World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i10.1123

World J Gastroenterol 2012 March 14; 18(10): 1123-1129
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Interleukin-8, a promising predictor for prognosis of pancreatic cancer

Ying Chen, Min Shi, Guan-Zhen Yu, Xiao-Ran Qin, Gang Jin, Peng Chen, Ming-Hua Zhu

Ying Chen, Min Shi, Ming-Hua Zhu, Department of Pathology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
Guan-Zhen Yu, Department of Oncology, Changzheng Hospital, Second Military Medical University, Shanghai 200433, China
Xiao-Ran Qin, Shanghai Chempartner Co., Ltd., Shanghai 201203, China
Gang Jin, Peng Chen, Department of General Surgery, Changzheng Hospital, Second Military Medical University, Shanghai 200433, China
Author contributions: Chen Y and Shi M contributed equally to this work; Chen Y, Shi M, Yu GZ, Jin G and Zhu MH designed the research; Chen Y, Shi M, Yu GZ, Qin XR, Jin G and Chen P performed the research; Shi M, Yu GZ and Chen P collected follow-up data; Chen Y, Jin G and Qin XR analyzed the data; Chen Y, Shi M and Zhu MH wrote the paper.
Supported by The National Key Project of Scientific and Technical Supporting Programs of China, No. 2006BAI02A14; National Natural Science Foundation of China, No. 30770996 and No. 30901776
Correspondence to: Ming-Hua Zhu, Professor, Department of Pathology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China. mhzhu2000@hotmail.com
Telephone: +86-21-81873000 Fax: +86-21-81873000
Received: May 9, 2011 Revised: November 17, 2011
Accepted: December 31, 2011
Published online: March 14, 2012

Abstract

AIM: To investigate the value of interleukin-8 (IL-8), a pro-inflammatory chemokine, in predicting the prognosis of pancreatic cancer.

METHODS: Expression of IL-8 and its receptor CXCR1 was assessed by immunohistochemistry in pancreatic cancer and chronic pancreatitis samples. Enzyme-linked immunosorbent assay was used to detect the serum IL-8 levels in pancreatic cancer patients. Human pancreatic cancer tissues were heterotopically transplanted to the immune-deficiency mice to evaluate the effect of serum IL-8 on the tumorigenesis of the cancer samples.

RESULTS: IL-8 and CXCR1 proteins were both over-expressed in pancreatic adenocarcinoma samples (55.6% and 65.4%, respectively) compared with the matched para-cancer tissues (25.9% and 12.3%, $P < 0.01$), or chronic pancreatitis (0% and 25%, $P < 0.05$). Serum IL-8 levels in pancreatic cancer patients (271.1 ± 187.7 ng/mL) were higher than in other digestive system tumors, such as gastric cancer (41.77 ± 9.11 ng/mL, $P = 0.025$), colorectal carcinoma (78.72 ± 80.60 ng/mL, $P = 0.032$) and hepatocellular carcinoma (59.60 ± 19.80 ng/mL, $P = 0.016$). *In vivo* tumorigenesis analysis further proved that tumor tissues from patients with higher serum IL-8 levels grew faster than those with lower IL-8 levels.

CONCLUSION: IL-8 can be a fine serum marker for predicting the prognosis pancreatic cancer.

Key words: Pancreatic cancer; Interleukin-8; CXCR1; Immunohistochemistry; Tumor implantation; Enzyme-linked immunosorbent assay

Peer reviewer: Lucia Ricci Vitiani, Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Viale Regina Elena, 299, Rome 00161, Italy

Chen Y, Shi M, Yu GZ, Qin XR, Jin G, Chen P, Zhu MH. Interleukin-8, a promising predictor for prognosis of pancreatic cancer. *World J Gastroenterol* 2012; 18(10): 1123-1129 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i10/1123.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i10.1123

INTRODUCTION

Interleukin-8 (IL-8) is a pro-inflammatory factor, belonging to CXC chemokine family. It was initially named neutrophil-activating peptide-1 for its potent chemotactic activity on granulocytes in inflammatory and immune

diseases[1,2]. Recently, it has been shown that IL-8 plays a critical role in cancer invasion, angiogenesis and metastasis[3-6] and is considered as an important component of tumor microenvironment[7,8]. The significance of tumor microenvironment, which can be described as the "soil" of cancer cells, has been emphasized, especially the cancer-stroma interaction, which has become critical determinants of cancer behavior[9]. Stromal cells can produce IL-8 to influence the ability of invasion or metastasis of cancer cells, and the cancer cells themselves can also secret IL-8 in an autocrine or paracrine manner, such as in breast cancer[10], gastric cancer[4], colon cancer[11], cervical cancer[12], pancreatic cancer[8,13] and leukemia[14,15].

