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Characteristics of the distribution of lectin receptors in intrahepatic cholangiocellular carcinoma

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Summary

The receptors of peanut agglutinin (PNA), *Dolichos biflorus* agglutinin (DBA) and *Ulex europaeus* agglutinin I (UEA-I) were localized in intrahepatic cholangiocellular carcinoma, hepatocellular carcinoma, intrahepatic bile ducts and normal, cirrhotic and pericarcinomatous liver using the avidin–biotin–peroxidase complex method. It was found that epithelial cells of normal bile ducts had many UEA-I receptors, fewer DBA receptors and no PNA receptors. The positive rates of PNA, UEA-I and DBA receptors in 18 cases of intrahepatic cholangiocellular carcinoma were 88.9%, 61.1% and 33.3% respectively, which were significantly higher than those in hepatocellular carcinoma (16.0%, 4.0% and 4.0% respectively). Hepatocytes in normal, cirrhotic and pericarcinomatous liver had no receptors for these three lectins. It is suggested that lectin receptor distribution in intrahepatic cholangiocellular carcinoma is obviously different from that in normal bile duct cells and in hepatocellular carcinoma, and might be used as an auxiliary index in its clinical diagnosis.

Introduction

Liver cancers are common human malignant tumours. With the discovery of α-foetoprotein (AFP) as a tumour marker and the continuous improvement of measuring it, the diagnosis of AFP-positive hepatocellular carcinoma, which is the most common of liver cancers, has become relatively easy. The investigation of the AFP variants, abnormal prothrombin, α-L-fucosidase and γ-glutamyltranspeptidase (γ-GT) (Liebman *et al.*, 1984; Dicioccio *et al.*, 1985; Buamah *et al.*, 1987) have led to an increase in the rate of diagnosis of hepatocellular carcinoma with a lower level of AFP or without AFP in the serum. However, the diagnosis of intrahepatic cholangiocellular carcinoma, another of the commoner liver cancers, is difficult because of the lack of a specific tumour marker.

Lectins are a group of proteins or glycoproteins isolated from plants, micro-organisms or animals. They can bind specifically to glycosyl residues on glycoconjugates. This binding has the specificity related to both monosaccharide and anomeric carbon on sugars, and is effected by the spatial conformation of glycoconjugates (Lis & Sharon, 1986; Damjanov, 1987). Therefore lectins can distinguish slight differences in saccharide structures of glycoconjugates. In this study, peanut agglutinin (PNA), *Ulex europaeus* agglutinin I (UEA-I) and *Dolichos biflorus* agglutinin (DBA) were used as probes. The lectin receptors in intrahepatic cholangiocellular carcinoma were detected histologically by the avidin–biotin–peroxidase complex (ABC) method. Normal intrahepatic bile ducts, hepatocellular carcinoma, and normal, cirrhotic and pericarcinomatous liver were also included. Changes in the glycoconjugate composition of bile duct cells after neoplastic transformation, and its clinical implication in the diagnosis of intrahepatic cholangiocellular carcinoma, were explored.

Materials and methods

Reagents

Biotinylated lectins (biotinyl-PNA, -UEA-I and -DBA) and an ABC kit were purchased from Vector Laboratories, USA; 3,3′-diaminobenzidine tetrahydrochloride (DAB) was from Fluka AG, Switzerland; *N*-acetylgalactosamine (GalNAc) was from J. T. Baker Chemical Co., USA; fucose (Fuc) was from Serva Fine Biochemica, West Germany. Galactose (Gal) was a product of Shanghai Second Reagents Factory, China.

Tissue samples and preparation of paraffin sections

Eighteen cases of intrahepatic cholangiocellular carcinoma (male, 10 cases; female, eight cases; with an average age of 45.3 years) were included in this study; among them, seven cases were well differentiated, six cases were moderately differentiated and five cases were poorly differentiated. Also 25 cases of hepatocellular carcinoma, five cases of liver

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cirrhosis and five cases of normal liver were studied with respect to their lectin receptors. These samples were obtained by surgical biopsies or resections in our institute, except for the normal liver tissues which were from normal subjects who had died in accidents. All tissues were fixed in formalin, dehydrated with graded ethanols and embedded in paraffin in the routine fashion to become paraffin blocks. Thirty serial sections, each 4–6 μm thick, were made from each paraffin tissue block.

