PDGFRβ expression in tumor stroma of pancreatic adenocarcinoma as a reliable prognostic marker

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Abstract

Pancreatic adenocarcinoma is a lethal disease that often develops a desmoplastic reaction in tumor stroma. In general, desmoplasia is thought to promote tumor growth. However, its molecular pathology and prognostic potential has not been fully investigated. Here we investigate 26 cases of pancreatic ductal adenocarcinoma, and examine the clinicopathological association between survival and expression levels of several molecular markers for stromal cells. These include alpha smooth muscle actin (SMA) and platelet-derived growth factor (PDGF) receptor β (PDGFRβ). Both are markers of activated fibroblasts or pancreatic stellate cells (PSCs) that play an important role in desmoplasia. The staining patterns of both molecular markers were not uniform so we categorized them into 3 grades (high, middle, and low) according to intensity. Interestingly, Kaplan-Meier analysis revealed that higher expression of PDGFRβ matched shorter prognosis ($p = 0.0287$, log-rank test) as well as lymphatic invasion and lymph node metastasis, whereas SMA did not ($p = 0.6122$). Our results suggest the prognostic potential of cancer stroma via PDGF-B signaling. Regulation of PDGF-B-associated signaling crosstalk between cancer cells and stroma cells, therefore, may indicate a possible therapeutic target for desmoplastic malignant tumors such as pancreatic adenocarcinoma.

Keywords: pancreatic adenocarcinoma, tumor stroma, prognosis, PDGFRβ, SMA

Introduction

Pancreatic adenocarcinoma is a lethal disease with a median survival time of approximately 6 months [1], but exactly why the disease is so difficult to treat is not fully understood.

Staging of the cancer, determined by tumor-node-metastasis (TNM) staging system, can be divided into prognostic subgroups [2], and several markers in tumor cells are also useful [3,4]. Lymph vessel invasion (ly) is useful as a histology marker, but neither intrapancreatic neural invasion (ne) nor blood vessel invasion (v) have proven clinically useful histology signs [5].

Histopathologically, pancreatic adenocarcinoma is often accompanied by a dense desmoplastic reaction [6], and this constitutes the characteristic stromal structure of the cancer. This desmoplasia forms approximately eighty percent of the tumor mass, and is thought to play an active role in carcinogenesis [7]. In human tumors in general, desmoplastic reaction is associated with recruitment and activation of fibroblasts and significant deposition of extracellular matrix (ECM) [8].

Normal fibroblasts, which are embedded in ECM, are activated by various stimuli that accompany insult to tissue. The differentiation between fibroblasts and myofibroblasts *in vivo* is made by positivity to alpha smooth muscle actin (SMA), and those that are positive are myofibroblasts [9]. Fibroblasts activate and differentiate into the myofibroblast phenotype as part of inflammation, where platelet-derived growth factor (PDGF) induces differentiation [10,11].

The receptor for PDGF-BB, PDGFRβ, has an important role in regulating mesenchymal cells, including pericytes, fibroblasts, and vascular smooth muscle cells, during development [12,13].

Activation of PDGFR may be involved in cancer progression via activation of these mesenchymal cells in most solid tumors [14], besides directly stimulating tumor cell growth: as reported for tumors responsive to Imatinib (Gleevec®), an inhibitor of PDGFR [15].

Expression of SMA and PDGFRβ may thus be important in the myofibroblasts of pancreatic adenocarcinoma stroma. However, their clinical significance has not been investigated in depth. Here we investigate the prognostic potential of tumor stroma markers in pancreatic cancer via immunostaining of SMA and PDGFRβ, and show that expression of PDGFRβ is the more valuable.

Materials and Methods

Patients

Twenty-six patients with primary pancreatic adenocarcinoma, who underwent pancreaticoduodenectomy (PD) or pylorus-preserving PD (PpPD) in the First Department of Surgery at the Hokkaido University Hospital, Japan, were included in this study. Of the total: 18 were male and 8 female, 14 had adjuvant chemotherapy and 12 did not. The patients ranged in age from 45-81Y. The samples were obtained under blanket, written informed consent, and the experiment approved by the Ethics Committee of Hokkaido University. The clinicopathology of the cases is summarized in Table 1. Pathology staging was made according to the Japan Pancreas Society [16] following TNM classification [17].

