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Expression of glycoconjugates in intrahepatic cholangiocellular carcinoma

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Summary. Twenty cases of intrahepatic cholangiocellular carcinoma (IHCCC) were studied by lectin histochemistry for their glycoconjugate expression. Combined alcian blue-peroxidic acid Schiff (ABPS) staining was also made for mucins in these tissues. Results showed that epithelial cells of intrahepatic bile ducts contained varied quantity of DBA, WGA, LCA, Con A, PHA, RCA_I, BLA_I, SJA, SBA, PSA and UEA_I receptors but no PNA receptors. Lectin receptors were present at varying rates; 100% (Con A), 95% (PSA), 90% (LCA), 95% (WGA), 30% (BSA_I), 35% (DBA), 65% (PHA), 74% (PNA), 85% (RCA_I), 35% (SBA), 25% (SJA) and 55% (UEA_I), respectively. The positive rates in well-differentiated IHCCC were significantly higher than those in either moderately or poorly-differentiated IHCCC. The distribution of lectin receptors in IHCCC was obviously different from that in peri-carcinomatous, cirrhotic and normal liver, and also differed from that in epithelial cells of normal intrahepatic bile ducts. All larger ducts were stained by ABPS and were mainly blue colour, while only 80% of IHCCC were positive for ABPS staining. Most of them were blue, others were red or purple. The results suggest that the expression of glycoconjugates had changed after the neoplastic transformation of bile duct cells.

Key words: Bile duct neoplasms – Lectins – Histocytochemistry – Liver – Glycoconjugates

Introduction

Lectins are a group of proteins or glycoproteins with different sugar-specificity (Barondes 1981) and cell differentiation, tumour formation and metastasis may be studied by using lectins to study glycoconjugates in tissues and cells (Damjanov 1987; Pang et al. 1987). They have been widely used in biology and medicine, especially in the diagnosis and differential diagnosis of tumours and have become prognostic indicators (Buaman et al. 1986, 1987; Allison 1986; Cooper 1984; Lis and Sharon 1986). Con A and LCA have been used routinely in the differentiation of hepatocellular carcinoma (HCC) from benign liver diseases which cause the elevation of serum alpha-fetoprotein. Ree and Kadin (1985) discovered that RCA_I receptors were present on normal histiocytes and absent on malignant histiocytes. The study by Irimura et al. (1987) showed that the pattern of UEA_I-reactive high molecular glycoproteins in carcinomas of the distal colon and rectum had a relationship to their metastatic potential. Levin et al. (1980) believed that PNA receptors on white blood cells of children with acute lymphoblastic leukaemia might be a prognostic marker for the disease.

Liver cancers are human common malignant tumours, especially in Africa and Asia (Hoofnagle et al. 1987; Popper et al. 1987). Pritchard and Butler (1988) have reported the lectin-binding characteristics of aflatoxin B_1 induced lesions in rat liver. Recently, we (Zhang et al. 1989b) have investigated lectin receptors in HCC. The expression of glycoconjugates in intrahepatic cholangiocellular carcinoma (IHCCC) was studied by the lectin histochemistry and alcian blue-peroxidic acid Schiff (ABPS) staining to explore its characteristics and possible clinical value.

Materials and methods

Tissue specimens. Twenty cases of IHCCC (male, 12 cases; female, 8 cases; with an average age of 46.90 years) were included in this study, among them, 7 cases were well-differentiated,

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7 moderately-differentiated and 6 poorly-differentiated. They were randomly selected from more than 1000 liver tumours. HCC, especially those of adenoid pattern were carefully excluded. No evidence of the infestation with Clonorchis or cirrhosis was found in these 20 IHCCC patients; there was no history of any other tumours before or for one year after the operation. Five cases of cirrhosis, 5 normal livers and the intrahepatic bile ducts in them were also studied for lectin receptors. These specimens were obtained by surgical biopsies or resections in our Institute. The normal liver tissues were obtained from normal subjects who died in accidents. All tissues were fixed in formalin, dehydrated with graded ethanols and embedded in paraffin in a routine fashion to become paraffin blocks. Thirty serial sections, each 4–6 μm thick, were made from each paraffin block.

