RESEARCH ARTICLE

RGS16 and FosB underexpressed in pancreatic cancer with lymph node metastasis promote tumor progression

**Ji Hyang Kim · Jin Young Lee · Kyu Taek Lee ·
Jong Kyoon Lee · Kwang Hyuck Lee · Kee-Taek Jang ·
Jin Seok Heo · Seong Ho Choi · Jong Chul Rhee**

Abstract Lymph node (LN) metastasis is one of the most important adverse prognostic factors for pancreatic cancer. The aim of this study was to identify novel lymphatic metastasis-associated markers for pancreatic cancer. DNA microarray analysis was used to determine and compare the expression profiles of 17 pancreatic cancer tissues with LN metastasis and 17 pancreatic cancer tissues without LN metastasis. The microarray results were validated by real-time reverse transcription-polymerase chain reaction and immuno-histochemistry. Only 58 genes were differentially expressed between the two groups with a difference in signal intensity ratio greater than a 1.5-fold change. Of these genes, 30 were significantly down-regulated in the LN metastasis group. Among five selected down-regulated genes for validation using real-time PCR, the expression of DST, FosB, RGS16, and CXCL12 was significantly lower in the LN metastasis group. Immunohistochemical analysis confirmed RGS16 and FosB underexpression in pancreatic cancer tissues with LN metastasis. RGS16 and FosB underexpression was associated with poor patient survival. Our findings show that RGS16 and FosB are underexpressed in pancreatic cancer with lymph node metastasis and associated with reduced survival, suggesting that RGS16 and FosB might be prognostic markers for pancreatic cancer.

Keywords Pancreatic cancer · Lymph node metastasis · RGS16 · FosB · DNA microarray

J. H. Kim
Health Promotion Center, Samsung Medical Center,
Sungkyunkwan University School of Medicine,
Seoul, South Korea

J. Y. Lee
Samsung Biomedical Research Institute, Samsung Medical Center,
Sungkyunkwan University School of Medicine,
Seoul, South Korea

K. T. Lee · J. K. Lee · K. H. Lee · J. C. Rhee
Department of Medicine, Samsung Medical Center,
Sungkyunkwan University School of Medicine,
Seoul, South Korea

K.-T. Jang
Department of Pathology, Samsung Medical Center,
Sungkyunkwan University School of Medicine,
Seoul, South Korea

J. S. Heo · S. H. Choi
Department of Surgery, Samsung Medical Center,
Sungkyunkwan University School of Medicine,
Seoul, South Korea

K. T. Lee (✉)
Department of Medicine, Samsung Medical Center,
Sungkyunkwan University School of Medicine,
50 Irwon-dong, Gangnam-gu,
Seoul, South Korea 135-710
e-mail: happymap@skku.edu

Introduction

Pancreatic cancer is one of the most clinically aggressive malignancies, with a 5-year survival rate of <20% after surgical resection of the primary tumor [1, 2]. Without active treatment, metastatic pancreatic cancer has a median survival of 3–5 months and 6–10 months for locally advanced disease, which increases to around 11–15 months with resectional surgery [3]. The late presentation and aggressive tumor biology of this disease mean that only a minority (10–15%) of patients can undergo potentially curative surgery [4]. Poor prognosis of pancreatic cancer is related to early metastasis to regional lymph nodes (LN) or

liver, as LN metastasis is an important prognostic factor for patients with pancreatic cancer. LN metastasis has been shown to be a major prognostic factor in resectable pancreatic cancer [5, 6]; however, the mechanisms involved in LN metastasis of pancreatic cancer are not fully understood. Therefore, identification of molecular markers that might predict LN metastasis of pancreatic cancer is important in selecting patients who would benefit from surgical treatment or molecular targeting therapy.

Gene expression profiles provide a lot of important information about the molecular characteristics of cancers and can be used to distinguish related cancer subtypes. Recently, several studies have used gene expression profiling technologies to identify differentially expressed genes in pancreatic cancer compared with normal pancreas [5–7]. In the present study, we focused on and identified the genes associated with LN metastasis, which is the most important prognostic factor in patients who undergo surgical resection for pancreatic cancer. Gene identification was accomplished by comparison of gene expression profiles between pancreatic cancer with and without LN metastasis.

