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• BASIC RESEARCH •

Loss of DPC4 expression and its correlation with clinicopathological parameters in pancreatic carcinoma

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Abstract

AIM: DPC4 is a tumor suppressor gene on chromosome 18q21.1 that has high mutant frequencies in pancreatic carcinogenesis. The purpose of this study was to investigate the role of DPC4 alterations in tumorigenesis and progression of pancreatic carcinomas.

METHODS: We studied the immunohistochemical markers of DPC4 in 34 adenocarcinomas and 16 nonmalignant specimens from the pancreas. The 16 nonmalignant specimens from the pancreas included 8 non-neoplastic cysts and 8 normal pancreatic tissues. The relationship between DPC4 alterations and various clinicopathological parameters was evaluated by chi-square test or Fisher's exact test. Survivals were calculated using Kaplan-Meier method (by a log-rank test).

RESULTS: All the 16 nonmalignant cases of the pancreas showed expression of DPC4 gene. Loss of DPC4 expression was seen in 8 of 34(23.5 %) pancreatic adenocarcinomas. The frequency of loss of DPC4 expression was higher in poorly differentiated adenocarcinoma (G3) than in well and moderately differentiated adenocarcinoma (G1 and G2) histologically (P=0.037). Loss of DPC4 expression of the patients at TNM stage IV was also higher than that of the patients at TNM stages I, II and III (60.0 % at stage IV, *versus* 14.3 % at stage I, 18.2 % at stage II, and 18.2 % at stage III) (P=0.223). The mean and median survival in patients with DPC4 expression was longer than those in patients with loss of DPC4 expression. Kaplan-Meier survival analysis demonstrated patients with DPC4 expression had a higher survival rate than patients with loss of DPC4 expression, but the difference did not reach statistical significance (P =0.879).

CONCLUSION: This study suggests that DPC4 is involved in the development of pancreatic carcinoma and is a late event in pancreatic carcinogenesis, DPC4 expression may be a molecular prognostic marker for pancreatic carcinoma.

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INTRODUCTION

The incidence of pancreatic carcinoma has increased in recent decades in the world, and this cancer has the lowest five-year survival rate among all cancers. The dismal survival of patients with pancreatic carcinomas is caused by the late diagnosis and low resection rates[1,2]. However, understanding the molecular pathogenesis of pancreatic carcinomas may be the foundation upon which to develop novel strategies for identifying genetic markers useful for the early diagnosis and treatment. An association has been demonstrated between pancreatic carcinomas and various genetic alterations including genes K-ras[3,4], Her-2/neu[5], p16[6], and p53[7]. Recently, DPC4 (deleted in pancreatic carcinoma, locus 4; Smad4) located on chromosome 18q21.1, has received special attention as its alterations may play a role in activation of pancreatic carcinogenesis[8].

DPC4 gene is a tumor suppressor gene, which has been shown to mediate the downstream effects of TGF-β superfamily signaling, resulting in growth inhibition[9]. Inactivation of DPC4 tumor-suppressor gene is relatively specific for pancreatic carcinoma, although it has been shown to occur in a small percentage of primary carcinomas of the esophagus[10,11], stomach[11,12], head and neck[13], breast, ovary[14], colon[15], and biliary tract[16]. DPC4 can be inactivated by one of the two identified mechanisms: intragenic mutation of one allele coupled with loss of the other allele, or deletion of both alleles (homozygous deletions). Both mutations and homozygous deletions of DPC4 gene have been observed in a high proportion of pancreatic carcinomas[8]. In contrast, the role of DPC4 in human pancreatic carcinoma remains less well defined. Recently, immunohistochemical labeling for the DPC4 gene product has become an extremely sensitive and specific marker for DPC4 gene alterations in pancreatic carcinomas, and has been shown to mirror the DPC4 genetic status of pancreatic carcinomas, because most mutations of DPC4 could result in a loss of the protein. Therefore, immunolabeling for DPC4 could provide a useful tool to examine genetic status in pancreatic adenocarcinomas[17,18].