For lectin histochemistry, tissue sections were deparaffinized with xylene and rehydrated with graded ethanols. In order to expose the penultimate carbohydrate residues (Gal) on glycoconjugates covered by SA residues, duplicate series of sections were pretreated with neuraminidase (EC 3.2.1.18; Sigma St. Louis, MO, USA) before stained by PNA, DBA, SBA, WGA, RCA_I, BSA_I and SJA. The sections were incubated with neuraminidase at a concentration of 0.5 IU/ml in 0.1 mol/L acetete buffer, pH 5.5 at 37° C for 30 min. The sections were then washed with TBS (0.85% NaCl, 0.05 mol/L Tris-HCl buffer, pH 7.5) 3 times, each for 5 min.

Endogenous peroxidase activity was destroyed by the immersion of sections in 0.3% H_2O_2 in absolute methanol for 30 min. After washing with TBS as above, the sections were immersed in 3% normal sheep serum in TBS and then washed with TBS to prevent the non-specific background.

Lectin receptors in tissues were localized by avidin-biotin-peroxidase complex (ABC) method (Hsu et al. 1981; Zhang et al. 1989a). Biotinylated lectins and ABC kit were purchased from Vector Laboratories, USA. The abbreviations, plant of origin and sugar specificity of the lectins used in this study are listed in Table 1. The treated sections were incubated with biotinylated lectins (10 μg/ml) at 37° C for 30 min and rinsed with TBS as above. The sections were then incubated with ABC reagent (prepared 30 min before use by mixing avidin and biotinylated peroxidase in a ratio of 1:1 and then diluted 100 times with TBS) at 37° C for 30 min. Following washing with TBS, the sections were colourized with 0.03% H_2O_2, 0.75 mg/ml of 3,3′-diaminobenzidine tetrahydrochloride (DAB, from Fluka AG., Switzerland) in TBS at room temperature for 5 min. After washing in runing water for 1 min, the sections were counterstained with haematoxylin, dehydrated with graded ethanols, cleared with xylene and mounted with neutral balsam.

For negative controls, biotinylated lectin and ABC reagent were replaced by TBS on two separate tissue sections. To contral non-specific staining treatment with 0.3% H_2O_2 in methanol and 3% normal sheep serum was omitted on two separate tissue sections. A specific inhibition test was performed by using biotinylated lectins with their corresponding sugar haptens (Table 1). The solid sugars were dissolved in biotinylated lectin solutions and fully mixed. After standing for 20 min at room temperature, the mixtures were ready to be used. One or two sections of each specimen were stained by H+E in order to make a pathological diagnosis.

Results of lectin staining were assessed by the observation of stained sections under a optical microscope. A section with brown staining was called positive. When the average incidence of brown-stained cells was less than 5%, the section was designated as (±); 5–30%, as (+); 30–70%, as (++); and more than 70%, as (+++).

The method of combined alcian blue-peroxidic acid Schiff (ABPS) staining was used for mucins in tissues (Mowry 1984). The main procedure was as follows: rehydrated sections were treated with a solution of 1% alcian blue in acetic acid for 5 min, followed by washing them well in distilled water. The sections were treated with 1% aqueous peroxidic acid for 5 min and washing again in distilled water. The sections were then

Table 1. Characteristics of twelve lectins used in this study

Abbreviation	Plant of origin	Sugar specificity	
		Nominal specificity	Inhibitor
I. Glc/Man-specific group			
Con A	Canavalia ensiformis	α-Man > α-Glc > GlcNAc	α-Glc, α-Meth-Man
LCA	Lens culinaris	α-Man > α-Glc > GlcNAc	α-Glc, α-Meth-Man
PSA	Pisum sativum	α-Man > α-Glc = GlcNAc	α-Glc, α-Meth-Man
II. GlcNAc-specific group			
WGA	Triticum vulgare	(GlcNAc 1,4 GlcNAc)$_{1-2}$ > β-GlcNAc > SA	GlcNAc, SA
III. Gal/GalNAc-specific group			
$BSA(GSA)_I$	Bandeiraea (griffonia) simplifolia	α-Gal > GalNAc	GalNAc, α-Gal
DBA	Dolichos biflorus	GalNAcα 1,3 GalNAc ≫ GalNAc > α-Gal	GalNAc, α-Gal
PHA	Phaseolus vulgaris	GalNAc > αGal	GalNAC, α-Gal
PNA	Arachis hypogaea	Galβ 1,3 GalNAc > α and β-Gal	α-Gal
RCA_I	Ricinus communis	β-Gal > α-Gal ≫ GalNAc	α-Gal, GalNAc
SBA	Glycine maximus	α and β-GalNAc > α and β-Gal	GalNAc, α-Gal
SJA	Sophora japonica	α and β-GalNAc > α and β-Gal	GalNAc, α-Gal
IV. Fuc-specific group			
UEA_I	Ulex europaeus	α-L-Fuc	α-L-Fuc

