Journal of Gastrointestinal Cancer (2019) 50:478–484
https://doi.org/10.1007/s12029-018-0103-6

ORIGINAL RESEARCH

Intratumoral Heterogeneity of SMAD4 Immunohistochemical Expression and Its Role in Prediction of Recurrence Pattern in Patients with Resectable Pancreatic Cancer

Ilya Pokataev[1] · Asel Kudaibergenova[2] · Anna Artemyeva[3] · Anna Popova[1] · Alexey Rumyantsev[1] · Danil Podluzhny[4] · Nikolay Kudashkin[4] · Mikhail Fedyanin[1] · Alexey Tryakin[1] · Sergey Tjulandin[1]

Published online: 20 April 2018

Abstract

Background The aim of our study was to evaluate consistency of SMAD4 expression in different tumor areas and its correlation with recurrence pattern in patients after resection for pancreatic cancer (PC).

Methods Records of patients who underwent resection for nonmetastatic PC between 2001 and 2015 were analyzed. Formalin-fixed, paraffin-embedded tissue sections from different areas of primary tumor and lymph node metastases were analyzed immunohistochemically (IHC) for SMAD4 expression using TMA technology.

Results SMAD4 expression was assessed in 356 tissue sections obtained from 91 patients. SMAD4 expression was positive in all assessed tumor slides only in 7 of 26 patients (26.9%). There were 54 recurrences (9 locoregional, 41 distant, and 4 both local and distant) with median follow-up of 21.7 months. There was no correlation between SMAD4 expression and locoregional recurrence pattern ($p = 0.30$). SMAD4 status influenced neither distant recurrence-free survival ($p = 0.99$) nor overall survival ($p = 0.13$).

Conclusions Different areas inside primary tumor and lymph node metastases express SMAD4 heterogeneously. SMAD4 IHC expression is not a biomarker of the recurrence pattern after surgical resection for PC.

Keywords Pancreatic neoplasms · Smad4 protein · Recurrence pattern

Introduction

Majority of patients with pancreatic cancer (PC) ultimately have disease progression after surgical treatment [1]. The most common sites of disease progression are distant metastases in liver, lungs, and peritoneum. However, there is a subset of patients with locoregional pattern of tumor progression. In ESPAC-1 trial, 35% of patients experienced only locoregional recurrences without evidence of distant metastases [2]. In addition, there are no established options for adjuvant treatment of PC patients but chemotherapy. Efficacy of adjuvant radiotherapy was not demonstrated in clinical trials [3]. Moreover, no clinical or molecular marker can predict locoregional pattern of recurrence. There is an unmet need in biomarkers to select patients for local adjuvant therapy.

SMAD4 mutations are one of the most common events in PC cells [4]. SMAD4 is a tumor suppressor gene, part of transforming growth factor-β (TGFβ) pathway. TGFβ signaling mediates epithelial-to-mesenchymal transition (EMT) in PC, and thus, SMAD4 deficiency enhances cell metastatic potential. Unaffected SMAD4 expression can be a factor of locoregional pattern of disease progression [5–7]. However, these data were obtained from patients with locally advanced PC or those who succumbed to the disease. Predictive role of SMAD4 after surgical treatment of PC is not clear. The aim of this study was to evaluate the

✉ Ilya Pokataev
pokia@mail.ru

[1] Department of Clinical Pharmacology and Chemotherapy, Federal State Budgetary Institution "N.N. Blokhin National Medical Research Center of Oncology" of the Ministry of Health of the Russian Federation, Moscow, Russia

[2] Department of Cytology, Federal State Budgetary Institution "N.N. Blokhin National Medical Research Center of Oncology" of the Ministry of Health of the Russian Federation, Moscow, Russia

[3] Department of Pathology, N.N. Petrov Cancer Research Institute, Saint Petersburg, Russia

[4] Department of Surgery #7, Federal State Budgetary Institution "N.N. Blokhin National Medical Research Center of Oncology" of the Ministry of Health of the Russian Federation, Moscow, Russia

impact of SMAD4 expression on the pattern of PC recurrence after surgery and patient outcomes.

