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Original Article

Nuclear translocation of fibroblast growth factor receptor 3 and its significance in pancreatic cancer

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Abstract: Nuclear translocation of fibroblast growth factor receptor 3 (FGFR3) was previously observed in some kinds of cancer. However, whether the phenomenon occurs in pancreatic cancer (PC), a malignancy with very dismal prognosis, remains unknown. In the present study, FGFR3 expression was firstly detected by Western blot and immunohistochemical staining in specimens of PC. Then, its correlations with clinicopathologic features and patient survival were evaluated. It was shown that FGFR3 was highly expressed in all the nuclear extracts, but in only one out of four whole tissue lysates, of tumor tissues, in contrast to those of non-tumor ones. Using immunohistochemistry, nuclear expression of FGFR3 was found to mainly locate in tumor cells, and was significantly associated with N stage. Furthermore, high FGFR3 nuclear expression was revealed to be associated with poor overall and disease-free survival in univariate analysis. For overall survival in the whole cohort and disease-free survival in patients with curative resection, high nuclear expression of FGFR3 was significant or marginally significant in multivariate analysis. However, its cytoplasmic expression was not related to clinical, pathologic variables and prognosis. These data suggest that nuclear translocation of FGFR3 is frequent and carries clinicopathologic as well as prognostic significances in PC.

Keywords: Fibroblast growth factor receptor 3, nuclear translocation, pancreatic cancer, prognosis

Introduction

Fibroblast growth factor receptor 3 (FGFR3) belongs to a high affinity cell surface-associated receptor family that is highly conserved throughout evolution [1]. The FGFR3 gene localizes on chromosome 4 p16.3, with 19 exons and 18 introns [2, 3]. It has been well documented that FGFR3 is expressed, amplified or mutated in some malignant tissues and cell lines, and is involved in the underlying mechanisms of tumor initiation and unfavorable biological behaviors, i.e. chemoresistance, growth and migration [4-16]. Interestingly, there might be different in subcellular localization of FGFR3 protein between tumor and adjacent non-tumor tissues in some kinds of cancer. In breast and bladder cancers, it was found that FGFR3 expression was predominantly localized in cytoplasm of normal cells and nuclei of malignant ones [17, 18]. These results provided evidence of nuclear translocation of the protein in cancer cells. However, further data remain to be accumulated.

The preliminary clue associated with FGFR3 in pancreatic cancer (PC), a well known lethal malignancy [19], was derived from our previous study showing that expression was obviously upregulated in three chemoresistant sub-lines, compared with that in the parent PC cell line, SW1990 [20]. Thus far, investigators mainly focused on its biological roles and regulation in PC [21, 22]. However, whether FGFR3 nuclear translocation occurs in PC has not been elucidated.

The aim of the present work is to investigate expression and location of FGFR3, and its clinicopathologic and prognostic significances in PC.

Materials and methods

Patients and samples

Liquid nitrogen-frozen samples from 4 patients (3 men and 1 woman, 38 to 65 years) were used in Western blot. There were 40 patients

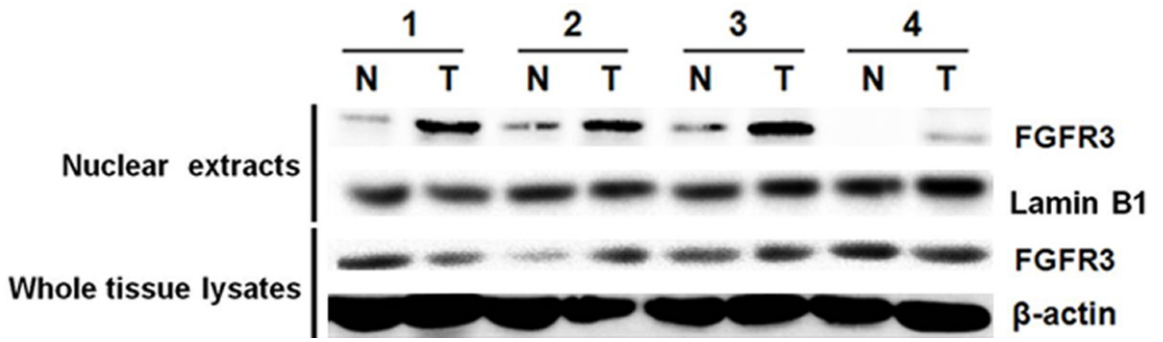

Figure 1. Expression of FGFR3 in pancreatic cancer (PC), detected by Western blot. FGFR3, fibroblast growth factor receptor 3; N, non-tuemor; T, tumor.