Abbreviations. Gal = galactose; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; Glc = glucose; SA = sialic acid; Fuc = fucose; α-Meth-Man = α-methyl-mannoside; α-Man = α-mannose

incubated with Schiff reagent for 15 min and washing them as above. Finally, these sections were counterstained, dehydrated, cleared and mounted as for lectin histochemistry. Acid mucins in tissues were stained in blue, neutral ones in red and mixtures in purple.

Results

Because of differences of glycoconjugate expression in different tumour cell colonies and the varied sugar specificity of lectins, the receptors of twelve lectins in IHCCC varied both qualitatively and quantitatively (Table 2). There were many differences in the distribution of lectin receptors, positivity rates, percentages of stained cancer cells and staining intensity among the lectins, even among tumour tissues. Positivity rates were related to tumour differentiation to a certain extent (Table 3). Statistically the total positivity of lectin receptors in well-differentiated IHCCC was significantly higher than that in moderately- or poorly-differentiated IHCCC and lower than that in normal bile ducts.

Table 2. Lectin receptors in IHCCC, HCC, bile ducts and hepatocytes of normal and cirrhotic liver tissues

Lectin	IHCCC (20)[a]					HCC (25)					Normal liver (5)[b]					Cirrhosis (5)[b]					Bile duct (15)[c]				
	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Con A	−	−	−	3	17	1	−	−	4	20	−	−	−	−	5	−	−	−	−	5	−	−	−	−	15
LCA	2	1	−	4	13	−	1	−	−	24	−	−	3	1	1	−	1	−	2	2	−	−	2	13	−
PSA	1	−	3	2	14	−	−	−	25	−	−	−	−	1	4	−	−	−	1	4	−	−	−	−	15
WGA	1	2	3	3	11	1	2	7	5	10	−	−	−	−	5	−	−	−	−	5	−	−	−	−	15
BSA_I	14	1	−	3	2	16	5	3	1	−	−	−	1	4	−	−	−	−	−	5	−	−	−	3	12
DBA	13	2	1	2	2	24	−	−	1	−	5	−	−	−	−	5	−	−	−	−	−	−	8	7	−
PHA	7	4	−	5	4	14	3	5	1	2	5	−	−	−	−	5	−	−	−	−	−	−	−	−	15
PNA	5	3	2	7	3	21	−	1	1	2	5	−	−	−	−	5	−	−	−	−	15	−	−	−	−
RCA_I	3	−	2	3	12	1	−	1	10	13	−	−	−	−	5	−	−	−	−	5	−	−	−	−	15
SBA	13	2	1	3	1	22	1	−	1	1	5	−	−	−	−	5	−	−	−	−	−	−	9	6	−
SJA	15	−	2	1	2	25	−	−	−	−	5	−	−	−	−	5	−	−	−	−	−	−	−	4	11
UEA_I	9	1	3	2	5	24	−	−	1	−	5	−	−	−	−	5	−	−	−	−	−	−	−	15	−

[a] Numerals in this table represent the number of cases

[b] Only reactions of lectins with hepatocytes of normal and cirrhotic liver tissues were counted, while the reactions with bile ducts within them were not in the two columns

[c] These cases refer to the normal, cirrhotic and pericarcinomatous liver tissue blocks which contain interlobular or larger bile ducts