Methods

Patients

We analyzed medical records of PC patients treated at the Department of Liver and Pancreatic Tumors of N.N. Blokhin Russian Cancer Research Center between 2001 and 2015. Eligibility criteria for this retrospective study were the following: (1) TNM stage I, II, or III; (2) pancreatic resection (pancreaticoduodenectomy, distal or total pancreatectomy) for PC; (3) no mortality within 2 months after surgery; (4) availability of formalin-fixed paraffin-embedded (FFPE) tissue blocks appropriate for IHC analysis; and (5) adenocarcinoma histological subtype.

We extracted clinicopathologic data from prospectively maintained database of N.N. Blokhin Russian Cancer Research Center, electronic and retained medical records. We evaluated the following clinical parameters: age, gender, clinical T&N stage, tumor location (head, body, or tail of the pancreas), and history of (neo)adjuvant chemo- or radio-therapy. We also evaluated the following pathologic parameters: tumor size, histological grade, margin status, and regional lymph node status. We collected all relevant information regarding long-term treatment results: date and the pattern of recurrence (locoregional, distant, or both) and date of death. We defined local recurrence as a retroperitoneal recurrence in the resection bed or locoregional lymph nodes. All other recurrence patterns were defined as distant.

The study was approved by the Institutional Review Board of N.N. Blokhin Russian Cancer Research Center (approval no. 01/2016).

Immunohistochemistry (IHC)

We collected FFPE tissue blocks of patients who underwent surgical resection for PC. Confirmation of PC diagnosis by an independent pathologist was mandatory before IHC analysis. We used tissue microarray technology (TMA) to provide uniform IHC staining of histological samples [8]. We selected at least four tumor blocks from each patient to assess intratumoral heterogeneity of SMAD4 expression. We evaluated SMAD4 expression in several slides from central areas of the primary tumor; in areas of tumor invasion into normal pancreatic tissue, the common bile duct, duodenum, and other tissues; and in regional lymph node metastases. Slides were prepared from selected blocks, stained by hematoxylin/eosin, then scanned by Pannoramic Flash III slide scanner, and analyzed with Pannoramic Viewer software. We marked all zones of interest in the digital slides. At least two such zones of 2 mm in diameter were chosen for each slide. We used TMA Grandmaster hardware to obtain samples of these zones for TMA-matrix production. Then, we microtomed the prepared TMA blocks and stained the slides by IHC.

We prepared 5-μm-thick slides, dried them in a thermostat for 18 h, and then kept at 60 °C for 30 min. Then, we deparaffinized slides with xylol, dehydrated them with absolute ethanol, and exposed them to 3% hydrogen peroxide to inhibit endogenous peroxidase activity. After that, we placed the slides in Ventana Ultra Autostainer for SMAD4 staining with a primary antibody against SMAD4 (B-8, sc-7966; Santa Cruz Bio, Santa Cruz, CA). We considered the SMAD4 expression positive in case of any staining intensity. We considered the slide SMAD4-negative when no tumor staining was detected, while normal cells had positive staining [7]. The pathologist performed blind SMAD4 expression analysis having no information regarding treatment or outcome. We categorized the IHC SMAD4-positive samples into three groups: 1+—mild expression in < 10% of tumor cells, 2+—weak or moderate staining in > 10% of tumor cells, and 3+—strong staining in > 10% of tumor cells.

