Expression of laminin in benign and malignant sclerosing lesions of extrahepatic bile ducts

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SUMMARY The distribution of laminin, a basement membrane glycoprotein, was studied by an immunohistochemical technique in six samples of benign sclerosing lesions of extrahepatic bile ducts and in 11 sclerosing cholangiocarcinomas. The expression of laminin showed that benign glandular structures were surrounded by a mainly intact basement membrane. In sclerosing cholangiocarcinomas laminin was irregularly distributed, and in large areas totally absent. The findings suggest that deposition of basement membrane material is defective. Sclerosing cholangitis may be extremely difficult to distinguish from well differentiated sclerosing cholangiocarcinoma. This sample was small, but the diseases studied were relatively rare.

Staining for laminin might be of additional use to surgical pathologists in the differential diagnosis of benign sclerosing lesions and sclerosing cholangiocarcinomas.

Disorders of the extrahepatic bile duct, which cause fibrous thickening of the wall and stenosis of the lumen, may be reactive to an inflammatory condition, or may represent a malignant disease. The condition presents a difficult diagnostic problem both to surgeons and surgical pathologists. Benign bile duct strictures may be due to primary sclerosing cholangitis, a rare condition of unknown aetiology, characterised by chronic fibrosis and inflammation, or to so called "secondary" sclerosing cholangitis, which may follow on from common bile duct stones or previous biliary surgery. In these conditions atypical glands are often present in the wall and may therefore be confused with well differentiated sclerosing cholangiocarcinoma, a variant of bile duct carcinoma.¹⁻⁴

Several studies have shown that loss of basement membrane integrity is characteristic of different types of malignant epithelial cells.⁵⁻¹³ Laminin is a large glycoprotein which is synthesised by many types of cells and is present in both epithelial and vascular basement membranes.^{14 15}

In this study we evaluated whether demonstration of basement membranes using laminin antibodies

might help to distinguish between benign and malignant sclerosing lesions of extrahepatic bile ducts.

Material and methods

Specimens obtained from extrahepatic sclerosing lesions were as follows: two of primary sclerosing cholangitis; four of secondary sclerosing cholangitis (two cases of postoperative fibrosis and two strictures following common bile duct stones); and 11 of sclerosing cholangiocarcinoma.

Routinely processed paraffin wax blocks of formaldehyde fixed tissues had been stored for one to 10 years. Dewaxed tissue sections were reacted with rabbit antibodies against mouse laminin, which cross-reacts with human laminin,^{16 17} and bound antibody was visualised by the avidin-biotin complex technique (ABC) (Vector Laboratories, Burlingame, California, USA), as described previously.¹¹ No nuclear counterstain for nuclei was used. Perivascular laminin staining was seen in all sections, which served as an internal positive control.¹⁸ Treatment with pepsin made it possible to detect laminin in formalin fixed tissues.¹⁸ The optimal dilution of the primary antibody was determined in a control series. Control staining with pre-immune serum was negative in all cases. Conventional staining techniques were used for routine histological examination.

Fig 1 Sclerosing cholangitis. Glandular structures are surrounded by a mainly intact layer of laminin. Blood vessels are positive for laminin (V). (Immunoperoxidase staining with laminin antibodies, bar = 100 μ m).

Fig 2 Well differentiated sclerosing cholangiocarcinoma. Malignant glands are surrounded by an irregular and discontinuous basement membrane. (Immunoperoxidase staining with laminin antibodies, bar = 100 μ m, V = blood vessels.)

Fig 3 Well differentiated sclerosing cholangiocarcinoma. In this specimen only patches of laminin was seen around glandular structures. (Immunoperoxidase staining with laminin antibodies, bar = 100 μ m, V = blood vessels.)

Results

In benign sclerosing lesions, both in primary sclerosing cholangitis and in secondary fibrosing lesions, glandular structures were surrounded by a mainly intact layer of laminin (fig 1). Only a few short breaks in continuity were seen. Nerves and vessel walls were positive for laminin. No positive staining was seen inside the cells.

In sclerosing cholangiocarcinomas there was an almost intact basement membrane around some well differentiated glands, but around most glands the basement membrane was irregular and discontinuous (fig 2). In places, even in well differentiated areas, only patches of laminin were seen (fig 3). In moderately differentiated areas the basement membrane was always clearly affected and in poorly differentiated areas only patches of laminin were seen, or laminin was totally absent. No intracellular staining was seen.

Discussion

The clinicopathological features of benign and malig-

nant disorders affecting the extrahepatic bile ducts may be similar. Anamnestic data may help to distinguish postoperative stenosis from other conditions, and endoscopic retrograde cholangiography (ERCP) or percutaneous cholangiography (PTC) may lead to a correct diagnosis, but in many cases laparotomy is needed. Benign sclerotic processes and sclerosing cholangiocarcinoma may be difficult to distinguish even from adequate biopsy specimens taken during laparotomy. Biopsy specimens are often small. Sclerosing cholangiocarcinomas are often extremely well differentiated. In benign disorders atypical intramural duct-like structures, involved in the sclerosing process, may histologically mimic neoplastic glands. Because the choice of treatment depends on the histopathological diagnosis, it is important to find methods which distinguish between these conditions.¹⁻⁴

Malignant transformation is accompanied by the loss of an intact basement membrane. This has been shown in different carcinomas using antisera against various basement membrane components.^{5-13 16} This study shows similar results for sclerosing cholangiocarcinomas. Possible mechanisms of basement

membrane deficiency are disturbed synthesis of basement membrane proteins, a lack of deposition of these proteins to form a basement membrane, a masking of the basement membrane proteins by unknown deposits, and proteolytic degradation of formed basement membranes. Several reports show that proteolytic activity is increased in malignant tumours.¹⁹⁻²¹ In pancreatic carcinomas intracellular laminin has been shown in the absence of extracellular deposits, suggesting that some cancer cells continue to synthesise laminin,¹¹ but in this study no intracellular laminin was seen in cholangiocarcinomas.

Our results show that basement membrane continuity is disturbed in sclerosing cholangiocarcinomas; only a few discontinuities are seen in benign sclerosing lesions. This study included only a few cases of benign lesions, because such specimens are difficult to obtain. Therefore, no definite conclusions can be drawn, but it seems that staining for laminin might be of some help to the surgical pathologists in cases where the differential diagnosis is unclear.

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