Pancreatic cancer is one of the most aggressive human malignancies and often with a dismal prognosis. The overall 5-year survival rate is only 5% or even less. Patients with pancreatic cancer have already been at advanced stage in their first visit to hospital. Around 15%-20% of the patients have the chance for tumor resection and the rest can only receive adjuvant therapies[16]. Although new drugs and techniques have been developed in treating pancreatic cancer, their therapeutic effects varied. Even the first-line anticancer drug, gemcitabine, still has not achieved satisfactory results in improving patients' outcome. The overall survival of pancreatic cancer patients is quite low, it is therefore, very important to predict the prognosis of the patients so as to enable more active treatment to prolong their lives.

Many studies[8] have revealed that pancreatic cancer highly produces IL-8, which can promote angiogenesis and invasion of tumors. Serum IL-8 levels were elevated in pancreatic cancer patients[17], suggesting the feasibility of IL-8 to be a fine marker in predicting the outcome of the patients. The main aim of the present study is to investigate the prognostic value of IL-8 in pancreatic cancer patients. We examined the expression and secretion levels of IL-8 in both tumor tissue and human blood. Furthermore, we implanted the cancer tissues from patients with various serum IL-8 levels subcutaneously to the nude mice and observed the growth of each xenograft. Based on these *in vitro* and *in vivo* analyses, we aim to more precisely define the role of IL-8 in predicting the prognosis of pancreatic cancer patients.

MATERIALS AND METHODS

Sample collection

Eighty-one pancreatic ductal adenocarcinoma (PDC) specimens with matched para-cancerous pancreas were collected during resection in the surgically treated patients. Five cases of chronic pancreatitis (CP) served as controls. All patients with pancreatic cancer had been followed up for survival and outcome until October 2008. Except for four patients who were alive till the end of follow-up, the rest all died.

The clinical information of the patients is presented in Table 1. Besides the patients with PDC, patients with chronic and acute pancreatitis (AP) were also included.

Table 1 Clinical data of patients subjected to enzyme-linked immunosorbent assay analysis for serum interleukin-8

Groups	Sex		Age (yr)	
	Male	Female	Range	Median
PDC	13	14	45-80	58
CP	13	2	9-58	40
AP	3	5	37-73	51
DA	3	5	47-65	58
GC	1	2	47-55	53
CRC	3	1	48-68	63
HCC	1	1	55-72	63.5

PDC: Pancreatic ductal carcinoma; CP: Chronic pancreatitis; AP: Acute pancreatitis; DA: Duodenal adenocarcinoma; GC: Gastric carcinoma; CRC: Colorectal carcinoma; HCC: Hepatocellular carcinoma.

Table 2 Background information of cell lines for interleukin-8 detection

Cell lines	Cell origin	Number in cell line library
BxPC3	Primary adenocarcinoma	CRL-1687 (ATCC)
CFPAC-1	Liver metastasis from adenocarcinoma	CRL-1918 (ATCC)
SW1990	Spleen metastasis from adenocarcinoma	CRL-2172 (ATCC)
Patu 8988s	Liver metastasis from adenocarcinoma	ACC 204 (DSMZ)

ATCC: American Type Culture Collection; DSMZ: German Collection of Microorganisms and Cell Cultures.

Those with other carcinomas from gastrointestinal system such as stomach, large bowel and liver were also investigated as controls. Diagnosis for all these patients was pathologically confirmed by HE staining. Supernatant from four pancreatic cancer cell lines (BxPC3, SW1990, CFPAC-1 and Patu 8988s) was collected after the cells were cultured for 48 h. Detailed information of these cell lines is shown in Table 2.

Ethics

This study was approved by the Ethics Committee of the Second Military Medical University (Shanghai, China). Informed consent was obtained from each patient before tissue specimen and blood samples were collected.

Immunohistochemical analysis

Unstained 3-μm sections were cut from the paraffin blocks and deparaffinized by routine procedures. Envision solution (K4065, DAKO, Denmark) was added to detect the primary antibody and 3,3'-diaminobenzidine was applied as chemicon. Sections were counterstained with hematoxylin. Primary antibodies against IL-8 (AHC0881) and CXCR1 (AHR1522Z) were purchased from BioSource (California, United States). In immunohistochemical (IHC) analysis, all the focal cases were considered negative and cases showing diffused expression were considered positive. Only the positive signals in epithelial cells were taken into account.

Enzyme-linked immunosorbent assay

The concentration of serum IL-8 in the patients was determined quantitatively with human IL-8 enzyme-linked immunosorbent assay (ELISA) detection kit provided by BioSource following the manufacturer's instructions. The up-limit of the test was 500 ng/mL.