Histological localization of lectin receptors

Using the ABC method of Hsu *et al.* (1981) with a slight modification, the procedure was as follows. (1) The sections were deparaffinized with xylene, and rehydrated with graded ethanols; (2) immersion of the sections in 0.3% H_2O_2 in methanol for 30 min to remove any endogenous peroxidase activity; (3) immersion in 3% normal sheep serum in TBS (0.85% NaCl, 0.05 M Tris/HCl buffer, pH 7.5) for 30 min to prevent non-specific absorption of proteins during labelling; (4) rinse three times with TBS, each for 3–5 min; (5) incubation with biotinyl-lectin (10 μg ml^{-1}) for 30 min at 37° C, followed by a TBS rinse as above; (6) incubation with ABC reagent (1 : 100 dilution) for 1 h at 37° C, and a TBS rinse as above; (7) coloration with 0.03% H_2O_2/0.75 mg ml^{-1} DAB in TBS for 5 min at room temperature; (8) after being rinsed for 1 min with tap water, the sections were counterstained with Haematoxylin, dehydrated with graded ethanols, cleared with xylene and mounted with neutral balsam.

Control experiments

(1) Negative control experiment: biotinyl-lectin and ABC reagent were respectively replaced by TBS on two separate tissue sections.

(2) Non-specific staining: the treatment with 0.3% H_2O_2 in methanol and 3% normal sheep serum was respectively omitted on two separate tissue sections.

(3) Specific inhibition test: biotinyl-UEA-I was replaced by biotinyl-UEA-I + Fuc; biotinyl-PNA by biotinyl-PNA + Gal; and biotinyl-DBA by biotinyl-DBA + GalNAc. The solid hapten sugars were dissolved in biotinyl-lectin solutions and fully mixed. After standing for 20 min at room temperature, the mixtures were ready to be used.

(4) H&E staining: one or two sections of each sample were stained by H&E in order to make a pathological diagnosis.

Assessment of the labelling results of lectin receptors

After excluding non-specific staining, a section with brown staining was called positive, otherwise it was negative (−). When the average rate of brown-stained cells was less than 5%, the section was designated as ±; 5–30%, as +; 30–70% as ++; and more than 70%, as +++.

Results

The distribution of lectin receptors in epithelial cells of normal intrahepatic bile ducts

Study of the distribution of lectin receptors in epithelial cells of bile ducts in the normal, cirrhotic and pericarcinomatous liver tissues investigated here revealed that bile duct cells had no PNA receptor, and DAB receptors were present in a small amount only in multiplied bile duct cells and on endomembranes of a few bile ducts, while a relatively large amount of UEA-I receptor existed in epithelial cells of both normal and multiplied bile ducts. Most of these were distributed in cytoplasm adjacent to the lumen of the bile ducts, and on their membranes (Fig. 1). However, the amount and distribution characteristics of lectin receptors in the bile duct cells varied to some extent with the size of the bile ducts.

The distribution of lectin receptors in intrahepatic cholangiocellular carcinoma

Positive receptors for each of three lectins in this carcinoma were different. They are shown in Tables 1 and 2. The positive rate of PNA receptors was the highest; out of 18 cases, 16 were positive. UEA-I receptors were present in 11 cases. The positive rate was the lowest for DBA receptors; only six cases had this receptor. The labelling result for the three lectins in one specimen was not always the same. There were five cases that were all positive when they were labelled with the lectins. Five cases contained two types of lectin receptors, and eight cases had one type of lectin receptor. All cases had at least one receptor for these three lectins (Table 1).

Similarly, the amount and distribution characteristics of one type of lectin receptor varied in different specimens. The staining rate of cancer cells varied from 0 to 100%. In the well differentiated carcinoma, the lectin receptors were mainly distributed on the membranes of the acinar lumen and in the cytoplasm adjacent to the lumen (Fig. 2). Inclusions of many acini were also stained by this procedure. The cancer cells of the poorly differentiated carcinoma appeared to be evenly stained in their overall cytoplasm (Fig. 3) or locally stained (Fig. 4). Plasma membranes of some cancer cells were stained very obviously in some cases (Fig. 5).

In addition, there were also differences in the distribution of lectin receptors between the groups of cancer cells in the same specimen. Some cancer cells had many lectin receptors, while some had none (Fig. 6). The amount and distribution characteristics of lectin receptors were not absolutely related to the differentiation grade or size of tumour, nor to the age or sex of the patients.

The distribution of lectin receptors in hepatocellular carcinoma and other liver tissues

The hepatocytes in normal, cirrhotic and pericarcinomatous liver lacked PNA, UEA-I and DBA receptors. The endothelial cells of blood vessels had many UEA-I receptors. Of 25 cases of hepatocellular carcinoma, PNA receptors were present in four cases, UEA-I in

