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Original Article

SFRP5 as a prognostic biomarker for patients with pancreatic ductal adenocarcinoma

Wence Zhou, Ming Tian, Jinjing Hu, Lihong Li, Yong He

Second Department of The General Surgery, The First Hospital of Lanzhou University, Lanzhou University, Lanzhou, China

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Abstract: Secreted frizzled-related protein 5 (SFRP5) is a member of the SFRP family and plays an important role in Wnt (wingless-type) signaling pathway. Aberrant expression of SFRP5 has recently been reported in several human cancers, but studies on human pancreatic ductal adenocarcinoma (PDAC) are lacking. Therefore, the aim of this study was to measure the expression of SFRP5 in pancreatic cancer patients and its correlation with prognosis. Semiquantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and Western blotting (WB) were used to explore the expression of SFRP5 in human PDAC tissues. Using immunohistochemistry to determine the expression of SFRP5 in 71 patients with PDAC. The results of qRT-PCR and WB showed that the expression of SFRP5 was lower in PDAC tissues than that of precancerous tissues. The expression of SFRP5 was correlated with distant metastasis (absent vs. present; $P<0.001$), TNM Stage (I-II vs. III-IV; $P=0.008$), first symptom (pain vs. others; $P=0.023$). Low expression of SFRP5 in PDAC was a poor prognostic factor for human PDAC. In conclusion, our data suggest that low expression of SFRP5 severs as a prognostic biomarker for PDAC therapy.

Keywords: SFRP5, PDAC, prognosis, biomarker

Introduction

Pancreatic cancer is one of the most common gastrointestinal tumors with the characteristics of hidden onset, high malignant degree, poor prognosis, low survival, and the lack of clinical typical presentation, the early diagnosis and treatment are more difficult [1]. Despite improvements in the treatment of pancreatic cancer, the 5-year survival of pancreatic cancer is still under 5% [2]. Given lack of early diagnosis is the primary reason, therefore, specific biomarkers are important for predicting prognosis for pancreatic cancer. SFRP5 acts as a soluble modulator of Wnt signaling pathway that contains a cysteine-rich domain homologous to the putative Wnt-binding site of Frizzled proteins [3]. With a 300 amino acids in length, SFRP5 has a frizzled-like cysteine-rich domain (CRD). The CRD could compete with the Frizzled receptors to bind Wnt ligands. Then, it will increase the free β-catenin levels [4]. The tumor cell cycle progression and proliferation can also be affected by SFRP5. It has been reported that several cancers has the low expression of SFRP5, such as prostate cancer, breast cancer, ovarian cancer, bladder cancer and gastric cancer [5]. However, the expression pattern and function of SFRP5 are still unknown in PDAC. In the present study, we examined 71 cases of pancreatic cancer from 2010 to 2013, we also analyzed the association between the SFRP5 expression and clinicopathological factors as well as prognosis. Then, immunohistochemistry is used to investigate the relation between SFRP5 expression and prognosis.

Materials and methods

Patients and tissue samples

Tumor and adjacent non-tumor pancreatic tissues (2 cm away from the tumor edge) were collected for immunochemical analysis from 71 patients with pancreatic cancer who underwent pancreaticoduodenectomy between 2010 to 2013 at the department of general surgery, the first affiliated hospital of Lanzhou University, Gansu, China. The criteria for case inclusion

Table 1. Correlations between Sfrp5 expression and clinicopathological data

Clinical pathology	Total	Sfrp5 immunohistochemical staining		*P* value
		Positive	Negative	
Age (year)				
<50	15	6	9	0.541
>50	56	24	32	
Gender				
Male	40	17	23	0.578
Female	31	13	18	
Tumor location				
Head	51	20	31	0.286
Others	20	10	10	
Degree of differentiation				
High	20	9	11	0.487
Low	51	21	30	
Distant metastasis				
Absent	15	1	14	0.001
Present	56	29	27	
TNM Stage				
I-II	29	23	6	0.000
III-IV	42	7	35	
Lymph node metastasis				
Absent	14	3	11	0.070
Present	57	27	30	
Prepancreatic invasion				
Absent	35	10	25	0.019
Present	36	20	16	
First symptom				
Abdominal	58	26	32	0.272
Pain and jaundice	13	4	9	

were as follows: (1) All the patients were treated by pancreat-oduodenectomy. (2) None of these patients received anticancer therapy before pancreat-oduodenectomy. (3) Abundant and accurate clinical details and follow-up data could achieve. The clinicopathological characteristics were carefully reviewed from pathology records. The study was approved by the Human Research Ethics Committee, the first hospital of Lanzhou University.

