Overexpression of inhibin βA and SPARC in pancreatic cancer patients with shorter disease free interval and survival

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Overexpression of inhibin βA and SPARC in pancreatic cancer patients with shorter disease free interval and survival

Directed by Professor Si Young Song

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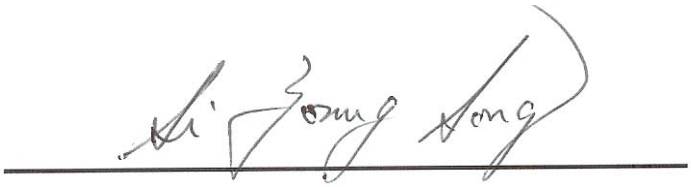

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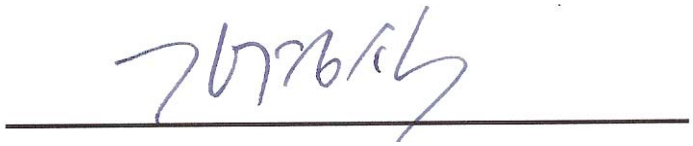

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Myoung Hwan Kim

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Overexpression of inhibin βA and SPARC in pancreatic cancer patients with shorter disease free interval and survival

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Inhibins βA belong to the transforming growth factor-β(TGF-β) superfamily of multifunctional cytokines that bind to transmembrane receptors with serine/threonine kinase activity. SPARC(Secreted protein acidic and rich in cysteine) is a phosphorylated, acidic, glycine-rich glycoprotein of 43 kDa that is secreted by endothelial cells. We characterized up-regulation of inhibin βA and SPARC using cDNA microarray originating from human pancreatic tissues. We aimed to validate expression of inhibin βA and SPRAC using immunohistochemistry with tissue microarray and evaluate that inhibin βA and SPARC can predict for survival and disease free interval (DFI) of patients with pancreatic cancer. Thirty nine patients who have underwent pancreatic cancer surgery included and clinical characteristics, recurrence and duration of survival were analyzed. We used tissue microarrays out of pancreatic cancer tissues resected from patients. Immunohistochemical stain with anti-inhibin βA-antibody and anti-SPARC-antibody would be used for validate the expression of inhibin βA and SPARC. The stains were reviewed by two pathologists blind to the original diagnosis and stained cells were scored semiquantitatively as negative, focal positive, diffuse positive, (0-2) using a

previously published method (Human pathol 2004;35:357-366). DFI and survival were calculated with the Kaplan-Meier method and their differences were evaluated by the log rank test. Of the 39 patients (28 men, 11 women; mean age 60.6 [8.4] years), stage(TNM staging) I was 3, stage II was 18, stage III was 10, and stage IV was 8, respectively. Immunohistochemical stain for inhibin βA was diffuse positive in 21 patients, focal positive in 12 patients and negative in 6 patients. Immunohistochemical stain for SPARC was diffuse positive in 9 patients, focal positive in 14 patients and negative in 16 patients. Patients with inhibin βA overexpression (diffuse positive) tended to be shorter duration of DFI than without inhibin βA overexpression (median 6 months vs 10 months, *p*=NS) and trended toward shorter survival (median 14 months vs 23 months) although this did not reach significance. Patients with SPARC overexpression tended to be shorter duration of DFI than without SPARC overexpression (median 7 months vs 9 months, *p*=NS) and trended toward shorter survival (median 10 months vs 19 months) although this did not reach significance. These data show inhibin βA and SPARC was overexpressed in patients with pancreatic ductal adenocarcinoma and suggest inhibin βA and SPARC overexpression tend to be correlated with DFI and survival.

Key Words: pancreatic cancer, inhibin beta A, SPARC

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Ⅰ. INTRODUCTION

Pancreatic cancer is one of the most malignant cancer and 4th leading cause of death in US.[1] In 2000, incidence of pancreatic cancer in Korea was 2.4% and it was 9th most common cancer among all type of cancer.[2] At present, no tumor specific marker for early detection are known that afford an opportunity of surgery, as the only chance of cure, and no effective treatments are reported at advanced disease. Furthermore, only 10% of individuals diagnosed with pancreatic cancer are candidates for resection. Even though after curative surgery, the recurrence rate is so high. So the research to elucidate the determinants for influencing on prognosis after curative surgery is required.

