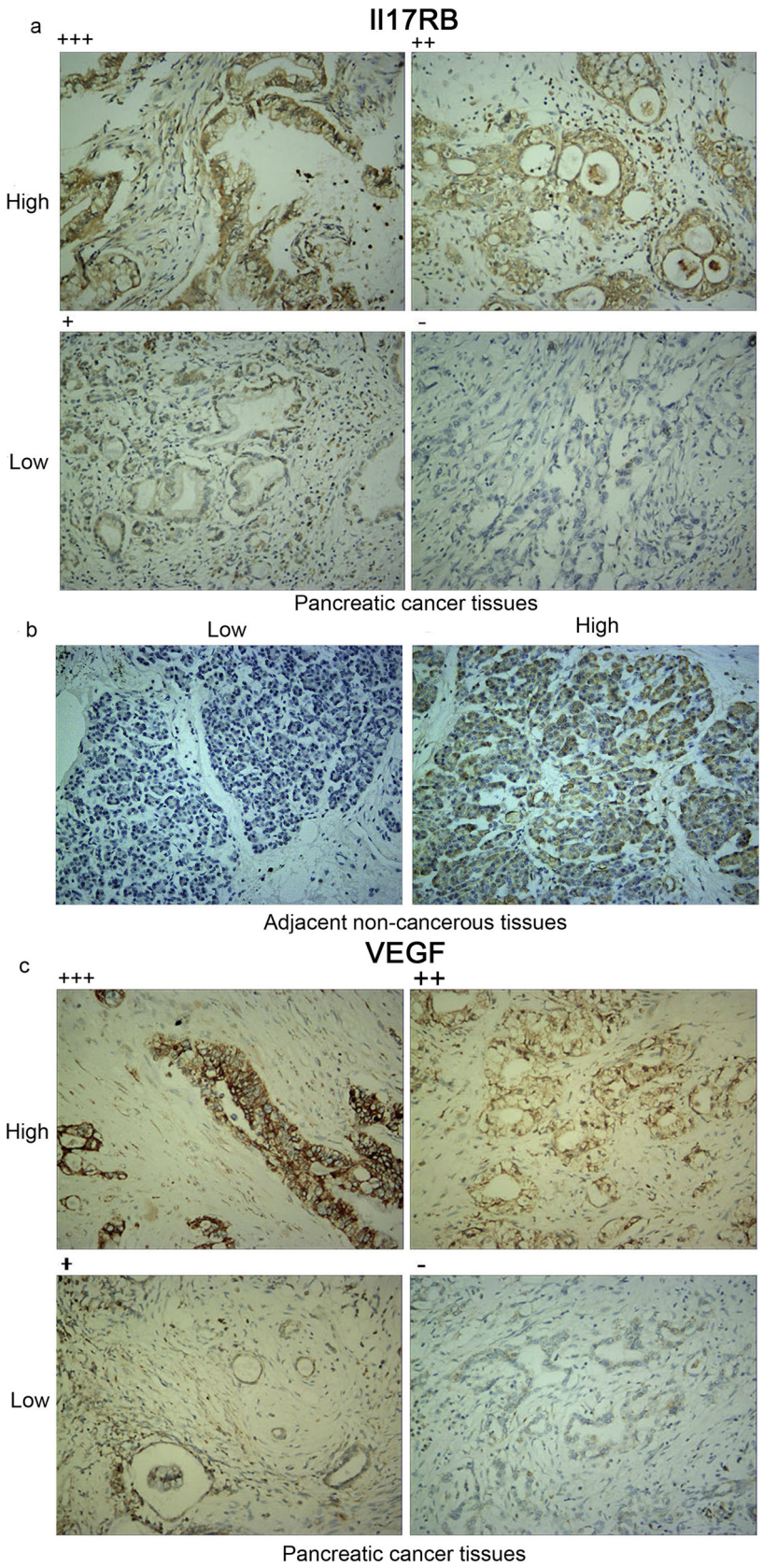

Fig. 1. IL17RB and VEGF expression in pancreatic cancer. a. Representative IL17RB staining quantified with scores of 0 to +3 according to staining intensity and percentage in pancreatic cancer tissues. (Original magnification × 200). b. Representative IL17RB staining quantified as either low or high expression according to staining intensity and percentage in matched adjacent non-cancerous tissues. (Original magnification × 200). c. Representative VEGF staining quantified with scores of 0 to +3 according to staining intensity and percentage (Original magnification × 200).