We applied large-scale analysis of gene expression profiling using cDNA microarray to evaluate the changes in survival-related gene expression profiles of pancreatic cancers with LN metastasis versus those without LN metastasis. These analyses were validated with real-time PCR and immunohistochemical staining in human pancreatic cancer tissue samples. Among the down-regulated genes identified, we focused on RGS16 and FosB expression as possible prognostic markers of pancreatic cancer.

Materials and methods

Clinical specimens

Thirty four fresh pancreatic cancer tissue specimens were selected from a tissue bank. We divided the samples into two groups according to the presence (group I) or absence (group II) of LN metastasis. The clinicopathologic characteristics of tumor stage and the survival data of each group are summarized in Table 1.

The cancer stage of each case was classified according to the tumor node metastasis (TNM) classification of the Union International Contre le Cancer. TNM was defined as the size or direct extent of the primary tumor (T, 1–4) that spreads to regional lymph nodes (N, 0–3) and develops distant metastases (M, 0/1). Clinical follow-up was obtained from the patient's medical record. Post-operative survival was measured from the day of surgery to the death or censored last follow-up visit. The patient's survival in group I with LN metastasis was 9 months, whereas that in group II without LN metastasis was 29 months. Most of the

Table 1 Clinicopathologic characteristics [a]

Characteristics	Group I (LN +)	Group II (LN-)
Age (years)	56.6±9.5	61±10.1
Gender		
Male	9	11
Female	8	6
Pathology		
T classification	T3	T3
N classification	N1	N0
Stage	IIB	IIA
Survival		
Alive	0	6
Dead	17	11
Median survival (months)	9	29

[a] These patients were selected in a blinded fashion

following clinicopathologic characteristics of the two groups, except for LN metastasis, were similar: surgical method, degree of differentiation of the cancer, tumor size, and the absence of distant metastasis.

Only tissues from patients who agreed with the aim and content of this study and provided written informed consent were included. Tissue samples were frozen in liquid nitrogen immediately after surgical removal and were maintained at a temperature of −80°C until required for RNA extraction. From each frozen tissue sample, serial sections were made and stained with hematoxylin and eosin. A pathologist reviewed the serial slides and selected representative sections that contained >70% of ductal adenocarcinomas in the tissue section volume.

Isolation of total RNA

Human pancreatic cancer tissue samples were dissolved in 1 mL of TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) and homogenized using a Micra D-8 (ART, Müllheim, Germany) homogenizer. The sample volume did not exceed 10% of the volume of TRIZOL reagent used for homogenization. The homogenized samples were incubated at a temperature of 15°C–30°C for 5 min in clear polypropylene tubes to permit the complete dissociation of the nucleoprotein complexes. Chloroform (0.2 mL) was added to the tube. After vigorous shaking, the mixture was incubated again at a temperature of 15°C–30°C for 2–3 min. After centrifugation at 12,000×g for 15 min at 4°C, the RNA in the aqueous phase was moved to a fresh RNA-free tube and mixed with 0.5-mL isopropyl alcohol. The samples were incubated at a temperature of 15°C–30°C for 10 min and centrifuged at 12,000×g for 10 min at a temperature of 4°C. The supernatant was removed and the RNA pellet was washed once with 75% ethanol, redissolved in diethylpyrocarbonate-

treated water, and stored at a temperature of −80°C. All the RNAs used in this study were exhaustively treated with TURBO DNA-free (Ambion, Austin, TX, USA) to remove residual DNA. The quality of the RNA was analyzed on an RNA chip by means of a bioanalyzer (model 2100; Agilent Technologies, Santa Clara, CA, USA). The 260/280 ratio of array-tested RNA was 1.8–2.0.