Table 3. The relationship of positivity of lectin receptors with the differentiation of IHCCC and its comparison with that in bile ducts

Tissue	No. of cases	Positive number of cases												Positivity (%)
		Con A	LCA	PSA	WGA	BSA_I	DBA	PHA	PNA	RCA_I	SBA	SJA	UEA_I	
IHCCC	20													
Differentiation														
well	7	7	7	7	7	4	3	6	5	6	3	4	5	76.19
moderate	7	7	6	6	6	1	2	5	5	6	3	1	4	61.90
poor	6	6	5	6	6	1	2	2	5	5	1	0	2	56.94
Positivity														
No.		20	18	19	19	6	7	13	15	17	7	5	11	
%		100	90	95	95	30	35	65	75	85	35	25	55	65.42
Normal bile ducts	15[a]													
Positivity														
No.		15	15	15	15	15	15	15	0	15	15	15	15	
%		100	100	100	100	100	100	100	0	100	100	100	100	91.67

[a] The number refers to the normal, cirrhotic and pericarcinomatous liver tissue blocks which contain interlobular or larger bile ducts

Glc/Man-specific lectins

Con A and PSA bound strongly to intrahepatic bile ducts. LCA was weaker than both Con A and PSA in the reaction with the bile ducts. Epithelial cells of the bile ducts were stained in a diffuse form by the three lectins (Fig. 1), some of them in a lumpy form. Hepatocytes in normal, cirrhotic and pericarcinomatous liver tissues contained these three lectin receptors. Con A, LCA and PSA had a relatively high affinity for IHCCC tissues. Positive rates of the lectin receptors were 100% (Con A), 95% (PSA) and 90% (LCA), respectively. These receptors in IHCCC were irregularly distributed mainly in cytoplasm of cancer cells as a non-granular staining (Fig. 2). Although both IHCCC and non-cancer liver tissues were positive for the lectin receptors, the irregular distribution in IHCCC still formed an obvious contrast with that in normal, cirrhotic and pericarcinomatous liver tissues, in which the distribution of the three lectin receptors was relatively even and regular.

GlcNAc-specific lectin

WGA can bind to both GlcNAc and SA. Its binding sites in tissues show the distribution of glycoconjugates containing these two sugar residues. Epithelial cells of intrahepatic bile ducts were rich in WGA receptors in their cytoplasm. WGA receptors in IHCCC were localized in cytoplasm and on plasma membranes of cancer cells (Fig. 3). Pretreatment of sections with neuraminidase abolished almost all stainings on the plasma membranes and reduced some in the cytoplasm. WGA receptors in normal, cirrhotic and pericarcinomatous liver tissues were localized in cytoplasm of hepatocytes in band-like distributed granular stainings and on plasma membranes. Endothelial lining cells of liver sinus were also stained. The distribution pattern of WGA receptors was obviously different between IHCCC and benign liver tissues though WGA could react with both of the tissues.

Gal/GalNAc-specific lectins

Epithelial cells of intrahepatic bile ducts had no PNA receptor. Some proliferating bile duct cells contained a few DBA receptors. Receptors of BSA_I, PHA, RCA_I, SBA and SJA were present in bile duct cells (Fig. 4). These receptors were mostly distributed in a polar form, that is, the receptors were localized in the cytoplasm and on the plasma membranes adjacent to lumens of bile ducts. Positive rates of RCA_I, PNA and PHA receptors in IHCCC were higher than those of other lectin receptors in Gal/GalNAc-specific group (Table 3). Lectin receptors in well-differentiated IHCCC were characteristic of the polar distribution and located in cytoplasm and on plasma membranes adjacent to acini of cancer cells (Fig. 5). Most of poorly-differentiated IHCCC had non-granular staining or dispersed granular stainings in cancer cells. Only RCA_I and BSA_I in Gal/GalNAc-specific lectins were positive for their receptors in hepatocytes of normal, cirrhotic and pericarcinomatous liver tissues. RCA_I receptors were granularly distributed in cytoplasm and on plasma membranes of hepatocytes and endothelial lining cells of liver sinus. Diffuse staining by BSA_I was found in dispersed hepatocytes. Pretreatment of sections with neuraminidase increased the positive rate of PNA receptors in both cancer and non-cancer tissues. However, the same treatment hardly changed positive rates of other lectin receptors in Gal/GalNAc-specific group.

