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Distribution of type IV collagen in pancreatic adenocarcinoma and chronic pancreatitis

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Summary. Changes in the basement membrane are present in various neoplastic conditions such as neurofibrosarcoma, cervical carcinoma, colorectal cancers and hepatoblastoma. This study examines the expression of type IV collagen in the basement membrane, using an immunohistochemical method, in the normal pancreas ($n=10$), chronic pancreatitis ($n=15$) and pancreatic adenocarcinoma ($n=30$). The formalin fixed, paraffin embedded tissue was sectioned and pretreated with protease prior to immunostaining for type IV collagen. There was a statistically significant difference in type IV collagen expression between pancreatic carcinoma and chronic pancreatitis ($P=0.0001$; χ^2 test with continuity correction). In pancreatic adenocarcinoma, type IV collagen distribution in the basement membrane was discontinuous and irregular or absent around individual or groups of neoplastic cells ($n=30$). Most cases of chronic pancreatitis showed continuous pattern of basement membrane type IV collagen around residual ducts ($n=10$). In the normal pancreas, only one of the ten cases showed discontinuous basement membrane around pancreatic ducts, while in the rest of the cases, the pattern was continuous. This study suggests that there is abnormal distribution of type IV collagen in the basement membrane in pancreatic carcinoma, which may be related to either abnormal deposition or degradation of the collagen. Immunostaining for type IV collagen may be of some diagnostic use for distinguishing pancreatic adenocarcinoma from problematic cases of chronic pancreatitis.

Keywords: type IV collagen, pancreatic cancers, chronic pancreatitis, immunohistochemistry

Epithelial alterations sometimes found in non-neoplastic pancreatic ducts and ductules, such as mucous cell hypertrophy, goblet metaplasia and epithelial hyperplasia (Oertel, 1989; Rode, 1990), can create diagnostic problems in routine histopathology. In addition, scattered aggregates of dilated ductules lined by cuboidal or goblet cells and surrounded by fibrous tissue, frequently found in chronic pancreatitis (Pour *et al*. 1982; Haglund *et al*. 1984), can occasionally be misinterpreted as carcinoma (Rode 1990).

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During normal embryological development of the pancreas, basement membrane is formed around the ducts and acini (Jamieson 1982). However, pancreatic

cells have been experimentally shown to lose this capacity to deposit basement membranes when they become malignant (Ingber *et al.* 1981). A number of basement membrane components, such as collagen IV and laminin, have been isolated and antibodies are currently available for their detection by immunohistochemical methods (Foellmer *et al.* 1983; Timpl & Martin 1982). Several studies have shown that basement membrane integrity is disrupted or lost in neoplastic cells (Albrechtsen *et al.* 1981; Barsky *et al*, 1983; Burtin *et al*, 1982; Compagno & Oertel 1978; Chanoki *et al.* 1991).

We used a monoclonal antibody directed against type IV collagen to see whether its expression may be helpful in distinguishing inflammatory conditions from adenocarcinoma in pancreatic tissue core biopsies.

Materials and methods

Pancreatic biopsies were obtained percutaneously, under ultrasonographic guidance, using a spring-loaded, Tru-cut type biopsy device, from a total of 47 patients who presented with masses in the pancreatic or peripancreatic region. Cases of chronic pancreatitis (n = 15) and pancreatic adenocarcinomas (n = 30) were investigated. A total of 10 cases of normal pancreas (two core biopsies and eight autopsy specimens) were included in the study. The adenocarcinomas investigated were histologically graded as well differentiated (n = 1), moderately differentiated (n = 2), moderately to poorly differentiated (n = 22) and poorly differentiated (n = 5). The moderately to poorly differentiated adenocarcinomas were identified as those with areas of both moderate glandular differentiation and anaplasia.

The tissues were formalin fixed and paraffin processed. After paraffin processing, 4-μm sections were cut, mounted on 5-aminopropyltriethoxysilane (AAS) coated slides and allowed to dry overnight. One section was used for immunostaining and another as negative control. After dewaxing in xylene, blocking of endogenous peroxidase, washing in phosphate-buffered saline (PBS), enzyme digestion for 20 minutes in 0.1% protease, and blocking of non-specific binding of secondary antibody with normal swine serum, routine streptavidin–biotin–peroxidase immunostaining with diaminobenzidine was applied to the sections incubated overnight with a monoclonal antibody against human collagen IV, PHM-12 (Silenus, cat no. 12HTTS03, clone 24.12.8) (1:400, dilution). The primary antibody was substituted with PBS in sections used as negative controls. The sections were counterstained with Harris haematoxylin.

