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Differential expression of CD44v6 in adenocarcinoma of the pancreas: an immunohistochemical study

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Abstract Alternative splicing gives rise to numerous CD44 isoforms, some of which seem to have a role in tumour metastasis. Specifically, a variant form of CD44 with sequences encoded by exon v6 (CD44v6) confers metastatic potential when transfected into a nonmetastasizing cell line of rat pancreatic adenocarcinoma. This study has investigated standard CD44 (CD44s) and CD44v6 expression immunohistochemically in 6 samples of normal pancreatic tissue, 4 of tissue affected by chronic pancreatitis, and 24 of tissue from metastasizing and nonmetastasizing pancreatic adenocarcinomas. In addition, 18 samples from lymph node or visceral metastases were included in the study. CD44s was expressed in nonneoplastic tissue and in tissue from pancreatic adenocarcinomas. In contrast, CD44v6 was not detected in any of the normal tissue or chronic pancreatitis specimens, whereas 54% of pancreatic adenocarcinomas and 55% of metastases expressed this variant exon. Although it is not clear whether CD44 isoforms containing exon v6 play a part in malignant progression in the human exocrine pancreas, it seems plausible that the expression of multiple isoforms containing this and other variant exon confers a selective advantage on pancreatic adenocarcinoma.

Key words Pancreatic adenocarcinoma · CD44s · CD44v6 · Immunohistochemistry · Metastasis

Introduction

The prognosis for pancreatic adenocarcinoma is extremely poor, because there are usually invasion of surrounding tissues and metastases to lymph nodes, liver or peritoneum at the time of diagnosis [10, 20]. The mechanisms underlying these invasive and metastatic capabilities have not yet been clarified.

The widely distributed surface glycoproteins known as CD44 are expressed in many types of tumours and normal tissues [4]. Alternative splicing and differential N- and O-linked glycosylation contribute to the heterogeneity of the family [3]. Alternative splicing of variant exons v1-v10 generates multiple isoforms [14]. The isoform lacking variant exons is referred to as CD44s (s for standard), which is the most common isoform in haematopoietic cells [8]. While CD44s is known to be implicated in various processes, such as lymphocyte homing, lymphocyte activation [2] and extracellular matrix adhesion [9], the functions of alternatively spliced isoforms are not clear.

The CD44 variant isoforms with the sequence encoded by exon 6 have been reported to confer metastatic potential when transfected into a nonmetastatic cell line of rat pancreatic adenocarcinoma [6]. Moreover, this effect can be blocked by anti-v6 antibodies [15]. In a recent work, non-Hodgkin lymphomas that expressed CD44v6 were associated with poorer survival than those that did not [13]. In human colorectal carcinoma [21] and intestinal type gastric adenocarcinoma [7] expression of CD44v6 is related to tumour progression.

In this study we have examined CD44s and CD44v6 expression immunohistochemically in nonneoplastic pancreatic tissues and pancreatic adenocarcinoma, in an attempt to evaluate the role of these adhesion molecules in malignant progression.

Materials and methods

Fifty-four samples of normal pancreatic tissue and tissue affected by chronic pancreatitis and metastasizing and nonmetastasizing adenocarcinoma were obtained from the archives of the Department of Pathology, "Germans Trias i Pujol" University Hospital. The samples were taken from a total of 28 patients (16 men and 12 women) aged between 34 and 82 years (mean, 71 years): 24 of them had ductal adenocarcinoma and 4, chronic pancreatitis. Six normal pancreatic samples were obtained from the nonneoplastic areas of pancreatic adenocarcinoma surgical specimens.

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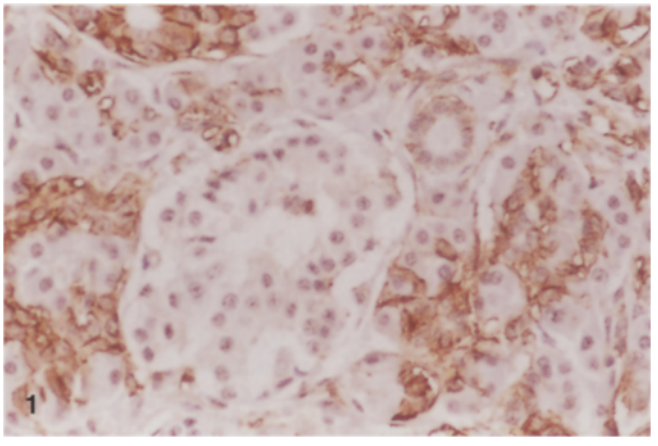
Of the 24 adenocarcinoma samples, 6 were from pancreatic adenocarcinomas with no evidence of metastasis at the time of diagnosis and 18 were from metastasizing pancreatic adenocarcinomas. In addition, 18 samples (1 per metastasizing case) from lymph node or visceral metastases were included in the study. According to the Cancer of the Pancreas Task Force stage classification the patients were distributed as follows: 4 in stage I; 2 in stage II; 10 in stage III; and 8 in stage IV. Degree of differentiation was evaluated following well established criteria. The results obtained with immunohistochemistry were correlated with grade of differentiation and tumour stage.