Fuc-specific lectin

UEA_I receptors existed in epithelial cells of intrahepatic bile ducts and in 55% of IHCCC. They were distributed in cytoplasm and on plasma membranes of cells. Some membranes of cancer cells were strongly stained by UEA_I. The distribution heterogeneity of UEA_I receptors in IHCCC was more obvious than that of other lectin receptors (Fig. 6). UEA_I receptors were distributed also in a polar form in some well-differentiated IHCCC and absent in hepatocytes of normal, cirrhotic and pericarcinomatous liver tissues.

Among the above twelve types of lectin receptors, six types were present in hepatocytes of non-cancer liver tissues and eleven in the epithelial cells of intrahepatic bile ducts. Lectin receptors varied in 20 cases of IHCCC. Three cases contained all types of lectin receptors studied; 1 had ten types; 3 had nine types; 3 had eight types; 6 had seven types; 1 had six types; 3 contained fewer than six types. Types of lectin receptors in well- and moderately-differentiated IHCCC were increased compaired with poorly-differentiated IHCCC. Average positive rates of lectin receptors among the four sugar-specific groups were Glc/Man = GlcNAc > Fuc > Gal/GalNAc. All staining by lectins could be completely eliminated by 0.5 mol/L of their corresponding sugar inhibitors (Table 1) but 1 mol/L of Glc was needed to extinguish Con A binding.

After the ABPS staining, the blue colour was found in epithelial cells of all interlobular and