Table 2
Correlation between IL17RB expression in tumor cells and clinic pathological factors.

Characteristics	IL17RB expression in tumor cells		
	Low n = 52	High n = 39	*P*-value
Age			
≤65y	33	22	
> 65y	19	17	P = 0.50
Sex			
Female	26	20	
Male	26	19	P = 0.90
Histological grade			
Well/moderately differentiated	38	24	
Poorly differentiated	14	15	P = 0.24
Vascular emboli			
present	16	14	
absent	36	25	P = 0.61
Perineural invasion			
present	39	27	
absent	13	12	P = 0.54
Tumor size			
≤3 cm	22	20	
> 3 cm	30	19	P = 0.40
Tumor stage			
I-IIA	29	16	
IIB-III	23	23	P = 0.16
Lymph node			
Absent, N0	29	13	
Present, N1	23	26	P = 0.03
VEGF expression			
Low	34	17	
High	18	22	P = 0.04

the core area of the tumor from the hematoxylin and eosin-stained slides and selected one representative FFPE archival block for each case. Core tissue biopsies (2 mm in diameter) were done using individual FFPE blocks (donor blocks) and arranged in recipient paraffin blocks (TMA blocks) using a trephine.

2.6. Immunohistochemistry

Immunohistochemical staining for IL17RB and VEGF were performed using the avidin-biotin-peroxidase complex method. The deparaffinized and hydrated tissues were heated for 10 min at 105 °C in Target Retrieval Solution (Dako, Carpinteria, CA, USA) and the slides were allowed to cool in a citrate buffer (PH = 6.3) for 10 min at 25 °C. Endogenous peroxidase was eliminated with 3% H_2O_2, followed by blocking with avidin for 20 min at 25 °C. Subsequently, the slides were incubated overnight at 4 °C with 5 μg/mL of rabbit polyclonal antibodies against IL17RB (TA351284, Origene, China) and Vascular Endothelial Growth Factor (VEGF) (MAB-0243, MXB, China). 24 h later, the slides were incubated with secondary antibodies at room temperature for half an hour. Tissue sections were stained with 3, 3-diaminobenzidine (DAB) for 10 min, counterstained with 10% Mayer's hematoxylin, dehydrated and mounted. For the negative control, the primary antibody was replaced with normal rabbit serum. Each section was independently evaluated by two pathologists.

2.7. Immunohistochemistry scoring

The TMA slides were captured digitally and stored as high-resolution image files using the Nano-Zoomer 2.0-HT slide scanner (hamatsu Photonics K.K., Hamutu city, Japan). The images were analyzed using the NDP view software. The immunostained tissue specimens were evaluated independently by two trained pathologists who were unaware of the clinical background and clinicopathological data of these specimens from patients. In the event of disagreement between the two observers, the slide was evaluated by a third anatomopathology staff in order to reach a consensus. IL17RB and VEGF expression was visually quantified on the basis of semi-quantitative treatment [16]. The results according to the staining intensity and number of positive cells: (1) Staining intensity: 0, recorded as negative expressin, no observed cell staining; 1, recorded as weakly positive, cells with light-brown cell staining; 2, recorded as moderately positive, cells stained brown with no background staining, or darkbrown stained cells with light-bown background staining; 3, recorded as strongly positive, cells stained darkbrown with no background staining. (2) Number of positive cells: 0, less than 5% positive cells; 1, between 5% and 25% positive cells; 2, between 25 and 50% positive cells; 3, more than 50% positive cells. The final scores were got by multiplying the two scores. The results were as follows: the score of 0, was considered as −; the score of 1–2, was considered as +; the score of 3–4, was considered as 2+; the score of 6–9, was considered as 3+. – and 1+ represented as low expression; 2+ and 3+ represented as high expression.

2.8. Statistical analyses

The primary outcome variables that were being evaluated were overall survival (OS) and disease-free survival (DFS). OS was calculated as the number of days from the date of surgery to the date of death. DFS was defined as the time between surgical resection and the date of the first recurrence. The cut-off date was 31 August 2017. A patient census was conducted during their last follow-up. All data analyses were carried out according to a pre-specified analysis plan. The $\chi 2$ -test was used to determine the significance of the differences between the covariates. Survival durations were calculated by the log-rank test to compare the cumulative survival durations of the patient groups. The Cox proportional hazards model was used for the univariate and multivariate analyses. One-way ANOVA analysis followed by the Dunnett test was used to compare the IL17RB expression in human pancreatic cancer cells after gemcitabine treatment. All analyses were performed using SPSS 16.0 software. A P-value < 0.05 was considered to represent statistical significance.

