Difference in tissue expression of tumour markers CA 19-9 and CA 50 in hepatocellular carcinoma and cholangiocarcinoma

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Summary  The expression of tumour markers CA 19-9 and CA 50, defined by the monoclonal antibodies 1116 NS 19-9 (19-9 antibody) and C 50, was studied by the immunoperoxidase technique in formalin-fixed, paraffin-embedded tissue sections from 11 hepatocellular carcinomas and 10 cholangiocarcinomas of the liver, and from specimens of normal liver and liver cirrhosis. The 19-9 and C 50 antibodies react with sialofucosylactotetraose, corresponding to sialylated blood group antigen Lewisα, and the C 50 antibody also with another sugar moiety, sialofucosylactotetraose. Neither marker was cancer specific. The CA 19-9 and CA 50 antigens are normal constituents of bile ducts. Nine out of 10 cholangiocarcinomas stained for CA 50, and eight out of 10 for CA 19-9. There was no apparent difference between the staining pattern of CA 19-9 and CA 50. Hepatocellular carcinomas were consistently negative for both markers. Thus, hepatocellular carcinomas and cholangiocarcinomas showed a clear difference in the reactivity for tumour marker antigens CA 19-9 and CA 50. This difference might be of clinical importance in the differential diagnosis between hepatocellular carcinoma and cholangiocarcinoma.

Immunohistochemically, the CA 19-9 antigen (Koprowski et al., 1979) has been demonstrated in various gastrointestinal carcinomas, such as pancreatic and biliary tract cancer, and also in many normal tissues, among others in pancreatic ducts, gallbladder, extra- and intrahepatic bile ducts, but not in hepatocytes (Atkinson et al., 1982; Arends et al., 1983; Haglund et al., 1986a). The CA 50 antigen (Lindholm et al., 1983) has been detected in tissue extracts from various gastrointestinal and non-gastrointestinal epithelial tumours: pancreatic, colorectal, gastric, gall bladder, lung, urinary bladder, and liver cell carcinomas, as well as carcinomas of the breast and uterine cervix (Nilsson et al., 1983). Benign and malignant pancreatic lesions express CA 50 (Haglund et al., 1986b), but CA 50 has not been reported in hepatobiliary lesions.

In this work, we have studied the tissue expression of CA 19-9 and CA 50 in hepatocellular carcinomas and intrahepatic cholangiocarcinomas, as well as in normal liver tissue and liver cirrhosis.

Materials and methods

Specimens

The following specimens were studied: 11 hepatocellular carcinomas; ten cholangiocarcinomas of the liver; four samples of liver cirrhosis; and five samples of normal liver tissue. In addition, histologically normal liver tissue was used in 16 specimens obtained from livers with carcinoma (all cholangiocarcinomas and half of the hepatocellular carcinomas). The samples were formalin-fixed, paraffin-embedded surgical specimens, which had been stored for between 1 and 11 years.

Antibodies

Tissue culture supernatants containing mouse monoclonal antibodies 1116 NS 19-9 (IgG1) and C 50 (IgM) were used for the CA 19-9 and CA 50 stainings. The antibodies were obtained from H. Koprowski (Philadelphia, USA) and L. Lindholm (Gotenburg, Sweden).

Staining procedure

Paraffin sections 5 μm thick were deparaffinised and treated with 0.4% pepsin (2500 FIP-U/g, Merck, Darmstadt, Germany) in 0.01 N HCl for 1 h at 37°C. All sections were then incubated in 0.5% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase, incubated with non-immune horse serum, diluted 1:20, and then reacted with the primary antibody supernatant; the C 50 antibody was diluted 1:20 and the 1116 NS 19-9 antibody 1:50. Bound antibody was visualized by the avidin-biotin complex assay (ABC) (Vectastain, Vector, Burlingame, CA): the sections were successively treated with biotinylated antimouse immunoglobulin antiserum, avidin, and biotinylated horse-radish peroxidase complex. Each step was followed by washing in phosphate-buffered saline (PBS). Finally, sections were incubated with 3-amino-9-ethyl-carbazole (AEC) and hydrogen peroxide, and then counterstained with haematoxylin. Sections stained with normal mouse serum and PBS served as negative controls.