Histological assessments of the basal lamina stained by collagen IV around neoplastic ducts and glands and acinar and ductal structures of non-neoplastic pancreatic tissue were made independently by two of the authors (CSL and JR). The pattern of staining was noted as either continuous, absent or discontinuous. In the histological assessment of stromal staining, the intensity was independently assessed on a semiquantitative 4-point scale as follows: 0, no staining; +, weak; + +, moderate; and + + +, intense staining. Cells with very weak equivocal staining were considered as negative.

Statistical analysis

Categorical variables were analysed using the chi-squared (χ^2) contingency tests. Only *P* values of less than 0.05 were considered significant.

Results (Table 1)

All cases of pancreatic adenocarcinoma ($n = 30$; 100%) showed abnormal pattern of collagen IV expression irrespective of the degree of differentiation. The basement membrane was either discontinuous and irregular ($n = 28$; 93%), or absent ($n = 2$; 7%) around ductal or glandular structures (Figure 1). Stromal staining was variable, ranging from mild to marked intensity, and was found in most cases ($n = 24$; 80%).

Most cases of chronic pancreatitis ($n = 10$; 67%) showed an intact, regular basement membrane around residual glandular structures (Figure 2), in contrast to adenocarcinoma ($P < 0.0001$, χ^2 test with continuity correction). Only a few cases ($n = 5$; 33%) showed abnormal collagen IV expression around ductal or glandular structures. Stromal staining was also variable, ranged from mild to moderate in intensity, and was found in most cases ($n = 12$; 80%).

Of the ten cases of normal pancreas studied, only one showed irregular and discontinuous basement membrane while the rest of the cases had regular, continuous basement membrane around normal ducts and acini. However, stromal staining for collagen IV was absent in two cases (Figure 3).

Discussion

Histological diagnosis of pancreatic cancer can be made in most instances when adequate material is available. However, differentiation between pancreatic carcinoma and chronic pancreatitis can be difficult when scattered neoplastic ducts are associated with secondary inflammation (Mackie *et al.* 1980) or in those cases where ductular changes of chronic pancreatitis can mimic adenocarcinoma (Rode 1990). The problem is com-

Table 1. Summary of collagen IV staining characteristics in benign and malignant conditions of the pancreas

Diagnosis	Number of cases	Staining pattern or number of cases						
		Basal lamina			Stroma			
		Cont.	Discont.	Absent	−	+	2+	3+
Adenocarcinoma	30							
Well differentiated	1	—	1	—	1	—	—	—
Moderately differentiated	2	—	2	—	—	1	1	—
Moderate to poorly differentiated	22	—	21	1	4	9	5	4
Poorly differentiated	5	—	4	1	1	2	2	—
Chronic pancreatitis	15	10	2	3	3	8	4	—
Normal pancreas	10	9	1	—	2	—	—	8

Cont., Continuous; Discont. discontinuous.

pounded if a biopsy is small. In view of these problems, we have been investigating means to diagnose pancreatic cancer in biopsy material with greater confidence.

Basement membranes are composed of a cross-linked network of type IV collagen, laminin and heparan sulphate proteoglycan (Timpl *et al*, 1979; Martinez-Hernandez & Amenta 1983; Abrahamson 1986). Alterations in these components are observed in a number of human malignancies. In neurofibromas, type IV collagen and laminin were found to surround tumour cells whereas this was not observed in neurofibrosarcoma (Chanoki *et al*. 1991). Abnormalities of the basement membrane are found in benign and malignant conditions of the ectocervix (Richards & Furness 1990). In benign cervical lesions, the squamous epithelium possesses a continuous basement membrane. Small breaks in the basement membrane occur when there is cytological atypia associated with either wart virus infection or cervical intraepithelial neoplasia (CIN) (Richards & Furness 1990). Fragmentation of the basement membrane is observed with invasive carcinoma of the cervix. A more recent immunohistochemical study of type IV collagen also shows that defects in the subepithelial basement membrane are present in both in-situ and invasive squamous cell carcinoma of the uterine cervix (Stewart & McNicol 1992).

In colorectal carcinomas, the distribution of type IV collagen immunoreactivity is sparse and this is most

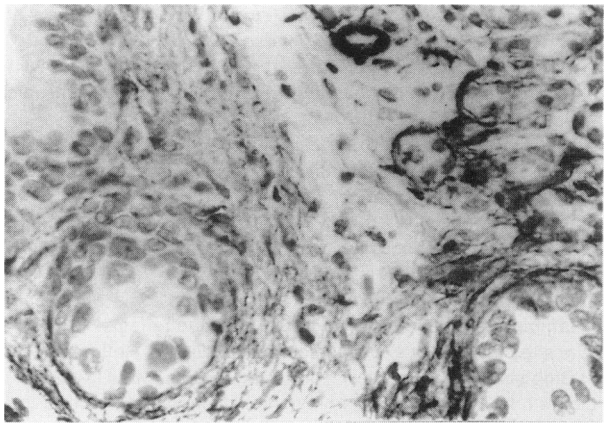

Figure 1. Discontinuous distribution of type IV collagen in the basement membrane around neoplastic glands in pancreatic adenocarcinoma (left). Some adjacent non-neoplastic acini and ductules (top right) showed regular and continuous distribution of surrounding basement membrane type IV collagen. Immunoperoxidase. × 360.