Study Design and Statistical Analysis

To assess intratumoral heterogeneity of SMAD4 expression, we compared SMAD4 expression in central areas of primary tumor and in areas of tumor invasion or regional lymph node

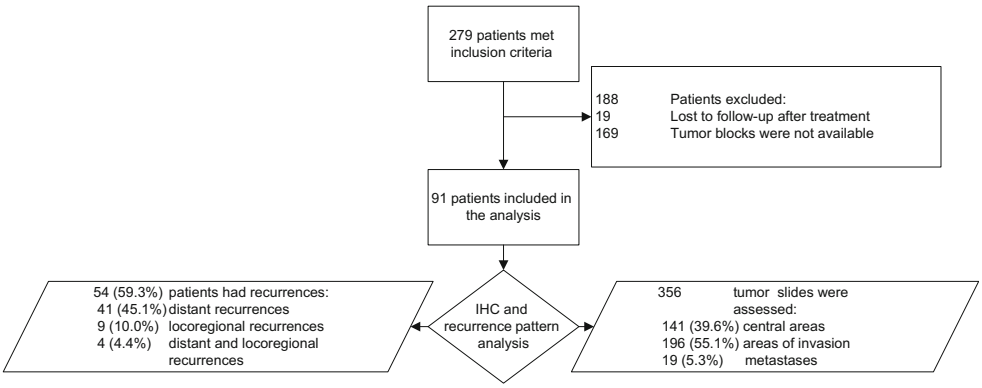

Fig. 1 Patients' flow chart

Table 1 Characteristics of included patients

Characteristics	Value ($n = 91$)
Gender, *n* (%)	
Male	42 (46.2%)
Female	49 (53.8%)
Age in years, median (range)	59 (35–75)
Adenocarcinoma subtype, *n* (%)	
Ductal	80 (87.9%)
Colloid (mucinous noncystic carcinoma)	1 (1.1%)
Clear cell carcinoma	1 (1.1%)
Undifferentiated carcinoma	2 (2.2%)
Mucinous cystadenocarcinoma	3 (3.3%)
Mixed acinar ductal carcinoma	1 (1.1%)
Serous cystadenocarcinoma	1 (1.1%)
Invasive intraductal papillary-mucinous carcinoma	1 (1.1%)
Hepatoid carcinoma	1 (1.1%)
Tumor grade (*G*), *n* (%)	
G1	22 (24.2%)
G2	53 (58.2%)
G3	16 (17.6%)
T stage, *n* (%)	
T1	2 (2.2%)
T2	6 (6.6%)
T3	68 (74.7%)
T4	15 (16.5%)
Regional lymph node metastases, *n* (%)	
Negative	49 (53.8%)
Positive	42 (46.2%)
Primary tumor site, *n* (%)	
Head	51 (77.3%)
Body or tail	15 (22.7%)
Tumor size, *n* (%)	
40 mm or less	34 (51.5%)
> 40 mm	32 (48.5%)
Resection margins (*R*), *n* (%)	
R0	69 (75.8%)
R1	18 (19.8%)
R2	4 (4.4%)
Pre- or postoperative therapy, *n* (%)	
Neoadjuvant chemotherapy (±adjuvant chemotherapy)	4 (4.4%)
Adjuvant chemotherapy	58 (68.2%)
Adjuvant chemoradiotherapy	7 (7.7%)
No pre- or postoperative therapy	22 (24.2%)

metastases. We combined results of SMAD4 expression in areas of invasion and in zones of metastases into one binary variable. We used the smallest values of SMAD4 expression. For example, if any of the several slides representing different areas of invasion or metastases inside one tumor showed negative SMAD4 expression, then the overall SMAD4 expression was considered negative in invasion/metastasis zones for this case.

We calculated Goodman and Kruskal's tau coefficient to determine if SMAD4 expression in the central tumor area predicts the expression in other tumor areas [9]. SMAD4 expression in central tumor areas was considered as the independent variable. Its expression in areas of tumor invasion to nearby tissues (e.g., common bile duct, duodenum) or involved lymph nodes was considered as the dependent variable.