Gene expression arrays

Human genome survey arrays (Applied Biosystems, Foster City, CA, USA) were used to analyze the transcriptional profiles of the pancreatic cancer tissue RNA samples. Digoxigenin-uridine triphosphate (Roche, Indianapolis, IN, USA)-labeled cRNA was generated and linearly amplified from 1 μg of total RNA using the chemiluminescent RT-IVT labeling kit (Applied Biosystems), according to the manufacturer's protocol. Array hybridization, chemiluminescence detection, and image acquisition and analysis were performed using a chemiluminescence detection kit (Applied Biosystems) and a chemiluminescent microarray analyzer (model 1700; Applied Biosystems), according to the manufacturer's protocol. Each microarray was first pre-hybridized at 55°C for 1 h in a hybridization buffer with a blocking reagent. Sixteen micrograms of labeled cRNA targets were first fragmented into 100–400 bases by means of incubation with fragmentation buffer at a temperature of 60°C for 30 min, mixed with an internal control target (ICT, 24-mer oligo labeled with LIZ fluorescent dye), and hybridized to each pre-hybridized microarray in a volume of 1.5 mL at 55°C for 16 h. After hybridization, the arrays were washed with hybridization wash buffer and chemiluminescence rinse buffer. Enhanced chemiluminescent signals were generated by first incubating the arrays with anti-digoxigenin-alkaline phosphatase (Roche Applied Science, Branford, CT, USA), enhanced with chemiluminescence-enhancing solution, and finally adding chemiluminescence substrate. Images were collected for each microarray using the Applied Biosystems 1700 analyzer, which is equipped with a high-resolution, large-format charge-coupled device camera, including "short" chemiluminescent images (5-s exposure each) and "long" chemiluminescent images (25-s exposure each) for gene expression analysis. The images were auto-gridded and the chemiluminescent signals were quantified, corrected for background and spot, and spatially normalized.

Microarray data analysis

The Applied Biosystems Expression System software was used to extract assay signals and assay signal-to-noise ratios from the microarray images. The bad spots flagged by the software were removed from the analysis. The raw data were exported to Excel files (Microsoft Corporation, Redmond, WA, USA). The exported data files were analyzed using Avadis software (StrandGenomics, Bangalore, India) and Arrayassist® (Stratagene, La Jolla, CA, USA). The assay signals of the remaining set of 99.5% of the genes were log-transformed. To select differentially expressed genes, the remaining sets of 99.5% of the genes were further filtered by means of a standard expression array system signal-to-noise threshold (signal-to-noise ratio was >3 in at least 1 sample) and a flag threshold (<5,000). The filtered genes were normalized using the quantile normalization method. Significant genes were calculated using one-way analysis of variance and the Tukey HSD test ($p<0.05$), and the fold-change method. Statistical significances were adjusted by the Benjamini–Hochberg FDR multiple-testing correction. Fold change was calculated using each group's average.

Real-time PCR for gene expression studies

Aliquots of 1 μg of total RNA were used to synthesize the first-strand cDNA using SuperScript III (Invitrogen). Subsequent PCRs were performed using TaqMan gene expression assays (Applied Biosystems). Freshly synthesized cDNAs (volume, 0.5 μL) were transferred to 19.5 μL of TaqMan universal PCR master mix (Applied Biosystems) containing AmpErase uracil-N-glycosylase, a 6-FAM-labeled TaqMan probe, and specific primers for each amplification. The TaqMan probe/primer sets were as follows: BRSK2, Hs00294694_m1; DST, Hs00391673_m1; FosB, Hs00171851_m1; RGS16, Hs00161399_m1; and CXCL12, Hs00171022_m1. Real-time PCR was performed in triplicate using an ABI Prism 7000 (Applied Biosystems). The standard relative curve method was performed for each target gene. The target amount was then divided by an endogenous reference amount (GAPDH; Applied Biosystems) to obtain a normalized target value.

Tissue material and immunohistochemistry

Tissue sections on glass slides were deparaffinized with xylene, rehydrated in serially diluted alcohol, and subsequently processed in a microwave for 15 min with Tris–EDTA (TE; pH 9) buffer for antigen retrieval. After blocking of endogenous peroxidase with 3% H_2O_2, the sections were immersed in 3% goat serum diluted with phosphate-buffered saline for 60 min. The slides then were incubated with mouse monoclonal anti-human RGS16 and FosB antibodies (1:30 dilution; R&D Systems Co., Cambridge, UK) for 90 min at room temperature. After rinsing three times with distilled water containing 0.1% Tween 20, the tissue sections were incubated with HRP-conjugated streptavidin for 20 min at room temperature. Slides were then washed, developed for 5 min with liquid 3,3′-diaminobenzidine tetrahydrochloride, counterstained with Meyer's hematoxylin, dehydrated, and mounted with Permount for histologic examination. The

results of immunostaining were recorded as an intensity score according to the estimated staining proportion (no, 0; weak, <10%; moderate, 10~50%; and strong, >50%).