with PC (22 males and 18 females, 42 to 77 years) whose specimens applied for immunohistochemical staining. Differentiation, T and N stages were determined by routine histological examinations after surgery. Thirty-four and six patients underwent curative (R0) and palliative (R1) resection, respectively. The Institutional Ethics Committee approval for the project was obtained.

Western blot

Proteins in nuclei and whole tissue lysates were extracted, respectively. Protein concentrations were determined by a BCA protein assay kit (Thermo Scientific, Meridian Rd, Rockford). Proteins were electrophoresed on polyacrylamide gels (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). Then, membranes were blocked with 5% non-fat dry milk, followed by the incubation with primary antibodies (against FGFR3, lamin B1 and β-actin, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) overnight at 4°C. Secondary antibodies were incubated at 37°C for 1 h. Protein bands were visualized by an ECL kit (Merck, Darmstadt, Germany).

Immunohistochemical staining and result evaluation

FGFR3 expression was detected immunohistochemically for paraffin-embedded samples. The PowerVision™ two-step staining kit (PV-6001, Beijing Zhongshan Biotech Co., China) was used for staining. In brief, 4 μm-thick sections were mounted, deparaffinized in xylene, rehydrated in ethanol. After washing in phosphate buffered saline (PBS) solution for 5 min, 3% hydrogen peroxide was used for 30 min to block endogenous peroxidase. Then antigen retrieval using microwave oven heating and 0.1% trypsin was performed. Afterwards, slides were incubated for 120 min at 37°C with the primary antibody at a dilution of 1:100. Following washing in PBS for three times, horseradish peroxidase (HRP)-labeled secondary antibody was added for an incubation of 30 min. Diaminobenzidine was used as a chromogen. Finally, slides were counterstained with hematoxylin. Non-immune rabbit serum at the same dilution was used as the negative control.

Two pathologists who had no prior knowledge of clinicopathologic and follow-up data (Z.Y. L. and W.X. Z.) independently evaluated nuclear and cytoplasmic staining of FGFR3, respectively. A discussion for consensus was performed when they were divergent. The brown coloration in cells was defined as positive. According to the criteria reported in a previous paper [18], the staining proportion of FGFR3 in cells was classified in four grades (0%=0, 0-10%=+, 10-50%=++, >50%=+++). Finally, FGFR3 expression was summarized as a simplified classification (low expression, 0, + and ++; high expression, +++). Same with the literature [18], the intensity of staining was not considered.

Follow-up

Forty patients whose samples were used for immunohistochemistry underwent follow-up at least one time. The follow-up time ranged from 1 to 34 months (median, 12 months). Totally, 17 patients have died, 12 patients censored