3. Results

3.1. Expression of IL17RB and VEGF

Tumors that were positive for IL17RB protein showed cytoplasmic staining. Typical images of positive immunostaining for IL17RB in cancer cells are shown in Fig. 1a. Upon evaluation of staining intensity, 33 cases had a score of 0, 19 cases had a score of 1+, 29 cases had a score of 2+, and 10 cases had a score of 3+. Thus, 39 cases (43%) were classified as high expressing IL17RB in pancreatic cancer tissue. Typical images of positive immunostaining for IL17RB in matched non-cancerous tissue samples were shown in Fig. 1b. Overall, 48 cases had a score of 0, 27 cases had a score of 1+, 16 cases had a score of 2+, and 0 cases had a score of 3+. Thus, 16 cases (13.3%) were categorized as high expressing IL17RB in matched adjacent non-cancerous tissues. Most of the stain in IL17RB positive tumors was observed in the cytoplasm, while two cases showed staining in both the membrane and the cytoplasm. In contrast, little to no staining was seen in the cytoplasm of pancreatic duct cells and acinar cells, and there was no staining in the membranes. Fig. 1c shows a representative picture of VEGF staining. Tumors positive for VEGF protein showed membrane staining. Upon evaluation for staining intensity, 28 cases had a score of 0, 23 cases had a score of 1+, 13 cases had a score of 2+, and 27 cases had a score of 3+. Thus, 40 cases (44%) were positive for VEGF overexpression.

3.2. Immunohistochemical expression of IL17RB and correlation with clinicopathological characteristics

Among the 39 patients with high IL17RB expression, 26 patients (66.7%) had lymph node metastasis whereas among the 52 patients

Table 3
OS and DFS: univariate analysis.

Variable	DFS			OS		
	HR	95% CI	P-value	HR	95% CI	P-value
Age						
≤65y	1			1		
> 65y	1.04	0.61–1.76	0.89	0.99	0.58–1.67	0.96
Sex						
Female	1					
male	151	0.90–2.53	0.11	1.37	0.82–2.30	0.23
Histological grade						
Well/moderate	1			1		
Poor	1.76	1.01–3.06	0.044	1.66	0.96–2.89	0.07
Vascular emboli						
present	1			1		
absent	1.13	0.67–1.94	0.65	1.17	0.69–2.00	0.56
Perineural invasion						
present	1			1		
absent	1.1	0.61–1.98	0.76	1	0.56–1.80	1.0
Tumor size						
≤3 cm	1			1		
> 3 cm	1.08	0.72–1.60	0.72	1.06	0.72–1.55	0.78
Tumor stage						
I-IIA	1			1		
IIB-III	1.56	0.93–2.62	0.092	1.65	1.04–2.78	0.06
Lymph node						
Absent, N0	1			1		
Present, N1	1.90	1.12–3.22	0.017	2.14	1.25–3.65	0.01
Adjuvant treatment						
Yes	1			1		
No	0.99	0.61–1.61	0.97	1.02	0.63–1.65	0.95
IL17RB						
High	1			1		
Low	0.48	0.28–0.80	0.005	0.5	0.30–0.84	0.01
Resection margins						
Negative, R0	1			1		
Positive, R1	1.55	0.90–2.67	0.11	1.57	0.91–2.69	0.10
Surgery						
Duodenopancreatectomy	1			1		
Distal pancreatectomy	1.07	0.48–2.35	0.88	1.11	0.50–2.44	0.80

Abbreviations: CI: confidence interval; DFS: disease-free survival; IL17RB: IL17B receptor; HR: hazard ratio; OS: overall survival.

Table 4
OS and DFS: multivariate analysis.

Variable	DFS			OS		
	HR	95% CI	P-value	HR	95% CI	P-value
Tumor stage						
I-IIA	1			1		
IIB-III	0.90	0.39–2.07	0.80	0.74	0.30–1.83	0.52
Lymph node						
Absent, N0	1			1		
Present, N1	1.94	0.81–4.64	0.14	2.59	1.01–6.64	0.047
IL17RB expression						
Yes	1			1		
No	0.53	0.32–0.90	0.02	0.57	0.34–0.97	0.039
Histological grade						
Well/moderate	1			1		
Poor	1.89	1.07–3.32	0.027	1.79	1.02–3.15	0.042

