Neoplasma

doi:10.4149/neo_2012_007

The Expression and Clinical Significance of pSTAT3, VEGF and VEGF-C in Pancreatic Adenocarcinoma

C. HUANG‡ [1,4,5], R. HUANG‡ [2], W. CHANG‡ [3], T. JIANG[1], K. HUANG[1], J. CAO[1], X. SUN* [1], Z. QIU* [1,4,5]

[1]Department of General Surgery, Affiliated First People's Hospital, Shanghai Jiao Tong University, Shanghai, P.R. China, 200080; [2]Digestive Endoscopy Center, Affiliated Huadong Hospital, Fudan University, Shanghai, P.R. China, 200040; [3]Department of Emergency, The First Affiliated Hospital of Soochow University, Suzhou, P.R. 215006; [4]Shanghai Key Laboratory of Pancreas Disease, Shanghai, P.R. China, 200080; [5]Pancreatic Cancer Center of Shanghai Jiao Tong University, Shanghai, P.R. China, 200080

**Correspondence: qiuwryb@online.sh.cn*
‡Contributed equally to this work.

Received July 9, 2011 / Accepted August 11, 2011

Signal transducers and activators of transcription 3 (STAT3) is a central cytoplasmic transcription factor and regulates a number of pathways important in tumorigenesis including cell cycle progression, apoptosis, tumor angiogenesis, invasion and metastasis.This study aims to investigate the expression of pSTAT3, VEGF and VEGF-C in pancreatic adenocarcinoma and their relations to the clinicopathological features, tumor angiogenesis and prognosis. In the present study, the expression of pSTAT3, VEGF and VEGF-C and microvascular density (MVD) were examined via immunohistochemistry. The clinicopathological information was collected and patients were regularly followed up. The relationship between the parameters and the clinicopathological features were analyzed, and the univariate and multivariate prognostic factors were also analyzed. The expression of pSTAT3 in tumor tissues was significantly higher in contrast to that in normal tissues, and pSTAT3 was related to VEGF and VEGF-C expression, MVD, tumor size, lymphogenous status and TNM staging ($P<0.05$). Survival analysis suggested that tumor size, TNM staging, pSTAT3 and VEGF expression were risk factors of prognosis, but no independent factors were found. We concluded that pSTAT3, which was a risk factor of prognosis, was abnormally expressed in pancreatic adenocarcinoma and related to tumor size, TNM staging and lymphatic metastasis. pSTAT3 may promote tumor angiogenesis via up-regulating VEGF on protein and even gene levels, and enhance the early lymphatic metastasis through VEGF-C. Better understanding of STAT3 signaling pathways in angiogenesis may contribute to the development of novel therapeutic strategies in angiogenesis and metastasis of pancreatic cancer.

Key words: Pancreatic adenocarcinoma, pSTAT3, VEGF, VEGF-C, Angiogenesis

Signal transducer and activator of transcription 3 (STAT3) is an important member of the STAT family. Initially, it was found as an acute reaction protein, induced by interleukin-6 (IL-6) [1]. The molecules of STAT family are composed of 7 domains, and they are amino terminal, coiled-coil, DNA-binding domain (DBD), linker (LK) domain, highly conservative SH2 domain, tyrosine activation site Y and transcriptional activation domain (TAD) of carboxyl terminal sequentially. Among these domains, the construction of TAD is the most variable, which is also the differentiated site among the members of STAT family. STAT3 is the key activator of transcription of the JAK-STAT3 signal transduction path. In the quiescent condition, STAT3 exists as an inactive dimeride in the cytoplasm. When the signal is present and activates the upstream molecule Janus kinase (JAK), STAT3 is recruited to JAK, followed by phosphorylation of the tyrosine residue Y and transforming to the active phospho-STAT3 (pSTAT3). pSTAT3 forms as a reverse double-stranded dimeride through its SH2 domain and enters into the nucleus, combining to the specific DNA succession. Thus, its TAD domain combines with coactivator CBP/p300 and becomes active, acting the role as transcriptional activation [2]. STAT3 expresses abnormally in various malignant tumor cells, such as multiple myeloma, Burkritt lymphoma, non-hodgkin lymphoma, melanoma, breast cancer, ovarial cancer, lung cancer, pancreatic adenocarcinoma, prostate cancer, etc. STAT3 can be activated by many oncogenes and protooncogenes, such as SRC, ABL, ROS, etc.

STAT3 has significant relationship with the tumor initiation, angiogenesis, invasion, and metastasis [3-4]. STAT3 can promote the expression of Bcl-xl, Mcl-1 of the Bcl-2 family, suppressing the cell apoptosis, and abnormally expressed STAT3 can up-regulate cyclin D1, promoting the generation of tumor cells. Meanwhile, it is indicated that STAT3 can suppress the maturation and differentiation of dendritic cells (DC), inducing the immune tolerance of T cells and promoting the immune invasion effect of tumor cells [5]. Besides, STAT3 can up-regulate matrix metalloproteinase-2, 9 (MMP-2, 9), which may degrade extracellular matrix and promote invasion and metastasis of tumor cells [6]. Neovessel angiogenesis is closely related with tumor progression and invasion. Nowadays, various kinds of factors can promote tumor neovessel angiogenesis, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), platelet-derived growth factor-BB (PDGF-BB), etc. Among these factors, VEGF is a significant factor of tumor neovessel angiogenesis. Besides, VEGF-C, also named as lymphatic generation factor, is a member of VEGF family, and it is the most important known lymphatic generation inducing factor up to date. VEGF-C can electively promote the hyperplasia and extension of lymphatics, enhance the permeability of lymphatics, pronouncedly promote the growth of lymphatics around tumors, make for the metastasis and dissemination of tumor cells through lymphatics and acts as an important factor which promote the invasion and metastasis of tumors [7]. In general, STAT3 acts an important role in the progression of various malignant tumors.