All biopsy specimens were fixed in 10% neutral formalin and embedded in paraffin at 57–60°C. Sections 5 μm thick were deparaffinized, placed in methanol containing 0.3% H_2O_2 for 30 min at room temperature, washed, heated in a microwave oven (3×3 min, with 2-min intervals) while in buffered citrate (0.1 M) (citric acid and sodium citrate, pH 6.0), washed, and incubated for 30 min with rabbit serum at 1:10 dilution. Subsequently, sections were incubated with anti-CD44s (clone 2C5; RD Systems, Abingdon, UK) and anti-CD44v6 (clone 2F-1D; RD Systems) mouse monoclonal antibodies at 1:1000 dilution for 22 h at room temperature. The slides were washed and incubated with biotinylated rabbit anti-mouse immunoglobulin antibodies at a 1:700 dilution and with avidin–biotin immunoperoxidase complex (Vector Laboratories, Burlingame, Calif.). After application of the chromogen 3,3′-diaminobenzidine tetrachloride at 0.5 mg/ml (Aldrich Chemical Co., Milwaukee, Wis.), sections were contrasted with haematoxylin, dehydrated and mounted with Permount. A nonimmune mouse serum was used as control instead of the specific monoclonal antibodies, and a non-small-cell lung carcinoma was used as positive control for CD44s and CD44v6.

Two features were taken into account for the evaluation of CD44 expression: the percentage of positively stained cells: negative <5%; focal between 5% and 50%; diffuse >50% and the intensity of staining: 0 no staining; 1 weak staining; 2 moderate staining; 3 strong staining.



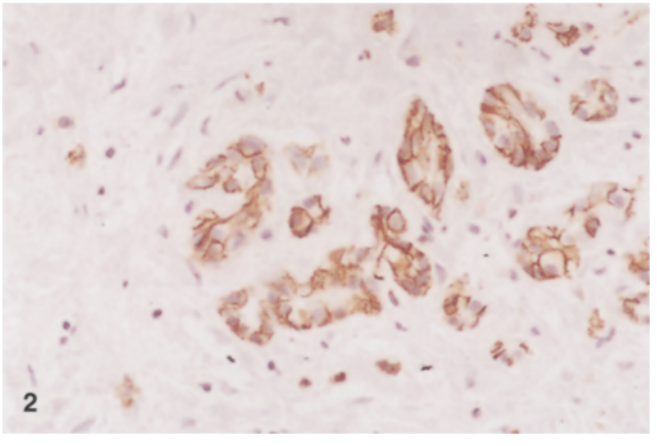
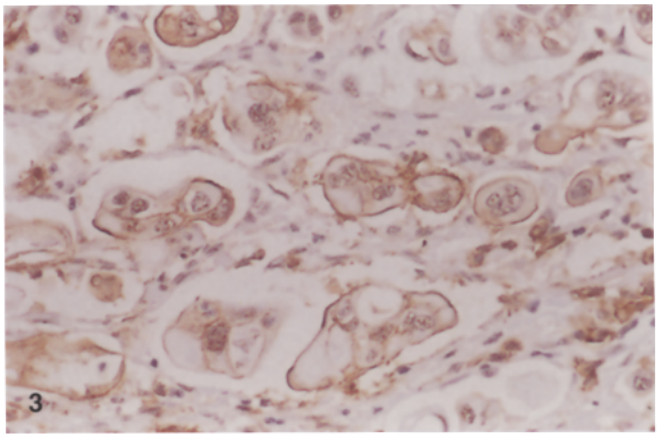
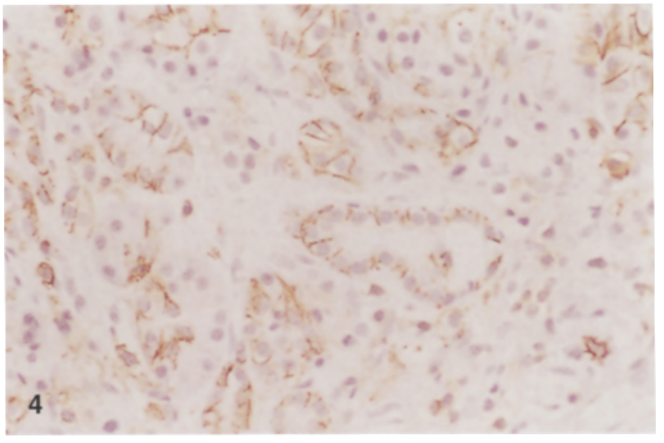