Abbreviations: CI: confidence interval; DFS: disease-free survival; IL17RB: IL17B receptor; HR: hazard ratio; OS: overall survival.
Adjusted for tumor size (≤3 cm or > 3 cm), adjuvant treatment (yes or no), resection margins (R0 vs R1) and surgery methods (duodenopancreatectomy or distal pancreatectomy).

with low IL17RB expression, only 23 patients (44%) had lymph node metastasis ($\chi 2$ test, P = 0.03). However, IL17RB expression had no significant correlation ship with the histological grade ($\chi 2$ test, P = 0.24) and stage ($\chi 2$ test, P = 0.16) of the tumor. Moreover, among the 39 patients with high IL17RB expression, 22 patients (56.4%) had stained positive for VEGF and among the 52 patients with low IL17RB expression, only 18 patients (34.6%) showed VEGF positive staining ($\chi 2$ test, P = 0.03) (Table 2).

3.3. IHC IL17RB expression and clinical outcomes

Using univariate analysis, we found that lymph node metastasis and high IL17RB expression were predictors of poor OS after pancreatic adenocarcinoma resection (P = 0.01 and P = 0.01, respectively). Poorly differentiated tumors and late stage tumors tended to have the poor OS, but no significant correlation was observed (P = 0.07 and P = 0.06, respectively). Lymph node metastasis, poorly differentiated tumors and high IL17RB expression were predictors of shorter DFS (P = 0.02, P = 0.04 and P = 0.005, respectively) (Table 3). Among cases in the high IL17RB expression after pancreatic adenocarcinoma resection category, median DFS was 7.3 months vs 10.4 months for those with low expression (P = 0.02), and median OS was 11.0 months (95% CI: 21.9–49.2) and 14.7 months (95% CI: 11.2–19.8), in the high and low IL17RB expression categories respectively (P = 0.01). Using the multivariate Cox proportional model, we found that high IL17RB expression, poorly differentiated tumor and lymph node invasion were significant negative prognostic factors for both OS and DFS (Table 4). Kaplan–Meier survival curves showed significantly reduced median DFS time for high IL17RB expressing patients as compared with low IL17RB expressing patients. Similarly, median OS time was also reduced in high IL17RB expressing patients (Fig. 2a).