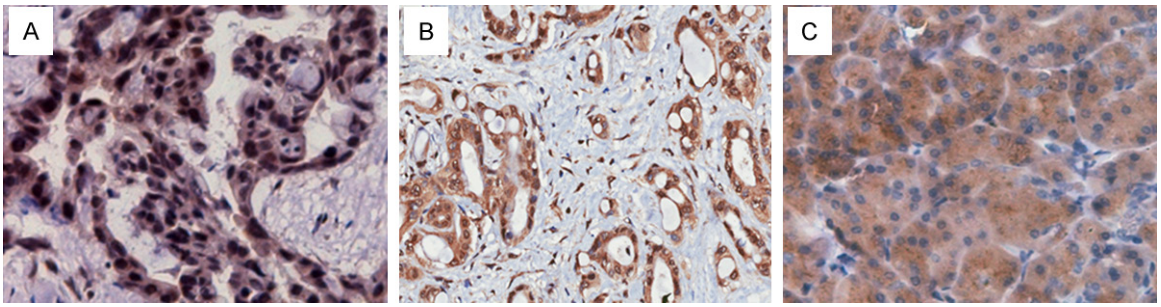

Figure 2. Expression of FGFR3 in PC, detected by immunohistochemical staining. A. High nuclear expression in tumor tissue (original magnification ×200); B. High nuclear and cytoplasmic expression in tumor tissue (original magnification ×200); C. High cytoplasmic expression in non-tumor tissue (original magnification ×200). FGFR3, fibroblast growth factor receptor 3.

Table 1. Tumoral FGFR3 expression and clinicopathologic features of PC

		Cytoplasmic expression			Nuclear expression		
Variables	n	High	Low	P	High	Low	P
Age				0.270			0.746
≥65 years	14	12	2		9	5	
<65 years	26	17	9		15	11	
Gender				0.723			0.526
Male	22	15	7		12	10	
Female	18	14	4		12	6	
Tumor location				0.469			0.746
Head	26	20	6		15	11	
Non-head	14	9	5		9	5	
Differentiation				1.000			1.000
G1	7	5	2		4	3	
G2-3	33	24	0		20	13	
T stage				0.162			1.000
T1-2	6	6	0		4	2	
T3	34	23	11		20	14	
N stage				0.079			0.022
N0	23	14	9		10	13	
N1	17	15	2		14	3	

NOTE: *P* values were derived from the Fisher's exact test (two-tailed).

during the follow-up, and 11 patients have lived 1 to 34 months.

Statistical analysis

Overall and disease-free survival served as endpoints. The McNemar and Chi-square tests were used to show differences of categorical variables. Patient survival and their differences were determined by Kaplan-Meier method and log-rank test. Cox regression (Proportional hazard model) was adopted for multivariate analysis of prognostic factors. Statistical software package SPSS11.5 (SPSS Inc, Chicago, IL) was employed for all analyses. Statistically significant *P* value was defined as <0.05.

Results

FGFR3 expression in liquid nitrogen-frozen PC samples

FGFR3 expression in tumor tissues, in contrast to non-tumor ones, was higher or lower in whole tissue lysates from 1 out of 4 patients, respectively, whereas it was not different in samples from other two patients (**Figure 1**). However, FGFR3 expression was much higher in nuclear extracts of tumor tissues than in those of non-tumor ones from all the patients (**Figure 1**).

FGFR3 expression in paraffin-embedded PC samples and its association with clinicopathological variables

High expression of FGFR3 was observed in nucleus and cytoplasm of tumor and non-tumor cells from 24, 29, 4 and 35 patients (60.0, 72.5, 10.0 and 87.5%) with PC, respectively (**Figure 2**). High nuclear FGFR3 expression was significantly more frequent in tumor than in non-tumor cells (P<0.001, McNemar test). Using Chi-square analysis, nuclear FGFR3 expression in tumor cells was significantly associated with N stage of PC (P=0.022; **Table 1**), whereas no other FGFR3-related parameters were of clinicopathologic implications (data not shown).

Factors influencing overall survival of PC after resection

Univariate analysis showed that patients with high nuclear expression of FGFR3 in tumor ce-