The expression of STAT3 in various tumor tissues has been reported in some pertinent literatures. But there are few researches focusing on the expression of STAT3 in pancreatic adenocarcinoma, the relationship between STAT3 and pathologic characteristics of pancreatic adenocarcinoma, and the relationship between STAT3 and prognosis of pancreatic adenocarcinoma. Pancreatic adenocarcinoma has a high malignancy, low resection rate and poor prognosis. And recently, its incidence rate has risen up yearly. In our study, we used immunohistochemical methods to measure the protein expression of pSTAT3, VEGF and VEGF-C in pancreatic adenocarcinoma tissues and to find the relationship of the expression of these proteins with microvascular density (MVD), pathologic characteristics and prognosis of the disease. This study aims at investigating the role and significance of STAT3 in the generation and progression of pancreatic adenocarcinoma, as well as the possible mechanism of STAT3 in tumor generation. We hope to offer a new target point and an idea of the molecule-targeted therapy in pancreatic adenocarcinoma.

Materials and methods

Specimen collection and case data. From 1993 to 2009, 71 pancreatic adenocarcinoma specimens from Department of General Surgery, Shanghai First Peoples' Hospital Affiliated to Shanghai Jiao Tong University were collected. Inclusion criteria: (1) All the patients suffered primary pancreatic adenocarcinoma. (2) None of the patients accepted preoperative radiotherapy or chemotherapy. (3) Postoperative pathologic diagnosis was confirmed as pancreatic ductal adenocarcinoma. (4) All the patients were residents of Mainland of China. Exclusion criteria: (1) The patients suffered relapsed tumor or other digestive systemic tumors. (2) Postoperative pathology proved as adenoma, benign lesions as inflammatory hyperplasia or nonepithelial sourced malignant tumor. (3) Those patients accepted preoperative radiotherapy or chemotherapy. Among the patients, 50 were male and 21 were female, aged from 40 to 80 years old, and the median age was 67 years old. According to the TNM staging (AJCC, 2002), 2 Ia cases, 16 Ib cases, 25 IIa cases, 23 IIb cases, 0 III case, 5 IV cases were enrolled into this study. 28 cases had regional lymphatic metastasis. Referring to the histological grade, 10 cases were G1, 45 cases were G2 and 14 cases were G3. Classified with the tumor location, 62 cases were carcinomas of head of pancreas, 9 cases were carcinomas of body and tail of pancreas. All specimens were fixed by formalin and slices were embedded by paraffin. Histologic characteristics were observed with HE staining, and expression level of target proteins were observed by immunohistochemisty. 10 normal pancreatic specimens were gained from the pancreatic tissues accessaried by dissection of donor livers.

Immunohistochemisty. Paraffin wax samples of pancreatic tumors and normal pancreatic tissue were cut into 4-μm-thick slices. These slices were dewaxed and the endogenous peroxidase activity was quenched after incubation in methanol containing 3% hydrogen peroxide for 10 minutes. The histologic sections were then incubated with rabbit anti-human pSTAT3 polyclonal antibody (Cell Signal, USA), rabbit anti-human CD34 antibody (Cell Signal, USA), mouse anti-human monoclonal VEGF antibody (Santa Cruz, USA), caprine anti-humanVEGF-C antibody (R&D Systems Company) or rabbit polyclonal IgG controls (Vector Laboratories, USA) in blocking buffer overnight at 4 °C. The sections were then rinsed in wash buffer (PBS containing 0.5% bovine serum albumin, 0.1% Tween-20) and incubated for 30 minutes with biotinylated goat anti-rabbit IgG (ABC staining kit, Santa Cruz, USA) diluted according to the manufacturer's protocol. Next, a solution of avidin-conjugated horseradish peroxidase (ABC staining kit) was applied for 30 minutes, according to the manufacturer's instruction. Peroxidase activity was developed in 0.5% (vol/vol) 3,3'-diaminobenzidine hydrochloride (DAB, Sigma, USA) in PBS containing 0.03% (vol/vol) hydrogen peroxide for 2 minutes. Sections were counterstained with Harris' hematoxylin and mounted in gelatin (Sigma, USA).

Assessment of outcomes. Randomized five visual fields were observed under high power lens (×400). Positive pSTAT3 was considered when buffied granules appeared in the nucleus. Positive VEGF and VEGF-C were considered when buffied granules appeared in the cytoplasm. Stained cells less than 5% were defined as negative (-), between 5% to 25% were defined

Table 1. The expression of pSTAT3, VEGF and VEGF-C in pancreatic adenocarcinoma and normal pancreatic tissues

Group (*n*)	pSTAT3 (+) (%)	VEGF (+) (%)	VEGF-C (+) (%)
Normal pancreas (10)	0 (0)	2 (20.00)	0 (0)
Pancreatic adenocarcinoma (71)	50 (70.42)	53 (74.64)	56 (78.87)

as weakly positive (+), between 26% to 50% were defined as positive (++), more than 50% were defined as strong positive (+++) [8]. Negative and weakly positive expression were defined as low expression, while postive and strong positive expression were defined as high expression. PBS acted as first antibodies were used as negative control, while known positive breast carcinoma specimens were used as positive control. Marking tumorous vascular endothelial cells with CD34 was used to measure microvessel density (MVD). First, the slice was overall observed to determine intensive areas of tumorous capillary under the lens (×100), named as "hotspot areas". Then, randomized three visual fields were observed under the lens (×200), and marked microvessel counts were counted respectively and the average was taken out. Under the lens, buffied endothelial cells or endothelial cell plexus were considered as a capillary. As long as the structure was unconnected, the branched structure was also considered as a vessel count.

Follow up. All the cases were followed up until August of 2009. The methods of follow up mainly included inquiring at Shanghai Disease Prevention Control Center, telephone calling and home visitation. Mean follow up time was 15.9 months (1-101 months) and 6 cases lose the follow up (8.5%).

