Distribution of vitamin B₁₂ R-binder in carcinomas of the digestive tract

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SUMMARY To evaluate whether the increase in serum transcobalamin I, seen in patients with carcinoma, is caused by synthesis of R-binder to tumour cells, the distribution of vitamin B₁₂ R-binder in 125 malignant growths of the digestive tract was studied. Positive staining for R-binder with immunoperoxidase was observed in 85 (70%) carcinomas. Positive staining for R-binder was observed in all four cholangiocarcinomas studied, but was absent in nine hepatocellular carcinomas.

These findings suggest that determination of R-binder in liver tumours may be of some value in differentiating hepatocellular carcinomas and cholangiocarcinoma, and that synthesis of R-binder by tumour cells causes an increase in serum transcobalamin I.

An increased concentration of serum transcobalamin I, a member of vitamin B₁₂ binding proteins called R-binder, is found in patients with myeloproliferative diseases.^{1,2} Similar increases in patients with carcinoma have also been noted, and it has been speculated that the increase was caused by the synthesis of R-binder in tumour cells.³⁻¹² Using an immunoperoxidase technique, we examined whether R-binder is detectable in various carcinomas of the digestive tract.

Material and methods

One hundred and twenty two carcinomas and three carcinoid tumours of the digestive tract were selected from the surgical pathology files of Kyoto University Hospital comprising: four squamous cell carcinomas of the oesophagus; 47 adenocarcinomas of the stomach; 21 adenocarcinomas and three carcinoid tumours of the colon; 24 carcinomas of the pancreas; 13 adenocarcinomas of the biliary tract; four cholangiocarcinomas; and nine hepatocellular carcinomas. Formalin fixed, paraffin embedded tissue blocks were available in all cases. Newly cut 4 µm thick sections were used for the study.

Immunostaining was performed using the indirect peroxidase labelled antibody technique as described previously.¹³ Deparaffinised sections were treated with

0.3% hydrogen peroxide in absolute methanol for 30 minutes to block the endogenous peroxidase activity; they were then incubated with 10% normal goat serum for 30 minutes to block non-specific binding of immunoglobulins. The sections were incubated with rabbit anti-R-binder antiserum (diluted at 1/200) overnight at 4°C. After washing with phosphate buffered saline (pH 7.2) the sections were incubated with peroxidase conjugated goat antirabbit IgG (Capel, Philadelphia, USA) diluted at 1/200 for 90 minutes at room temperature. The histochemical reaction for peroxidase was carried out according to the method of Graham and Karnovsky.¹⁴ The specificity of R-binder staining was confirmed by replacing the primary antiserum with antiserum preincubated with an excess of purified R-binder.¹³

The gastric carcinomas were classified histologically according to the criteria of the Japanese Research Society for Gastric Cancer.¹⁵ Carcinomas of the colon and biliary tract were classified according to WHO criteria^{16,17} and were graded as well, moderately or poorly differentiated. Pancreatic carcinomas were classified according to the criteria of Morohoshi *et al.*¹⁸

Results

The results of this study are summarised in the table. Positive staining for R-binder was observed in 85 of 122 carcinomas of the digestive tract (70%). None of the three carcinoid tumours of the colon showed positive staining. Among the carcinomas of the

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Table Immunoreactive R-binder in carcinomas of the digestive tract

Histological classification	No Positive/Total
Oesophagus:	
Squamous cell carcinoma	0/4
Stomach:	
Papillary adenocarcinoma	5/7
Tubular adenocarcinoma	
Well differentiated	7/7
Moderately differentiated	9/10
Poorly differentiated adenocarcinoma	4/7
Mucinous adenocarcinoma	8/9
Signet ring cell carcinoma	5/7
Colon:	
Well differentiated adenocarcinoma	7/7
Moderately differentiated adenocarcinoma	6/6
Poorly differentiated adenocarcinoma	3/3
Mucinous adenocarcinoma	3/5
Carcinoid tumour	0/3
Biliary tract:	
Well differentiated adenocarcinoma	5/6
Moderately differentiated adenocarcinoma	2/6
Poorly differentiated adenocarcinoma	0/1
Pancreas:	
Ductal adenocarcinoma	
Well differentiated	6/8
Moderately differentiated	4/4
Mucinous adenocarcinoma	1/1
Adenosquamous carcinoma	3/3
Solid and cystic tumour	0/1
Acinar cell carcinoma	0/1
Ampullary carcinoma	3/3
Undifferentiated carcinoma	0/3
Liver:	
Hepatocellular carcinoma	0/9
Cholangiocarcinoma	4/4

stomach, colon, pancreas and biliary tract (including cholangiocarcinomas), R-binder was detected in 65% to 90% of each organ, whereas none of the squamous cell carcinomas of the oesophagus or hepatocellular carcinomas showed positive staining for R-binder. In most of the well or moderately differentiated adenocarcinomas there was luminal staining of tumour glands and staining of the secretory material within lumens (figs 1 and 2). Cytoplasmic staining was also found in some cases (fig 3). In poorly differentiated or mucin producing carcinomas R-binder was detected in the cytoplasm or mucin of some tumour cells with varying degrees of intensity (fig 4). No significant correlation was observed between the incidence of R-binder expression and tumour differentiation in carcinomas of the stomach, colon, and pancreas. In biliary tract carcinomas, however, the incidence of R-binder expression was reduced with progressive dedifferentiation.