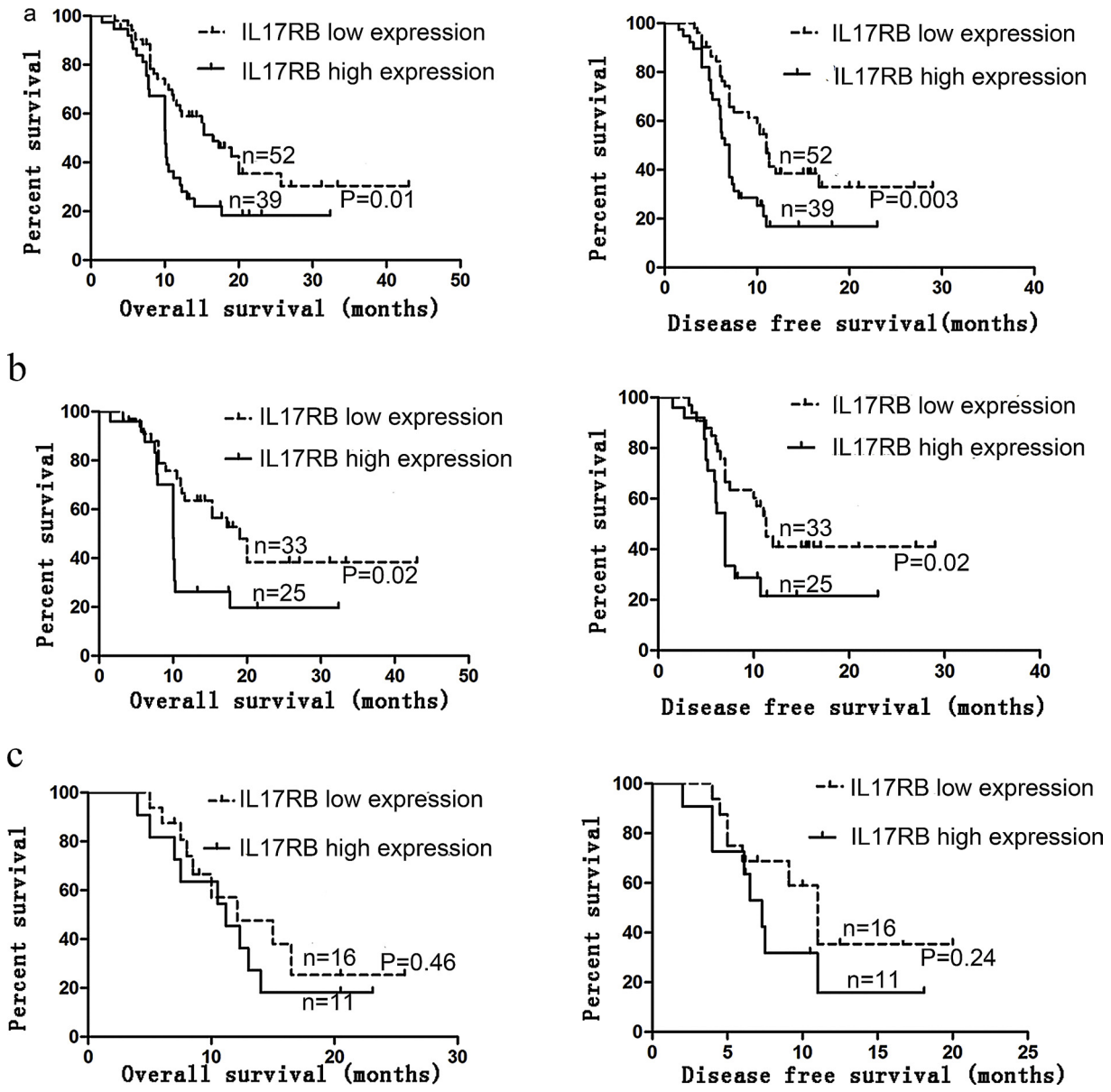

Fig. 2. Survival and IL17RB expression in pancreatic cancer. a. OS and DFS curves according to IL17RB expression in tumor cells. b. OS and DFS curves according to IL17RB expression in patients who received gemcitabine-based adjuvant chemotherapy. c. OS and DFS curves according to IL17RB expression in patients who did not receive gemcitabine. Solid line represents high IL17RB expression the dotted line represents low expression.

3.4. IL17RB expression could predict benefits from adjuvant gemcitabine therapy

We investigated whether IL17RB expression had an effect on the benefits from adjuvant gemcitabine-based treatment. In order to do so, we tested the correlation between adjuvant treatment with gemcitabine-based chemotherapy and IL17RB expression using a multivariate Cox proportional hazard model that adjusted for stage, differentiation, resection margins and lymph node metastasis of the tumors. We found that high IL17RB expression was significant negative prognostic factors for OS (P = 0.042). This result was consistent with the Kaplan–Meier curves (Fig. 2b). Low IL17RB expression was associated with a longer OS as compared with the OS in high IL17RB expressing patients who also received gemcitabine-based adjuvant chemotherapy (n = 58; log-rank test P = 0.02). There was no significant difference between the OS in low IL17RB expressing patients and high IL17RB patients who did not receive gemcitabine (n = 27; log-rank test P = 0.46). Furthermore, low IL17RB expression was associated with a longer DFS as compared to the DFS in high IL17RB expressing patients who also received gemcitabine-based adjuvant chemotherapy (n = 58; log-rank test P = 0.02). There was no significant difference between DFS in low IL17RB expression patients and high IL17RB expression patients who did not receive gemcitabine (n = 27; log-rank test P = 0.24) (Fig. 2b,c). Futhermore, we investigated the IL17RB expression in human pancreatic cell lines, gemcitabine enhanced IL17RB mRNA and protein expression in Miapaca-2 and Panc-1 cell lines (Fig. 3a, b).

4. Discussion

Our study evaluated the impact of the IL17R component, IL17RB, in PDAC after curative resection using TMA technology and IHC methods. Our data showed a strong relationship between IL17RB protein abundance in cancer cells and OS and DFS outcome in patients with resected PDAC. We confirmed this result using univariate and multivariate analysis after adjustment for standard clinicopathological prognostic factors similar to our previous study [2]. We demonstrated that among