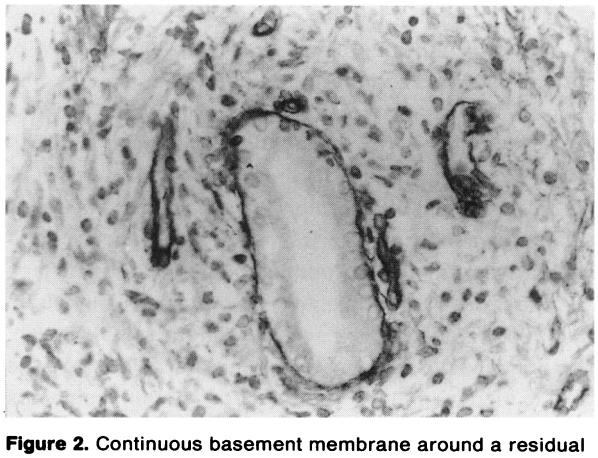

Figure 2. Continuous basement membrane around a residual gland (middle) in chronic pancreatitis as highlighted by type IV collagen immunostaining. Blood vessels adjacent to the gland were also stained positive. Immunoperoxidase. × 360.

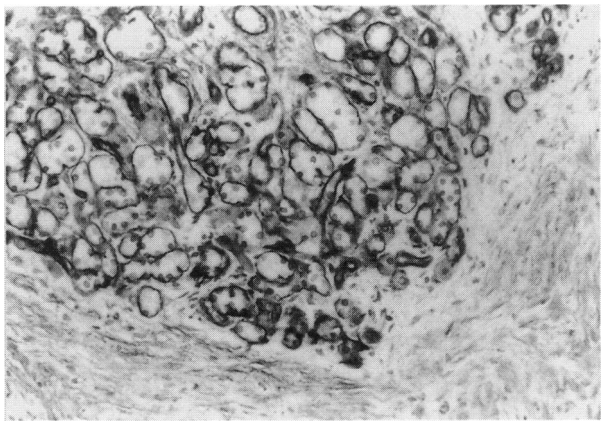

Figure 3. Continuous distribution of type IV collagen in the basement membrane around the acini and a few ducts present in normal pancreatic tissue. Immunoperoxidase. × 180.

severe at the invasive margin of the tumour (Hewitt *et al.* 1992). On the other hand, epithelial basement membrane collagen-IV immunostaining is abundant in normal mucosa and benign adenomas of the colorectum (Hewitt *et al.* 1992). In hepatoblastomas, increased amounts of type IV collagen are present in the persinusoidal space around the cords of tumour cells, while this is not observed in the normal liver (Ruck & Kaiserling 1992).

Basement membrane formation is essential for the development of the pancreas (Jamieson 1982). However, early studies on pancreatic cell lines and human pancreatic cancers showed that neoplastic transformation of pancreatic cells was associated with loss or abnormal production of basement membrane components (Ingber *et al.* 1981; Barsky *et al.* 1983; Haglund *et al.* 1984). In our study, similar findings were obtained in which most cases of pancreatic adenocarcinoma showed irregular or deficient type IV collagen expression around neoplastic ducts. On the other hand, the majority of cases of normal pancreas and chronic pancreatitis show continuous and regular basement membrane. This difference in type IV collagen expression was statistically significant ($P = 0.0001$; χ^2 test with continuity correction).

The abnormal expression of type IV collagen in pancreatic adenocarcinomas may be related to a combination of enzymatic degradation of the collagen by type IV collagenase, and defective or reduced synthesis of collagen-IV, described earlier by Liotta *et al.* (1988). Tumour cells possess laminin receptors which allow the cells to bind to laminin. The attached laminin then binds to type IV collagen in basement membranes which enables the neoplastic cell to invade through the basement membrane into the stromal extracellular matrix (Liotta, 1984; 1986; Liotta *et al.* 1988). During the process of tumour infiltration, type IV collagenase, a metalloproteinase produced by the neoplastic cells, degrades the basement membrane collagen-IV (Liotta *et al.* 1988).

In conclusion, this study shows that there is discontinuity or absence of basement membrane type IV collagen in most cases of pancreatic adenocarcinoma, in contrast to chronic pancreatitis and the normal pancreas. Consequently, immunostaining for type IV collagen may be of some diagnostic use for distinguishing pancreatic adenocarcinoma from problematic cases of chronic pancreatitis.

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