So the method to identifying high risk group of pancreatic cancer should be developed and we should clearly understand the histologic characteristics and molecular mechanism of precursor lesion of pancreatic cancer.[3] Recently, various method of analysis of gene expression such as cDNA microarray, SAGE(Serial Analysis of Gene Expression), oligoncleotide microassay, has led to identified novel tumor marker in various type of cacner.[4] Genes expressed at levels greater in the pancreatic cancers as compared to normal

tissues were identified in pancreatic cancer cell line and pancreatic cancer tissue using cDNA microarray and validation of those gene with overexpression was performed by immunohistochemical staining and *in situ* hybridization in pancreatic cancer.[5-7]

To validate the specific gene overexpression among many upregulated genes using whole tissue section is not efficient because it needs much time and high cost. Recently developed tissue microarray (TMA) technology has the potential to significantly accelerate studies seeking for associations between molecular changes and clinical endpoints.[8,9] In pancreatic cancer, it is TMA technology provides valid and cost-effective means to screen large numbers of novel tumor markers, even in tumors such as pancreatic adenocarcinomas that characteristically have abundant desmoplastic stroma.[10]

Inhibins and activins are polypeptide hormones which belong to the transforming growth factor-β(TGF-β) superfamily. Inhibins and activins are involved in regulating diverse functions during embryonic development and adult tissue homeostasis.[11] Pancreatic cancer samples markedly over-expressed the activin/inhibin βA subunit, whereas the βB subunit was only moderately increased in comparison to normal pancreatic samples.[12]

SPARC (Secreted protein acidic and rich in cysteine or osteonectin) is a phosphorylated, acidic, glycine-rich glycoprotein of 43 kDa that is secreted by endothelial cells and is present in large amounts in the parietal endoderm of mouse embryos and in human placenta. SPARC is a frequent target for aberrant methylation in pancreatic cancer and that SPARC expression in fibroblasts adjacent to pancreatic cancer cells is regulated through tumor-stromal interactions because SPARC mRNA expression in fibroblasts is markedly augmented as a result of soluble factors released from pancreatic cancer cells. Immunohistochemical labeling revealed that the SPARC protein was overexpressed in the stromal fibroblasts immediately adjacent to the neoplastic epithelium in primary pancreatic cancers, but rarely expressed in

the cancers themselves.[13]

In this study, we assumed that inhibin βA and SPARC was overexpressed in patients with pancreatic cancer and we aimed to validate expression using immunohistochemistry with TMA and evaluate that inhibin βA can predict for survival and disease free interval (DFI) of patients with pancreatic cancer This study will be helpful to find new prognostic indicator after surgical resection.

Ⅱ. MATERIAL AND METHOD

1. Subjects

The study included 39 patients diagnosed as pancreatic cancer and have received curative surgical resection and that had complete clinical data and follow up. The median follow-up duration was 16 months(2-73).

There were 28 males and 11 females. The minimum and maximum ages were 41 and 78 years respectively with the average age of 60.6±8.4 years.

2. Selection of upregulated gene in cDNA microarray

cDNA microarray was performed according to methods described in the Affymetrix® Genechip Expression Analysis Technical Manul (Affymetrix, Santa Clara, CA, USA).

Comparison of the gene expression profile between 25 pancreatic adenocarcinoma tissues and 18 normal pancreatic tissues showed significant and consistent expression changes. In pancreatic cancer, 892 genes were overexpressed at least two fold more than in normal tissues. Among those genes, we selected inhibin beta A and SPARC(Secreted Protein Acid Rich Cysteine, osteonectin).

3. Tissue microarray and immunohistochemistry

Accumax™ array (ISU ABXIS inc., Seoul, Korea) was used as tissue microarray consisted of resected pancreatic cancer tissue and matched nontumor pancreatic tissue. The diameter of each spot on the slide was 1.0 mm.

Pancreas cancer tissues of 64 different cases and corresponding 58 non-neoplastic tissues(2 spots for 64 cases (128 spots) and 58 non-neoplastic spots (4 spots)). Each tissue section is extracted from various donor blocks and transferred into a ready-made recipient block. The donor blocks are generally conventional, formalin-fixed, paraffin-embedded blocks.