Statistics. SPSS 17.0 statistical software was used to analyzing the data. χ^2 test or Fisher exact test was adopted as analysis of accounts. Spearman test was adopted as correlation analysis. *t* test was adopted as mean analysis, while rank sum test was adopted when the data was not consistent with normal distribution. Kaplan-Meier survival curve was adopted as survival analysis and median survival was accounted. Logrank test was adopted as univariate survival analysis. Cox regression test was adopted as multivariate survival analysis. $P<0.05$ was considered as statistical significance.

Results

pSTAT3, VEGF, VEGF-C were highly expressed in pancreatic adenocarcinoma tissues. Among 71 pancreatic adenocarcinoma tissues, positive expression of pSTAT3, VEGF and VEGF-C protein were 50 (70.42%), 53 (74.64%) and 56 (78.87%) cases, respectively. While in control group of normal pancreatic tissues, VEGF presented weakly positive in only two cases and no positive expression of pSTAT3 and VEGF-C was found (Fig. 1). Positive expression levels of pSTAT3, VEGF and VEGF-C in pancreatic adenocarcinoma were obviously higher than that in normal control group ($P<0.05$, Tab. 1).

MVD in pancreatic adenocarcinoma tissues was obviously higher than that in normal pancreatic tissues. Count of CD34 stained microvessel counts under the lens (×200) was considered as MVD index. MVD in pancreatic adenocarcinoma tissues was 24.8±9.2, while MVD in normal pancreatic tissues was 14.3±5.6. The statistical results showed that MVD in pancreatic adenocarcinoma tissues was obviously higher than that in normal pancreatic tissues ($P<0.05$, Fig 2A). It was thus clear that the blood supply of pancreatic adenocarcinoma tissues was obviously more than normal pancreatic tissues.

The expression of pSTAT3 and MVD, as well as VEGF and MVD had positive correlation in pancreatic adenocarcinoma tissues. In 71 cases in which pSTAT3 protein expression arranged from (-) to (+++), MVD was 16.1±4.6, 24.5±3.6, 25.7±5.8 and 37.6±8.0, respectively. Except that MVD expression between the (+) pSTAT3 expressed group and the (++) pSTAT3 expressed group was not statistically significant, MVD was statistically significant among other groups ($P<0.05$). In the four groups which VEGF protein expression arranged from (-) to (+++), MVD was 17.6±6.7, 24.6±8.3, 25.9±6.1 and 40.0±8.0, respectively. MVD expression between the (+) VEGF expressed group and the (++) VEGF expressed group was not statistically significant, while MVD was statistically significant among other groups ($P<0.05$). It could be seen that the positive correlation of protein expression existed between pSTAT3 and MVD, as well as VEGF and MVD in pancreatic adenocarcinoma tissues (Fig 2B and Fig 2C)

The expression of pSTAT3 and VEGF, as well as pSTAT3 and VEGF-C had positive correlation in pancreatic adenocarcinoma tissues. Among 39 high expressed pSTAT3 pancreatic adenocarcinoma tissue slices, VEGF protein was highly expressed in 29 cases and VEGF-C protein was highly expressed in 22 cases. While in 32 low expressed pSTAT3 cases, only 6 cases showed highly expressed VEGF protein and 9 cases showed highly expressed VEGF-C protein, while the rest showed low expression of whatever VEGF protein or VEGF-C protein. It could be seen that the positive correlation of protein expression existed between pSTAT3 and VEGF, as well as pSTAT3 and VEGF-C ($P<0.05$, Tab. 2).

Table 2. The relationship between the pSTAT3 and VEGF expression in pancreatic adenocarcinoma

Group	VEGF (low expression)	VEGF (high expression)	VEGF-C (low expression)	VEGF-C (high expression)
pSTAT3 (low expression)	26	6	23	9
PSTAT3 (high expression)	10	29	17	22