Antibodies and Immunohistochemistry

Tissue sections were prepared from paraffin blocks and stained with hematoxylin and eosin (HE): double immunohistochemical staining was performed using the primary antibodies for PDGFRβ (CST Japan, Tokyo), or SMA (DAKO Japan Co., Kyoto, Japan) and CD31 (DAKO) followed by detection of the antibody with alkaline phosphatase-conjugated Fast Blue BB / Naphthol AS-MX-phosphate readout system for PDGFRβ and SMA in red, or a peroxidase-conjugated streptavidin-DAB readout system (DAKO) for CD31 in brown, with nuclear counter staining in blue.

Stain intensity of SMA and PDGFRβ in cancer stroma was divided into three grades: weak

(1+), intermediate (2+) and strong (3+). Density of CD31 positive vasculature was evaluated in the same manner as with SMA or PDGFRβ. The histological factors ly (lymphatic invasion), v (vascular invasion), ne (neural invasion) were determined according to routine pathological diagnostic protocol [16].

Statistical Analysis

χ^2 test was used for pre-screening candidate factors, with $p < 0.05$ as statistical significance. Survival curves were calculated using Kaplan and Meier method, and differences between curves were analyzed using the log-rank test, with $p < 0.05$ as statistical significance.

Results

In addition to those factors for routine pathological diagnosis, ly, v, ne, T, N, and M, we divided the stroma samples into three grades (1 to 3) based on immunohistochemistry: that is, positivity for SMA or PDGFRβ, and microvascular density determined by CD31 positive structure. Fig. 1 shows four representative cases with overall survival (OS) in months (m). Expression levels of SMA and PDGFRβ did not necessarily correlate.

We then compared distribution values for ly, v, ne, T, N, CD31 density, SMA, or PDGFRβ, by χ^2 test between patient groups with shorter prognosis than the median period of 15 months (n = 14), plus the group that survived for more than 15 months (n = 12). The results are shown in Figure 2. Analysis revealed that stromal positivity for PDGFRβ was a factor, with a difference of $p < 0.05$ between the two groups, although SMA was not statistically significant. Staging and ly were confirmed to be also statistically significant, as reported previously [2,5]. We also checked for relationship of adjuvant chemotherapy to survival, but there was none (Table 1).

We therefore tested for prognostic impact of PDGFRβ positivity in stroma in pancreatic adenocarcinoma using Kaplan-Meier survival analysis. Patients strongly positive for PDGFRβ (3+) were significantly worse off in terms of survival than those with intermediate or weak positivity (2+ and 1+) ($p = 0.0287$ by log-rank test): Figure 3A. Median survival rates were 22.5 months for lower PDGFRβ expression and 13.0 months for higher PDGFRβ expression). On the other hand, strength

of SMA expression was not significant ($p = 0.6122$: Figure 3B. Median survival rates were 17.0 months for lower SMA expression and 15.0 months for higher SMA expression.

Discussion

Pancreatic adenocarcinoma is a devastating disease characterized by tumor desmoplasia in stroma: a significant increase in connective tissue that envelopes tumor cell nests [18]. In this patient study we show that more expression of PDGFRβ in stroma of pancreatic adenocarcinoma correlates with a worse prognosis, while expression levels of SMA do not.

Although the general impact of tumor stroma in pancreatic cancer is poorly understood, there is previous work on stroma as a prognostic marker in pancreatic ductal adenocarcinoma [19]. In this work, ratio of SMA-stained area to collagen-stained area is usefully defined as the "activated stroma index", and a combination of high "stromal activity" and low collagen deposition is linked with a worse prognosis, whereas a combination of high collagen deposition and low stromal activity is linked with a better outcome. However, to our knowledge, there is no report as yet linking PDGFRβ expression and "stromal activity".