Statistical analysis

Results are expressed as the mean±standard error of the mean (SEM), unless indicated otherwise. Group differences were statistically analyzed using the Student's t test. Correlations between RGS16 and FosB expression and lymph node metastasis were analyzed using the Mann–Whitney U test. The Kaplan–Meier method was used to generate survival curves, and differences in survival were analyzed using the log-rank test, based on the RGS16 and FosB expression status. Probability values <0.05 were considered statistically significant.

Results

Identification of genes differentially expressed between pancreatic cancer tissues with and without lymph node metastasis

To minimize the number of falsely significant genes in which the signal-to-noise ratio was >3 in at least 1 sample and the flag value was <500,074 were filtered, leaving 15,357 genes from the original 30,469 genes. Of the 15,357 genes that were analyzed on the cDNA arrays, 184 genes (t test, $p<0.05$) were found to be differentially expressed between pancreatic cancer tissues with LN metastasis and those without LN metastasis. For the two groups we analyzed, only 58 genes were differentially expressed, with a difference in signal intensity ratio greater than a 1.5-fold

Table 2 Representative down-regulated genes that were>1.5-fold expressed in pancreatic cancer tissue with lymph node metastasis

Probe_ID	GB_Accession	Gene_Symbol	Gene_Name	Chromosome	Fold (G1/G2)
234890	BC036847	NEUROG2	neurogenin 2	4q25	−1.52
181512	AL118502	RPS10	ribosomal protein S10	6p21.31	−1.54
163612	BC003625	IER2	immediate early response 2	19p13.13	−1.56
216847	U78557	MTMR11	myotubularin related protein 11	1q12-q21	−1.59
164565	BC027875	MYO15B	myosin XVB pseudogene	17	−1.72
123273	BC006175	JUN	v-jun sarcoma virus 17 oncogene homolog (avian)	1p32-p31	−1.72
219483	AB004885	TLK1	tousled-like kinase 1	2q31.1	−1.73
211614	BC063858	ATP8B2	ATPase, Class I, type 8B, member 2	1q22	−1.75
181676	BC006791	RPL10A	ribosomal protein L10a	6p21.3-p21.2	−1.80
194171	AB011104	COH1	Cohen syndrome 1	8q22.2	−1.85
179827	BC009693	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)	19q13.1	−1.87
144113	BC063042	FOSB	FBJ murine osteosarcoma viral oncogene homolog B	19q13.32	−1.94
205128	BC004490	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	14q24.3	−1.97
139004	BC007080	TMEM14B	transmembrane protein 14B	6p25.1-p23	−2.00
204023	BC036447	RHPN2	rhophilin, Rho GTPase binding protein 2	19q13.12	−2.12
234978	BC069587	STN2	stonin 2	14q31.1	−2.14
138880	BC004896	MRPL39	mitochondrial ribosomal protein L39	21q21.3	−2.19
118740	L36033	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	10q11.1	−2.23
136039	AK094988	CTSLL3	cathepsin L-like 3	9	−2.25
173025	U94829	RGS16	regulator of G-protein signaling 16	1	−2.39
235090	AY302538	ATP8B3	ATPase, Class I, type 8B, member 3	19p13.3	−2.63
105390	BC040197	FOSB	FBJ murine osteosarcoma viral oncogene homolog B	19q13.32	−2.90
137081	AF257505	BTN3A2	butyrophilin, subfamily 3, member A2	6p22.1	−3.35
234743	BC037292	FAM62C	family with sequence similarity 62 (C2 domain containing), member C	3q22.3	−3.37
199956	BC030997	METT5D1	methyltransferase 5 domain containing 1	11p14.1	−4.31
141235	BC010850	FLJ20397	hypothetical protein FLJ20397	7p22.3	−4.32
229604	BC051771	FLJ30707	hypothetical protein FLJ30707	13q14.13	−4.54
179781	AK090476	NOD3	NOD3 protein	16p13.3	−4.80
234535	AK096713	DST	dystonin	6p12-p11	−5.78
103153	AK074411	BRSK2	BR serine/threonine kinase 2	11p15.5	−7.23

change. Of these genes sets, 30 were significantly down-regulated in pancreatic cancer tissues with LN metastasis compared with those without LN metastasis. Table 2 contains complete lists of down-regulated genes in pancreatic cancer tissues with LN metastasis with the name, description, and chromosome location.