Animal models and heterotopic implantation of human tumor tissues

Eleven severe combined immunodeficiency (SCID) nude mice aged 6-7 wk were housed under specific pathogen-free conditions. Animals were inoculated with subcutaneously (sc) transplanted fresh tissue cubes (1 mL) from pancreatic cancer patients with a detected serum IL-8 level. Animals were monitored daily by general clinical observation throughout the study period. Tumor volumes were calculated based on the following formula: tumor volume = (length × width^2)/2. Animals were euthanized when their tumors reached an appropriate size (0.3 cm^3) and the latent period was recorded. All experimental manipulations were undertaken in accordance with the NIH Guide for the Care and Use of Laboratory Animals, with the approval of the Biomedical Ethics Committee of the Second Military Medical University (Shanghai, China).

Histological analysis of implanted tumor tissues

After implanted with human tumor tissues heterotopically, animals with growing tumor were sacrificed by craniocervical dislocation. The mice were then weighed, and the sc tumor tissues were excised. Part of the tumor tissues underwent routine histological examination and the rest was stored in liquid nitrogen for future use. Histology of the corresponding human tumor tissues was also reviewed.

Statistical analysis

The IHC results were analyzed by χ^2 test. And the ELISA assay results were expressed as arithmetic mean ± SD. The comparisons between groups of patients were made using analysis of variance, and Fisher's Least Significant Difference test was used for animal models. The difference was considered significant at $P < 0.05$. SPSS 10.0 was applied as a statistical tool. Survival rates were calculated by the Kaplan-Meier method.

RESULTS

IL-8 and CXCR1 expression in PDC

IL-8 was expressed in 55.6% (45/81) of pancreatic cancer specimens, whereas in 25.9% (21/81) of non-cancer tissues ($P < 0.01$, $\chi^2 = 14.727$) (Table 3). IL-8 immunoreactivity was absent in CP (0%, 0/5) ($P = 0.016$, $\chi^2 = 5.827$) (Table 3). The positive signal was localized in the cytoplasm of cancerous or normal ductal cells (Figure 1). Focal stromal cells could express both IL-8 and CXCR1. The relationship between the expression of IL-8 or CXCR1 and the histological grading of pancreatic cancer is shown in Table 3. The CXCR1 expression level was significantly higher in pancreatic cancer samples (65.4%,

Table 3 Interleukin-8 and CXCR1 expression in pancreatic cancer, matched para-cancer tissue and chronic pancreatitis

	Pancreatic cancer differentiation			Matched para-cancer tissue	Chronic pancreatitis
	Well	Moderate	Poor		
IL-8					
+	5	33	7	21	0
-	11	22	3	60	5
CXCR1					
+	10	35	8	10	1
-	6	20	2	71	4

IL-8: Interleukin-8.