Discussion

R-binder, an immunologically identical vitamin B₁₂ binding protein, occurs in various body fluids, granulocytes, and serum. We previously examined the distribution of R-binder in the normal digestive

Fig 1 Moderately differentiated adenocarcinoma of colon. There is luminal staining of tumour glands and staining of secretory material within lumens. (Immunoperoxidase and methyl green.)

Fig 2 Ductal adenocarcinoma of pancreas. Positive staining for R-binder is found on luminal border of gland-forming tumour cells. (Immunoperoxidase and methyl green.)

Fig 3 Cholangiocarcinoma of liver. R-binder is detected in cytoplasm of tumour cells. (Immunoperoxidase and methyl green.)

Fig 4 Mucinous adenocarcinoma of stomach. Positive staining for R-binder is observed in cytoplasm of some tumour cells and in mucin. (Immunoperoxidase and methyl green.)

system using an immunoperoxidase technique¹³ and showed that R-binder was present in the epithelia of the bile ducts, gallbladder, pancreatic ducts, large and small intestine, and in the gastric mucosa with intestinal metaplasia. In this study we have shown that R-binder was present not only in normal epithelia of the digestive tract but in carcinomas of the various digestive organs.

It has been reported that an increased concentration of serum R-binder—that is, TCI, is occasionally found in patients with carcinoma. Waxman and Gilbert reported a high increase in serum vitamin B₁₂ binding protein in some patients with hepatocellular carcinoma.⁴ Carmel *et al* indicated that eight of 139 patients with non-haematological malignant disease showed high TCI concentrations.⁹ Sheppard *et al* observed that the tumour tissues of a patient with disseminated carcinoma of unknown origin, whose serum TCI was very high, contained considerably higher concentration of R-binder than normal tissues.¹² These findings suggest that the increase in serum TCI in patients with carcinoma is due to the synthesis of R-binder by tumour cells. The results of this study provide additional evidence that R-binder is produced in carcinoma cells.

The incidence of carcinomas containing R-binder in this series was much higher than that of the cases with a high concentration of serum TCI reported by Carmel *et al*.⁹ This discrepancy may be because extensive malignant disease and a large number of tumour cells are needed before an increase in serum TCI concentration occurs. Indeed, almost all cases with high serum TCI had generalised metastases, especially in the liver.^{3,8,9,12} The increase in serum TCI in patients with carcinoma may have been caused by increased production of R-binder in the tumour cells and by decreased degradation in the liver.^{3,8,9,12}

Among adenocarcinomas of the stomach, colon, pancreas and biliary tract, the prevalence of R-binder was high; no squamous cell carcinomas of the oesophagus or hepatocellular carcinomas examined in this series expressed R-binder. Acinar cell carcinomas and solid and cystic tumours of the pancreas, considered to have arisen from acinar cells of the pancreas,¹⁸ also stained negatively. In our previous study R-binder was not detected in the squamous epithelium of the oesophagus, in hepatocytes, or in acinar cells of the pancreas.¹³ These findings indicate that carcinomas arising from tissue negative for R-binder do not produce R-binder. Some reports, however,^{4,6,10,11} have documented an increase in serum TCI in some patients with hepatocellular carcinomas. It has been noted that these cases had certain clinical features—that is, they did not show an increased concentration of serum α -fetoprotein, had no underlying cirrhosis, and most of the patients were adolescents or young adults.^{4,5,10,11} Paradinas *et al* reported that seven of 10 patients with these features, including high serum unsaturated vitamin B₁₂ binding capacity, had the fibrolamellar variant of hepatocellular carcinoma.¹¹ The reason why R-binder was not detected in hepatocellular carcinomas in this study may be that the tissues examined were all from hepatocellular carcinomas with cirrhosis and did not include any of the fibrolamellar variant. Van Tonder *et al* reported an absence of abnormal vitamin B₁₂ binding protein in a study of 242 South African blacks with hepatocellular carcinoma.¹⁹ Thus hepatocellular carcinoma producing R-binder seems to be uncommon. Our present results on liver tumours suggest that R-binder detection in liver tumours may be of some value in differentiating cholangiocarcinoma from hepatocellular carcinoma except for the fibrolamellar variant.

In this study we also investigated whether there was an association between R-binder expression and the differentiation of carcinomas, but we could not show a clear association, except for the correlation between the incidence of R-binder expression and differentiation of the biliary tract carcinomas; not only well differentiated carcinomas but poorly differentiated carcinomas in the stomach, colon, and pancreas showed a high incidence of R-binder positivity. Moreover, staining intensity did not correlate with tumour differentiation. We could not find any association between R-binder and a particular histological type of carcinoma.

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