Validation of microarray data by real-time PCR

The differences in gene expression found by microarray analyses were validated by using real-time PCR. We chose genes that were >1.5-fold down-regulated according to our microarray results (Table 2). Among the genes, we evaluated the levels of expression of five selected down-regulated genes in pancreatic cancer tissues with LN metastasis. The expression of DST, FosB, RGS16, and CXCL12 was significantly lower in pancreatic cancer tissues with LN metastasis than in pancreatic cancer tissues without LN metastasis (Fig. 1a). There was no statistically significant difference in the levels of BRSK2 in pancreatic cancer tissues with LN metastasis compared with pancreatic cancer tissues without LN metastasis. Although some variation concerning the degree of regulation was observed, the data obtained with microarrays were substantially confirmed for four selective genes by real-time PCR (Fig. 1b). In summary, microarrays together with real-time PCR validation results clearly demonstrate that the expression of DST, FosB, RGS16, and CXCL12 were confirmed to be significantly lower in pancreatic cancer tissues with LN metastasis than in pancreatic cancer tissues without LN metastasis.

Immunohistologic analysis of RGS16 and FosB expression

Among down-regulated genes, RGS16 and FosB were selected for further investigation because RGS16 and FosB have never been studied in pancreatic cancer. To confirm the underexpression of RGS16 and FosB proteins in pancreatic cancer tissues with LN metastasis, we performed immunohistochemical staining using anti-RGS16 and anti-FosB antibodies and validated underexpression of RGS16 and FosB proteins in pancreatic cancer tissues with LN metastasis (Fig. 2). For further analysis, the immunostaining results were grouped into two categories. The tissues that exhibited >10% immunoreactivity were classified as positive, whereas those tissues with no or <10% immunoreactivity were classified as negative. In pancreatic cancer tissues with LN metastasis, positive expression of RGS16 was demonstrated in 5.9% (one of 17) and negative in 94.1% (16 of 17). We observed that 70.6% (12 of 17) of pancreatic cancer tissues without LN metastasis were positive, of which staining was moderate in 47.1%, and weak in 23.5%. In pancreatic cancer tissues with LN
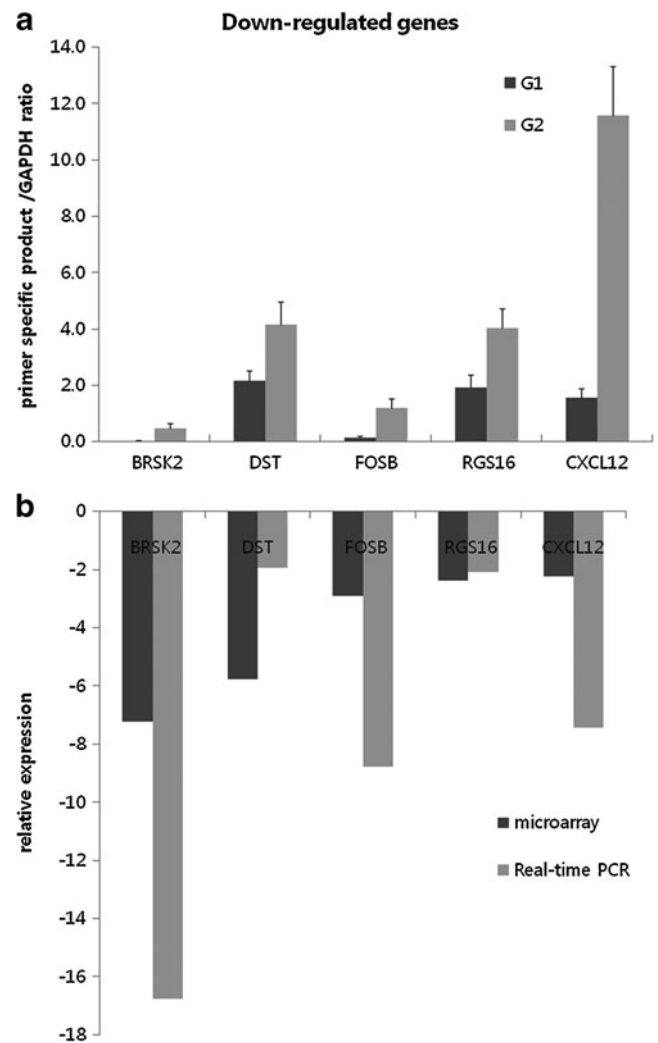