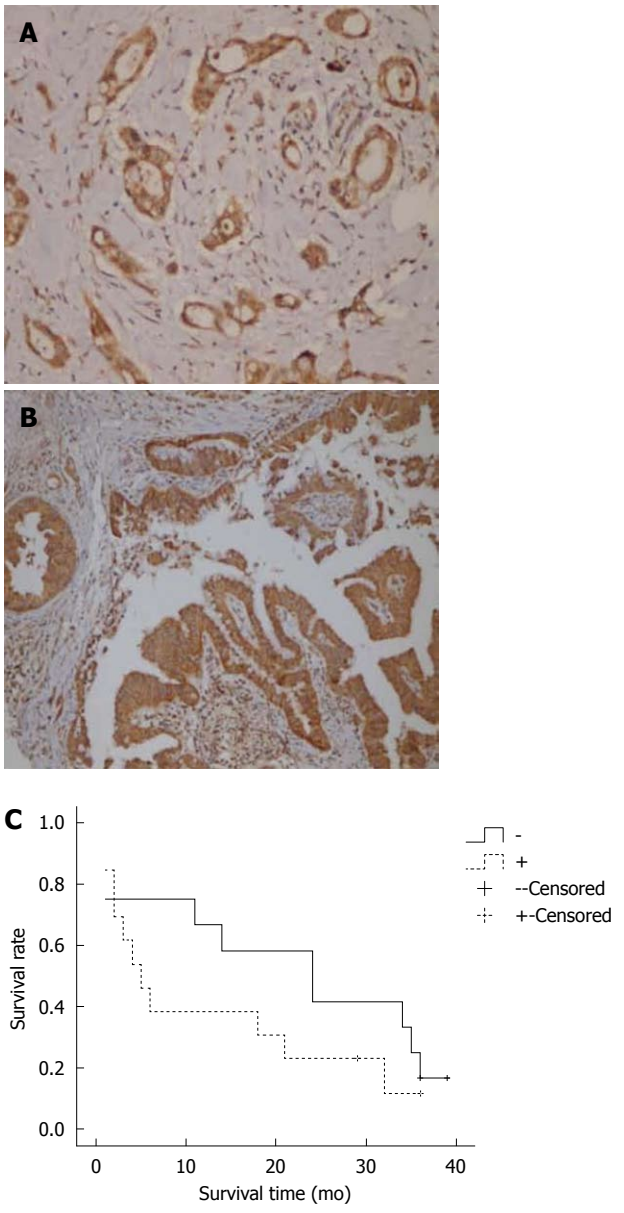

Figure 1 Interleukin-8 and CXCR1 expression in pancreatic cancer. A: Interleukin-8 (IL-8) expression in pancreatic cancer (× 200); B: CXCR1 expression in pancreatic cancer (× 200); The positive signals of IL-8 and CXCR1 were mainly located in the cytoplasm of cancer cells. For IL-8, focal positivity was found in the fibroblasts; C: Survival analysis of pancreatic cancer patients. "Censored" means patients were still alive till the end of follow-up study. The patients without IL-8 expression survived longer than those IL-8 positive.

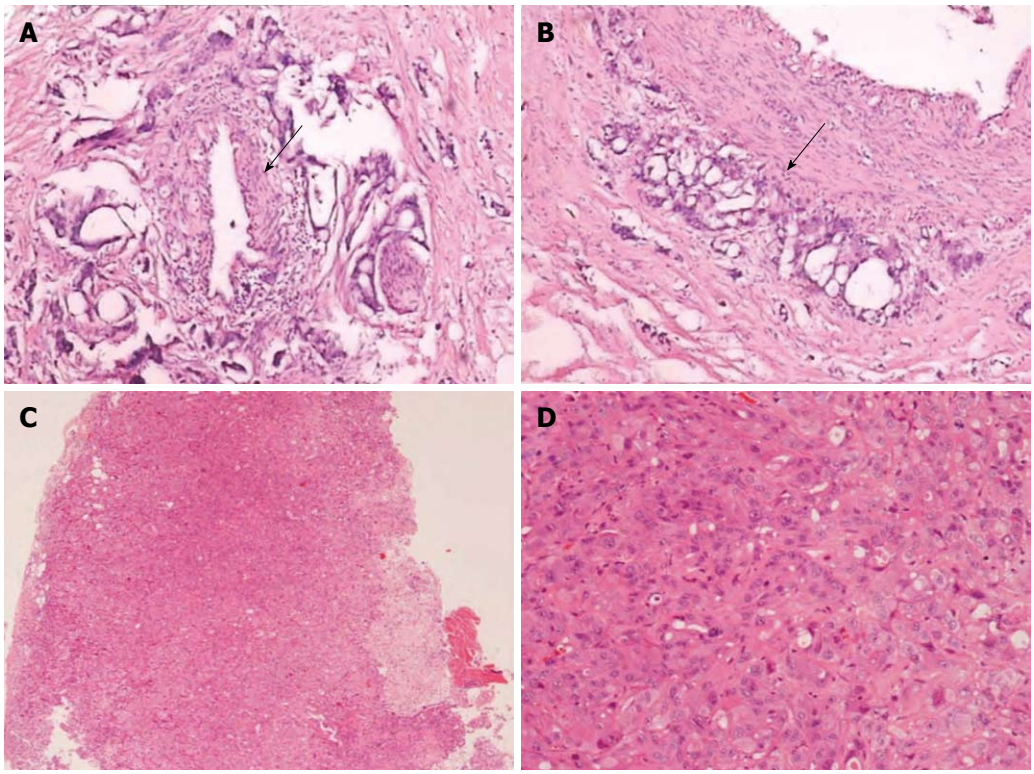

Figure 2 Histology of human pancreatic cancer tissue and transplanted tumor in SCID mice. A: Both vascular (arrow) and neural invasion (cross) were seen in the original human pancreatic ductal adenocarcinoma (PDC) (× 100); B: Cancer cells were infiltrating the medium-sized vessel in human PDC tissues (× 100) (arrow). Desmoplastic response was obvious in the human PDC tissues; C, D: Transplanted cancer tissue in SCID mice in low power (C, × 40) and in high power (D, × 200). Cancer cells were almost clustered and poorly differentiated. In some areas, primitive gland formation could be seen (arrow). Cells grew into sheet with very less stromal reaction than the original human tissues.

Table 4 Interleukin-8 concentration in the supernatant of pancreatic cancer cell lines

Cell line	IL-8 concentration (ng/mL)
BxPC3	81.38
CFPAC-1	3.906
SW1990	> 500
Patu 8988s	498.9

SW1990 and Patu8988 originated from metastatic sites showed much higher levels of interleukin-8 (IL-8) than BxPC3 and CFPAC-1.