Paraffin sections of thickness 5mm were dewaxed in xylene, and hydrated in

graded ethanol. They were then allowed to cool for 20 min and rinsed in phosphate-buffured saline (PBS). Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol. Antigen retrieval was carried out by autoclaving in 0.01 M trisodium citrate buffer (pH=6.0) at 60℃ for 3 minutes. Non-specific binding sites blocked with normal 10% donkey serum in PBS at room temperature for 30 min. Antibodies used were as follows: rabbit anti-SPARC polyclonal antibody (1:200, SC-25574, Santa Cruz Biotechnology, Inc., CA, USA), purified goat anti-inhibin βA antibody (1:1000, SC-6308, Santa Cruz Biotechnology, Inc., CA, USA), followed by secondary antibodies conjugated to biotin. Peroxidase-conjugated streptavidin was used with 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma) as chromogen for detection. All slides were counterstained with hematoxylin.

Two pathologists who did not have clinical information graded immunoreactivity. The stains were reviewed by two pathologists blind to the original diagnosis and stained cells were scored semiquantitatively as negative, focal positive, diffuse positive, (0-2) using a previously published method (Human pathol 2004;35:357-366). negative (<1% neoplastic cells or peritumoral desmoplastic tissue with moderate or strong labeling), focal positive (1% to 25% of neoplastic cells or peritumoral fibroblasts with moderate or strong labeling), and diffuse positive (26% to 100% of neoplastic cells or peritumoral desmoplasia with moderate or strong labeling

4. Statistical analysis.

Survival was calculated from the date of resection to the date of death or date of the latest follow-up. Disease-free interval and overall survival were calculated with the Kaplan-Meier method and their differences were evaluated by the log rank test. The correlation between inhibin βA and SPARC expressions and various clinicopathologic characteristics was analyzed by using either the chi-square test or Student t-test. The statistical analyses were performed SPSS software version 11.5 (SPSS Inc, Chicago, Ill). A *p*-value

less than .05 was regarded as statistically significant.

Ⅱ. RESULT

1. General characteristics of subjects

From January 1997 to December 2002, total 52 patients diagnosed as pancreatic cancer and have received curative surgical resection. Of the 52 patients, 39 patients that had complete clinical data and follow up enrolled. Of the 39 patients, 28 were male and 11 were female and the male to female ratio is 2.5:1. The mean age at the time of surgery was 60.6± 8.4 years.

The locations of pancreatic cancer were head (26 cases, 66.7%), body (2 cases, 5.1%), and tail of pancreas (11 cases, 28.2%). Three patients (7.7%) had stage I disease, 18 (46.2%) had stage II disease, 10 (25.6%) had stage III disease, and 8 (20.5%) had stage IV disease.

The duration of follow-up period was from 2 months to 73 months (median 16 months) and median survival was 13.8 months. Postoperative recurrence was occurred in 20/39 (51.3%) patients. The duration of postoperative recurrences was 2 months to 23 months (mean 7.6±5.5 months). Peritoneal and distant metastasis occurred in 4 and 5 patients, respectively. Local recurrence including lymph node metastasis was found in 8 patients. Concomitant local and peritoneal recurrence occurred in two patients.

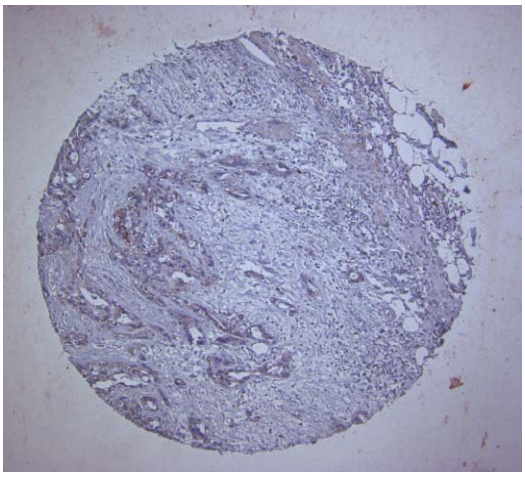
2. Verification of inhibin βA and SPARC expression at the protein level by IHC using TMA

The cores from tissue microarray included pancreatic cancer and nontumor tissue from the same patient on the same slide, for comparison of expression at the protein level and localization by IHC staining.