Fig. 1 Gene expression analysis of selected down-regulated genes in pancreatic cancer tissues with and without lymph node metastasis. **a** Down-regulated gene analysis between pancreatic cancer tissues with and without lymph node metastasis by real-time PCR. **b** Real-time PCR validation of microarray data. For each sample, the amount of target and endogenous reference was determined from the appropriate standard curve. The target amount was then divided by an endogenous reference (GAPDH) amount to obtain a normalized target value. The *black bar* represents the fold (Group II/Group I) values obtained in DNA microarray; the *gray bar* represents the fold (Group II/Group I) values obtained in real-time PCR

metastasis, positive expression of FosB was found in 5.9% (one of 17) and negative in 94.1% (16 of 17). We observed that 58.9% (10 of 17) of pancreatic cancer tissues without LN metastasis were positive, of which staining was moderate in 11.8% and weak in 47.1%. Overall, staining was significantly stronger in pancreatic cancer tissues without LN metastasis than in pancreatic cancer tissues with LN metastasis for RGS16 and FosB expression. Overall survival analysis using the Kaplan–Meier method revealed that the prognosis of patients with tumors expressing negative RGS16 and FosB was significantly

Fig. 2 Underexpression of RGS16 and FosB in pancreatic cancer tissues with lymph node metastasis as determined by immunohistochemistry. Immunohistochemical analysis of RGS16 and FosB in pancreatic cancer tissues. **a** A representative negative staining in pancreatic cancer tissues with lymph node metastasis. Magnification, ×200. **b**, a representative positive staining in pancreatic cancer tissues without lymph node metastasis. Magnification, ×200
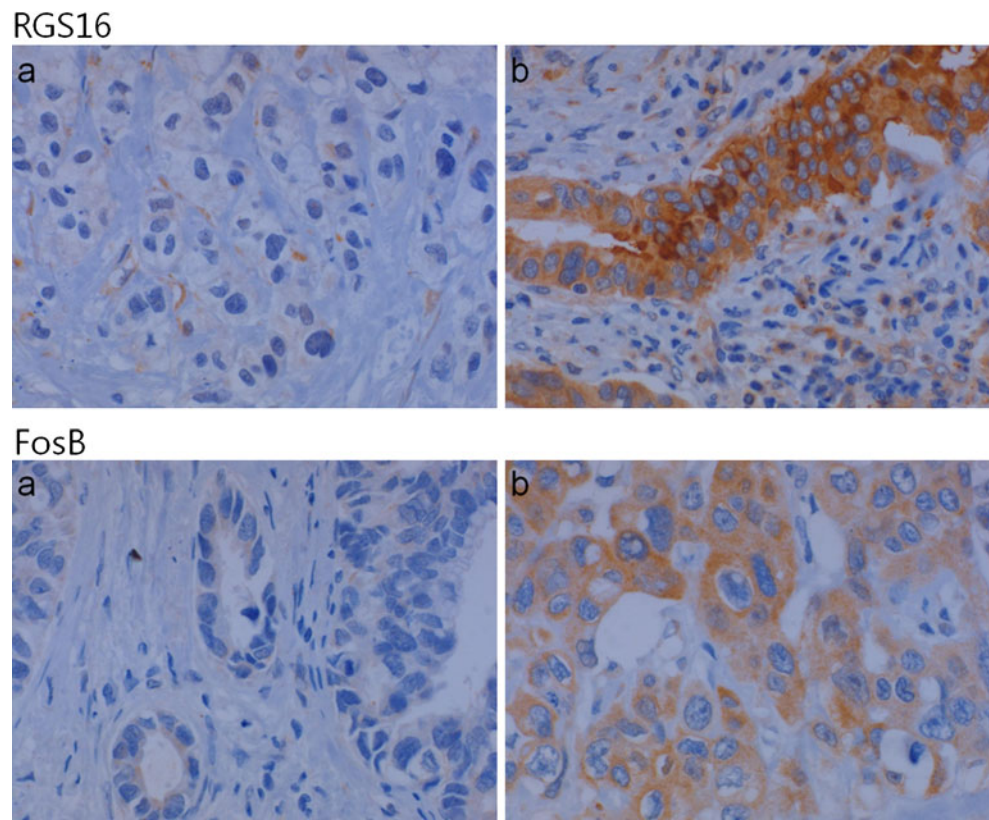
poorer than tumors expressing positive RGS16 and FosB (Fig. 3, $p=0.001$ and $p=0.043$ by log-rank test, respectively). These findings are consistent with the hypothesis that RGS16- and FosB underexpressing cancers are somewhat more aggressive than RGS16- and FosB-positive cancers.