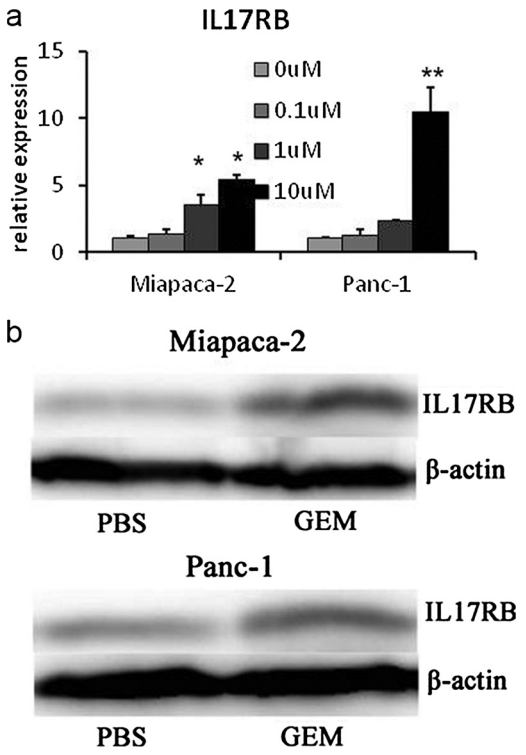

Fig. 3. Gemcitabine-induced IL17RB expression in human pancreatic cancer cell lines. a. Miapaca-2 and Panc-1 cells were treated with the indicated concentrations of gemcitabine for 48 h, total RNA was extracted to determine IL17RB mRNA levels by qRT-PCR. *, $p < 0.05$; **, $p < 0.01$. b. Miapaca-2 and Panc-1 cells were treated with 10 μM gemcitabine for 48 h, total protein was extracted to determine CXCL8 protein levels by western blot.

the patients who received the gemcitabine treatment, OS and DFS were longer in patients with low IL17RB expression than in patients with high IL17RB expression. However, this was not the case in patients who did not receive the gemcitabine treatment. Cumulatively, our results confirmed that IL17RB protein expression has a significant clinical benefit in PDAC patients who receive the gemcitabine adjuvent therapy. However, since the population of PDAC patients who did not receive the gemcitabine adjuvant therapy was small, a larger population of PDAC patients was needed to confirm the prognostic value of IL17RB.

Previous univariate and multivariate analyses yielded similar results - IL17RB expression was a positive prognostic marker in PDAC [6]. However, our study is different on several parameters. First, we only focused on the R0 and R1 surgical patients, and not on the R2 and unresectable patients. Hence, we could establish a standard baseline before they received the adjuvant therapy. Second, accurate data from the adjuvant chemotherapy regiment was collected, and OS and DFS were evaluated after they received the gemcitabine treatment. Furthermore, we evaluated the relationship between the IL17RB and VEGF expression.

IL17RB, the receptor of IL17B, induces tumorgenesis and chemoresistance in several tumors. Lee et al. reported that IL17RB/IL17B axis signaling promoted breast tumorigenicity by activating NF-κB to upregulate antiapoptotic factor Bcl-2 [11]. In pancreatic cancer, the IL-17RB/IL17B axis correlates with postoperative metastasis in patients, through inducing CCL20, CXCL1, CXCL8, TFF1 chemokine expression via the ERK1/2 pathway to promote cancer cell invasion in distant organs [6]. Furthermore, in gastric cancer, lung cancer and thyroid tumor IL17RB expression tightly correlates with tumor formation [17–20]. Even in breast cancer, IL17RB expression is tightly correlated with chemoresistance [8,9].

We found IL17RB expression significantly correlates with OS and DFS in patients and especially in cases where adjuvent therapy with gemcitabine is administered. Song *et.al* found that gemcitabine could induce CXCL8 and CXCL1 expression by activating the NF-κB pathway, which acts against the epithelial cells to induce the tumor angiogenesis and growth. Furthermore, gemcitabine combined with anti-CXCL8 antibody treatment can inhibit pancreatic cancer growth [14]. Meanwhile, gemcitabine in combination with anti-NF-κB can inhibit pancreatic tumor growth [21,22]. In pancreatic cancer, the IL17RB/IL17B pathway through TRAF6/NF-κB pathway can induce CXCL8, CCL20, CXCL1, TFF1 expression which in turn induces cell metastasis and survival through inducing tumor angiogenesis [6]. IL17RB, through activation of NF-κB, may induce chemokine secretion and gemcitabine-resistance. IL17RB may also play an important role in the prognosis of patient who receive gemcitabine adjuvant therapy. Futhermore, we also found the IL17RB expression was enhanced after gemcitabine treated in human pancreatic cancer. IL17RB might play a potential role in the gemcitabine resistance in human pancreatic cancer cells. VEGF, as a key mediator of angiogenesis, that played a key role in pancreatic cancer progression and metastasis and even in chemoresistance [23,24]. Our study showed that IL17RB IHC expression correlated significantly with tumor growth, lymphatic metastasis and VEGF protein expression. Previous studies demonstrated that high IL17RB expression correlated with cancer progression and metastasis by inducing angiogenesis and stemness [7]. Hence, IL17RB too might play an important role in gemcitabine-resistance through inducing angiogenesis.