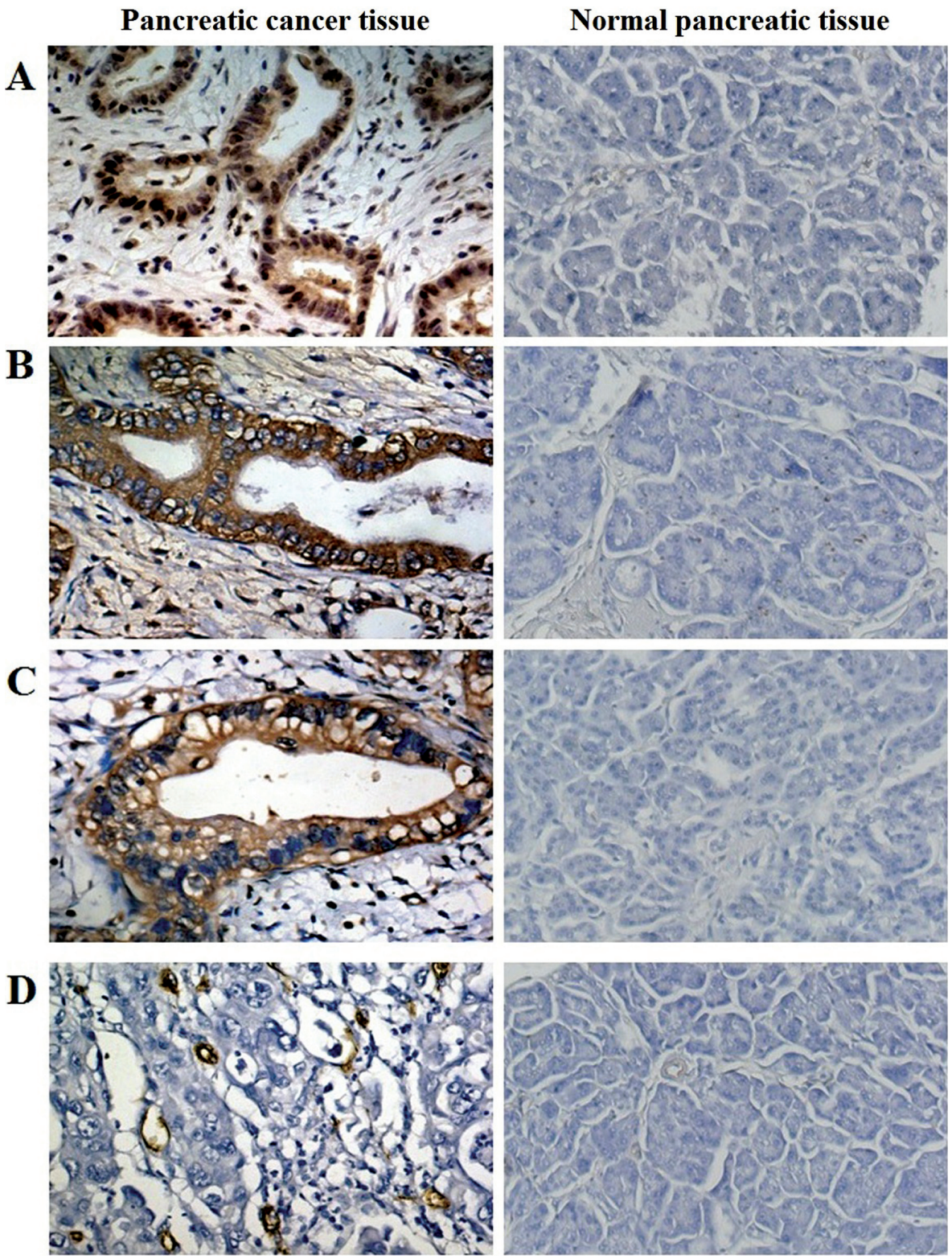

Figure 1. Analysis of pSTAT3, VEGF, VEGF-C, CD34 protein expression in pancreatic cancer and normal pancreatic tissue by immunohistochemistry.
(A) pSTAT3 staining , in nucleus; (B) VEGF staining , in cytoplasma;
(C) VEGF-C staining , in cytoplasma; (D) CD34 staining , in vascular endothelium.

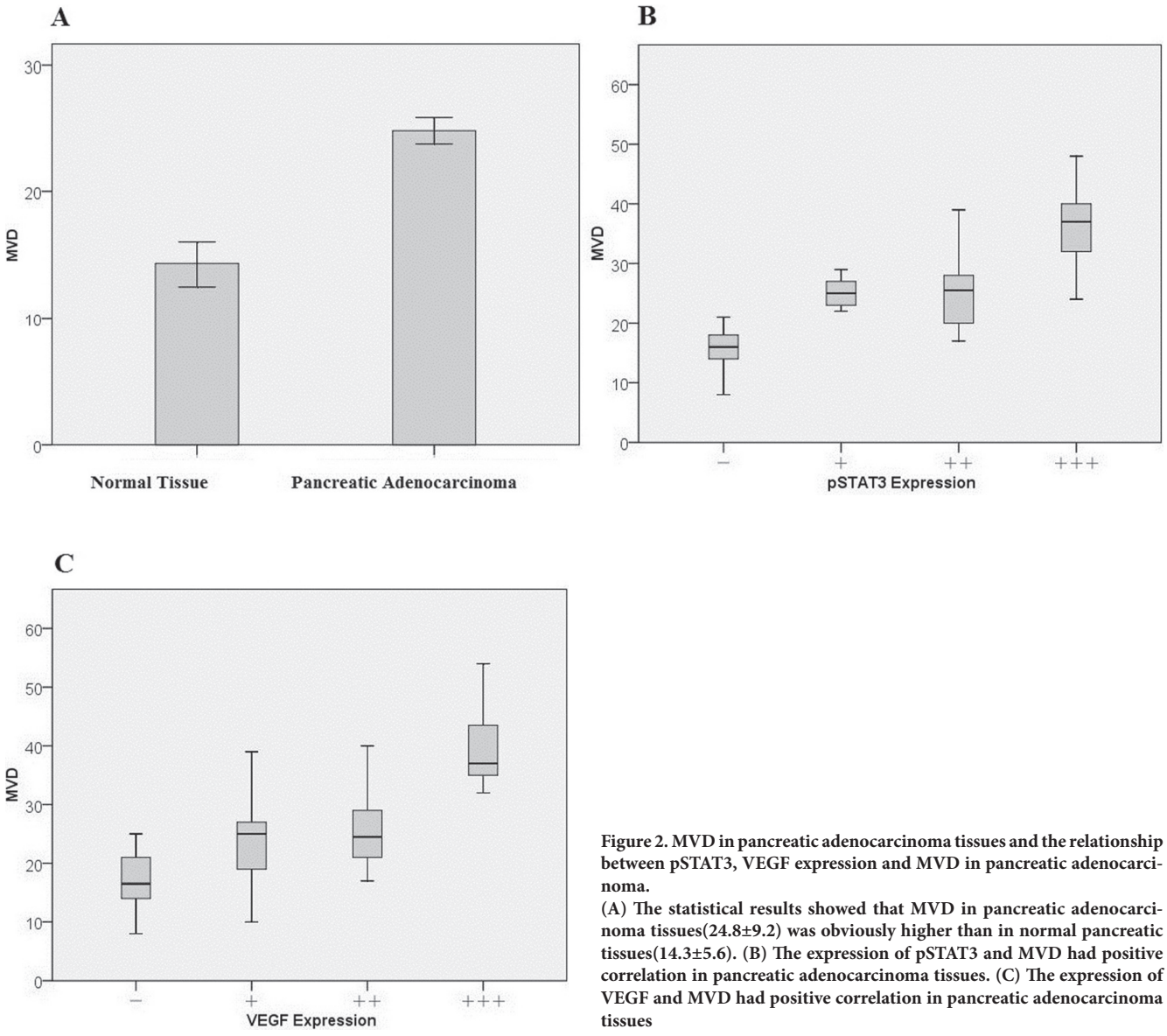

Figure 2. MVD in pancreatic adenocarcinoma tissues and the relationship between pSTAT3, VEGF expression and MVD in pancreatic adenocarcinoma.
(A) The statistical results showed that MVD in pancreatic adenocarcinoma tissues(24.8±9.2) was obviously higher than in normal pancreatic tissues(14.3±5.6). (B) The expression of pSTAT3 and MVD had positive correlation in pancreatic adenocarcinoma tissues. (C) The expression of VEGF and MVD had positive correlation in pancreatic adenocarcinoma tissues