In the present study, we examined DPC4 expression in 34 adenocarcinomas and 16 nonmalignant specimens from the pancreas using a monoclonal antibody to human DPC4 protein by means of immunohistochemistry and studied the relation between expression of DPC4 and various clinicopathological parameters in order to elucidate whether altered DPC4 expression played a role in the tumorigenesis and progression of pancreatic carcinomas.

MATERIALS AND METHODS

Patients and samples

Thirty-four specimens of pancreatic adenocarcinomas were retrieved from the pathology archives of China-Japan Friendship Hospital between 1984 and 2000. There were 22 males and 12 females with pancreatic carcinomas, and the average age of the patients was 55.18±11.29 years (mean±SD), with a range of 30-75 years. Twenty-eight patients were followed up until death or until the time of this study.

Histopathological grade and clinical staging were evaluated according to the criteria by Klöppel for pancreatic tumors[19] and the International Union Against Cancer (UICC) TNM classification[20]. Histopathologic examination revealed well differentiated adenocarcinoma in 10 patients, moderately differentiated adenocarcinoma in 15 patients, and poorly differentiated adenocarcinoma in 9 patients. Seven patients were at UICC stages I, 11 at stages II, 11 at stage III, and 5 at stage IV. In addition, 16 nonmalignant specimens from the pancreas including 8 non-neoplastic cysts and 8 normal pancreatic tissues were used as controls.

Immunohistochemistry

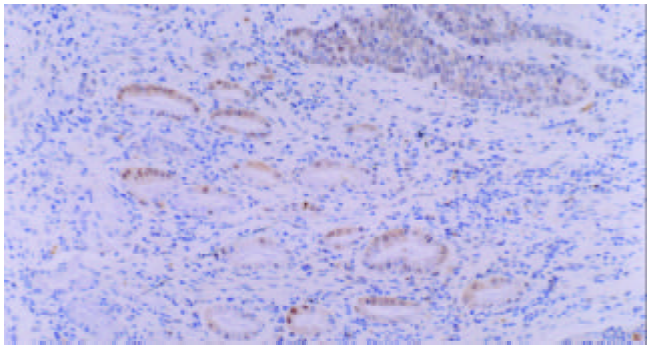
Tissues were routinely fixed in neutral formalin, embedded in paraffin, and 5 μm thick consecutive sections were cut. After deparaffinized, the slides were placed in a solution of 3 % hydrogen peroxide (1:1) for 10 minutes to block the activity of endogenous peroxidase, and then heated in a microwave for 5 minutes at 100 ℃. After the slides were cooled for 30 minutes, nonspecific binding was blocked with a protein solution for 10 minutes, and then each slide was labeled with a 1:100 dilution of monoclonal antibody to DPC4 (clone B8, Santa Cruz, CA). Anti-DPC4 antibody was detected by adding biotinylated secondary antibodies, avidin-biotin complex, and 3,3' -diaminobenzidine. The sections were counterstained with hematoxylin. Positive cells were stained dark brown in the nuclei and/or cytoplasms, and the staining was graded into four categories: 0, no staining, 1+, weak staining, 2+, moderate staining, 3+, heavy staining. Positive staining was considered as expression of DPC4. Normal pancreatic ducts, islets of Langerhans, acini, lymphocytes, and stromal fibroblasts showing moderate to strong expression of DPC4 gene, served as positive internal controls for each section. For negative controls, the primary antibody was replaced with phosphate buffered solution (PBS).

Statistical analysis

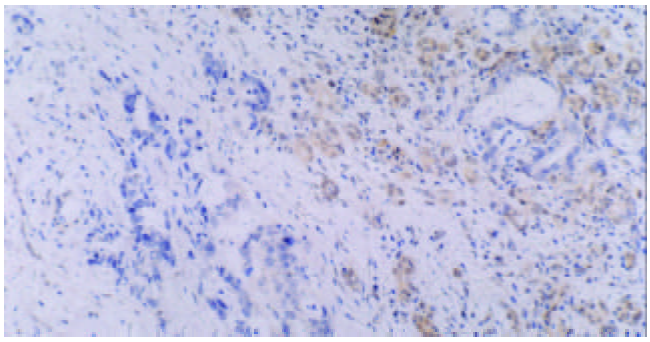
The data were analyzed with chi-square test or Fisher' s exact test to compare the differences between the two subgroups of patients based on the results of DPC4 staining. All of the tests were two-tailed. Survivals were calculated using Kaplan-Meier method (by a log-rank test).