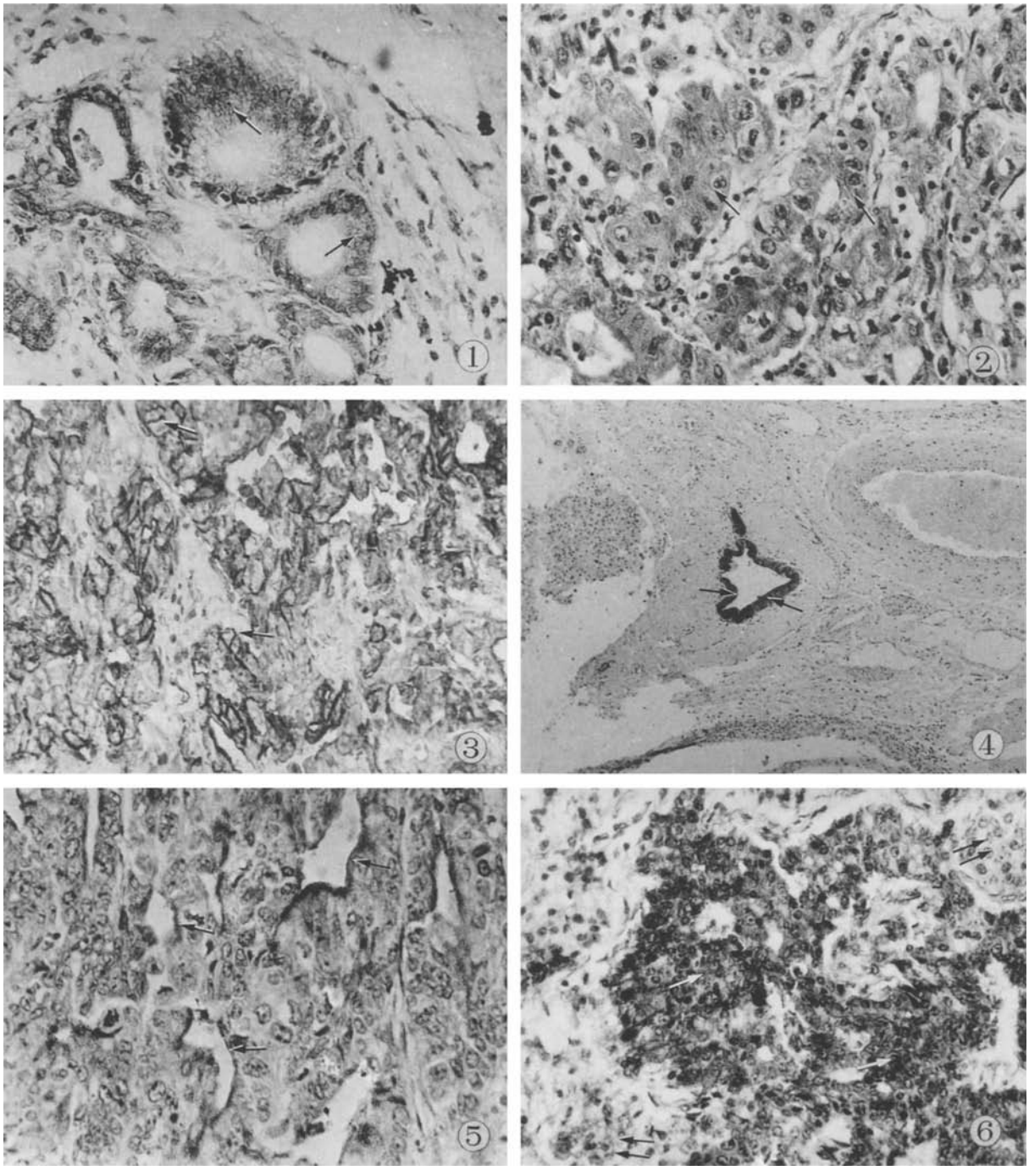

Fig. 1. Diffuse staining (*arrow*) in cytoplasm of epithelial cells of bile ducts with Con A (× 132)

Fig. 2. Cytoplasmic staining of cancer cells in IHCCC with PSA (× 528)

Fig. 3. WGA receptors (*arrow*) on plasma membranes of cancer cells in IHCCC (× 528)

Fig. 4. Strong staining of bile duct cells (*arrow*) with SJA (× 132)

Fig. 5. The polar distribution of BSA_I receptors (*arrow*) in IHCCC (× 528)

Fig. 6. Heterogeneity of UEA_I receptor distribution in IHCCC (× 528). Some of cancer cells were strongly stained (*arrow*); some were not (*double arrow*)

larger bile ducts, while bile ductuli in portal canals were weaklier or not stained by ABPS. Among 20 cases of IHCCC, 4 cases were negative for ABPS staining, 3 weakly positive, 8 moderately positive and 5 strongly positive. The result coincides with previous report of Edmondson (1958). The stainings varied in their colour and location. They were distributed in cytoplasm and tissue spaces, on plasma membranes and within acini of IHCCC. The red and purple stainings were mainly located in cytoplasm, while the blue stainings were mainly distributed around and in acini as well as in tissue space of IHCCC, which corresponded to the position of most lectin receptors. Generally, the stronger the staining by ABPS, the more lectin receptors in IHCCC.

Discussion

It is well known that HCC has a close relationship with HBV infection and aflatoxin. However, causes for IHCCC are less well defined. Some believe that IHCCC may be related to infestation by Clonorchis; this was not confirmed in our study. In addition, none of these patients had cirrhosis, in contrast with HCC patients (among these, 50–90% had cirrhosis).

The epithelial cells of intrahepatic bile ducts are not only involved in the transportation and concentration of bile, but also take part in the synthesis and secretion of mucus which is rich in glycoproteins (Reuben 1984). Lectins are sugar-binding proteins. Therefore, these bile duct cells contain many types of lectin receptors. Among twelve lectin receptors investigated in the study, eleven were present in bile duct cells, which is much more than that found in hepatocytes (Table 2 and 3) and also more than in HCC (Zhang et al. 1989b).

In order to compare the location and quantity of lectin receptors with mucins and observe the change of mucins in IHCCC, combined alcian blue-peroxidic acid Schiff (ABPS) staining was made. The results suggest that most lectin receptors in normal bile ducts and IHCCC had the same position as the mucins. The structure of the mucins changed greatly in IHCCC. Epithelial cells of bile ducts contained acid mucins in their cytoplasm, while cancer cells of IHCCC mainly had neutral or mixed mucins, which might be caused by the loss or decrease of the sialylation function in IHCCC. The hypothesis was confirmed by lectin histochemistry. Although ABPS staining can differentiate neutral mucins from acid or mixed ones by their colours it can not identify the structures of these glycoconjugates and lectin histochemistry is superior to it in this regard.