Controls

The following controls were used: (1) A known CA 19-9 and CA 50 positive specimen of pancreatic carcinoma served as positive control in each series. (2) Sialic acid in the sialofucosyl-lactotetraose residue was removed with neuraminidase. Sections were incubated with 0.3 U ml⁻¹ Vibrio cholerae neuraminidase (1 U ml⁻¹) (Behringwerke, Marburg, Germany), diluted with PBS containing 0.9 mM Ca²⁺ and 0.5 mM Mg²⁺ for 2 h at 37°C to remove sialic acid before incubation with the primary antibodies. Sections incubated with buffer only served as negative controls.

Results

Normal liver

In all five specimens, many large and middle size bile ducts stained for CA 19-9 and CA 50 (Figure 1a and b). In addition, small foci of positive cells were seen inside the liver parenchyma. These cells do not look like hepatocytes and probably represent interlobular bile ducts. In most specimens the CA 50 staining was more intense.

In histologically normal liver tissue adjacent to carcinoma, small biliary ducts and part of the large bile ducts stained positively for CA 50 in all specimens. CA 19-9 was expressed in some small bile ducts in one specimen. In all other speci-
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Figure 1 Normal liver tissue. Immunoperoxidase staining with the 1116 NS 19-9 a, and C 50 b antibodies, counterstained with hematoxylin. Small interlobular bile ducts are positive for CA 19-9 and CA 50 and in addition one bile duct in the portal area for CA 50. Bar = 100 μm.

Figure 2 Liver cirrhosis. Immunoperoxidase staining with the 1116 NS 19-9 a, and C 50 b antibodies, counterstained with hematoxylin. Bile ducts stain for CA 19-9 and CA 50. Bar = 100 μm.

Figure 3 Hepatocellular carcinoma. Immunoperoxidase staining with the 1116 NS 19-9 a, and C 50 b antibodies, counterstained with hematoxylin. A large bile duct stain for CA 19-9 and CA 50, whereas small bile ducts within the carcinoma (arrow) stain positively only for CA 50. Hepatocellular carcinoma cells are negative. Bar = 100 μm.

A mens a positive CA 19-9 staining was observed in part of the large bile ducts only, but not in the small interlobular bile ducts.

Liver cirrhosis

In areas of proliferation of bile ducts, the ductal epithelium stained strongly for both CA 50 and CA 19-9 (Figure 2a and b). No clear difference was seen between the markers. The interlobular bile ducts expressed CA 19-9 and CA-50 like in normal liver.

Hepatocellular carcinomas

Large bile ducts between foci of hepatocellular carcinoma stained positively both for CA 50 and CA 19-9. The staining for CA 50 was usually more uniform. Benign looking structures within the carcinoma stained positively for CA 50 in five out of 11 specimens and for CA 19-9 in one specimen. These structures are similar to those structures which stained positively in normal liver. Tumour cells of hepatocellular carcinomas were consistently negative for both makers (Figure 3a and b).

Cholangiocarcinomas

Nine out of 10 cholangiocarcinomas stained positively for CA 50, and eight out of 10 for CA 19-9. The positivity was predominantly seen in the apical part of the carcinoma cells, but in many cells a diffuse intracytoplasmic staining was seen (Figure 4a and b). There was no apparent difference between the expression of CA 19-9 and CA 50.

Neuraminidase treatment

Incubation of specimen with 0.3 U ml⁻¹ neuraminidase abolished the CA 19-9 and CA 50 staining reactions.
carcinomas did not express these antigens at all. On the other hand, most cholangiocarcinomas (80–90%) stained for both CA 19-9 and CA 50. The staining pattern was similar to that of pancreatic carcinomas (Haglund et al., 1986a,b). Thus it seems that the difference between hepatocellular carcinoma and cholangiocarcinoma is more pronounced when using staining for CA 19-9 or CA 50 than it is with carcinoembryonic antigen (CEA) or alpha-foetoprotein (AFP). CEA can be found in both cholangiocarcinomas and hepatocellular carcinomas (Kojiro et al., 1981; Goodman et al., 1985). Alpha-foetoprotein is expressed in 35–73% of hepatocellular carcinomas, but not in cholangiocarcinomas (Thung et al., 1979; Kojiro et al., 1981; Goodman et al., 1985).