RESULTS

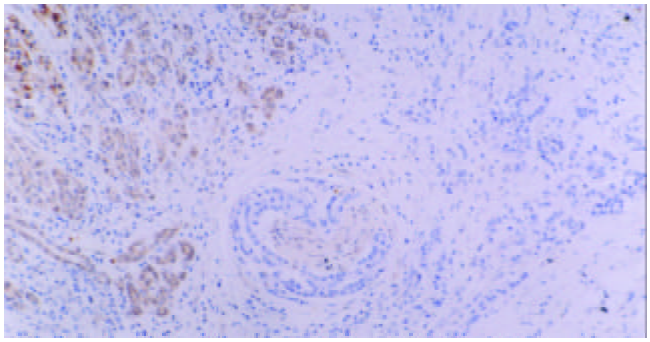
The results of DPC4 protein immunohistochemistry are summarized in Table 1, and typical examples of the positive and negative groups are shown in Figure 1 (A-E). It was observed that pancreatic carcinoma showed loss of DPC4 expression, whereas the adjacent normal pancreatic tissue had DPC4 expression (Figures 1B and C).



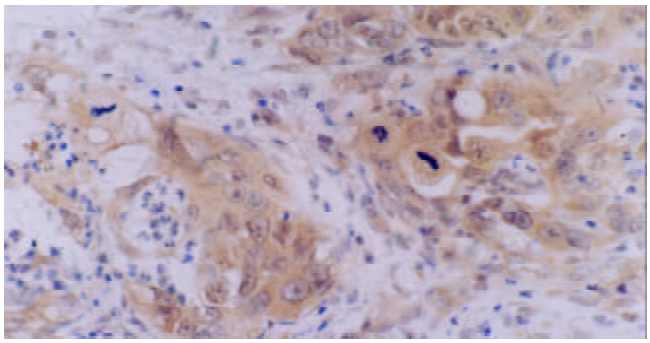
A: Well-differentiated pancreatic carcinoma showed DPC4 expression. hematoxylin counterstain. original magnification, ×100.



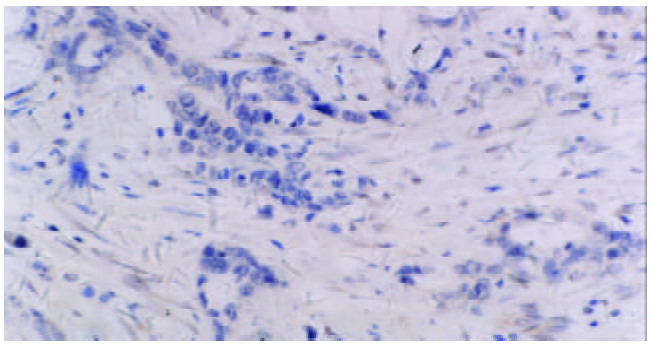
B: Well-differentiated pancreatic carcinoma showed loss of DPC4 expression (left), whereas the adjacent normal pancreatic tissue had DPC4 expression (right). hematoxylin counterstain. original magnification, ×200.



C: Moderately-differentiated pancreatic carcinoma showed loss of DPC4 expression (right), whereas the adjacent normal pancreatic tissue had DPC4 expression (left). Vortex in the middle shows invasion of pancreatic nerve. hematoxylin counterstain. original magnification, ×160.



D: Poorly-differentiated pancreatic carcinoma showed DPC4 expression. Hematoxylin counterstain. original magnification, ×400.



E: Poorly-differentiated pancreatic carcinoma showed loss of DPC4 expression. hematoxylin counterstain. original magnification, ×400.