We have reported lectin receptors activity in HCC earlier (Zhang et al. 1989b). Comparing lectin receptors in IHCCC with those in HCC, it was found that IHCCC had more types of lectin receptors than HCC. The positive rates of Glc/Man- and GlcNAc-specific lectin receptors were similar in these two common malignant tumours of liver, whereas the positive rates of Gal- and Fuc-specific lectin receptors were significantly higher in IHCCC than in HCC (Table 2). This may be related to their origin; IHCCC comes from intrahepatic bile duct cells which contained more lectin receptors than hepatocytes and tumours from these ducts may maintain the function of synthesizing some lectin receptors (glycoconjugates) as do normal bile ducts. In addition, the polar distribution of lectin receptors in well- and moderately-differentiated IHCCC tissues could differentiate them from HCC and normal liver tissues. However, it was difficult to differentiate poorly-differentiated IHCCC from HCC.

During the neoplastic transformation of bile duct cells, glycoconjugates in the cells undergo many changes in their composition and structure (Hakomori 1985). Our results suggest that changes of glycoconjugate expression might include the appearance of new lectin binding sites. PNA can bind to Tn antigen which has a sequence of β-D-Gal(1 $\rightarrow$ 3)-D-GalNAc-(Springer et al. 1979) and is a middle product of more complex glycoconjugates. The accumulation of Tn antigen might be caused either by the increase of its synthesis or by the decrease of its breaking down. It might also result from either the decrease of sialyl transferase activity or the increase of sialydase activity. The mechanism of the appearance of Tn antigen in some IHCCC remains to be further studied.

Changes may also include a decrease of lectin binding sites in IHCCC. Of twelve types of lectin receptors investigated, eleven existed in epithelial cells of intrahepatic bile ducts. However, among 20 cases of IHCCC, seventeen cases had lectin receptors less than eleven types. Receptors of DBA, BSA_I, SBA and SJA were present in less than 50% of IHCCC. There were negative cases for all lectin studied except Con A. The results indicate that cancer cells of IHCCC had various defects in the ability to synthesize glycoconjugates though this ability still remained in most of cases.

Increase of lectin binding sites might also occur since staining for some lectins (DBA and UEA_I) in normal bile duct cells were very light, while they were rather strong in some IHCCC.

Finally, a change of lectin receptor distribution may occur in IHCCC. Most of the lectin receptors were localized in the cytoplasm and on plasma membranes adjacent to the lumens of bile ducts. This polar distribution could also be seen in well-differentiated IHCCC, whereas the distribution of lectin receptors in most of IHCCC was irregular.

Heterogeneity of tumour cells has been confirmed by many researchers (Nicolson 1984). Our study demonstrated that heterogeneity of glycoconjugate expression also existed in tumours. The quantity of one type of lectin receptor varied among cancer cell groups in the same specimen (Fig. 6). Many researchers have commented that changes in glycoconjugates on cancer cell surfaces were closely related to the metastatic potentials of tumours (Nicolson 1982; Miner et al. 1982; Steck and Nicolson 1983). It is unclear whether cancer cells with different glycoconjugates in IHCCC have a different metastatic potential or not.

From the results of specific inhibition tests, we can see that the binding of lectins to glycoconjugates in tissues is specific. The differences of sugar concentrations required for the complete inhibition of the binding result from differences in the affinity of lectins for saccharides, which is also effected by spatial structures of glycoconjugates. Thus, lectins with the same sugar specificity have different staining results in an identical specimen. After excluding non-specific staining, the binding of one lectin to tissue confirms the presence of glycoconjugate(s) with sugar hapten(s) of this lectin. Conversely, we can not exclude their presence.