The relationships between the expression of pSTAT3, VEGF, VEGF-C and clinicopathological features of pancreatic adenocarcinoma. It was concluded that the expression levels of pSTAT3, VEGF and VEGF-C were not related with the factors such as patients' age, gender, tumor locations and pathological grade, while the expression levels of pSTAT3, VEGF and VEGF-C were significantly related with tumor sizes, TNM staging and lymphatic metastasis ($P<0.05$, Tab. 3).

Survival analysis

Univariate survival analysis. Follow-up showed that the median life span of the 71 patients was 13.3 months (95% CI 10.4-16.2), and the longest follow up time was 101 months. Univariate survival analysis showed that the clinicopathological related factors, which affected the survival of patients, included tumor sizes and TNM staging ($P<0.05$). The median life spans of the patients whose tumor limited in the body of pancreas and exceeded the body of pancreas were 15.0 months (95% CI 11.9-18.1) and 11.1 months (95% CI 5.9-16.3), respectively. The median life spans of the patients with TNM Stage I, Stage II and Stage III +Stage IV were 15.0 months (95% CI 1.0-35.1), 11.7 months (95% CI 8.2-15.2) and 6.0 months (95% CI 5.5-6.5), respectively. The results of immunohistochemistry showed that the expression levels of pSTAT3 and VEGF in pancreatic adenocarcinoma tissues were closely related with the prognosis of the patients ($P<0.05$). Postoperative median life spans of patients with highly and lowly expressed pSTAT3 were 8.9 months (95%

Table 3. The relationship between pSTAT3, VEGF and VEGF-C expression in pancreatic adenocarcinoma and clinicopathological features

Group	*n*	pSTAT3		VEGF		VEGF-C	
		High expression	*P*	High expression	*P*	High expression	*P*
Age							
≥65yr	39	20	0.495	18	0.559	19	0.343
<65yr	32	19		17		12	
Gender							
Male	50	29	0.422	27	0.221	24	0.255
Female	21	10		8		7	
Tumor sizes							
Within glands	21	2	<0.05*	3	<0.05*	5	<0.05*
Beyond glands	50	37		32		26	
Tumor location							
Head	62	35	0.499	30	0.688	26	0.441
Body & tail	9	4		5		5	
Pathologic classification							
Poorly differentiated	10	8	0.263	3	0.343	4	0.649
Moderately differentiated	45	24		25		22	
Well differentiated	14	7		7		5	
Clinical staging							
Ia+Ib	18	1	<0.05*	1	<0.05*	3	<0.05*
IIa+IIb	48	33		29		25	
III+IV	5	5		5		2	
Lymphatic metastasis							
(-)	43	15	<0.05*	14	<0.05*	10	<0.05*
(+)	28	24		21		25	

CI 6.2-11.6) and 14.0 months (95% CI 11.9-16.1), respectively. While postoperative median life spans of patients with highly and lowly expressed VEGF were 8.9 months (95% CI 7.1-10.7) and 14.0 months (95% CI 3.4-24.6), respectively.(Fig. 3)

Multivariate survival analysis. Cox regression analysis was carried out on tumor sizes, TNM staging, the expression levels of pSTAT3 and VEGF in pancreatic adenocarcinoma tissues, which aimed to analyze the influence of clinicopathological features of pancreatic adenocarcinoma, and the expression of pSTAT3 and VEGF in pancreatic adenocarcinoma tissues on the prognosis of the disease. It was regretful that this study didn't find an independent factor which affected the prognosis of the patients with pancreatic adenocarcinoma. Neither the expression of pSTAT3 nor VEGF in pancreatic adenocarcinoma was an independent factor of prognosis, and none of the factors affected the prognosis of the patients with statistical significance (Tab. 4).

Discussion

STAT3 is a key activating transcription factor in the body. STAT3 can be activated by various cytokines and oncogenes

Tab. 4 The univariate and multivariate analysis of prognosis of the pancreatic adenocarcinoma patients

Variables	Univariate analyasis	Multivariate analysis		
	P-value	*P*-value	RR	95% CI
Gender (male vs. female)	0.662			
Age (<65yr vs. ≥65yr)	0.880			
Tumor size (within vs. beyond)	0.026*	0.067	1.201	0.987-1.461
Tumor location (head vs. body & tail)	0.702			
Histological grade (G1/G2/G3)	0.803			
TNM staging (Ia/Ib/IIa/IIb/III/IV)	0.041*	0.603	1.092	0.783-1.524
Lymph node status (negative vs. positive)	0.135			
pSTAT3 (high vs. low)	0.021*	0.492	1.153	0.768-1.733
VEGF (high vs. low)	0.012*	0.180	1.346	0.871-2.079