Figure 1 Representative immunostaining results of DPC4 in pancreatic carcinoma (A-E). Positive cells were stained dark brown in the nuclei and/or cytoplasms (A-D).

All the 16 nonmalignant cases of the pancreas showed expression of DPC4 gene products. Loss of DPC4 expression was seen in 8 of 34 (23.5 %) pancreatic adenocarcinomas. The results of immunostaining of DPC4 expression in 34 pancreatic carcinomas and the correlation with various clinicopathological parameters are shown in Table 2. A significant difference was found in the frequency of loss of DPC4 expression between well and moderately differentiated adenocarcinomas (G1 and G2) and poorly differentiated adenocarcinoma (G3) histologically (P =0.037). Although loss of DPC4 expression in the patients at TNM staging IV was higher than that in those at stages I, II and III (60.0 % at stage IV, versus 14.3 % at stage I, 18.2 % at stage II, and 18.2 % at stage III), the difference did not reach any statistical significance (P=0.223). In addition, a higher frequency of loss of DPC4 expression in patients with lymph node-metastasis was also revealed, however the difference was not significant (P=0.228). The mean and median survival in patients with DPC4 expression was longer than that in patients with loss of DPC4 expression (Table 3). Kaplan-Meier survival analysis demonstrated patients with DPC4 expression had a higher survival rate than those with loss of DPC4 expression, but the difference did not reach any statistical significance (P=0.879) (Figure 2).

Table 1 Loss of DPC4 expression in pancreatic tissues (%)

Tissues	*n*	Loss expression of DPC4 (%)
Normal pancreas	8	0 (0)
Non-neoplastic cysts	8	0 (0)
Pancreatic carcinoma	34	8 (23.5)

Table 2 Correlations between loss expression of DPC4 and clinicopathological parameters in pancreatic carcinoma

Parameters	*n*	Loss expression of DPC4 (%)	*P*
Age (y)			
≥60	17	2 (11.8)	0.268
45≤x<60	11	4 (36.4)	
<45	6	2 (33.3)	
Sex			
Male	22	6 (27.3)	0.681
Female	12	2 (16.7)	
Pathological grade			
G1+G2	27	4 (14.8)	0.037
G3	7	4 (57.1)	
Tumor diameter			
≤4.5 cm	19	4 (21.2)	1.000
>4.5 cm	15	4 (26.7)	
Tumor location			
Head	24	6 (25.0)	0.842
Body and tail	10	2 (20.0)	
Lymph node			
Negative	20	3 (15.0)	0.228
Positive	14	5 (35.7)	
TNM staging			
I	7	1 (14.3)	0.223
II	11	2 (18.2)	
III	11	2 (18.2)	
IV	5	3 (60.00)	

G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Table 3 Mean and median survivals in pancreatic carcinomas

	Mean survival (days)	Median survival (days)
DPC4 expression	329.94±41.54	319.00±30.32
Loss of DPC4 expression	300.00±61.88	206.00±88.39

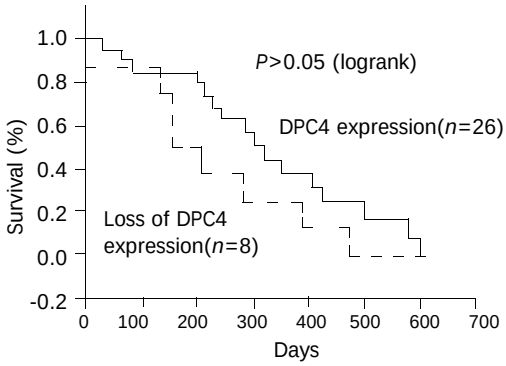


Figure 2 Kaplan-Meier survival curves comparing patients with DPC4 expression and patients with loss of DPC4 expression. Although patients with DPC4 expression had a higher survival rate than those with loss of DPC4 expression, the difference did not reach ang statistical significance.