Discussion

Recent clinical studies have revealed that the most important prognostic factor in pancreatic cancer is the presence of lymph node metastasis in patients with pancreatic cancer who have undergone surgery [1, 2, 8, 9]. The search for novel prognostic and therapeutic targets for pancreatic cancer has been extensively performed. Expression of certain genes, such as stanniocalcin1 (STC1), p8, and LIMK2, correlate well with the number of metastatic lymph nodes involved [10, 11]. AP2α-low expression and MUC17-high expression were associated with LN metastasis of pancreatic cancer and poor survival [12]. Several other serum biomarker candidates for pancreatic cancer have emerged from studies investigating genes or proteins differentially expressed in human pancreatic cancer, including CEACAM1, osteopontin, macrophage inhibitory cytokine 1, MUC4, and hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein 1 [13]. However, there remain no effective molecular markers of clinical utility in pancreatic cancer.

In the current study, a DNA microarray gene expression profiling approach was used to identify novel lymphatic metastasis-associated genes in pancreatic cancer tissues with LN metastasis versus pancreatic cancer tissues without LN metastasis. From this screening, we identified 30 genes that were down-regulated in pancreatic cancer tissues with LN metastasis. Of these genes, RGS16 and FosB, two of the largest differences in expression between two groups, were focused on further investigation because RGS16 and FosB have never been studied in pancreatic cancer. Microarray, together with real-time PCR validation results, clearly demonstrated that RGS16 and FosB were down-regulated in pancreatic cancer tissues with LN metastasis. These results led us to examine the significance of RGS16 and FosB expression in pancreatic cancer. Immunohistochemical analysis showed that pancreatic cancer tissues with LN metastasis had significantly lower levels of RGS16 and FosB than pancreatic cancer tissues without LN metastasis, consistent with mRNA expression levels observed in patient tumors through microarray and real-time PCR. RGS16 and FosB underexpression in pancreatic cancer tissues with LN metastasis was shown to be associated with poor patient survival in this report, suggesting that RGS16 and FosB underexpression might be important for acquiring malignant potential in pancreatic cancer. Therefore, these results suggest that RGS16 and FosB underexpression could be a useful prognostic marker for the pancreas cancer patients.
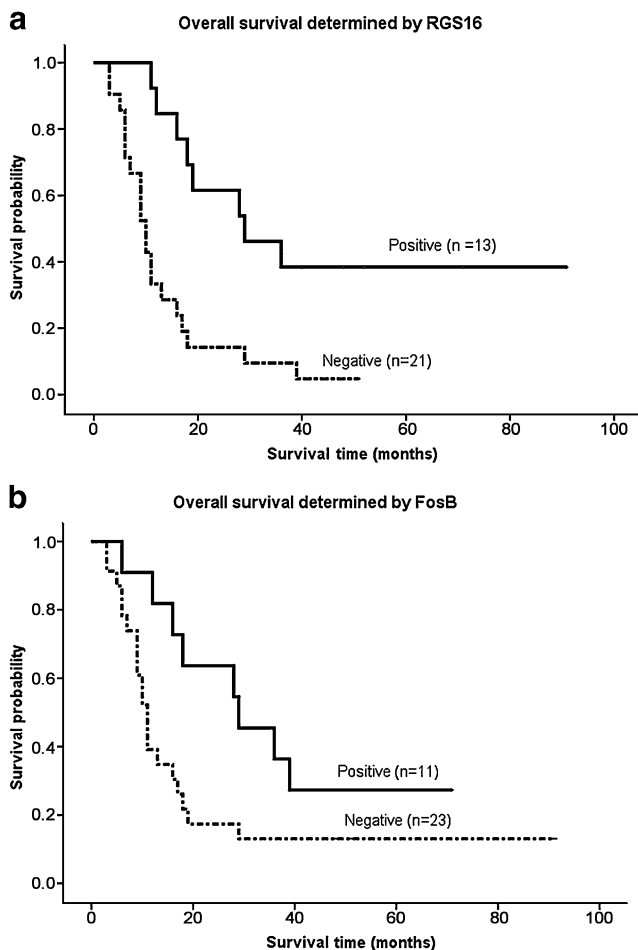
Fig. 3 Survival curves of patients with positive and negative for RGS16 and FosB expression. Kaplan–Meier analysis of overall survival for patients with pancreatic cancer ($n=34$). **a** RGS16 expression—negative cases ($n=21$) had significantly poorer prognosis than expression—positive cases ($n=13$). **b** FosB expression—negative cases ($n=23$) had significantly poorer prognosis than expression—positive cases ($n=11$)