In tumor stroma, desmoplastic reaction is associated with activated fibroblasts positive for SMA, plus significant deposition of ECM including collagen [8]. In the case of pancreatic adenocarcinoma, pancreatic stellate cells (PSCs) are considered another key player in desmoplasia, together with activated myofibroblasts [18,20,21]. Both cells are positive for SMA and collagen [22]. In the pancreas, insult or inflammation stimulates quiescent PSCs to undergo morphological and functional change and become myofibroblast-like cells that express SMA [21]. Pancreatic tumor cells

induce proliferation of PSCs positive for PDGFRβ, and induce PSC production of ECM proteins via signaling that includes PDGF-BB [23]. PSCs *in vitro* also increases proliferation, invasion, and colony formation of pancreatic tumor cells, and protect them from attack by radiation and gemcitabine [24,25]. Furthermore, they create a tumor-supportive microenvironment by producing ECM [21]. In fact, coinjection of cancer cells with PSCs *in vivo* increase tumor size in a subcutaneous xenograft model [23] plus tumor incidence, growth, and metastasis in orthotopic models of pancreatic cancer [24,25].

It is interesting, therefore, that SMA does not correlate significantly with prognosis, while PDGFRβ does, although both SMA and PDGFRβ are important markers of activated myofibroblasts or PSCs. PDGFRβ positivity may therefore relate directly to interactive functions of myofibroblasts or PSCs with tumor cells and other stroma cells, while SMA may relate to some autonomous function such as contractile ability. The prognostic impact of PDGFRβ expression in tumor stroma has been reported for prostate and breast cancer [12,26]. PDGF-BB, the ligand of PDGFRβ functions primarily via paracrine mechanisms involving other cell types, such as fibroblasts and endothelial cells [27]. PDGF-BB induces proliferation of fibroblasts, but does not induce acquisition of an activated phenotype associated with excessive ECM deposition [28]. PDGF-BB are released from injured epithelial cells and infiltrate immune cells as part of the normal wound healing process [29]. Although PDGF-BB is secreted by cancer cells and correlates with cancer progression [30], most cancer cells do not express PDGFRβ. Therefore, it is possible that PDGFRβ expression in tumor

stroma may be a universal marker of tumor activity and correlate with disease prognosis.

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Conflict of Interests: None.

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Figures legends

Figure 1. Microphotographs of serial sections of four representative cases, with overall survival (OS) in months (m). Hematoxylin-eosin (HE) staining and immunostaining of alpha-smooth muscle actin (SMA) and platelet-derived growth factor receptor β (PDGFRβ) (red) together with CD31 staining (brown). Bars: 100 μm.

Figure 2. Histograms of various clinicopathological factors for two groups of cases divided at the median survival period of 15 months. P values were calculated by χ^2 test. *: $p < 0.05$.

Figure 3. Kaplan-Meier curves for PDGFRβ (A) and SMA (B). P values were calculated by log-rank test. *: $p < 0.05$.

Table 1. The clinicopathology of the cases.

The clinicopathological characteristics of the cases			
Sex			%
	Male	18	69.2
	Female	8	30.8
Median age at operation (years)			
	Average ± SD	62.8 ± 10.4	
	Median	61.5	
	Range	45-81	
Death			
	Yes	22	84.6
	No	4	15.4
Overall survival (months)			
	average ± SD	22.3 ± 19.7	
	Median	15	
	range	3-86	
Ly			
	0	8	30.8
	1	10	38.5
	2	6	23.1
	3	2	7.7
V			
	0	5	19.2
	1	8	30.8
	2	7	26.9
	3	6	23.1
Ne			
	0	2	7.7
	1	5	19.2
	2	11	42.3
	3	7	26.9
	x	1	3.8
T			
	0	0	0.0
	is	0	0.0
	1	1	3.8
	2	2	7.7
	3	11	42.3
	4	12	46.2
N			
	0	11	42.3
	1	5	19.2
	2	7	26.9
	3	3	11.5
M			
	0	2	7.7
	1	24	92.3
stage			
	I	1	3.8
	II	1	3.8
	III	8	30.8
	IV a	10	38.5
	IV b	6	23.1
CD31			
	1	4	15.4
	2	11	42.3
	3	11	42.3
SMA			
	1	4	15.4
	2	7	26.9
	3	15	57.7
PDGFRβ			
	1	8	30.8
	2	5	19.2
	3	13	50.0
Adjuvant chemotherapy			
	Yes	14	53.8

	No	12	46.2
	Median survival: 19m vs. 14m, p = 0.1351 (log-rank test).		