Immunohistochemical analysis

Four micron-thick sections were deparaffinized by xylene and then subjected to antigen retrieval (citrate buffer, PH=6.0) for twenty minutes. Block endogenous peroxidase by hydrogenperoxide incubating 30 minutes. Sections were incubated for 60 minutes with mouse anti-human SFRP5 antibody (1:200 dilution; Abcam, catalog number: ab198206). Reaction products were visualized with 3,3'-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin showed a relatively homogenous staining across each paracancerous section. We measured the staining intensity of SFRP5 as: 0, negative; 1, weak; 2, moderate; 3, strong; The job was assessed by three independent investigators who were blinded to patient characteristics, and comparisons were made between tumor and precancerous tissues.

Western blot analysis

Briefly, total tissue protein were separated and loaded in the 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer to polyvinylidenedifluoride (PVDF) membranes. After the membranes were washed and blocked, incubated with the primary antibody mouse anti-human SFRP5 antibody (1:1000 dilution; Abcam) and Horseradish peroxidase (HRP)-conjugated secondary antibodies. Antibody binding was detected by enhanced chemiluminescence (ECL) assays. Betaactin (1:2000 dilution, Sigma-Aldrich) was used as the loading control.

Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from tumor specimens using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. qRT-PCR was done by a SYBR PrimeScript RT-PCR kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. GAPDH was used as an internal control. The primers were as follows: SFRP5-Forward: 5'-ACGGTATTGGGGAGTATATC-3', SFRP5-Reverse: 5'-CCAATAAAAAATAATCCGA-3', GAPDH-Forward: 5'-CGCATCCTGGGCTACACTGA-3', GAPDH-Reverse: 5'-GTGGTCGTTGAGGGCAATG-3'. The relative expression of SFRP5 was compared to GAPDH by using the equation: $2^{-\Delta Ct}$ [ΔCt = Ct (SFRP5) - Ct (GAPDH)]. All experiments were done in triplicate.