In conclusion, IL17RB overexpression is a negative prognostic factor in resected pancreatic cancer patients treated with adjuvant gemcitabine. These results can be confirmed through experiments using a bigger sample size. Further mechanistic studies are warranted to identify interactions that could exist between IL17RB expression and gemcitabine activity.

Funding

This study was funded by Doctoral Fund of Ministry of Education of Jiangsu Province (grant numbers: 2018K256C).

Contributions

Song Y. and Huang H. conceived and designed the study, analyzed the data and wrote, edited and reviewed the manuscript. Jiang C. X. and Ji B. researched, analyzed data, and reviewed the manuscript. Yao N.H. and Chen Z.M. researched and collected the data. All authors gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgment

Not applicable.

References

[1] A.S. Paulson, T.H. Cao, M.A. Tempero, A.M. Lowy, Therapeutic advances in pancreatic cancer, Gastroenterology 144 (2013) 1316–1326.

[2] S.P. Cleary, R. Gryfe, M. Guindi, P. Greig, L. Smith, R. Mackenzie, S. Strasberg, S. Hanna, B. Taylor, et al., Prognostic factors in resected pancreatic adenocarcinoma: analysis of actual 5-year survivors, J. Am. Coll. Surg. 198 (2004) 722–731.

[3] T.A. Sohn, C.J. Yeo, J.L. Cameron, L. Koniaris, S. Kaushal, R.A. Abrams, P.K. Sauter, J. Coleman, R.H. Hruban, K.D. Lillemoe, Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators, J. Gastrointest. Surg. 4 (2000) 567–579.

[4] C. Paola, C. Roberta, M. Gianluca, R. Simona, T. Elisabetta, N. Francesco, IL17 favours carcinogenesis and limits the anti-tumor responses in pancreatic cancer. [Abstract], Proceedings of the 106th Annual Meeting of the American Association for Cancer Research, Apr 18–22, Philadelphia, PA. Philadelphia (PA), 2015 AACR; Cancer Res, 75(15 Suppl.): Abstract nr 3170.