To assess correlation between pattern of recurrence and SMAD4 expression status in the tumor, we divided all cases into 3 groups according to their SMAD4 expression status: SMAD4-positive, SMAD4-heterogeneous, and SMAD4-negative. SMAD4-positive cases were defined as positive SMAD4 expression in all assessed tumor slides. SMAD4-heterogeneous cases were defined as positive and negative SMAD4 expression in different slides of one tumor sample. SMAD4-negative cases were defined as negative SMAD4 expression in all assessed tumor slides. The correlation was assessed using Goodman and Kruskal's statistics. We also compared distant recurrence-free survival (DRFS) and overall survival (OS) by SMAD4 expression status.

We defined DRFS as the time from tumor resection to the first distant recurrence. The cases of death in patients without distant recurrence were censored at the time of death. OS was calculated from date of resection to death (or last contact with patient for censored cases). We used Kaplan-Meier method to assess these end points. All survival comparisons were made by the log-rank test.

All statistics were two-tailed and a p value of < 0.05 indicated statistical significance. Statistical analysis was conducted using Microsoft Excel 2007 and IBM SPSS Statistics v. 20.

Results

Patient Characteristics

A total of 279 patients underwent resection for PC between 2001 and 2015. Of them, 188 patients were excluded from the analysis: 19 patients were lost to follow-up and other 169 patients not included due to lack of archival FFPE blocks appropriate for analysis. Overall, 91 patients were included in this study (Fig. 1). Their characteristics are presented in Table 1.

SMAD4 expression was assessed in 356 tissue slides prepared from resected tumors of 91 patients: 141 (39.6%) slides represented central areas of primary tumors; 196 (55.1%) slides, areas of tumor invasion to other tissues; and 19 (5.3%) slides, regional lymph node metastases.

Table 2 SMAD4 expression in different areas of resected tumor sample

Location	SMAD4 expression score			
	0	1+	2+	3+
Central areas of the primary tumor ($n = 141$)	120 (85.1%)	12 (13.2%)	8 (8.8%)	1 (1.1%)
Areas of invasion ($n = 196$)	166 (84.7%)	25 (13.8%)	2 (1.0%)	2 (1.0%)
Lymph node metastases ($n = 19$)	17 (89.5%)	2 (10.5%)	0	0

Intratumoral Heterogeneity of SMAD4 Expression

SMAD4 expression was positive in 52 of 356 slides (15%) obtained from 26 patients. In most cases, SMAD4 expression had mild intensity (1+) (Table 2).

Further, we evaluated correlation between SMAD4 expression in central tumor areas and areas of invasion or metastases (Table 3). Only 18 samples had positive SMAD4 expression in tumor central areas (Table 3). Only 7 samples (38.9%) of them had positive SMAD4 expression inside all evaluated areas of invasion or metastases while other 11 samples (61.1%) were SMAD4 negative in these areas. SMAD4-negative expression in tumor central areas was associated with the same pattern of expression in areas of invasion or metastases in 67 of 73 cases (91.8%). These data demonstrate low but statistically significant association between tumor central areas and other tumor areas (Goodman-Kruskal tau coefficient = 0.122 ± 0.087, $p = 0.001$). Statistically significant association mostly related to strong correlation of SMAD4-negative expression in tumor central areas and areas of invasion/metastases.

Association of SMAD4 Expression and Pattern of Recurrence

With median follow-up of 21.7 months (range 2.0–85 months), disease progression occurred in 54 of 91 patients (59.3%). The pattern of recurrence was local, distant, or both in 9 (10.0%), 41 (45.1%), and 4 (4.4%) cases, respectively. Other 38 (41.8%) patients had no recurrences at the time of analysis. Median OS was 21.2 months (95% CI 19.1–23.5 months).

We evaluated the impact of SMAD4 expression on the recurrence pattern in patients with documented disease progression ($n = 54$). Patients were divided into 3 subgroups according to their SMAD4 expression status (Table 4).