Most cholangiocarcinomas show immunohistochemical reactivity for CA 19-9 and CA 50. This is in concordance with the expression of these antigens in serum. Elevated serum levels of CA 19-9 (67–73%) and CA 50 (58%) are frequently seen in patients with bile duct carcinomas (Jalanko et al., 1984; Ritts et al., 1984; Kuusela et al., 1987). Hepatocellular carcinomas do not stain for either marker. Yet, some patients with hepatocellular carcinoma have elevated CA 19-9 (22%) and CA 50 levels (54–78%) in serum (Jalanko et al., 1984; Chan et al., 1985; Habib et al., 1986; Kuusela et al., 1987). An explanation for elevated CA 50 and CA 19-9 levels in serum of patients with hepatocellular carcinomas might be that CA 50 and CA 19-9 is produced by the tumour, but that the serum levels are more sensitive than immunohistochemical staining. However, previous works on pancreatic cancer speak against that theory, as some patients with pancreatic cancer had a strong tissue expression of CA 19-9 and CA 50, but normal serum levels (Haglund et al., 1986a,b). Furthermore, the percentage of positive serum samples in patients with hepatocellular carcinomas is not greater than the percentage of positive samples in patients with benign liver diseases (Jalanko et al., 1984; Chan et al., 1985; Haglund et al., 1987; Kuusela et al., 1987). Therefore, we do not think that hepatocellular carcinomas produce neither CA 19-9 nor CA 50.

The reason for the higher frequency of elevated CA 50 than CA 19-9 serum values in liver cirrhosis is not known. Immunohistochemically, both CA 50 and CA 19-9 were strongly and widely expressed, and no difference between these markers could be demonstrated. The C 50 antibody reacts, in addition to sialylated Lewis' with at least one other antigenic determinant, sialosylactotetraose (Nilsson et al., 1985). This carbohydrate structure could be shed into the blood stream and explain the difference between the expression of the markers.

The reactivity for CA 19-9 and CA 50 in cholangiocarcinomas may be of importance in the differential diagnosis with hepatocellular carcinoma. On the other hand, cholangiocarcinomas of the liver cannot be distinguished from metastatic liver disease. Other gastrointestinal tumours, such as pancreatic, extrapancreatic bile duct, gastric and colorectal carcinomas often express CA 19-9 and CA 50 (Atkinson et al., 1982; Arends et al., 1983; Nilsson et al., 1983; Haglund et al., 1986a,b), and primary tumours of these organs must be excluded by other diagnostic methods.

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Figure 4 Cholangiocarcinoma of the liver. Immunoperoxidase staining with the 1116 NS 19-9 a, and C 50 b antibodies, counterstained with hematoxylin. Tumour cells stain for CA 19-9 and CA 50. Bar = 100 μm.

Discussion

Monoclonal antibodies 1116 NS 19-9 (19-9-antibody) and C 50 have been obtained after immunisation of mice with human colonic adenocarcinoma cell lines SW 1116 and COLO 205, respectively (Koprowski et al., 1979; Lindholm et al., 1983). Both antibodies react with sialosyl-fucosyl-lactotetraose, corresponding to sialylated blood group antigen Lewis' (Magnani et al., 1982; Månsson et al., 1985). In addition, the C 50 antibody reacts with a least one other carbohydrate structure, the sialosyl-lactotetraose, which lacks the fucose moiety of sialylated Lewis' (Nilsson et al., 1985). Thus, the C 50 antibody does not require the fucosyl moiety of the sugar chain of the Le' structure for binding.

Immunoperoxidase staining has previously been shown to be a reliable method of demonstrating the CA 19-9 and CA 50 antigens in formalin-fixed, paraffin-embedded specimens. The optimal staining result is obtained after enzyme pretreatment (Haglund et al., 1986a,b).

Our results show that the CA 19-9 and CA 50 antigens are normal constituents of bile ducts. There was a slightly stronger expression of CA 50, which might reflect the broader reactivity of the C 50 antibody.

Hepatocellular carcinomas and cholangiocarcinomas showed a clear difference in the expression of tumour marker antigens CA 19-9 and CA 50. Tumour cells of hepatocellular
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