53/81) than in samples from the adjacent non-cancerous pancreas (12.3%, 10/81) ($P < 0.001$, $\chi^2 = 48.026$). No statistical difference was observed between histological grading and IL-8 or CXCR1 expression.

Overall survival analysis was performed in the patients. Most people died within 3 years after surgery and only four were alive till the end of follow-up. The median survival was 5 mo (range, 1-36 mo) in the IL-8 positive group and 24 mo (range, 1-39 mo) in the IL-8 negative group. Although IL-8 positive patients seemed to live shorter than IL-8 negative ones, no significant difference was observed by Kaplan-Meier method ($P = 0.245$).

Serum IL-8 levels in pancreatic cancer patients and pancreatic cancer cell lines

Serum IL-8 level was measured in patients with PDC ($n = 27$), AP ($n = 8$), CP ($n = 15$) and other kinds of cancer from digestive system, such as duodenal adenocarcinoma (DA, $n = 4$), gastric carcinoma (GC, $n = 3$), rectal colonic carcinoma (CRC, $n = 4$), and hepatocellular carcinoma (HCC, $n = 2$). Blood was collected before surgery in the cancer patients. The serum IL-8 levels were significantly higher in PDC (271.1 ± 187.7 ng/mL) than in CP (97.02 ± 130 ng/mL, $P = 0.002$), AP (133.6 ± 162.9 ng/mL, $P = 0.041$), GC (41.77 ± 9.11 ng/mL, $P = 0.025$), CRC (78.72 ± 80.6 ng/mL, $P = 0.032$) or HCC (59.6 ± 19.8 ng/mL, $P = 0.016$). No significant difference was observed between PDC and DA (168.7 ± 212.4 ng/mL, $P = 0.247$). Two cancer cell lines originated from metastatic site (SW1990 and Patu 8988s) showed a much high level of IL-8 secretion (Table 4).

Histology of human tumor tissues implanted into SCID mice

Tumors subcutaneously implanted into the SCID mice were mostly composed of strands of cells, and glandular architectures were not very obvious (Figure 2). Intracellular vacuoles could be observed in some regions as immature or primary glands, indicating their adeno-epithelial origin. Unlike cancers growing in human pancreas, the desmoplastic reaction was not so remarkable and only several fibroblastoid spindle cells appeared in the implanted cancer tissues. Nerve and vessel invasion by can-

cer cells was easily observed in human cancer tissues, but none was detected in transplanted cancer tissues.

In vivo growth of heterotopically transplanted tumor tissues and its correlation with serum IL-8 levels

Among all the 11 cases of human tumor tissues implanted into SCID mice, seven tumors grew subcutaneously in animals. We set the terminal goal of growth at 0.3 cm^3 and recorded the latent period. The slowest growing period was 93 d and the fastest was 44 d. Seven animals with tumors were divided into three groups based on the median latent period of 61 d for analysis: group 1, failed to form tumor, $n = 4$; group 2, latent period < 61 d, $n = 3$; and group 3, latent period ⩾ 61 d, $n = 4$. Serum IL-8 level in group 2 (140.1 ± 33.4 ng/mL) was much higher than in group 1 (23.4 ± 17.4 ng/mL) and group 3 (23.2 ± 16.1 ng/mL) ($P < 0.001$). There was no significant difference between group 1 and group 3 ($P = 0.998$).

DISCUSSION

In this study, the expression level of IL-8 and its receptor CXCR1 was elevated in pancreatic cancer and serum IL-8 level was significantly higher in patients with pancreatic cancer than in those with pancreatitis and also higher than in the patients with other kinds of tumors from digestive system. *In vivo* tumor tissues from pancreatic cancer patients with a higher serum IL-8 level grew faster and behaved more aggressively than those with low serum IL-8 levels. Follow-up data showed that patients with high serum IL-8 levels had a relatively lower survival than those with low serum IL-8 levels. Due to the insufficient number of patients involved in the follow-up analysis, no statistical difference was achieved in the two groups.