The staining pattern of inhibin βA demonstrated prominent staining with the cytoplasm of tumor cell(Fig. 1). Normal ductal epithelium & acinar cell was negative. The internal positive control was pancreatic islet cells.

The pattern for SPARC expression in tumor tissue showed prominent staining of stromal fibroblasts both within the tumor and adjacent to it. Tumor cells staining were predominantly noted in the cytoplasm(Fig. 2). Acinar cell was negative. The internal positive control was pancreatic islet cells.

(a)



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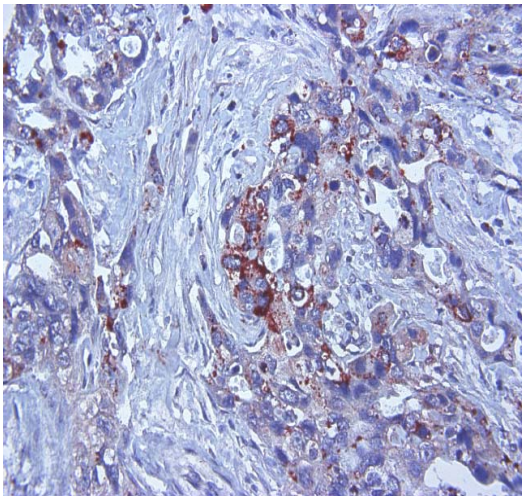
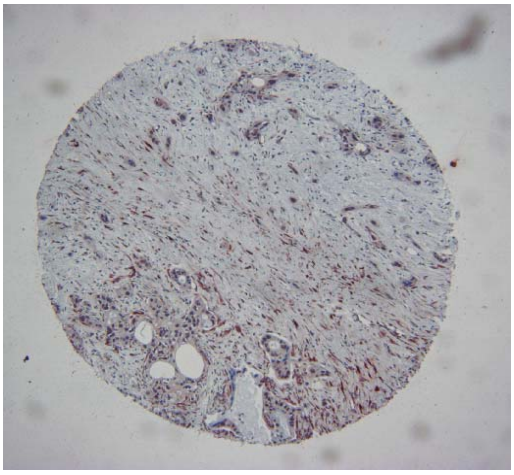


Figure 1. Immunohistochemical staining for inhibin βA in TMA in patients with pancreatic adenocarcinoma. (a) and (b) show that mainly cytoplasmic expression of inhibin βA (a, x100 ; b, x400).

(a)



(b)

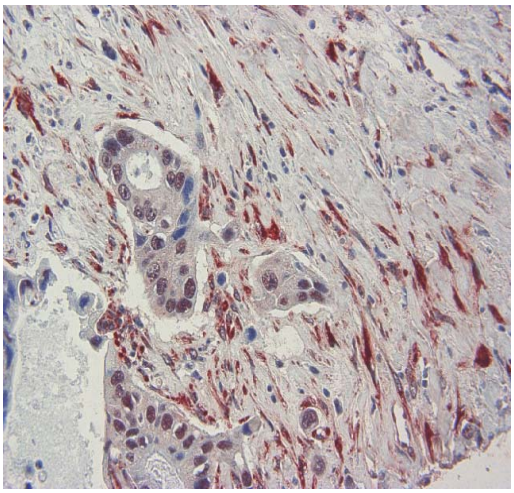


Figure 2. Immunohistochemical staining for SPARC in TMA in patients with pancreatic adenocarcinoma. (a) and (b) show that show that prominent staining of nuclus and stroma within the tumor(a, x100 ; b, x400).

Immunohistochemical stain for inhibin βA was diffuse positive in 21 patients, focal positive in 11 patients and negative in 7 patients. Most non-neoplastic pancreatic duct cells were not stained with the anti-inhibin βA antibody. In contrast, the tumor cell of ductal carcinoma showed strong cytoplasmic expression. Thirty two of 39 pancreatic cancers (82%) were positive for inhibin βA. No significant correlation was found between inhibin βA expression and age, sex, T state, N stage, M stage and overall staging (Table 1). Immunohistochemical stain for SPARC was diffuse positive in 9 patients, focal positive in 14 patients and negative in 16 patients. Twenty three of 39 pancreatic cancers (59%) were positive for SPARC. No significant correlation was found between SPARC expression and age, sex, T stage, N stage, M stage and overall staging (Table 2).