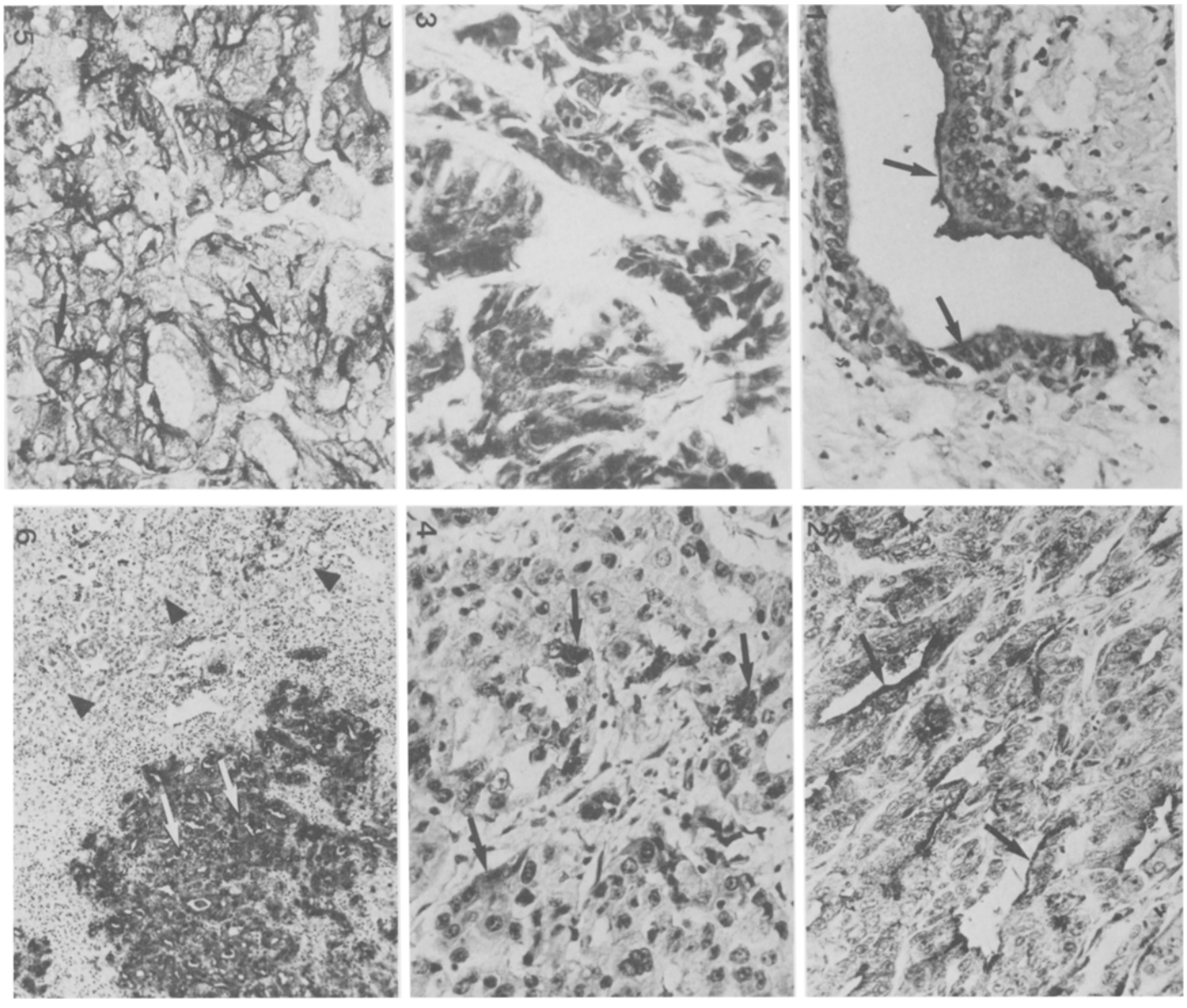

Fig. 1. UEA-I receptors (arrows) on the plasma membranes and cytoplasm of bile duct cells, which were adjacent to the lumen (×528).

Fig. 2. The polarized distribution of UEA-I receptors (arrows) in a well differentiated intrahepatic cholangiocellular carcinoma (×528).

Fig. 3. Staining of the whole cytoplasm of a poorly differentiated intrahepatic cholangiocellular carcinoma after DBA labelling (×528).

Fig. 4. Localized staining (arrows) in cytoplasm of a moderately differentiated intrahepatic cholangiocellular carcinoma after PNA labelling (×528).

Fig. 5. UEA-I receptors (arrows) outlining the plasma membranes of cancer cells (×528).

Fig. 6. Heterogeneous distribution of UEA-I receptors in intrahepatic cholangiocellular carcinoma (×132). Some groups of cancer cells were strongly stained (arrows), while others were not (arrowheads).

Table 1. Positive number of intrahepatic cholangiocellular and hepatocellular carcinoma for the three lectin receptors.

Tissue	Total cases	All lectins	Combination of two lectins			Single lectin			
			PNA + DBA	PNA + UEA-I	UEA-I + DBA	PNA	DBA	UEA-I	None
Intrahepatic cholangiocellular carcinoma	18	5	5	10	5	16	6	11	0
Hepatocellular carcinoma	25	0	1	0	0	4	1	1	20

Table 2. Comparison of positive rates of lectin receptors in intrahepatic cholangiocellular and hepatocellular carcinoma.