IL-8 is a member of the CXC chemokine family and is a chemotactic factor for T cells, neutrophils, and basophils. Besides its pro-inflammatory role, IL-8 has been evaluated as a pro-oncogenic effector in various types of human cancers, including leukemia, astrocytoma, melanoma, breast cancer, ovarian cancer, lung cancer, prostate cancer, colon cancer, renal cell carcinoma, gastric cancer and pancreatic cancer[18]. The most critical effect of IL-8 on cancer cells is its strong angiogenic potential and ability of promoting invasion and metastasis[19-21]. *In vitro* IL-8 can enhance the proliferation and survival of endothelial cells and through its receptor CXCR1 can upregulate the expression of matrix protein, MMP-2 and MMP-9[22]. IL-8 can mimic the role of vascular endothelial growth factor (VEGF), transactivate VEGFR2 and promote angiogenesis[23]. Various signals or pathways can induce IL-8 expression in cancers[24-26] and the whole IL-8-involved network is very complicated. It has been confirmed that nuclear factors, NF-κB and AP-1 are the upstream regulators of transcription of IL-8 mRNA, both of which may cooperate with each other in the production of IL-8. In our study, a higher expression level of IL-8 and CXCR1 was detected in pancreatic cancer tissues than in para-cancerous pancreas. Although IL-8 could be elevated in colon and gastric cancers, the serum IL-8 level in pancreatic cancer was remarkably higher than in colon and gastric cancers. Moreover, IL-8 level was even higher in acute pancreatitis, in which IL-8 is considered to be a reliable indicator in evaluating the severity of inflammation and necrosis[27]. These data suggest that pancreatic cancer cells have a higher capability to produce chemokines than inflammatory cells, and function in an autocrine manner, which fulfills the composition of tumor microenvironment.

We have no direct experimental information on which cells are responsible for the secretion of IL-8, but according to the IHC analysis, IL-8 expression occurred mostly inside cancer cells, indicating that they might be the main source of IL-8. Pancreatic cancer cell lines have been investigated for IL-8 secretion and its mRNA expression, and quite a number of these cell lines showed high levels of IL-8 in the supernatant and mRNA expression[28]. We also analyzed IL-8 levels in the cultured supernatant of four pancreatic cancer cell lines, and found that those cell lines originated from the primary tumor site produced less IL-8 than those selected from metastatic site. Nomura *et al*[29] demonstrated similar results using two sublines of pancreatic cancer, both of which were sequentially selected from the parental cell line and possessed a high potential of organ metastasis, and the high IL-8 expression was closely correlated with the aggressive behavior of cancer cells. Besides cancer cells, inflammatory cells in the tumor stroma is also one of the important sources of IL-8 secretion. Neutrophils can not only promote tumor destruction[30], but also increase the growth of tumor cells[31]. Neutrophil-dependent release of VEGF-A leads to subsequent recruitment of neutrophils, resulting in angiogenesis.

Our survival analysis showed that IL-8 positive pancreatic cancer patients had a lower survival than IL-8 negative ones, whereas no significant difference was observed between the two groups possibly due to the insufficient number of cases involved. *In vivo* preclinical experiments of animal models on IL-8 have been conducted by many labs using various tumor cell lines, such as prostate cancer, lung cancer, breast cancer and pancreatic cancer[18]. In these cell lines, a high level of IL-8 is directly correlated with tumor growth, angiogenesis and metastasis in nude mice. But in ovarian cancer, IL-8 was negatively involved during tumorigenesis in animal models. Lee *et al*[30] demonstrated that the inverse regulation of IL-8 on xenograft growth was mainly mediated by its induction of neutrophil infiltration. In our animal model, we for the first time used the clinically resected pancreatic cancer tissues and transplanted them sc into SCID mice. The infiltration of inflammatory cells was not as evident as that observed by Lee *et al*[30] in ovarian cancer. The growth rate of tumors was significantly correlated with the serum level of IL-8 of the corresponding patients. The higher serum IL-8 level in the patients, the faster the tumor grew, indicating the more aggressive phenotype of this tumor, the poorer prognosis of the patients. IL-8

level can be measured in the tumor tissues resected surgically and in the patients' sera, it can also be detected in other kinds of clinical samples, including pancreatic juice obtained from the duodenum. Moreover, IL-8 concentration in pancreatic juice could be used to discriminate between normal pancreas and pancreatic cancer[32].

In summary, IL-8 was highly expressed in pancreatic cancer in both tumor tissues and blood samples. *In vivo* analysis showed that IL-8 would be a sensitive marker in predicting prognosis and monitoring disease progression of the pancreatic cancer patients. The patients with high serum levels of IL-8 should receive more active treatment due to the more aggressive biology of their cancer. Besides its prognostic value, IL-8 may represent a promising target for the development of adjuvant therapy for pancreatic cancer.

COMMENTS

Background

Many studies have revealed that pancreatic cancer highly produces IL-8 and IL-8 can promote angiogenesis and invasion of tumors. Serum IL-8 level was elevated in pancreatic cancer patients, suggesting the feasibility of IL-8 to be a fine marker in predicting outcomes of pancreatic cancer patients.