Table 1. Comparison of inhibin βA expression and clinicopathologic characteristics

	Inhibin βA			p
	Diffuse Positive (n=21)	Focal Pogative (n=11)	Negative (n=7)	
Sex				0.18
Male	14	7	7	
Female	7	4	0	
Age	59.1 ± 9.7	62.4 ± 6.8	62.0 ± 6.0	0.52
Invasiveness				0.45
T1+T2	1	2	1	
T3+T4	20	9	6	
N stage				0.07
N0	17	4	4	
N1	4	7	3	
TNM stage				0.08
I	1	1	1	
II	13	2	3	
III	1	6	3	
IV	6	2	0	

Table 2. Comparison of SPARC expression and clinicopathologic characteristics

	SPARC			p
	Diffuse Positive (n=9)	Focal Pogative (n=14)	Negative (n=16)	
Sex				0.37
Male	8	10	10	
Female	1	4	6	
Age	58.1 ± 8.4	58.5 ± 8.8	63.8 ± 7.3	0.07
Invasiveness				0.15
T1+T2	1	3	0	
T3+T4	8	11	16	
N stage				0.29
N0	7	10	8	
N1	2	4	8	
TNM stage				0.07
I	0	3	0	
II	5	5	8	
III	1	2	7	
IV	3	4	1	

3. Correlation of inhibin βA and SPARC expression with prognosis

At the time of this analysis, the median follow-up time was 16 months, ranging from 2-73 months. Postoperative recurrence was occurred in 20/39 (51.3%) patients. The duration of postoperative recurrences was 2 months to 23 months (mean 7.6±5.5 months).

Thirty-one out of thirty eight (81.6%) patients died of pancreatic cancer and the median survival time was 13.8 months, ranging from 2-73 months. The difference in disease free interval and overall survival between patients with inhibin βA positive tumors and those with inhibin βA negative tumors was calculated using the Kaplan Meier analysis.

Patients with inhibin βA overexpression tended to be shorter duration of DFI than without inhibin βA overexpression (median 6 months vs 10 months, *p*=NS) and trended toward shorter survival (median 14 months vs 23 months) although this did not reach significance. Patients with SPARC overexpression tended to be shorter duration of DFI than without SPARC overexpression (median 7 months vs 9 months, *p*=NS) and trended toward shorter survival (median 10 months vs 19 months) although this did not reach significance. Patients with both of inhibin βA and SPARC overexpression tended to be shorter duration of DFI than with neither inhibin βA or SPARC overexpression (median 6 months vs 12 months, *p*=NS) and trended toward shorter survival (median 10 months vs 13 months) although this did not reach significance.

Ⅰ Ⅴ. DISCUSSION

Activins and inhibins belong to the transforming growth factor- β(TGF- β) superfamily of secreted signaling molecules, which mediate important events in normal growth and development. Three subunits, inhibin-βA, inhibin-βB and inhibin-αβ, have been identified as the major components of the activin/inhibin family. Activins/inhibins signal through a family of transmembrane serine-threonine kinases with 2 distinct subgroups, known as type I and type II activin receptors.[12] An important component of this signaling cascade is represented by members of the Smad family of proteins. Activated activin type I receptor phosphorylates Smad2 and/or Smad3, which leads to the separate heterodimerization of these Smads with Smad4 (DPC4) and subsequent translocation of the complexes into the nucleus, where they effect transcriptional regulation.[3,12] It is known that the α subunit is a tumor suppressor gene in endocrine tumors and ovarian tumor such as stromal/granulosa cell tumors and also expression of inhibin βA was increased.[14-15] The absence of the activin/inhibin α subunit and the over-expression of activin/inhibin β subunits are thought to contribute to the development of these tumors, raising the possibility that perturbations in activin/inhibin expression may contribute to neoplastic transformation. [12]