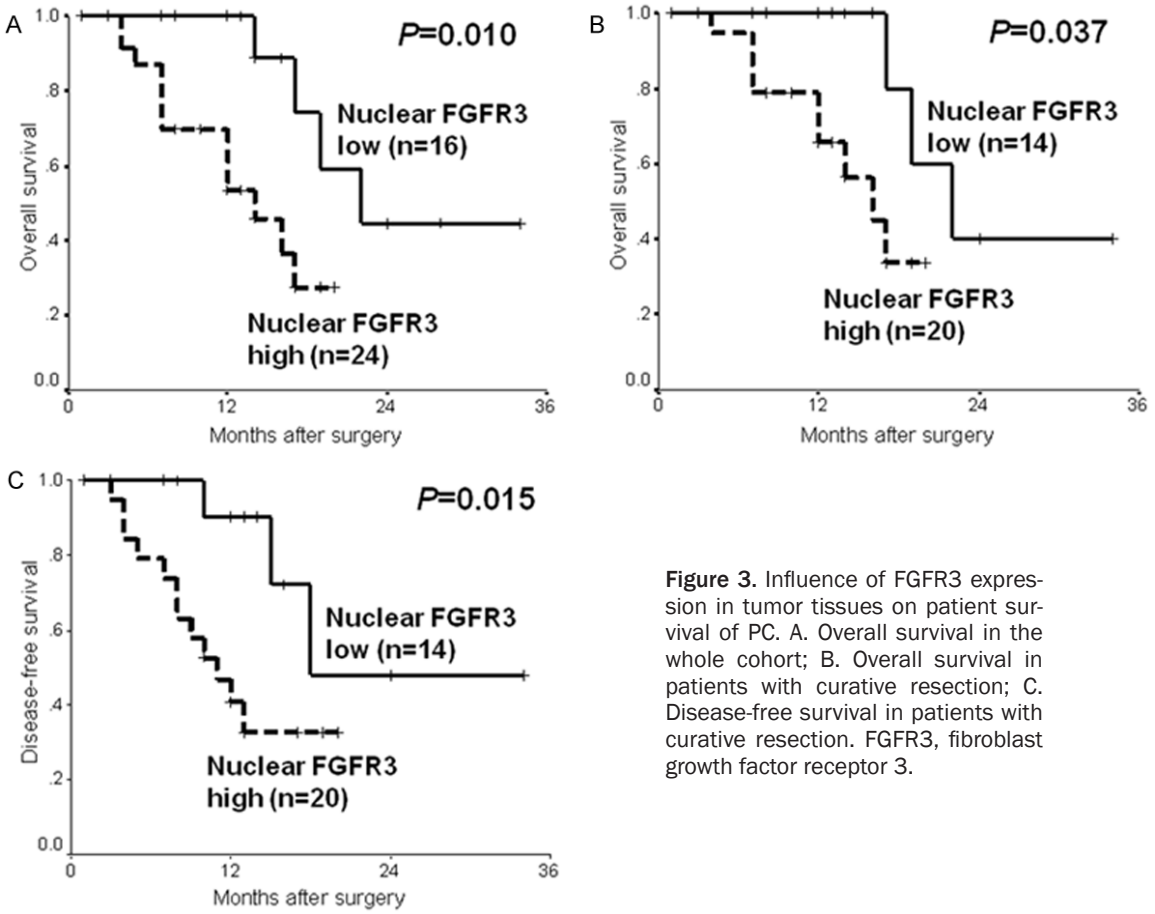

Figure 3. Influence of FGFR3 expression in tumor tissues on patient survival of PC. A. Overall survival in the whole cohort; B. Overall survival in patients with curative resection; C. Disease-free survival in patients with curative resection. FGFR3, fibroblast growth factor receptor 3.

lls had significantly poorer overall survival (P= 0.010; **Figure 3A** and **Table 2**). Besides, the survival benefits were also found in those with N0 stage and underwent curative resection (P< 0.05; **Table 2**), whereas others, including cytoplasmic FGFR3 expression, were not prognostic (P>0.05; **Table 2**). Multivariate Cox regression analysis identified nuclear FGFR3 expression as the single independent prognosticator for overall survival of PC after surgical resection (P=0.022; **Table 2**).

Factors influencing overall survival of PC after curative resection

By univariate analysis, high nuclear expression of FGFR3, but not cytoplasmic FGFR3 expression, in tumor cells, was linked to significantly poorer overall survival after curative resection (P=0.037; **Figure 3B** and **Table 3**). Besides, N stage was also shown to be of prognostic relevance (P=0.026; **Table 3**). However, none of them was significant in multivariate Cox regression analysis (P>0.05; **Table 3**).