Tissue	No. of cases	Positive rate of lectin receptors (%)		
		PNA	UEA-I	DBA
IHCCC	18	88.89	61.11	33.33
HCC	25	16.00	4.00	4.00

one case, and DBA in one case. Not only were the labelled cells fewer, but also the positive rates of lectin receptors were much lower than those in intrahepatic cholangiocellular carcinoma (Table 2). Statistical results showed significant differences in the positive rates of PNA, UEA-I and DBA receptors between the two carcinomas.

The result of specific inhibition tests showed that the positive staining by PNA, UEA-I and DBA was inhibited by 0.5 M Gal, Fuc and GalNAc respectively.

Discussion

Epithelial cells of bile ducts play an important role in the transportation and concentration of bile as well as in the synthesis and secretion of bile mucus. They contain a large amount of glycoconjugates. These cells not only have UEA-I and DBA receptors, but are also rich in other lectin receptors (the results are not included here). The presence of these lectin receptors is closely related to the complex sugar components in glycoconjugates and their spatial structure. During the neoplastic transformation of cells, they undergo many morphological changes. The structures of the glycoconjugates also change (Hakamori, 1985). Our study with lectins revealed that there were obvious differences in the glycoconjugates of normal bile ducts and intrahepatic cholangiocellular carcinoma.

UEA-I is a lectin with high specificity for the sequence Fucα1→2Galβ1→4(Fucα1→3)GlcNAc-. It has been widely used in the study of glycoconjugates in epithelial cells of human intestine, and it is thought that the increases and decreases in UEA-I receptor expression are respectively associated with the neoplastic transformation of colon epithelial cells and the metastasis of colon cancer (Irimura *et al.*, 1987). PNA can specifically bind to Galβ1→3GalNAc-. Both UEA-I and PNA are considered to be useful lectins in the pathological diagnosis of tumours (Cooper, 1984; Allison, 1986). DBA has a specificity for GalNAc. Because these three lectins have different sugar-binding specificities, the distribution of their binding sites in cells of normal bile ducts and intrahepatic cholangiocellular carcinoma were not the same. From normal bile ducts to carcinoma, PNA receptors changed from negativity to positivity, DBA receptors increased significantly, while UEA-I receptors underwent a change in their distribution, which reflected the obvious differences in the structure of glycoconjugates between the cells of normal bile ducts and the carcinoma. The results suggest that the following changes might be involved during the neoplastic transformation of bile duct cells. (1) The appearance of new antigens, mainly T antigen which can bind PNA. This might be related to a decrease in sialylation in cancer cells, which changes the terminal oligosaccharide residue Sialα2→3Galβ1→3GalNAc- on glycoconjugates to Galβ1→3GalNAc-. This inference was confirmed by the treatment of tissues with neuraminidase, which removed the sialyl residue and, therefore, exposed the PNA-binding sites. (2) The increase in DBA-binding sites, which reflects the difference in the quantity of GalNAc residues between the normal bile ducts and the carcinoma. However, this increase was only present in some carcinomas. (3) The change in distribution of lectin receptors. The lectin receptors in epithelial cells of bile ducts were distributed in a polar fashion. They were mainly on plasma membranes and in cytoplasms that were adjacent to the lumens of the bile ducts. This distribution is related to the synthesis and secretion of mucin by bile duct cells. Although the lectin receptors were also distributed in a polar fashion in some well-differentiated carcinomas, the distribution of these receptors in most intrahepatic cholangiocellular carcinomas underwent a change and was very irregular. (4) The heterogeneity of glycoconjugates in cancer cells. Lectin receptors in some carcinomas had qualitative and quantitative differences between neighbouring groups of cancer cells (Fig. 6). The heterogeneity might be produced by differences in the glycosylation function between these cells. It

remains to be further investigated whether the cells with different glycosylation functions are related to the metastasis of tumours or not.

In addition, the receptors of PNA, UEA-I and DBA were absent from the hepatocytes of normal, cirrhotic and pericarcinomatous liver, and the positive rates of the three lectin receptors in intrahepatic cholangiocellular carcinoma were much higher than those in hepatocellular carcinoma. Every case of intrahepatic cholangiocellular carcinoma was positive for at least one and usually for two or three lectin receptors. The results suggest that a negative result for all three lectin receptors in a tissue might exclude the presence of intrahepatic cholangiocellular carcinoma. Therefore, the three lectin receptors in hepatocytes might be useful for the pathological diagnosis of this carcinoma and its differentiation from hepatocellular carcinoma. However, the differentiation of intrahepatic cholangiocellular carcinoma from liver metastatic tumours might be more difficult. Because of the lack of enough specimens, we have only examined three cases of liver metastatic tumours with respect to their lectin receptors. No conclusive result has been obtained. A further study on this problem would be valuable.

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