Pancreatic cancer samples markedly overexpressed the activin/inhibin βA subunit.[12] Inhibin βA expression was confirmed at the protein level by immunohistochemical labeling of tissue microarrays and/or at the transcript level by RT-PCR.[16] The overexpression of inhibin βA was not the result of *SMAD4*/*DPC4* mutations, and it is unclear whether and/or how loss of expression of Dpc4 protein upregulates inhibin βA. Inhibin βA also stimulates inflammatory corneal angiogenesis by increasing vascular endothelial growth factor (VEGF) levels.[17] Overexpression of VEGF has been shown to be associated with the aggressive biology of human pancreatic adenocarcinoma.[18] Whether inhibin βA is involved in regulating VEGF

overexpression in pancreatic cancers needs to be established.[16]

In our study, the staining pattern of inhibin βA was cytoplasmic staining of tumor cells. We verified that inhibin βA expression in 82% patients with pancreatic cancer at the protein level by IHC using TMA. We could not identified that statistically significant differences of DFI and survival between patients with inhibin βA overexpression and patients without inhibin βA overexpression. Cellular differentiation was poorer in patients with inhibin βA overexpression than that of patients without inhibin βA overexpression. This study suggested that the inhibin βA expression might be associated with biology of human pancreatic adenocarcinoma, although the relationship between inhibin βA expression and prognosis is not established.

SPARC, also known as osteonectin or BM-40 (basement membrane), is a calcium-binding and collagen-binding glycoprotein associated with stress-related exteracelluar matrix. SPARC is a matricellular glycoprotein involved in diverse biological processes, including tissue remodeling, wound repair, morphogenesis, cellular differentiation, cell proliferation, cell migration, and angiogenesis. Similar to wound healing, substantial ECM turnover and rearrangement also occur during tissue invasion by tumor cells. High levels of SPARC are often associated with metastatic tumors. Angiogenesis, the growth of new vessels from extant vasculature, is a major factor in tumor growth and metastasis. Since neovascularization includes endothelial cell invasion and ECM remodeling, it was not surprising to find that SPARC is expressed by endothelial cells in culture and in tissues.[19] SPARC overexpressed not only in various gastrointestinal cancers including gastric cancer, esophageal cancer, hepatocellular carcinoma but also in breast cancer and lung cancer and is associated with progression and poor prognosis.[20,21,22,23,24] SPARC is strongly expressed by the stromal myofibroblasts of human HCC, especially of high grade. This expression could play a role in tumor progression.[15] SPARC was specifically expressed within the juxtatumoral stromal cells, indicating a critical “regionality” of gene expression within the stromal response itself.[16]

SPARC is a frequent target for aberrant methylation in pancreatic cancer and that SPARC expression in fibroblasts adjacent to pancreatic cancer cells is regulated through tumor-stromal interactions because SPARC mRNA expression in fibroblasts is markedly augmented as a result of soluble factors released from pancreatic cancer cells. Immunohistochemical labeling revealed that the SPARC protein was overexpressed in the stromal fibroblasts immediately adjacent to the neoplastic epithelium in primary pancreatic cancers, but rarely expressed in the cancers themselves.[13]

In our studies, the pattern for SPARC expression in tumor tissue showed prominent staining of stromal fibroblasts both within the tumor and adjacent stromal tissues. We verified that SPARC expression in 59% patients with pancreatic cancers at the protein level by IHC using TMA. But we could not identified that statistically significant differences of DFI and survival between patients with SPARC overexpression and patients without SPARC overexpression. Limitation of this study showed that evaluation of SPARC applied to patients with respectable pancreatic ductal adenocarcinomas. Whether SPARC status is important in patients with unresectable disease is not known. This study suggested that the peritumoral stroma may play a role in the phenotypic behavior of a malignancy, although the relationship between stromal SPARC expression and prognosis is not known. Further studies are needed to evaluate their role and mechanism.

Ⅴ. CONCLUSION

Thirty nine patients with pancreatic caner who had undergone surgical resection of pancreas in Severance Hospital of Yonsei University, college of medicine between January 1997 and December 2002 were evaluated in order to study the relationship between inhibin βA and SPARC expression and clinicopathological findings, and investigate the prognostic significance of inhibin βA and SPARC in patients with pancreatic cancer and the results as below were obtained.

Of the 39 patients, 28 were male and 11 were female and the male to female ratio is 2.5:1. The mean age at the time of surgery was 60.6± 8.4 years. The locations of pancreatic cancer were head (26 cases, 66.7%), body (2 cases, 5.1%), and tail of pancreas (11 cases, 28.2%). Three patients (7.7%) had stage I disease, 18 (46.2%) had stage II disease, 10 (25.6%) had stage III disease, and 8 (20.5%) had stage IV disease.