In SMAD4-positive subgroup, only 1 (20%) patient had local recurrence, while other 4 (80%) patients experienced disease progression with distant recurrences. Approximately the same proportion of patients with SMAD4-negative tumors had distant recurrences (72.2%). In the SMAD4-heterogeneous subgroup, all patients developed distant recurrences. These data contradict the hypothesis of predictive significance of SMAD4 expression for locoregional recurrence pattern after tumor resection (Goodman-Kruskal tau coefficient = 0.045 ± 0.016, $p = 0.300$). There was no statistically significant difference in DRFS (Fig. 2) nor in OS (Fig. 3) between subgroups of patients. In patients with SMAD4-positive, SMAD4-heterogeneous, and SMAD4-negative tumors, median DRFS was 7.1, 19.5, and 11.8 months, respectively ($p = 0.987$), and median OS was 15.2, 32.6, and 20.5 months, respectively ($p = 0.131$).

Discussion

PC still portends dismal prognosis. Even in subgroup of patients (15–20%) eligible for potentially curative resection, 5-year OS rate is approximately 29% [1, 10]. Surgical resection followed by adjuvant chemotherapy is the current standard treatment for patients who underwent surgical treatment. Other options, such as adjuvant radiotherapy, did not demonstrate any benefit in most clinical trials [2, 11–13]. Probable reason for local therapy failure is distant progression of PC after surgery in most cases. On the other hand, results of prospective clinical trials indicate that 15–35% of patients will develop only locoregional recurrences [2, 11]. Therefore, detection of biomarkers capable to predict the recurrence pattern is a major clinical issue. Key proteins of EMT should be studied as potential biomarkers of tumor metastatic potential.

Published data indicate that dysregulation of TGFβ signaling pathway is responsible for EMT process in

Table 3 Correlation of SMAD4 expression in central tumor areas and in areas of invasion or metastases

SMAD4 in central tumor areas	SMAD4 in areas of invasion or metastases		Goodman and Kruskal's tau test
	Positive	Negative	
Positive ($n = 18$)	7 (38.9%)	11 (61.1%)	Coefficient = 0.122 ± 0.087, $p = 0.001$
Negative ($n = 73$)	6 (8.2%)	67 (91.8%)	

Table 4 Association between SMAD4 expression and a pattern of recurrence in patients with documented disease progression

SMAD4 expression	Pattern of recurrence		Goodman and Kruskal's tau test
	Locoregional	Distant	
Positive ($n = 5$)	1 (20%)	4 (80%)	Coefficient = 0.045 ± 0.016, $p = 0.300$
Heterogeneous ($n = 10$)	0 (0%)	10 (100%)	
Negative ($n = 39$)	8 (20.5%)	31 (72.2%)	

PC. This dysregulation is often caused by loss of SMAD4 (DPC4) protein function [5]. The whole-genome sequencing of 100 pancreatic ductal adenocarcinomas shows that the mutation in *SMAD4* gene is one of the most common mutations in PC representing in 31% of cases [4]. Epigenetic changes may play an important role in SMAD4 deficiency because lack of IHC SMAD4 expression is even more common finding than *SMAD4* mutations detected by gene sequencing [14].

Current data suggest possible value of SMAD4 IHC expression in prediction of locoregional pattern of PC progression [6, 7]. However, these data were obtained from patients with locally advanced disease and those who died of PC. Therefore, these data cannot be extrapolated to patients with resectable PC. Patients, who had resection for PC, represent different population, and their tumors can have other genomic alterations. It follows that validation of this biomarker in resectable PC is necessary.

Results of published studies are contradictory. Winter et al. [15] analyzed correlation between SMAD4 expression in tumor and recurrence pattern in 127 patients with PC after surgical treatment. The loss of SMAD4 expression was found in 32% of cases. The results demonstrated no difference in the recurrence pattern regardless of SMAD4 expression. They also demonstrated that SMAD4 expression was not a prognostic factor [15].