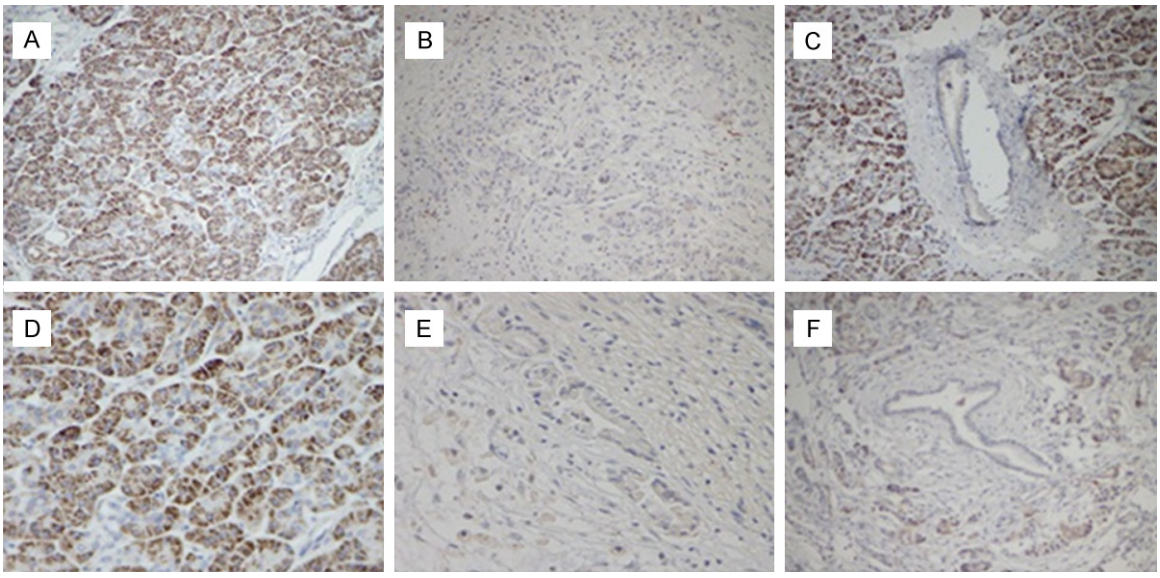

Figure 1. Immunohistochemistry analysis Sfrp5 expression in PDAC tissues and paracancerous tissues. A, D. High expression of Sfrp5 in adjacent noncancerous tissues; B, E. Low expression of Sfrp5 in PC tissues; C, F. High expression of Sfrp5 in adjacent noncancerous tissues and Low expression of Sfrp5 in PC tissues. A, B with ×200 magnification, D, E with ×400 magnification, C, F with ×100 magnification.

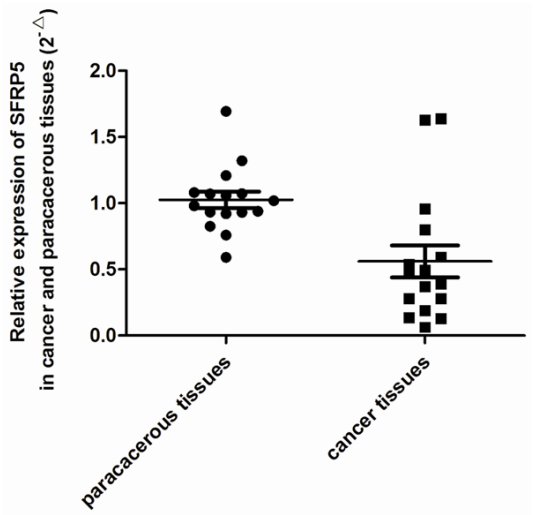

Figure 2. Expression of Sfrp5 mRNA in PDAC tissues and paracancerous tissues.

Statistical analysis

The X^2 and Fisher's exact test was used to analyze the SFRP5 expression and clinical characteristics. The prognostic value of SFRP5 was analyzed by Kaplan-Meier's method. Univariate and multivariate Cox regression models were used to calculate hazard ratios. Then independent prognostic factors were identified from 71 PADC patients. *P* value less than 0.05 regarded as significant and statistical data were obtained by SPSS software (version 20.0, SPSS, Chicago, IL, USA).

Results

Associations between SFRP5 expression and clinicopathological characteristics

The patient clinical and pathological characteristics are summarized in **Table 1**. The median age was 57 years (range 24-75 years). 41 (57.7%) patients have a low SFRP5 expression. The expression of SFRP5 was lower in PADC tissues than that of paracancerous tissue (**Figure 1**). There were significant differences in the expression of SFRP5 when comparing other clinicopathological characteristics such as Distant metastasis (*P*=0.001), TNM Stage (*P*<0.001) and Prepancreatic invasion (*P*=0.019).

Low expression of SFRP5 in PADC by qRT-PCR and Western blot

We used qRT-PCR and Western blot to measure the expression of SFRP5 from mRNA and protein level. PDAC tissues showed lower mRNA expression levels of SFRP5 compared with paracancerous tissues (P<0.001, **Figure 2**). The protein expression of SFRP5 were detected

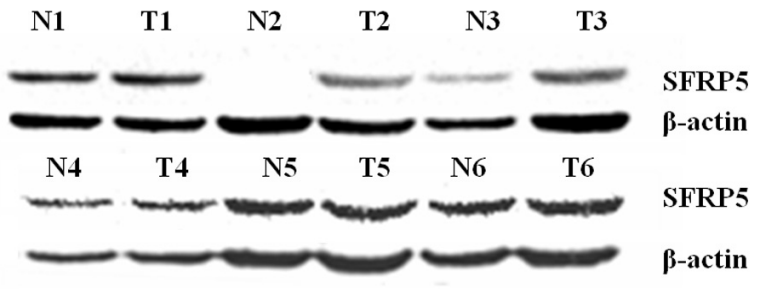

Figure 3. WB analysis of Sfrp5 expression in PDAC tissues and paracancerous tissues.