Factors influencing disease-free survival of PC after curative resection

Univariate analysis found that patients with high nuclear FGFR3 expression in tumor cells possessed significantly poorer disease-free survival (P=0.015; **Figure 3C** and **Table 4**). Besides, N stage also impacted disease-free survival (P=0.006; **Table 4**), while other variables were not prognostic (P>0.05; **Table 4**). Using multivariate Cox regression analysis, N stage and nuclear FGFR3 expression in tumor cells were all marginally significant (P=0.062 and 0.092; **Table 4**).

Discussion

Sustaining proliferative signaling has been regarded as one of hallmarks of cancer [23]. Therefore, many molecules that promote cancer cell growth, such as growth factors and their receptors [24-26], were thought as proto-oncogenes. As one of the underlying mechanisms, some receptors could translocate to cell nuclei and regulate, as transcriptional regula-

Table 2. Factors associated with overall survival in patients with PC after resection

		Univariate			Multivariate		
Variables	n	median ± SE	95% CI	P	HR	95% CI	P
Age				0.263			
≥65 years	14	17.6±1.3	15.1-20.1				
<65 years	26	18.4±2.4	13.6-23.2				
Gender				0.442			
Male	22	18.4±2.8	13.0-23.8				
Female	18	17.6±1.8	14.2-22.1				
Tumor location				0.191			
Head	26	21.7±2.7	16.4-27.0				
Non-head	14	14.0±1.7	10.6-17.3				
Differentiation				0.241			
G1	7	20.0±6.4	7.5-32.5				
G2-3	33	17.0±1.6	13.8-20.2				
T stage				0.102			
T1-2	6	21.7±1.9	17.9-25.4				
T3	34	18.1±2.3	13.6-22.6				
N stage				0.013			0.326
N0	23	23.4±3.0	17.5-29.2		1		
N1	17	13.8±2.2	9.5-18.1		1.757	0.570-5.412	
Curability				0.039			0.068
R0	34	20.8±2.5	16.0-25.6		1		
R1	6	11.7±3.3	5.2-18.2		3.172	0.916-10.982	
Nuclear FGFR3				0.010			0.022
High	24	14.0±3.2	7.7-20.3		5.043	1.258-20.213	
Low	16	22.0±3.3	15.6-28.4		1		
Cytoplasmic FGFR3				0.107			
High	29	16.4±2.7	11.1-21.7				
Low	11	22.0±4.4	13.3-30.7				

NOTE: *P* values were derived from the Log-rank test (univariate) and Cox regression analysis (multivariate).

tors, expression of proliferation-related genes [27, 28]. For FGFR3, nuclear localization was also found in breast and bladder cancers [17, 18]. However, evidence about this phenomenon in PC is still lacked. The present study first found, by Western blot, that FGFR3 expression was much higher in nuclear extracts of tumor tissues than in those of non-tumor ones from all the four patients, unlike it in whole tissue lysates (**Figure 1**). This finding preliminarily suggests the presence of FGFR3 nuclear translocation in PC. Further results from immunohistochemical staining found much higher ratio (60%) of nuclear expression of FGFR3 in tumor cells (**Figure 2**), compared with that in non-tumor ones (10%), unlike cytoplasmic expression. Based on aforementioned evidence, it mi ght be summarized that nuclear FGFR3 expression was frequent in PC, similar with previous reports [17, 18]. More importantly, tumoral nuclear, but not cytoplasmic, FGFR3 expression was correlated with N stage (**Table 1**), a well known marker of progression and predictor of poor prognosis in PC [29, 30]. Therefore, it could be speculated that nuclear translocation of FGFR3 might play a crucial role in biological behaviors of PC. It was previously shown that nuclear translocation of FGFR1, a receptor in the same family with FGFR3, induced c-Jun and was involved in the regulation of cell proliferation [31]. Whether FGFR3 has similar effects in the nuclei of tumor cells in PC, or whether other mechanisms exist, needs further mechanistic studies.

Because of extremely gloomy prognosis of PC, its prognostic markers are long of interest.