[5] C.M. Liang, C.C. Liao, S.M. Liang, Refolded albumin inhibits IL-17B-driven pancreatic cancer migration. [Abstract], Proceedings of the 107th Annual Meeting of the American Association for Cancer Research, Apr 16–20; New Orleans, LA. Philadelphia (PA), 2016 AACR; Cancer Res, 76(14 Suppl.): Abstract nr 3855.
[6] H.H. Wu, W.W. Hwang-Verslues, W.H. Lee, C.K. Huang, P.C. Wei, C.L. Chen, J.Y. Shew, E.Y. Lee, Y.M. Jeng, et al., Targeting IL-17B-IL-17RB signaling with an anti-IL-17RB antibody blocks pancreatic cancer metastasis by silencing multiple chemokines, J. Exp. Med. 212 (2015) 333–349.
[7] Q. Bie, C. Jin, B. Zhang, H. Dong, IL-17B: A new area of study in the IL-17 family, Mol. Immunol. 90 (2017) 50–56.
[8] V. Alinejad, M. Hossein Somi, B. Baradaran, P. Akbarzadeh, F. Atyabi, H. Kazerooni, H. Samadi Kafil, L. Aghebati Maleki, H. Siah Mansouri, M. Yousefi, Co-delivery of IL17RB siRNA and doxorubicin by chitosan-based nanoparticles for enhanced anticancer efficacy in breast cancer cells, Biomed. Pharmacother. 83 (2016) 229–240.
[9] E. Laprevotte, S. Cochaud, S. du Manoir, M. Lapierre, C. Dejou, M. Philippe, J. Giustiniani, K.A. Frewer, A.J. Sanders, et al., The IL-17B-IL-17 receptor B pathway promotes resistance to paclitaxel in breast tumors through activation of the ERK1/2 pathway, Oncotarget 8 (2017) 113360–113372.
[10] S.L. Gaffen, Structure and signalling in the IL-17 receptor family, Nat. Rev. Immunol. 9 (2009) 556–567.
[11] C.K. Huang, C.Y. Yang, Y.M. Jeng, C.L. Chen, H.H. Wu, Y.C. Chang, C. Ma, W.H. Kuo, K.J. Chang, et al., Autocrine/paracrine mechanism of interleukin-17B receptor promotes breast tumorigenesis through NF-κB-mediated antiapoptotic pathway, Oncogene 33 (2014) 2968–2977.
[12] Z. Wang, Y. Li, A. Ahmad, S. Banerjee, A.S. Azmi, D. Kong, F.H. Sarkar, Pancreatic cancer. Understanding and overcoming chemoresistance, Nat. Rev. Gastroenterol. Hepatol. 8 (2011) 27–33.
[13] R. Warsame, A. Grothey, Treatment options for advanced pancreatic cancer. A review, Expert Rev. Anticancer Ther. 12 (2012) 1327–1336.
[14] Y. Song, T. Baba, Y.Y. Li, K. Furukawa, Y. Tanabe, S. Matsugo, S. Sasaki, N. Mukaida, Gemcitabine-induced CXCL8 expression counteracts its actions by inducing tumor neovascularization, Biochem. Biophys. Res. Commun. 458 (2015) 341–346.
[15] S. Isaji, Y. Kawarada, S. Uemoto, Classification of pancreatic cancer:comparison of Japanese and UICC classifications, Pancreas 28 (2004) 231–234.
[16] S.Q. Fan, J. Ma, J. Zhou, W. Xiong, B.Y. Xiao, W.L. Zhang, C. Tan, X.L. Li, S.R. Shen, et al., Differential expression of Epstein-Barr virus-encoded RNA and several tumor-related genes in various types of nasopharyngeal epithelial lesions and nasopharyngeal carcinoma using tissue microarray analysis, Hum. Pathol. 37 (2006) 593–605.
[17] L. Ren, Y. Xu, C. Liu, S. Wang, G. Qin, IL-17RB enhances thyroid cancer cell invasion and metastasis via ERK1/2 pathway-mediated MMP-9 expression, Mol. Immunol. 90 (2017) 126–135.
[18] Y.F. Yang, Y.C. Lee, S. Lo, Y.N. Chung, Y.C. Hsieh, W.C. Chiu, S.F. Yuan, A positive feedback loop of IL-17B-IL-17RB activates ERK/β-catenin to promote lung cancer metastasis, Cancer Lett. 422 (2018) 44–55.
[19] Q. Bie, C. Sun, A. Gong, C. Li, Z. Su, D. Zheng, X. Ji, Y. Wu, Q. Guo, et al., Non-tumor tissue derived interleukin-17B activates IL-17RB/AKT/beta-catenin pathway to enhance the stemness of gastric cancer, Sci. Rep. 6 (2016) 25447.
[20] Q. Bie, B. Zhang, C. Sun, X. Ji, P.A. Barnie, C. Qi, J. Peng, D. Zhang, D. Zheng, et al., IL-17B activated mesenchymal stem cells enhance proliferation and migration of gastric cancer cells, Oncotarget 8 (2017) 18914–18923.
[21] X. Pan, T. Arumugam, T. Yamamoto, P.A. Levin, V. Ramachandran, B. Ji, G. Lopez-Berestein, P.E. Vivas-Mejia, A.K. Sood, et al., Nuclear factor-kappaB p65/relA silencing induces apoptosis and increases gemcitabine effectiveness in a subset of pancreatic cancer cells, Clin. Cancer Res. 14 (2008) 8143–8151.
[22] S. Arora, A. Bhardwaj, S. Singh, S.K. Srivastava, S. McClellan, C.S. Nirodi, G.A. Piazza, W.E. Grizzle, L.B. Owen, A.P. Singh, An undesired effect of chemotherapy: gemcitabine promotes pancreatic cancer cell invasiveness through reactive oxygen species-dependent, nuclear factor κB- and hypoxia-inducible factor 1α-mediated up-regulation of CXCR4, J. Biol. Chem. 288 (2013) 21197–21200.
[23] W.A. Spannuth, A.M. Nick, N.B. Jennings, G.N. Armaiz-Pena, L.S. Mangala, C.G. Danes, Y.G. Lin, W.M. Merritt, P.H. Thaker, et al., Functional significance of VEGFR-2 on ovarian cancer cells, Int. J. Cancer 124 (2009) 1045–1053.
[24] J.S. Wey, M.J. Gray, F. Fan, A. Belcheva, M.F. McCarty, O. Stoeltzing, R. Somcio, W. Liu, D.B. Evans, et al., Overexpression of neuropilin-1 promotes constitutive MAPK signalling and chemoresistance in pancreatic cancer cells, Br. J. Cancer 93 (2005) 233–241.