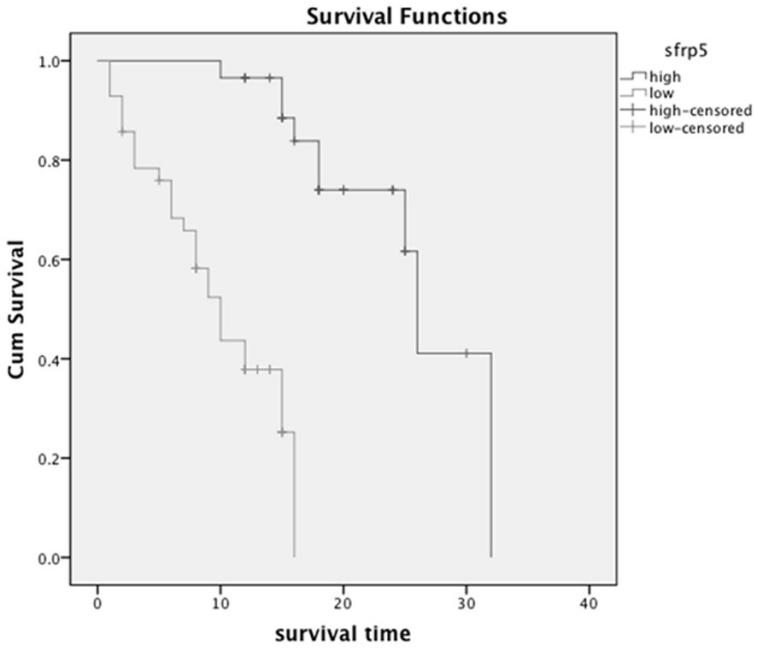

Figure 4. K-M survival analysis of Sfrp5 expression in 71 PC patients.

from six pairs of PDAC tissues, and found that SFRP5 protein level was lower in PDAC tissues than paracancerous tissues (**Figure 3**).

Univariate and multivariate analysis

Kaplan-Meier analysis and log-rank test were used to explore whether SFRP5 was a prognostic factor (**Figure 4**). We found the correlation of the SFRP5 expression with overall survival in 71 PADC patients. The results of univariate and multivariate analysis for overall survival are summarized in **Table 2**. Univariate analysis showed that distant metastasis, TNM Stage, Lymph node metastasis, Prepancreatic invasion as well as SFRP5 could influence overall survival. The multivariate analysis showed that distant metastasis, TNM Stage and SFRP5 were independent prognostic factors for overall survival. In a word, low expression of SFRP5 was an independent prognostic factor for PADC patients.

Association of SFRP5 expression with survival.

Discussion

PDAC is the most frequent type of pancreatic cancer accounting for almost 90% of all pancreatic tumors [6]. The most notable feature of PDAC is poor prognosis, as well as aggressive tumor growth [7]. Presently pancreatectomy remained the only potential for cure. Those who received pancreatectomy had a median survival of 12.6 months, who did not receive the surgery had the median survival of 3.5 months [8]. Therefore, the effective therapeutic and early detection strategies are greatly needed for PDAC.

In recent years, downregulation of SFRP5 had been reported in many tumors such as hepatocellular carcinoma [9], prostate cancer [10], gastric cancer [11], colorectal cancer [12] and breast cancer [13]. The SFRP5 function as tumor suppressor gene has an important implication in carcinogenesis, where they are downregulated in many tumors [14]. In 168 primary breast carcinomas, JurgenVeeck et al, found that down regulation of SFRP5 was associated with reduced overall survival (OS) (P=0.045) and was an independent risk factor affecting OS in a multivariate Cox proportional hazard model. They indicated that SFRP5 might be a novel biomarker potentially useful in clinical breast cancer treatment [15]. Her-Young Su [16] suggested that epigenetic silencing of SFRP5 leaded to oncogenic activation and contributed to ovarian cancer progression and che-

Table 2. Univariate and multivariate analysis of prognostic factors for overall survival in PADC patients