Authors of a large study from South Korea analyzed correlation between SMAD4 IHC expression and recurrence pattern in 641 patients who had surgical resection for PC. They revealed SMAD4 inactivation in 74% of cases. It correlated with higher rate of distant metastases. Positive SMAD4 expression was associated with locoregional recurrence in 56% of cases, whereas lack of expression was associated with locoregional recurrence only in 23% of cases. SMAD4 expression status also correlated with progression-free survival and OS [16].

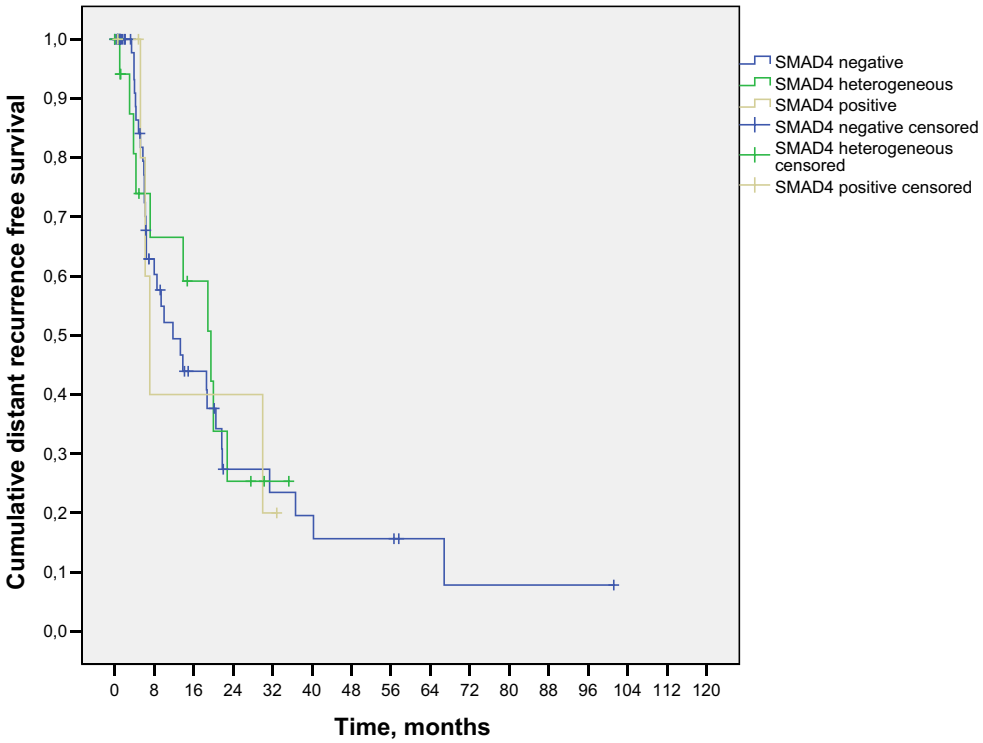

Fig. 2 Distant recurrence-free survival stratified by SMAD4 expression status

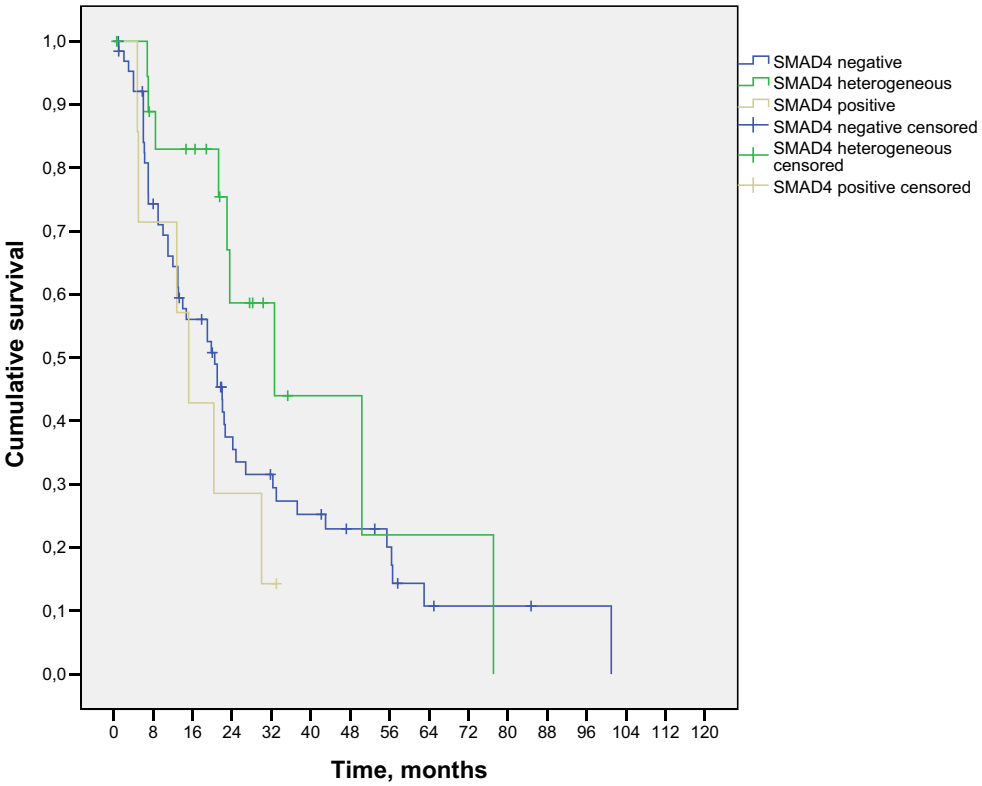

Fig. 3 Overall survival stratified by SMAD4 expression status

We revealed isolated locoregional recurrences only in 10% of cases. Other patients had distant metastases as first events of cancer progression. In our study, expression of SMAD4 did not correlate with the pattern of PC recurrence. Rates of locoregional recurrences in patients with positive and negative SMAD4 expression were equal.

It is reasonable to assess not only development of distant metastases but also DRFS in surgically treated patients with PC. Our data demonstrated that SMAD4 expression affected neither DRFS nor OS. Although low number of patients limited the power of such analysis, we did not find even a trend towards the SMAD4 expression influence on these end points. Median DRFS and OS in the group with heterogeneous SMAD4 expression were the highest among all groups. But in the group with fully SMAD4-positive expression, median DRFS and OS were lower than in other groups.