Thirty two of 39 pancreatic cancers (82%) were positive for inhibin βA. No significant correlation was found between inhibin βA expression and age, sex, T state, N stage, M stage and overall staging. The degree of cellular differentiation was significantly correlated to inhibin βA positivity (p=0.01) Twenty three of 39 pancreatic cancers (59%) were positive for SPARC. No significant correlation was found between SPARC expression and age, sex, T state, N stage, M stage and overall staging.

But we could not identify that statistically significant differences of DFI and survival between patients with inhibin βA or SPARC overexpression and patients without inhibin βA or SPARC overexpression, respectively.

In conclusion, this study demonstrated that inhibin βA and SPARC were overexpressed in patients with pancreatic ductal adenocarcinoma. Although additional studies are needed to clarity that inhibin βA and SPARC were statistically significant as prognostic marker for survival and recurrence, this result suggest that inhibin βA and SPARC overexpression tend to be

correlated with DFI and survival.

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국문요약

췌장암에서 inhibin βA와SPARC의 발현양상과 임상적 특징과의 상관관계분석

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Inhibin βA는 serine/threonine 활성효소 기능을 가지는 세포막간 수용체에 결합하는 다기능의 싸이토카인으로 구성된 TGF-β superfamily에 속하며 췌장암에서 발현이 증가된다. SPARC(Secreted protein acidic and rich in cysteine)는 내피세포에 의해 분비되는 당단백질로 췌장암에서 흔한 비정상 메틸화의 대상이 된다. 저자 등은 췌장암 조직에서 cDNA microarray를 이용하여 Inhibin βA와 SPARC의 유전자 발현을 확인하였고 조직미세배열기술(Tissue microarray; TMA)과 면역화학염색법을 이용하여 Inhibin βA와 SPARC의 유전자의 단백질 발현을 확인하고 Inhibin βA와 SPARC의 발현이 췌장암 환자의 생존과 재발을 예측할 수 있는지 알아보고자 하였다.

수술을 시행한 췌장암 환자 39명의 임상적 특징, 재발과 생존기간에 대해 조사하였다. 췌장암 환자의 조직에서부터 TMA을 제작하였고 항inhibin βA항체와 항SPARC항체를 사용하여 면역화학염색을 시행하여 inhibin βA와 SPARC의 단백질 발현을 확인하였고 임상적 특징과의 상관관계를 분석하여 다음과 같은 결과를 얻었다.

39명의 환자 중 28명은 남자, 11명은 여자였고 평균연령은 60.6±

8.4세였다. 췌장암의 위치는 두부가 26예(66.7%), 췌부가 2예(5.1%), 미부가 11예(28.2%)였고 췌장암의 병리학적 병기는 1기가 3예(7.7%), 2기가 18예(46.2%), 3기가 10예(25.6%), 4기가 8예(20.5%)였다.

39명의 환자 중 32명(82%)에서 inhibin βA 양성이었으며 inhibin βA의 발현과 나이, 성별, T병기, N병기, M병기, TNM병기는 유의한 상관관계가 없었다. 39명의 환자 중 23명(59%)에서 SPARC양성이었으며 SPARC의 발현과 나이, 성별, T병기, N병기, M병기, TNM병기는 유의한 상관관계가 없었다. inhibin βA와 SPARC의 발현과 췌장암 환자의 생존기간과 재발기간과의 유의한 상관관계는 나타나지 않았으나 inhibin βA와 SPARC이 발현이 나타난 환자에서 inhibin βA와 SPARC이 발현이 환자에 비해 췌장암 환자의 생존기간과 재발기간이 좀 더 긴 경향을 보였다.

이상의 결과를 종합하면 본 연구에서 췌장암환자에서 inhibin βA와 SPARC발현이 증가함을 확인하였고 inhibin βA와 SPARC가 췌장암 환자에서 생존과 재발을 예측할 수 있는 예후인자로서의 가능성을 추측할 수 있으며 추가적인 연구를 통해 이를 증명하는 것이 필요할 것으로 판단된다.

핵심되는 말: 췌장암, inhibin beta A, SPARC