The RGS family, comprising 22 homologues of proteins, plays a role in cellular proliferation, differentiation, membrane trafficking, and embryonic development through the involvement of the mitogenoactivated protein kinase signaling pathway [14, 15]. RGS16 is expressed in several tissues, such as the retina, pituitary gland, bone marrow, and liver [16, 17]. A previous study showed that RGS16 is overexpressed abnormally in high hyperdiploid acute lymphoblastic leukemia, indicating involvement of over-expression of the RGS16 gene in hematologic malignancies [18]. RGS16 expression was higher in colorectal cancer tissue and related to a poorer overall survival rate [19]. On the other hand, a substantial percentage of breast tumors have a RGS16 mutation and reduced RGS16 protein expression. The loss of RGS16 in some breast tumors enhances PI3K signaling elicited by growth factors, and thereby promotes proliferation and TKI evasion down-stream of HER activation [20]. The altered expression of RGS is associated with ischemia or hypoxia, suggesting that pharmaceutical modulation of RGS activity impacts the treatment of diseases associated with abnormal G coupled protein receptor output [21]. The activity and specificity of Rgs16 protein are regulated through phosphorylation, in which several oncogenes are involved, by modulating the phosphorylation on a tyrosine residue in the RGS box [22].

The Fos family of transcription factors includes c-Fos, FosB, Fra-1, Fra-2, as well as the smaller FosB splice variants, FosB2 and deltaFosB2. Together with the Jun family members, the Fos family of transcription factors form the group of AP-1 proteins which, after dimerization, bind to so-called TPA-responsive elements in the promoter and enhancer regions of target genes [23]. In contrast to Jun proteins, Fos family members are not able to form homodimers, but hetero-dimerize with Jun partners, giving rise to various trans-activating or trans-repressing complexes with different biochemical properties [24]. The expression of Fos proteins might be crucial for the activity of AP-1-regulated genes [23]. Recent results suggest that Fos proteins might be involved in the progression of many tumor types. Some breast cancers have been shown to exhibit very high FosB expression [25]. Overexpression of FosB in K562 myeloid cells significantly increased sensitivity to VPA, which suggested that FosB can be a potential marker of VPA sensitivity in AML [26]. Deregulated Fra-1 expression was also found by cDNA arrays in a highly metastatic rat pancreatic carcinoma cell line compared to its non-metastatic counterpart [27]. In contrast, increased cell motility of a hamster pancreatic carcinoma cell line was accompanied by a high induction of c-Fos, which was a necessary step in the development of cell motility in anti-sense experiments. In human pancreatic tumors, c-Fos mRNA and protein overexpression was found in the majority of carcinomas [28]. In contrast to the bulk of data on the function of c-Fos and Fra-1, far less is known about the role of FosB, which is often expressed more strongly than Fra-1 in clinical cancer tissues.

The present study revealed that RGS16 and FosB are underexpressed in pancreatic cancer tissues with LN metastasis. Decreased RGS16 and FosB levels were associated with decreased patient survival, suggesting that RGS16 and FosB might be promising prognostic markers for pancreatic cancer.

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