Research frontiers

The present study is to confirm the prognostic value of IL-8 in pancreatic cancer patients. The authors examined the expression and secretion levels in tumor tissues and human blood, respectively. Cancer tissues from patients with various serum IL-8 levels were implanted subcutaneously into nude mice to observe the growth of each xenograft.

Innovations and breakthroughs

ELISA was used to detect the serum IL-8 levels in pancreatic cancer patients. Human pancreatic cancer tissues were transplanted heterotopically into immune-deficiency mice to evaluate the effect of serum IL-8 on the tumorigenesis of cancer samples. Besides its prognostic value, IL-8 may represent a promising target for the development of adjuvant therapy for pancreatic cancer.

Peer review

This issue is of great interest and is definitely offering the opportunity to more accurately predict prognosis of patients affected by pancreatic carcinoma and to monitor the disease progression.

REFERENCES

1 **Baggiolini M**, Imboden P, Detmers P. Neutrophil activation and the effects of interleukin-8/neutrophil-activating peptide 1 (IL-8/NAP-1). *Cytokines* 1992; **4**: 1-17
2 **Mukaida N**, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. *Cytokine Growth Factor Rev* 1998; **9**: 9-23
3 **Iguchi H**, Ono M, Matsushima K, Kuwano M. Overproduction of IL-8 results in suppression of bone metastasis by lung cancer cells in vivo. *Int J Oncol* 2000; **17**: 329-333
4 **Konno H**, Ohta M, Baba M, Suzuki S, Nakamura S. The role of circulating IL-8 and VEGF protein in the progression of gastric cancer. *Cancer Sci* 2003; **94**: 735-740
5 **Kuwada Y**, Sasaki T, Morinaka K, Kitadai Y, Mukaida N, Chayama K. Potential involvement of IL-8 and its receptors in the invasiveness of pancreatic cancer cells. *Int J Oncol* 2003; **22**: 765-771
6 **Kozłowski L**, Zakrzewska I, Tokajuk P, Wojtukiewicz MZ. Concentration of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in blood serum of breast cancer patients. *Rocz Akad Med Bialymst* 2003; **48**: 82-84
7 **Kogan-Sakin I**, Cohen M, Paland N, Madar S, Solomon H, Molchadsky A, Brosh R, Buganim Y, Goldfinger N, Klocker H, Schalken JA, Rotter V. Prostate stromal cells produce CXCL-1, CXCL-2, CXCL-3 and IL-8 in response to epithelia-secreted IL-1. *Carcinogenesis* 2009; **30**: 698-705
8 **Matsuo Y**, Ochi N, Sawai H, Yasuda A, Takahashi H, Funahashi H, Takeyama H, Tong Z, Guha S. CXCL8/IL-8 and CXCL12/SDF-1alpha co-operatively promote invasiveness and angiogenesis in pancreatic cancer. *Int J Cancer* 2009; **124**: 853-861
9 **Farrow B**, Albo D, Berger DH. The role of the tumor microenvironment in the progression of pancreatic cancer. *J Surg Res* 2008; **149**: 319-328
10 **Derin D**, Soydinc HO, Guney N, Tas F, Camlica H, Duranyildiz D, Yasasever V, Topuz E. Serum IL-8 and IL-12 levels in breast cancer. *Med Oncol* 2007; **24**: 163-168
11 **Lurje G**, Zhang W, Schultheis AM, Yang D, Groshen S, Hendifar AE, Husain H, Gordon MA, Nagashima F, Chang HM, Lenz HJ. Polymorphisms in VEGF and IL-8 predict tumor recurrence in stage III colon cancer. *Ann Oncol* 2008; **19**: 1734-1741
12 **Tjiong MY**, van der Vange N, ten Kate FJ, Tjong-A-Hung SP, ter Schegget J, Burger MP, Out TA. Increased IL-6 and IL-8 levels in cervicovaginal secretions of patients with cervical cancer. *Gynecol Oncol* 1999; **73**: 285-291
13 **Zhou DH**, Trauzold A, Röder C, Pan G, Zheng C, Kalthoff H. The potential molecular mechanism of overexpression of uPA, IL-8, MMP-7 and MMP-9 induced by TRAIL in pancreatic cancer cell. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 201-209
14 **Bauer S**, Adrian N, Siebenborn U, Fadle N, Plesko M, Fischer E, Wüest T, Stenner F, Mertens JC, Knuth A, Ritter G, Old LJ, Renner C. Sequential cancer immunotherapy: targeted activity of dimeric TNF and IL-8. *Cancer Immun* 2009; **9**: 2
15 **Vinante F**, Rigo A, Vincenzi C, Ricetti MM, Marrocchella R, Chilosi M, Cassatella MA, Bonazzi L, Pizzolo G. IL-8 mRNA expression and IL-8 production by acute myeloid leukemia cells. *Leukemia* 1993; **7**: 1552-1556
16 **Li D**, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet* 2004; **363**: 1049-1057
17 **Wigmore SJ**, Fearon KC, Sangster K, Maingay JP, Garden OJ, Ross JA. Cytokine regulation of constitutive production of interleukin-8 and -6 by human pancreatic cancer cell lines and serum cytokine concentrations in patients with pancreatic cancer. *Int J Oncol* 2002; **21**: 881-886
18 **Xie K**. Interleukin-8 and human cancer biology. *Cytokine Growth Factor Rev* 2001; **12**: 375-391
19 **Matsuo Y**, Sawai H, Funahashi H, Takahashi H, Sakamoto M, Yamamoto M, Okada Y, Hayakawa T, Manabe T. Enhanced angiogenesis due to inflammatory cytokines from pancreatic cancer cell lines and relation to metastatic potential. *Pancreas* 2004; **28**: 344-352
20 **Miyamoto M**, Shimizu Y, Okada K, Kashii Y, Higuchi K, Watanabe A. Effect of interleukin-8 on production of tumor-associated substances and autocrine growth of human liver and pancreatic cancer cells. *Cancer Immunol Immunother* 1998; **47**: 47-57
21 **Li M**, Zhang Y, Feurino LW, Wang H, Fisher WE, Brunicardi FC, Chen C, Yao Q. Interleukin-8 increases vascular endothelial growth factor and neuropilin expression and stimulates ERK activation in human pancreatic cancer. *Cancer Sci* 2008; **99**: 733-737
22 **Li A**, Dubey S, Varney ML, Dave BJ, Singh RK. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *J Immunol* 2003; **170**: 3369-3376
23 **Petreaca ML**, Yao M, Liu Y, Defea K, Martins-Green M. Transactivation of vascular endothelial growth factor receptor-2 by interleukin-8 (IL-8/CXCL8) is required for IL-8/CXCL8-induced endothelial permeability. *Mol Biol Cell* 2007; **18**: 5014-5023