Figure 1

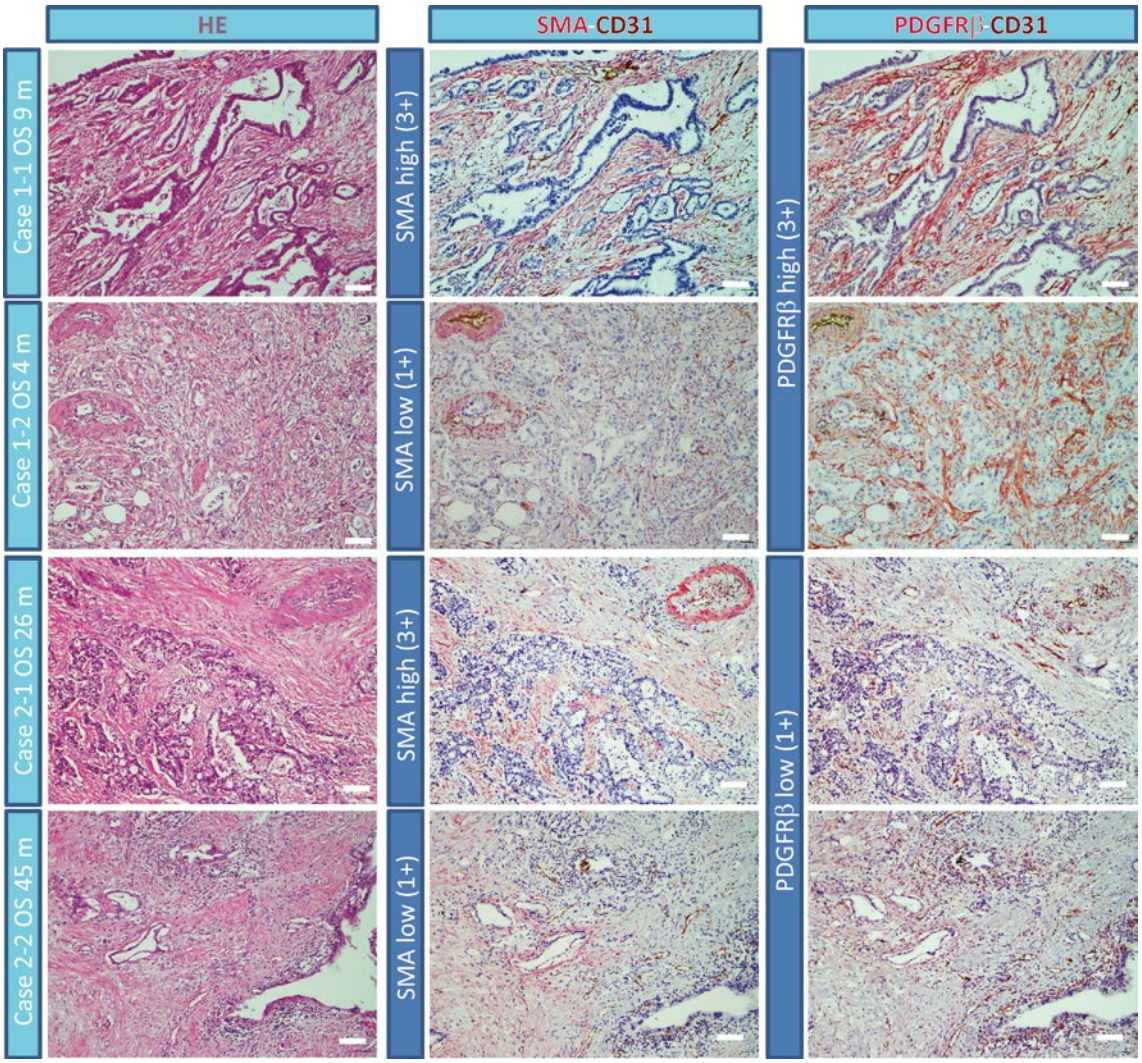

HE
SMA-CD31
PDGFRβ-CD31
Case 1-1 OS 9 m
Case 1-2 OS 4 m
Case 2-1 OS 26 m
Case 2-2 OS 45 m
SMA high (3+)
SMA low (1+)
SMA high (3+)
SMA low (1+)
PDGFRβ high (3+)
PDGFRβ low (1+)