Fig. 1 CD44s expression in normal epithelial and acinar pancreatic cells. ×400

Fig. 2 CD44s immunoreactivity in chronic pancreatitis. ×250





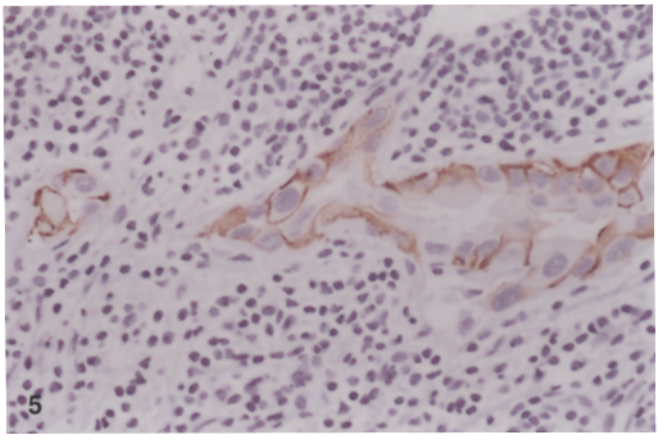


Fig. 3 CD44s diffuse immunostaining of adenocarcinoma cell membranes. ×400

Fig. 4 Primary pancreatic adenocarcinoma with CD44v6 focal immunoreactivity. ×250

Fig. 5 CD44v6 immunostaining of cell membranes in lymph node metastases of pancreatic adenocarcinoma. ×400

Table 1 CD44s expression in nonneoplastic pancreas and in pancreatic adenocarcinoma (*F* focal, *D* diffuse)

	No. of cases	No. of positive cases	Distribution		Intensity		
			F	D	1	2	3
Normal pancreas	6	6 (100%)	6 (100%)	0	0	6 (100%)	0
Chronic pancreatitis	4	4 (100%)	2 (50%)	2 (50%)	0	3 (75%)	1 (25%)
Nonmetastasizing adenocarcinoma	6	4 (66%)	3 (75%)	1 (25%)	0	2 (50%)	2 (50%)
Metastasizing adenocarcinoma	18	13 (72%)	7 (53%)	6 (47%)	0	5 (38%)	8 (61%)
Total adenocarcinomas	24	17 (70%)	10 (58%)	7 (42%)	0	7 (41%)	10 (58%)
Metastasis	18	13 (72%)	6 (46%)	7 (53%)	0	7 (53%)	6 (46%)

Table 2 CD44v6 expression in nonneoplastic pancreas and in pancreatic adenocarcinoma (*F* focal, *D* diffuse)

	No. of cases	No. of positive cases	Distribution		Intensity		
			F	D	1	2	3
Normal pancreas	6	0					
Chronic pancreatitis	4	0					
Nometastasizing adenocarcinoma	6	3 (50%)	3 (100%)	0	1 (33%)	2 (66%)	0
Metastasizing adenocarcinoma	18	10 (55%)	7 (70%)	3 (30%)	3 (30%)	6 (60%)	1 (10%)
Total adenocarcinomas	24	13 (54%)	10 (76%)	3 (23%)	4 (30%)	8 (61%)	1 (8%)
Metastasis	18	13 (72%)	11 (84%)	2 (15%)	5 (38%)	7 (53%)	1 (8%)

Two of the authors evaluated the slides independently. Those on which they did not initially agree were reviewed to reach a consensus. Frequency tables were analysed by Chi-square or Fisher's exact test.

Results

All normal pancreatic tissue samples examined showed focal immunoreactivity of moderate intensity for CD44s in the ductal epithelium and in acinar cells, whereas endocrine cells seemed to be negative (Fig. 1). Similarly, ducts enmeshed in fibrous tissue from chronic pancreatitis cases were focally (50%) or diffusely (50%) positive for CD44s in all cases studied (Fig. 2). The intensity of plasma membrane staining was moderate in 75% of cases and strong in 25% (Table 1). In contrast, CD44v6 was not expressed in any of the normal pancreatic tissue or chronic pancreatitis samples (Table 2). Of the adenocarcinomas, 70% were positive for CD44s (66% of non metastasizing cases and 72% of metastasizing cases). The distribution of CD44s staining was predominantly focal in non-metastasizing neoplasms (75%), while in metastasizing tumours it was focal in 53% of cases and diffuse in 47% (Fig. 3).