24 **Chelouche-Lev D**, Miller CP, Tellez C, Ruiz M, Bar-Eli M, Price JE. Different signalling pathways regulate VEGF and IL-8 expression in breast cancer: implications for therapy. *Eur J Cancer* 2004; **40**: 2509-2518

25 **Grzesiak JJ**, Smith KC, Burton DW, Deftos LJ, Bouvet M. Integrin-mediated laminin-1 adhesion upregulates CXCR4 and IL-8 expression in pancreatic cancer cells. *Surgery* 2007; **141**: 804-814

26 **Fong YC**, Maa MC, Tsai FJ, Chen WC, Lin JG, Jeng LB, Yang RS, Fu WM, Tang CH. Osteoblast-derived TGF-beta1 stimulates IL-8 release through AP-1 and NF-kappaB in human cancer cells. *J Bone Miner Res* 2008; **23**: 961-970

27 **Pooran N**, Indaram A, Singh P, Bank S. Cytokines (IL-6, IL-8, TNF): early and reliable predictors of severe acute pancreatitis. *J Clin Gastroenterol* 2003; **37**: 263-266

28 **Bellone G**, Smirne C, Mauri FA, Tonel E, Carbone A, Buffolino A, Dughera L, Robecchi A, Pirisi M, Emanuelli G. Cytokine expression profile in human pancreatic carcinoma cells and in surgical specimens: implications for survival. *Cancer Immunol Immunother* 2006; **55**: 684-698

29 **Nomura H**, Nishimori H, Yasoshima T, Hata F, Tanaka H, Nakajima F, Honma T, Araya J, Kamiguchi K, Isomura H, Sato N, Denno R, Hirata K. A new liver metastatic and peritoneal dissemination model established from the same human pancreatic cancer cell line: analysis using cDNA macroarray. *Clin Exp Metastasis* 2002; **19**: 391-399

30 **Lee LF**, Hellendall RP, Wang Y, Haskill JS, Mukaida N, Matsushima K, Ting JP. IL-8 reduced tumorigenicity of human ovarian cancer in vivo due to neutrophil infiltration. *J Immunol* 2000; **164**: 2769-2775

31 **Albini A**, Sporn MB. The tumour microenvironment as a target for chemoprevention. *Nat Rev Cancer* 2007; **7**: 139-147

32 **Noh KW,** Pungpapong S, Wallace MB, Woodward TA, Raimondo M. Do cytokine concentrations in pancreatic juice predict the presence of pancreatic diseases? *Clin Gastroenterol Hepatol* 2006; **4**: 782-789

S- Editor Gou SX **L- Editor** Ma JY **E- Editor** Zheng XM