Figure 2

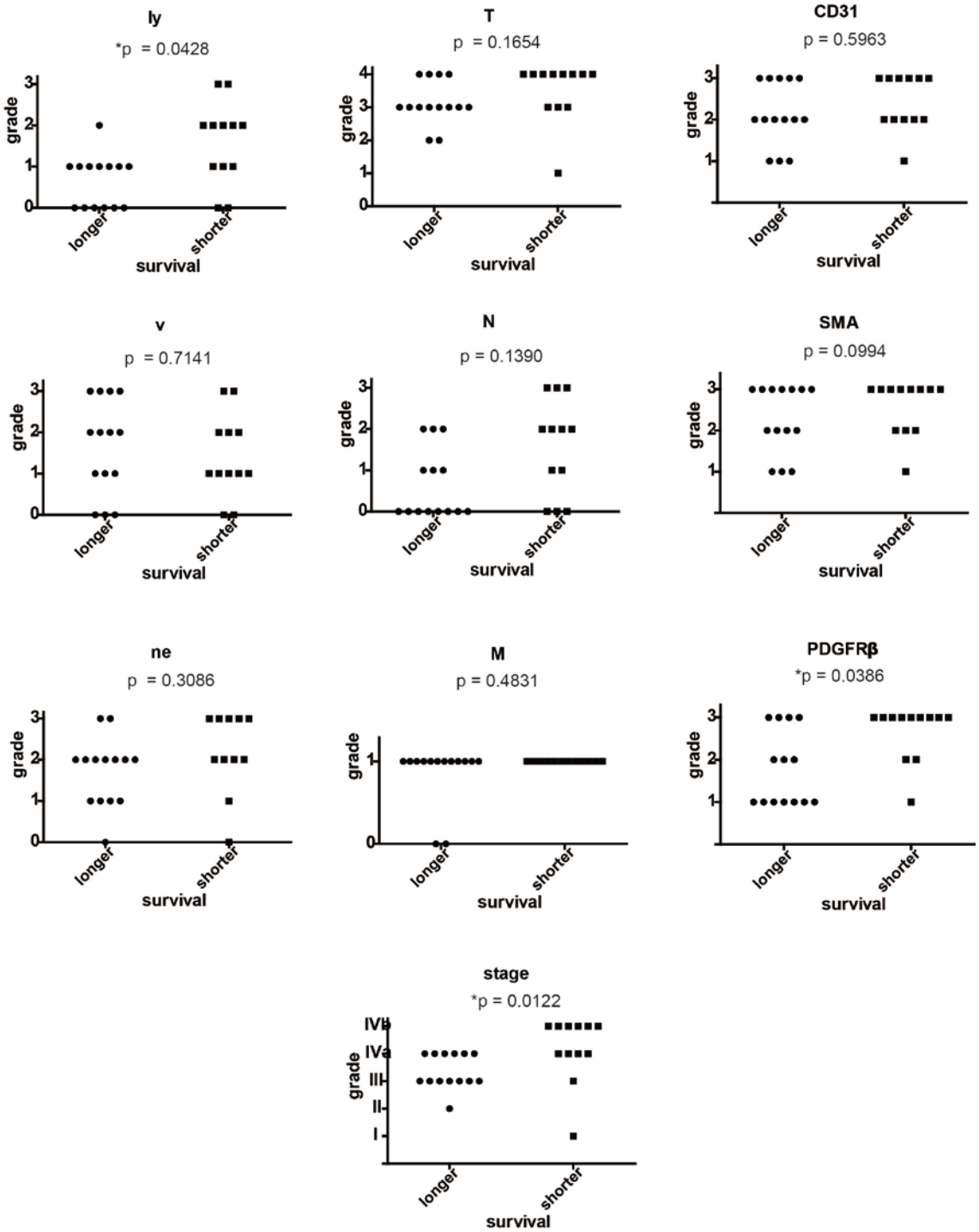
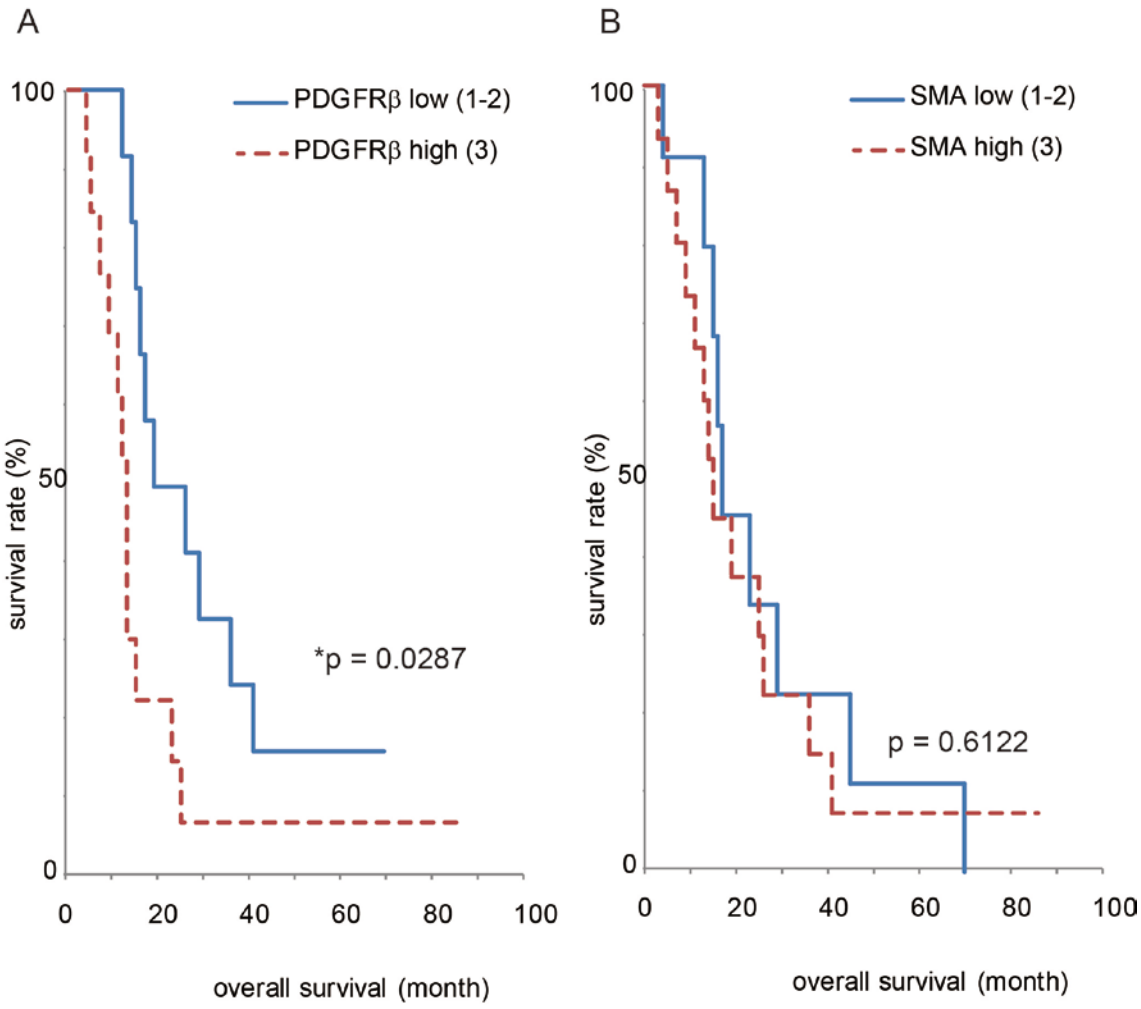

ly
*p = 0.0428
T
p = 0.1654
CD31
p = 0.5963
v
p = 0.7141
N
p = 0.1390
SMA
p = 0.0994
ne
p = 0.3086
M
p = 0.4831
PDGFRβ
*p = 0.0386
stage
*p = 0.0122
grade
survival
longer
shorter
IVb
IVa
III
II
I

Figure 3


A
100
50
0
survival rate (%)
PDGFRβ low (1-2)
PDGFRβ high (3)
*p = 0.0287
0
20
40
60
80
100
overall survival (month)
B
100
50
0
survival rate (%)
SMA low (1-2)
SMA high (3)
p = 0.6122
0
20
40
60
80
100
overall survival (month)