SMAD4 expression in our study was positive in 29% of patients with PC, but even in these cases, the staining was weak. The rate of positive SMAD4 expression in our study is significantly lower than in the study of Winter et al. (73%), but these discrepancies are consistent with other published data [17]. The reported frequency of SMAD4 IHC expression in PC cells varies from 15 to 76% [16, 18]. We assume that weak staining and low rate of positive SMAD4 expression in our study refers to the use of formalin-fixed and paraffin-embedded tumor blocks instead of fresh tissue. But we also assessed blocks with normal tissue as positive control for all samples.

We have also found out that SMAD4 staining in different tumor areas is highly heterogeneous. Only one third of patients with positive SMAD4 expression in the central areas of tumor were SMAD4-positive in areas of invasion or regional lymph nodes. It means that positive SMAD4 expression revealed by tumor core biopsy is insufficient to draw a firm conclusion about SMAD4 expression in other tumor areas. This fact limits the clinical use of SMAD4 status.

The retrospective nature, relatively short follow-up period, and failure to obtain tissue samples for most of patients are the main limitations of our study. At the time of analysis, only 59.3% patients had disease progression. This, as well as the abovementioned small number of tissue samples, decreases the power of statistical analyses in our study.

In summary, these limitations of our study and contradictory results of other studies need future trials aiming to assess predictive or prognostic role of SMAD4 expression status. Our data show significant intratumoral heterogeneity of SMAD4 expression. Therefore, assessing SMAD4 expression in tumor biopsy specimens seems to be equivocal, because it is not representative for the whole tumor.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Financial Support The study was funded by N.N. Blokhin Russian Cancer Research Center. No other support was done.