Characteristics	Univariate analysis			Multivariate analysis		
	H	95% CI	*P*	HR	95% CI	*P*
Age (<50, >50)	1.491	0.577-3.851	0.409	1.304	0.328-5.183	0.706
Gender (male, female)	1.976	0.993-3.932	0.052	1.003	0.458-2.195	0.995
Tumor location (Head, others)	0.986	0.482-2.017	0.969	1.194	0.482-2.956	0.701
Degree of differentiation (high, low)	1.152	0.539-2.461	0.716	1.291	0.416-4.006	0.658
Distant metastasis (absent, present)	0.110	0.052-0.232	<0.001	0.070	0.019-0.250	<0.001
TNM Stage (I-II, III-IV)	20.740	4.780-89.987	<0.001	9.065	1.770-46.426	0.008
Lymph node metastasis (No, Yes)	0.309	0.140-0.681	0.004	2.414	0.793-7.349	0.121
Prepancreatic invasion (No, Yes)	0.128	0.053-0.312	<0.001	0.098	0.029-0.327	0.098
First symptom (pain, others)	1.671	0.751-3.718	0.208	3.410	1.185-9.813	0.023
SFRP5 (low, high)	0.071	0.023-0.221	<0.001	0.240	0.063-0.910	0.036

moresistance. It was reported that SFRP5 was methylated in 60% of prostate cancer cell lines [17]. All the research indicates that SFRP5 will be a potential biomarker for PDAC.

However, little is known about the role of SFRP5 in PDAC. Low expression of SFRP5 was found in the majority of the 60 PDAC samples by a research group. It was observed that the expression loss of SFRP5 in PDAC samples were significantly higher than those in the paracancerous tissue samples [18]. In our study, the mRNA and protein level of SFRP5 had been explored to be lower in PDAC tissues than paired paracancerous tissues. We also found that the expression of SFRP5 was associated with distant metastasis, TNM stage and prepancreatic invasion.

Early PDAC diagnostic criteria are as follow: tumor diameter less than 2 cm, confined to the pancreatic parenchyma, no peripancreatic invasion and metastasis [19]. Early diagnosis of this study was only 5.6% (4/71), but the average survival time for PDAC patients has reached 26 months of those who received radical surgery. In the early diagnosis of PDAC, the current clinical application is CA19-9 and imaging examination [20]. Over the past 20 years, studies found that many PDAC biomarkers, including CA242 [21], TATI [22], POA [23] and MMP7 [24]. Although the sensitivity and specificity of these markers have reached more than 70% [25], but the effect is not as good as CA19-9, therefore, the study on these markers need to be continued further. The researches on PDAC had accumulated a database form, which combined with serum proteomics and molecular bioinformatics were expected to find a high sensitivity and specificity of biomarker. Further functions of SFRP5 would be pivotal in finding possible novel therapeutics for cancer through targeting SFRP5. In our study, SFRP5 is the independent prognostic factor in clinical patient samples. In the future, the role of SFRP5 is needed to prove its prognostic value.

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Disclosure of conflict of interest

None.

Address correspondence to: Wence Zhou, Second Department of The General Surgery, The First Hospital of Lanzhou University, Lanzhou University, Lanzhou 730000, China. E-mail: zhouwc129@163.com (WCZ); tianm13@lzu.edu.cn (MT)

References

[1] Siegel RL, Kimberly D, Ahmedin J. Global cancer Statistics 2015. CA Cancer J Clin 2015; 65: 5-29.

[2] Maitra A, Hruban RH. Pancreatic cancer. Annu Rev Pathol 2008; 3: 157-188.

[3] Rao TP, Kühl M. An updated overview on Wnt signaling pathways: a prelude for more. Circ Res 2010; 106: 1798-806.

[4] Niehrs C. The complex world of WNT receptor signaling. Nat Rev Mol Cell Biol 2012; 13: 767-779.

[5] Surana R, Sikka S, Cai W, Shin EM, Warrier SR, Tan HJ, Arfuso F, Fox SA, Dharmarajan AM, Kumar AP. Secreted frizzled related proteins: Implications in cancers. Biochim Biophys Acta 2014; 1845: 53-65.

[6] Fesinmeyer MD, Austin MA, Li CI, De Roos AJ, Bowen DJ. Differences in survival by histologic type of pancreatic cancer. Cancer Epidemiol Biomarkers Prev 2005; 14: 1766-1773.

[7] Hidalgo M. Pancreatic cancer. N Engl J Med 2010; 362: 1605-1617.

[8] Bilimoria KY, Bentrem DJ, Ko CY, Ritchey J, Stewart AK, Winchester DP, Talamonti MS. Validation of the 6th edition AJCC Pancreatic Cancer Staging System: report from the National Cancer Database. Cancer 2007; 110: 738-44.