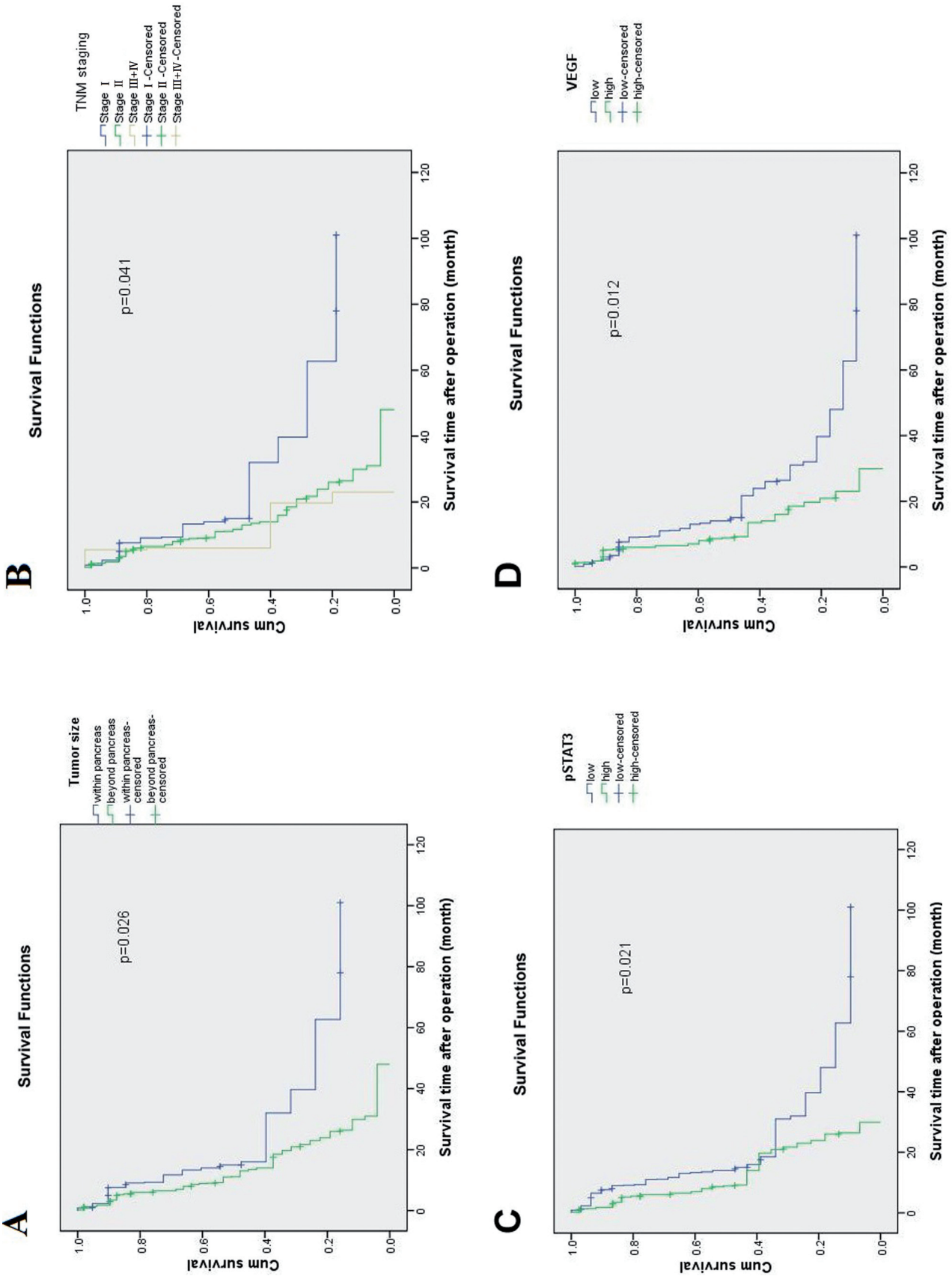

Figure 3. The survival analysis of the prognostic risk factors.
(A) tumor size; (B) TNM staging; (C) pSTAT3 expression; (D) VEGF expression were closely related with the prognosis of the patients.

and plays a role in transcriptional activation, inhibiting tumor cells apoptosis, enhancing tumor cells proliferation, angiogenesis, invasion and metastasis [5].

This study used immunohistochemisty to detect pSTAT3, VEGF, VEGF-C and MVD in pancreatic adenocarcinoma specimens, investigating the relationship between pSTAT3 and VEGF, which is a key factor of tumor angiogenesis, as well as pSTAT3 and VEGF-C, which is an important factor of lymphatic generation. Further, we explored the relationship between pSTAT3 and neovessel angiogenesis. Survival of the patients was followed up. We studied the relationship between clinical tumorous pathological characteristics and pSTAT3, VEGF as well as VEGF-C, and the influences of clinical tumorous pathological characteristics and varied expression of pSTAT3 and VEGF in tumor tissues on the prognosis of patients.

The statistical results showed that pSTAT3 was closely related with the expression of VEGF ($P<0.05$). Meanwhile, the expression of pSTAT3 and MVD, as well as VEGF and MVD had positive correlation in pancreatic adenocarcinoma ($P<0.05$). VEGF is an important factor of regulating tumorous neovessel angiogenesis. Thus, pSTAT3 may promote tumorous neovessel angiogenesis through the regulation of VEGF. Funamoto et al [9] found that STAT3 could promote the expression of VEGF-mRNA in normal myocardial cells of mice, which promoted the angiogenesis. Wei et al[10] thought that acting as an activating transcription factor, STAT3 could be directly combined with the definite sequence of promoter in VEGF-DNA and promote its transcription. The combining site might be the -TTCCCAAA- sequence of -848 position of the upper stream. It showed that STAT3 could regulate the expression of VEGF at genic level and promoted tumorous neovessel angiogenesis. Hypoxia inducible factor-1α (HIF-1α) is a newly found activating transcription factor in recent years, and HIF-1α plays an important role in generation and progression of tumors. The downstream regulatory factors of HIF-1α include VEGF, carbornic anhydrase 9 (CA-9) and glucose transporter 1 (GLUT-1), etc. It has been reported that STAT3 may indirectly regulate the expression of VEGF through HIF-1α. However, the relevant mechanism which is the effect on HIF-1α from STAT3 is still unclear nowadays. Jung et al [11] thought that STAT3 could be competitively bound to HIF-1α against pVHL, inhibiting the degradation of ubiquitin-proteasomes path and enhancing the protein level of HIF-1α. STAT3 doesn't directly combine with the oxygen-dependant degradation domain (ODDD) which controls the degradation of HIF-1α, instead, it combines with its downstream segments. The authors presumed that the large STAT3 molecule could cover and protect ODDD from being degraded (Fig. 5). Niu et al [12] found that STAT3 could be combined with the definite sequence of promoter in HIF-1α-DNA and promoted its transcription and the sequence might be at -355 to -363 positon. However, Gray et al [13] found that the expression of VEGF and HIF-1α promoted by STAT3 were two correspondingly independent processes. Regardless of inhibiting STAT3 or HIF-1α, it could not decrease the activity of the other factor for transferring into the nucleus, and both factors had synergistic effects. Immunoprecipitation indicated that HIF-1α, STAT3, CBP/p300 and sym-transcription factor Ref-1/Ape formed as a transcription complex, which combined with the promoter of VEGF, and then the maximum activation was achieved [14].