References

1. Neoptolemos JP, Palmer DH, Ghaneh P, Ghaneh P, Psarelli EE, Valle JW, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. Lancet. 2017;389(10073):1011–24.
2. Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med. 2004;350:1200–10.
3. Ducreux M, Cuhna AS, Caramella C, Hollebecque A, Burtin P, Goéré D, et al. Cancer of the pancreas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26(Supplement 5):v56–68.
4. Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015;518(7540):495–501.
5. Deckers M, van Dinther M, Buijs J, Que I, Löwik C, van der Pluijm G, et al. The tumor suppressor Smad4 is required for transforming growth factor beta-induced epithelial to mesenchymal transition and bone metastasis of breast cancer cells. Cancer Res. 2006;66:2202–9.
6. Crane CH, Varadhachary GR, Yordy JS, Staerkel GA, Javle MM, Safran H, et al. Phase II trial of cetuximab, gemcitabine, and oxaliplatin followed by chemoradiation with cetuximab for locally advanced (T4) pancreatic adenocarcinoma: correlation of Smad4 (Dpc4) immunostaining with pattern of disease progression. J Clin Oncol. 2011;29:3037–43.
7. Iacobuzio-Donahue CA, Fu B, Yachida S, Luo M, Abe H, Henderson CM, et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. J Clin Oncol. 2009;27:1806–13.
8. Chen W, Foran DJ. Advances in cancer tissue microarray technology: towards improved understanding and diagnostics. Anal Chim Acta. 2006;564(1):74–81.
9. Goodman LA, Kruskal WH. Measures of association for cross classifications. Part I. J Am Stat Assoc. 1954;49:732–64.
10. Bockhorn M, Uzunoglu FG, Adham M, Imrie C, Milicevic M, Sandberg AA, et al. Borderline resectable pancreatic cancer: a consensus statement by the International Study Group of Pancreatic Surgery (ISGPS). Surgery. 2014;155:977–88.
11. Klinkenbijl JH, Jeekel J, Sahmoud T, van Pel R, Couvreur ML, Veenhof CH, et al. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region. Ann Surg. 1999;230:776–84.
12. Van Laethem J, Hammel P, Mornex F, et al. Adjuvant gemcitabine alone versus gemcitabine-based chemoradiotherapy after curative resection for pancreatic cancer: a randomized EORTC-40013-22012/FFCD-9203/GERCOR phase II study. J Clin Oncol. 2010;28:4450–6.
13. Schmidt J, Abel U, Debus J, et al. Open-label, multicenter, randomized phase III trial of adjuvant chemoradiation plus interferon alfa-2b versus fluorouracil and folinic acid for patients with resected pancreatic adenocarcinoma. J Clin Oncol. 2012;30:4077–83.
14. Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, et al. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science (Wash. DC). 1996;271:350–3.
15. Winter JM, Tang LH, Klimstra DS, Liu W, Linkov I, Brennan MF, et al. Failure patterns in resected pancreas adenocarcinoma. Lack of predicted benefit to SMAD4 expression. Ann Surg. 2013;258:331–5.
16. Shin SH, Kim HJ, Hwang DW, Lee JH, Song KB, Jun E, et al. The DPC4/SMAD4 genetic status determines recurrence patterns and treatment outcomes in resected pancreatic ductal adenocarcinoma: a prospective cohort study. Oncotarget. 2017;8:17945–59.
17. Toga T, Nio Y, Hashimoto K, Higami T, Maruyama R. The dissociated expression of protein and messenger RNA of DPC4 in human invasive ductal carcinoma of the pancreas and their implication for patient outcome. Anticancer Res. 2004;24(2C):1173–8.
18. Shugang X, Hongfa Y, Jianpeng LXZ, Jingqi F, Xiangxiang L, et al. Prognostic value of SMAD4 in pancreatic cancer: a meta-analysis. Transl Oncol. 2016;9(1):1–7.