DISCUSSION

This study aimed to clarify the role of DPC4 in the development of pancreatic carcinoma. The product of DPC4 gene belongs to the evolutionally conserved family of Smad proteins which are linked to TGF-β superfamily of cytokines. Smad proteins are involved in the regulation of cell differentiation as well as the inhibition of cell proliferation, and their alterations could confer resistance to TGF-β and thereby contribute to tumorigenesis[21,22]. DPC4 gene produces a 64-KD protein that influences gene transcription and growth arrest. In fact, DPC4 protein has three distinguishable domains, and mutations in each of these domains could lead to the loss of DPC4 function[8,23,24].

There were different findings about the frequency of DPC4 alterations in pancreatic carcinomas in previous reports (9 %-55 %)[8,25]. The discrepancies between studies might be due to differences in the study populations, techniques, or the statistical method. In our study, eight of the 34-pancreatic carcinoma specimens were immunohistochemically labeled for the loss of DPC4 protein (23.5 %). However, DPC4 immunohistochemical staining was found in all of the 16 nonmalignant specimens from the pancreas. This finding suggested that DPC4 might be involved in the tumorigenesis and development of pancreatic carcinoma.

Our study showed loss of DPC4 expression was correlated with the histological grade in patients with pancreatic carcinoma. Loss of DPC4 expression in those with poorly differentiated adenocarcinomas was significantly higher than that in those with well and moderately differentiated adenocarcinomas, which implied that DPC4 gene might preserve phenotypic characteristics under normal conditions and control the malignant progression of pancreatic carcinomas.

There was a trend toward a higher frequency of loss of DPC4 expression in patients at TNM staging IV in this study. When stratified by stage, the highest percentage of loss of DPC4 reactivity was found in carcinomas at stage IV (60.0 %), compared with 14.3 % at stage I, 18.2 % at stage II, and 18.2 % at stage III carcinomas. A higher frequency of loss of DPC4 expression in patients with lymph node-metastasis was also revealed. Survival analysis demonstrated patients with DPC4 expression had a higher survival rate than those with loss of DPC4 expression.

The data in our study were correlated fairly well with what has been reported. The results from Wilentz *et al* showed that DPC4 expression in duct lesions with a histologically low-grade (PanIN-1 and -2) was significantly higher than that in those with a histologically high-grade (PanIN-3)[26]. Another study showed that DPC4 expression in PanIN could be

predictive of DPC4 expression in the subsequent invasive ductal adenocarcinoma. Additionally, DPC4 expression could be used to differentiate recurrent or persistent adenocarcinomas from a second primary adenocarcinoma. A recent study found that survival of patients whose tumors expressed DPC4 protein was significantly longer (19.2 months) as compared with 14.7 months of those without DPC4 protein expression, and DPC4 expression was correlated with a better prognosis of pancreatic carcinomas[18]. Biankin *et al* also found DPC4/Smad4 expression had a potential as a prognostic indicator in patients with pancreatic carcinoma, and loss of DPC4 expression was associated with improved survival after resection, whereas resection did not improve the survival in patients whose tumor expressed DPC4.

These findings suggest that loss of DPC4 expression occurs biologically late in the neoplastic progression leading to the development of infiltrating pancreatic carcinoma, and indicates a poor prognosis for patients. It is reasonable to postulate that DPC4 plays a pivotal role in regulating all TGF-β superfamily signal pathways. Abrogation of DPC4 function might cause a breakdown in this signaling pathway and loss of transcription of genes critical to cell-cycle control. Cells might therefore become TGF-β resistant and escape from TGF-β-mediated growth control and apoptosis. Experimental evidences indicated that DPC4 could regulate an angiogenic switch by decreasing the expression of vascular endothelial growth factor (VEGF) and increasing the levels of angiogenesis inhibitor thrombospondin-1 (TSP-1).

In conclusion, our study shows that loss of DPC4 expression is involved in the carcinogenesis and development of pancreatic carcinoma and is a late event in pancreatic carcinogenesis. DPC4 expression may be a molecular prognostic marker for pancreatic carcinoma.

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