References

Allison RT (1986) Lectins in diagnostic histopathology. Med Lab Sci 43:369–376

Barondes SH (1981) Lectin: their multiple endogenous cellular functions. Ann Rev Biochem 50:207–231

Buamah PK, Harris R, James OFW, Skillen AW (1986) Lentil-lectin-reactive alpha-fetoprotein in the differential diagnosis of benign and malignant liver disease. Clin Chem 32:2083–2084

Buamah PK, Cornell C, Cassells-Smith AJ, Skillen AW (1987) Differential reactivity of α-fetoprotein with lectins and its usefulness in the diagnosis of various liver diseases. Clin Chim Acta 168:69–73

Cooper HS (1984) Lectins as probes in histochemistry and immunohistochemistry: the peanut (Arachis hypogaea) lectin. Human Pathol 15:904–906

Damjanov I (1987) Lectin cytochemistry and histochemistry. Lab Invest 57:5–20

Edmondson HA (1958) Tumors of the liver and intrahepatic bile ducts. Atlas of tumor pathology Section VII-Fascicle 25. Armed Fores Institute of Pathology, Washington DC, pp 80–88

Hakomori S-I (1985) Aberrant glycosylation in cancer cell membranes as focused on glycolipids: overview and perspectives. Cancer Res 45:2405–2414

Hoofnagle JH, Shafritz DA, Popper H (1987) Chronic type B hepatitis and the "healthy" HBsAg carrier state. Hepatology 7:758–763

Hsu S-M, Raine L (1981) Protein A, avidin and biotin in immunohistochemistry. J Histochem Cytochem 29:1349–1353

Irimura T, Ota DM, Cleary KR (1987) Ulex europeus agglutinin I-reactive high molecular weight glycoproteins of adenocarcinoma of distal colon and rectum and their possible relationship with metastatic potential. Cancer Res 47:881–889

Levin S, Russell EC, Blanchard D, McWilliams NB, Maurer HM, Mohanakamar T (1980) Receptors for peanut agglutinin (Arachis-hypogaea) in childhood acute lymphocytic leukemia: possible clinical significance. Blood 55:37–39

Lis H, Sharon N (1986) Lectins as molecules and as tools. Ann Rev Biochem 55:35–67

Miner KM, Kawaguchi T, Uba GW, Nicolson GL (1982) Clonal drift of cell surface, melanogenic and experimental metastatic properties of in vivo-selected, brain meninges-colonizing murine B16 melanoma. Cancer Res 42:4631–4636

Morry RW (1984) Combined alcian blue-PAS technique. In: Bancroft JD, Cook HC (eds) Manual of histological techniques. Churchill Livingstone, Edinburgh London Melbourne New York, pp 111–112

Nicolson GL (1982) Cancer metastasis. Organ colonization and the cell-surface properties of malignant cells. Biochim Biophys Acta 695:113–176

Nicolson GI (1984) Cell surface molecules and metastasis. Regulation of metastatic phenotypic diversity. Exp Cell Res 150:3–22

Pang KY, Bresson JL, Walker WA (1987) Development of gastrointestinal surface. VIII Lectin identification of carbohydrate differences. Am J Physiol 252:G685–G691

Popper H, Shafritz DA, Hoofnagle JH (1987) Relation of the hepatitis B virus carrier state to hepatocellular carcinoma. Hepatology 7:764–772

Pritchard DJ, Butler WH (1988) The lectin binding characteristics of aflatoxin B_1 induced lesions in rat liver. J Pathol 154:269–276

Ree HJ, Kadin ME (1985) Lectin distinction of benign from malignant histiocytes. Cancer 56:2046–2050

Reuben A (1984) Biliary proteins. Hepatology 4:465–505

Springer GF, Desai PR, Murphy MS, Yang HJ, Scanlon FF (1979) Precursors of the blood group MN antigens as human carcinoma associated antigens. Transfusion 19:233–249

Steck PA, Nicolson GL (1983) Cell surface glycoproteins of 13762 NF mammary adenocarcinoma clones of differing metastatic potentials. Exp Cell Res 147:255–267

Zhang S, Wu M, Chen H, Zhang X (1989a) Localization of WGA receptors in liver tumor tissues. J Med Coll PLA 4:37–40

Zhang S, Wu M, Chen H, Zhang X (1989b) Expression of glycoconjugates in hepatocellular carcinoma. Histochemistry 92: (in press)

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