Table 3. Factors associated with overall survival in patients with PC after curative resection

		Univariate			Multivariate		
Variables	n	median±SE	95% CI	P	HR	95% CI	P
Age				0.570			
≥65 years	14	17.6±1.3	15.1-20.1				
<65 years	20	20.0±2.8	14.5-25.5				
Gender				0.974			
Male	16	20.7±3.3	14.3-27.2				
Female	18	17.6±1.8	14.2-22.1				
Tumor location				0.240			
Head	22	22.6±3.0	16.6-28.5				
Non-head	12	15.3±1.7	12.0-18.7				
Differentiation				0.256			
G1	7	20.0±6.4	7.5-32.5				
G2-3	27	17.0±0.7	15.7-18.3				
T stage				0.158			
T1-2	6	21.7±1.9	17.9-25.4				
T3	28	17.0±2.1	12.9-21.1				
N stage				0.026			0.133
N0	22	24.3±3.1	18.2-30.4		1		
N1	12	14.2±2.2	9.9-18.6		2.501	0.756-8.277	
Nuclear FGFR3				0.037			0.143
High	20	16.0±1.9	12.2-19.8		3.320	0.667-16.534	
Low	14	22.0±3.3	15.6-28.4		1		
Cytoplasmic FGFR3				0.196			
High	25	17.0±2.0	13.1-20.9				
Low	9	22.0±4.1	14.0-30.0				

NOTE: *P* values were derived from the Log-rank test (univariate) and Cox regression analysis (multivariate).

Except for conventional clinical and pathologic variables identified [29, 30, 32-34], molecular prognostic factors in PC have recently been extensively investigated and summarized [35, 36]. Here, we provided a novel candidate, FGFR3. Previously, the impact of FGFR3 on cancer prognosis remains controversial, although in data from the same kind of malignancy [37, 38]. Our results from immunohistochemistry support FGFR3 as an indicator of adverse outcome in PC, on the basis of its association with patient survival (**Figure 3** and **Tables 2**-**4**). What calls for special attention is that nuclear, but not cytoplasmic, FGFR3 has prognostic implication. In view of the relationship between nuclear FGFR3 expression and N stage, its prognostic relevance might be easily understood. However, further validation in large scale studies might be needed, because nuclear FGFR3 lost its significances in multivariate analysis for patients with curative resection (**Tables 2**-**4**).

Collectively, our data suggest that nuclear translocation of FGFR3 is frequent and carries clinicopathologic as well as prognostic significances in PC. These findings expand the spectrum of cancers in that FGFR3 has the power to be oncogenic through nuclear translocation.

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Disclosure of conflict of interest

None.

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Table 4. Factors associated with disease-free survival in patients with PC after curative resection

		Univariate			Multivariate		
Variables	n	median±SE	95% CI	P	HR	95% CI	P
Age				0.612			
≥65 years	14	14.8±1.7	11.5-18.2				
<65 years	20	18.7±3.2	12.4-24.9				
Gender				0.939			
Male	16	19.3±3.7	12.0-26.6				
Female	18	14.4±1.5	11.4-17.4				
Tumor location				0.326			
Head	22	21.4±3.1	15.4-27.4				
Non-head	12	11.5±1.6	8.5-14.5				
Differentiation				0.215			
G1	7	18.0±6.0	6.2-29.8				
G2-3	27	13.0±2.6	7.8-18.2				
T stage				0.125			
T1-2	6	18.2±2.8	12.7-23.7				
T3	28	15.1±2.4	10.5-19.8				
N stage				0.006			0.062
N0	22	21.4±3.2	15.2-27.6		1		
N1	12	10.2±1.7	6.9-13.4		2.549	0.954-6.814	
Nuclear FGFR3				0.015			0.092
High	20	11.1±2.0	7.0-15.0		2.724	0.848-8.752	
Low	14	18.0±3.0	12.2-23.8		1		
Cytoplasmic FGFR3				0.351			
High	25	13.0±1.5	10.1-15.9				
Low	9	18.0±4.6	9.1-26.9				

NOTE: *P* values were derived from the Log-rank test (univariate) and Cox regression analysis (multivariate).

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