[9] Takagi H, Sasaki S, Suzuki H, Toyota M, Maruyama R, Nojima M, Yamamoto H, Omata M, Tokino T, Imai K, Shinomura Y. Frequent epigenetic inactivation of SFRP genes in hepatocellular carcinoma. J Gastroenterol 2008; 43: 378-389.

[10] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009; 59: 225-249.

[11] Cheng YY, Yu J, Wong YP, Man EP, To KF, Jin VX, Li J, Tao Q, Sung JJ, Chan FK, Leung WK. Frequent epigenetic inactivation of secreted frizzled-related protein 2 (SFRP2) by promoter methylation in human gastric cancer. Br J Cancer 2007; 97: 895-901.

[12] Qi J, Zhu YQ, Luo J, Tao WH. Hypermethylation and expression regulation of secreted frizzled-related protein genes in colorectal tumor. World J Gastroenterol 2006; 12: 7113-7117.

[13] Veeck J, Niederacher D, An H. Aberrant methylation of the Wnt antagonist SFRP1 in breast cancer is associated with unfavourable prognosis. Oncogene 2006; 25: 3479-3488.

[14] Yang Z, Wang Y, Fang J, Chen F, Liu J, Wu J, Wang Y. Expression and aberrant promoter methylation of Wnt inhibitory factor-1 in human astrocytomas. J Exp Clin Cancer Res 2010; 29: 23-26.

[15] Veeck J, Geisler C, Noetzel E, Alkaya S, Hartmann A, Knüchel R, Dahl E. Epigenetic inactivation of the secreted frizzled-related protein-5 (SFRP5) gene in human breast cancer is associated with unfavorable prognosis. Carcinogenesis 2008; 29: 991-998.

[16] Su HY, Lai HC, Lin YW, Liu CY, Chen CK, Chou YC, Lin SP, Lin WC, Lee HY, Yu MH. Epigenetic silencing of SFRP5 is related to malignant phenotype and chemoresistance of ovarian cancer through Wnt signaling pathway. Int J Cancer 2010; 127: 555-567.

[17] Zi XL, Guo Y, Simoneau AR, Hope C, Xie J, Holcombe RF, Hoang BH. Expression of Frzb/secreted Frizzled-Related Protein 3, a secreted Wnt antagonist, in human androgen-independent prostate cancer PC-3 cells suppresses tumor growth and cellular invasiveness. Cancer Res 2005; 65: 9762-9770.

[18] Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008; 321: 1801-1806 .

[19] Bilimoria KY, Bentrem DJ, Ko CY, Ritchey J, Stewart AK, Winchester DP, Talamonti MS. Validation of the 6th edition AJCC Pancreatic Cancer Staging System: report from the National Cancer Database. Cancer 2007; 110: 738-744.

[20] Ardengh JC, de Paulo GA, Ferrari AP. Pancreatic carcinomas smaller than 3.0 cm: endosonography (EUS) in diagnosis, staging and prediction of resectability. HPB (Oxford) 2003; 5: 226-230

[21] Lamerz R. Role of tumour markers: cytogenetics. Ann Oncol 1999; 10: 145-149.

[22] Pasanen PA, Eskelinen M, Partanen K, Pikkarainen P, Penttilä I, Alhava E. Tumor-associated trypsin inhibitor in the diagnosis of pancreatic carcinoma. J Cancer Res Clin Oncol 1994; 120: 494-497.

[23] Shahangian S, Fritsche JH Jr, Hughes JI, Gelder FB. Pancreatic oncofetal antigen and carbohydrate antigen 19-9 in sera of patients with cancer of the pancreas. Clin Chem 1989; 35: 405-408.

[24] Kuhlmann KF, van Till JW, Boermeester MA, de Reuver PR, Tzvetanova ID, Offerhaus GJ, Ten Kate FJ, Busch OR, van Gulik TM, Gouma DJ, Crawford HC. Evaluation of matrix metalloproteinase 7 in plasma and pancreatic juice as a biomarker for pancreatic cancer. Cancer Epidemiol Biomarkers Prev 2007; 16: 886-891.

[25] Goggins M. Molecular markers of early pancreatic cancer. J Clin Oncol 2005; 23: 4524-4531.