In a word, researches indicated that STAT3 could promote the expression of VEGF-mRNA and proteins through various paths, thus it promoted tumorous neovessel angiogenesis. In this study, the expression of pSTAT3 was positively correlated with VEGF and MVD, which suggested that STAT3 played a promotive role in neovessel angiogenesis of pancreatic adenocarcinoma. It may inhibit tumorous neovessel angiogenesis and improve the patients' survival rate by cutting off the signal transduction path of STAT3.

Cancer cells also need to acquire phenotypes of lymph-angiogenesis beside angiogenesis to growth and metastasis in vivo. VEGF-C is known to be a potent lymph-angiogenesis mitogen that plays an important role in tumor lymph-angiogenesis and metastasis [7]. Kabashima et al [15] found that the over-expression of VEGF-C in prophase gastric carcinoma was related with lymphatic metastasis, which indicated VEGF-C played an important role in lymphatic metastasis of prophase carcinomas. VEGF-C can be regulated by various factors such as NF-κB signal transduction path, cyclooxygenase-2(COX-2), etc [16]. This study adopted immunohistochemisty to research the relationship between the expression of STAT3 and VEGF-C, and the results showed that pSTAT3 could promote the expression of VEGF-C. STAT3 may promote the generation of tumorous lymph nodes, thus it promotes the early metastasis of tumors. The discovery of our study can provide a new mechanism for STAT3 promoted invasion of tumors. However, the mechanism needs more additional cytological and in vivo experiments to be verified.

The statistical results indicated that tumor sizes, tumor staging, the expression levels of pSTAT3 and VEGF were all the risk factors which affected the prognosis of pancreatic adenocarcinoma ($P<0.05$). It is regretful that the independent risk factor which affects the prognosis of pancreatic adenocarcinoma has still not been found today. While in other types of tumor cells, the expression of pSTAT3 is related to the prognosis as well. Ijichi [17] assayed the expression of pSTAT3 in 303 gastric carcinoma specimens, positive rate of pSTAT3 was 26.1% (79 cases). In univariate analysis, both disease free survival (DFS) and overall survival (OS) of positively pSTAT3 expressed tumor patients were lower than that of pSTAT3 negatively expressed tumor patients. The research indicated that pSTAT3 was one of the risk factors of prognosis in gastric carcinoma as well. Although both belonged to digestive systemic malignancies, the positive expression of pSTAT3 in gastric carcinoma specimens is lower than that in pancreatic adenocarcinoma specimens, which may be related to organ specificity, sample capacity and malignant levels of tumors. Sheen-Chen et al [18] used immunohistochemisty to

detect the expression of pSTAT3 in primary invasive breast carcinoma specimens. The results suggested that pSTAT3 was not related to patients' ages, ER levels, tissue classification, tumor classification, lymphatic metastasis or TNM staging. In multivariate analysis, the expression of pSTAT3 was related to the five-year overall survival rate of patients. In pancreatic adenocarcinoma, pSTAT3 is a correlation factor of the prognosis and it is related to varied clinicopathological features such as tumor sizes, TNM staging and lymphatic metastasis, etc. While in breast carcinoma, similar relationship has not been found. Takemoto [19] studied the expression of pSTAT3 in cervical cancer and found pSTAT3 was closely related to lymphatic metastasis, lymphatic invasion and tumor sizes. However, in multivariate analysis, the independent prognosis factor related to overall survival rate was not found. Although pSTAT3 acted as a risk factor of prognosis, which was identical with our study, was not an independent prognosis factor. It promoted us to search other molecules acting as the markers of prognosis. From this, pSTAT3 is over-expressed in other types of tumor cells and the expression of pSTAT3 is closely related to the prognosis. The high expression of pSTAT3 often indicates the poor prognosis. It is suggested that pSTAT3 signal transduction path plays an important role in the generation and progression of tumors. VEGF acting as a downstream target of pSTAT3, is also one of the risk factors affecting prognosis, which indicates that cutting off the generation of tumor neovessels, inhibiting the tumor proliferation and promoting the apoptosis of tumor cells from the upstream may be effective therapies.

Conclusion

From this study, we found that pSTAT3, VEGF and VEGF-C present abnormal expression in pancreatic adenocarcinoma and MVD expression in pancreatic adenocarcinoma tissues is obviously higher than that in normal pancreatic tissues. The expression of pSTAT3 is related to VEGF, VEGF-C, tumor sizes, TNM staging and lymphatic metastasis. Tumor sizes, TNM staging and the expression of pSTAT3 and VEGF are the risk factors of prognosis. However, Up to date, the independent risk factor of prognosis has not been found. pSTAT3 can regulate the expression of VEGF through various paths. pSTAT3 presents over expression in polytype tumors and acts as an important risk factor of prognosis.