Most metastases (72% or 13 of 18) stained positively for CD44s, the distribution being diffuse in 53% of cases and focal in the rest (Table 1). The intensity of CD44s staining varied from moderate to strong in malignant tumour samples (Table 1).

CD44v6 immunostaining was positive in 13 of 24 (54%) adenocarcinomas (50% of nonmetastasizing cases (Fig. 4) and 55% of metastasizing cases) and in 72% of the samples taken from metastases (Fig. 5). CD44v6 positivity was predominantly focal and staining intensity was weak or moderate in most cases of adenocarcinoma and metastasis. These results are summarized in Table 2.

The differences in CD44v6 expression between metastasizing and nonmetastasizing pancreatic adenocarcinoma and between primary tumours and their lymph node metastases did not reach statistical significance.

No significant correlation could be demonstrated between CD44v6 expression and presence of metastasis at the time of diagnosis, degree of histological differentiation, or tumour stage. The intensity and distribution of immunoexpression did not correlate with the histopathological variables analysed.

Discussion

Ductal adenocarcinoma of the pancreas is the fifth leading cause of death from cancer in Western countries [19]. In most cases the diagnosis is made when the tumour has already spread beyond the pancreas and has metastasized to lymph nodes. Metastases to nodal groups occur very early in the course of the disease, and some of these groups are not usually removed by the standard Whipple

procedure [1], which greatly jeopardizes the outcome of seemingly appropriate treatment.

Takada et al. [17] have recently shown, by flow cytometry, that CD44 levels in pancreatic carcinoma cells are over 100 times those in normal pancreatic cells. In the same study, four human pancreatic adenocarcinoma cell lines were investigated with anti-human CD44 and showed similar positive results. In another study, Takada et al. [18] also demonstrated that proliferation of human pancreatic adenocarcinoma cells is not affected by anti-CD44 antibody, whereas the invasive capacity of these cells is suppressed by this antibody.

However, Rall et al. [12], using reverse transcriptase protein chain reaction (RT-PCR) methods, have reported no differences in CD44 levels in specimens of primary or metastatic adenocarcinoma and control pancreata. In contrast, CD44v6 is more frequently expressed in adenocarcinoma samples than in control specimens. Moreover, Gansauge et al. [5] have demonstrated, by immunohistochemistry and RT-PCR analysis, that production of CD44 splice isoforms carrying variant exons v5-v10 is enhanced in pancreatic adenocarcinoma.

Our results are partly in concordance with the data previously reported on this subject. We have not found statistically significant differences in CD44s expression in normal pancreatic tissues, tissue affected by chronic pancreatitis, and metastatic and nonmetastatic carcinoma. The results reported by Rall et al. [12] and Gansauge et al. [5] are in agreement with our data in this regard.

We have obtained positive reactions with anti-CD44v6 monoclonal antibody in pancreatic nonmetastasizing and metastasizing adenocarcinomas, as well as in samples from metastatic tumours. Normal and inflamed tissues showed no reactivity for this variant exon in our hands. Rall et al. [12], using RT-PCR/Southern blot analysis, found two CD44 isoforms that hybridize with radiolabelled CD44v6 in malignant tissues. The differences between metastasizing and nonmetastasizing carcinomas observed in their study, like our results, were not statistically significant. This may be explained by the fact that pancreatic adenocarcinoma, as stated above, metastasizes very early to lymph node groups that are not always removed by the usual Whipple procedure [1]. In view of this, carcinomas considered to be nonmetastasizing are presumably metastatic at the time of diagnosis. More recently, Gansauge et al. [5], basing their statement on immunohistochemical, Western blot and RT-PCR techniques, have reported that in pancreatic adenocarcinomas exon v6 is associated with several other variant exons, leading to an expression of multiple splice isoforms that contain at least v4–v10, whereas CD44v6 immunohistochemical expression in normal ductal cells is the translation of a splice isoform consisting only of the variant exon v6. These authors did not specify whether the adenocarcinomas included in their study were metastasizing or not. Our results, like those of Rall et al. [12], seem to indicate that CD44v6 overexpression in human pancreatic adenocarcinoma may be related to a possible role of this variant isoform in the invasive and metastatic mechanisms of this neoplasm. This is supported by several studies suggesting that surface expression of exon 6-containing isoforms diminishes binding to hyaluronate and thus permits the maligant cell to detach from the basement membrane or from adjacent cells [16]. In view of Gansauge et al.'s results [5], further investigations are needed to determine whether detaching and invasive capabilities are conferred on neoplastic cells by exon v6 itself or rather by CD44 isoforms with large chains containing several variant exons.

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