Acknowledgements: This work was supported by a grant (No. 09QA1404600) awarded by fund for scientific research of Science and Technology Commission of Shanghai Municipality and grants (No. 81101844 and No. 30972952) from the National Natural Science Foundation of China.

References

[1] AKIRA S, NISHIO Y, INOUE M, WANG XJ, WEI S et al. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell. 1994; 77: 63-71. http://dx.doi.org/10.1016/0092-8674(94)90235-6

[2] SCHINDLER C, LEVY DE, DECKER T JAK-STAT Signaling: From Interferons to Cytokines. J. Biol. Chem. 2007; 282: 20059-63. http://dx.doi.org/10.1074/jbc.R700016200

[3] QIU ZJ, HUANG C, SUN J, QIU W, ZHANG JF, LI HM, JIANG T, HUANG KJ, CAO J RNA interference-mediated signal transducers and activators of transcription 3 gene silencing inhibits invasion and metastasis of human pancreatic cancer cells. Cancer Sci 2007; 98:1099-1106. http://dx.doi.org/10.1111/j.1349-7006.2007.00485.x

[4] HUANG C, JIANG T, ZHU L, LIU J, CAO J et al. STAT3-targeting RNA interference inhibits pancreatic cancer angiogenesis in vitro and in vivo. Int J Oncol 2011;38:1637-1644.

[5] YU H, JOVE R The Stats of Cancer-New Molecular Targets Come of Age. Nat. Rev. Cancer. 2004; 4: 97-105. http://dx.doi.org/10.1038/nrc1275

[6] HUANG C, CAO J, HUANG KJ, ZHANG F, JIANG T et al. Inhibition of STAT3 activity with AG490 decreases the invasion of human pancreatic cancer cell in vitro. Cancer Science. 2006; 97:1417-1423. http://dx.doi.org/10.1111/j.1349-7006.2006.00340.x

[7] KARKKAINEN MJ, PETROVA TV Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. Oncogene. 2000; 19: 5598-605. http://dx.doi.org/10.1038/sj.onc.1203855

[8] CAMPBELL CL, JIANG Z, SAVARESE DMF, SAVARESE TM Increased Expression of the Interleukin-11 Receptor and Evidence of STAT3 Activation in Prostate Carcinoma. Am. J. Pathol. 2001; 158: 25-32. http://dx.doi.org/10.1016/S0002-9440(10)63940-5

[9] FUNAMOTO M, FUJIO Y, KUNISADA K, NEGORO S, TONE E et al. Signal Transducer and Activator of Transcription 3 Is Required for Glycoprotein 130-mediated Induction of Vascular Endothelial Growth Factor in Cardiac Myocytes. J. Biol. Chem. 2000; 275: 10561-6. http://dx.doi.org/10.1074/jbc.275.14.10561

[10] WEI D, LE X, ZHENG L, WANG L, FREY JA et al. Stat3 Activation Regulates The Expression of Vascular Endothelial Growth Factor And Human Pancreatic Cancer Angiogenesis And Metastasis. Oncogene. 2003; 22: 319-29. http://dx.doi.org/10.1038/sj.onc.1206122

[11] JUNG JE, LEE HG, CHO IH, CHUNG DH, YOON SH et al. STAT3 is a potential modulator of HIF-1-mediated VEGF expression in human renal carcinoma cells. FASEB J. 2005; 19: 1296-8.

[12] NIU G, BRIGGS J, DENG J, MA Y, LEE H et al. Signal Transducer and Activator of Transcription 3 Is Required for Hypoxia-Inducible Factor-1A RNA Expression in Both Tumor Cells and Tumor-Associated Myeloid Cells. Mol. Cancer Res. 2008; 6: 1099-105. http://dx.doi.org/10.1158/1541-7786.MCR-07-2177

[13] NIU G, WRIGHT KL, HUANG M, SONG L, HAURA E et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene. 2002; 21: 2000-8. http://dx.doi.org/10.1038/sj.onc.1205260

[14] GRAY MJ, ZHANG J, ELLIS LM, SEMENZA GL, EVANS DB et al. HIF-1a, STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas. Oncogene. 2005; 24: 3110-20. http://dx.doi.org/10.1038/sj.onc.1208513

[15] KABASHIMA A, MAEHARA Y, KAKEJI Y Overexpression of vascular endothelial growth factor C is related to lymphogenous matastasis in early gastric carcinima. Oncology. 2001; 60: 146-50. http://dx.doi.org/10.1159/000055312

[16] TSAI PW, SHIAH SG, LIN MT Up-regulation of vascular growth factor C in breast cancer cells by heregulin-beta 1. A critical role of p38/nuclear-kappa B signaling pathway. J Biol Chem. 2003; 278: 5750-9. http://dx.doi.org/10.1074/jbc.M204863200

[17] LEE J, KANG WK, PARK JO, PARK SH, PARK YS et al. Expression of Activated Signal Transducer and Activator of Transcription 3 Predicts Poor Clinical Outcome in Gastric Adenocarcinoma. APMIS. 2009; 117: 598-606. http://dx.doi.org/10.1111/j.1600-0463.2009.02512.x

[18] SHEEN-CHEN SM, HUANG CC, TANG PR, CHOU FF, ENG HL Prognostic Value of Signal Transducers and Activators of Transcription 3 in Breast Cancer. Cancer Epidemiology, Biomarks & Prevention. 2008; 17: 2286-90. http://dx.doi.org/10.1158/1055-9965.EPI-08-0089

[19] TAKEMOTO S, USHIJIMA K, KAWANO K, YAMAGUCHI T, TERADA A et al. Expression of activated signal transducer and activator of transcription-3 predicts poor prognosis in cervical squamous-cell carcinoma. British Journal of Cancer. 2009; 101: 967-72. http://dx.doi.org/10.1038/sj.bjc.6605212