HISTOCHEMICAL STUDIES OF FRUCTOSE 1,6-DIPHOSPHATASE IN HUMAN HEPATOCELLULAR CARCINOMA¹

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The activities of fructose 1,6-diphosphatase in hepatocellular carcinoma tissues were studied histochemically. The formazan deposition, which represents the activity of fructose 1,6-diphosphatase, was found to be localized in the cytoplasm of hepatoma cells. The degree of the formazan deposition was proportional to the activity obtained by a kinetic method with tissue extracts.

The three hepatoma cases studied were all well differentiated hepatocellular carcinoma and had varying FDPase activities, ranging from near normal to undetectable levels, even though their degrees of histochemical differentiation were quite similar.

Evidences of decreasing enzyme activities of fructose 1,6-diphosphatase (FDPase) and glucose 6-phosphatase (G6Pase) in accordance with the increasing growth rate of experimental rat hepatomas have been reported (8). Decreased activities and virtual absence of FDPase and G6Pase in human hepatoma tissues have been also obtained by Boxer and Shonk (3) and in our laboratory (7). It is not convincing, however, that the small activities of the above two gluconeogenic enzymes demonstrated with tissue homogenates reside in the hepatoma cells and not in the liver cells which might be included in some tumor tissues. In an attempt to solve this problem, a histochemical technique was employed in the present study to demonstrate the localization of FDPase activities in hepatoma tissues. The results on G6Pase have been reported elsewhere (5).

On three cases of hepatocellular carcinoma studied in the present work, FDPase activity was demonstrated in hepatoma cells, and the results obtained with histochemical and biochemical methods agreed well with each other. Comparison of FDPase activities in the hepatomas with the degrees of their histological differentiation also constitutes a part of the present work.

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MATERIALS AND METHODS

Liver carcinoma specimens were obtained at operations from three patients with clinical diagnosis of primary hepatoma (Cases 1–3), one patient with metastatic carcinoma of the stomach to the liver (Case 4) and one patient with cholelithiasis as a control.

Case 1. A 26 year old female was hospitalized because of lethargic condition with a complaint of fullness in the upper abdomen. Physical findings were unremarkable except the palpable liver of increased consistency. Significant laboratory findings were a decreased fasting blood sugar level of 31 mg/dl with a diabetic type of glucose tolerance test and slight elevated serum transaminase activities (GOT 160 K. U. and GPT 225 K. U.). Liver scan showed a space occupying region in the right lobe. Exploratory laparotomy was done and right lobe was successfully removed with a tumor. The patient was in good health for one year and a half after the operation.

Case 2. A 35 year old female was pointed out at abnormal region in the upper abdomen at the mass survey examination of the stomach. She was suspected of having a hepatic cancer due to a positive α -fetoprotein reaction in serum and an abnormal finding of liver scan. Other laboratory findings were within normal limits. Physical examination revealed tenderness and enlargement of the liver with nodular surface at the left costal margin. The presence of a tumor of the left lobe of the liver was confirmed by peritoneoscopic examination. Operation was performed with successful removal of the left lobe with a hepatoma.

Case 3. A 52 year old male was admitted because of general weakness and loss of appetite for 7 month duration. He had a history of duodenal ulcer in 1945 and an operation of subtotal gastrectomy and blood transfusion in 1970. In August 1974, he had the above complaints and slightly increased levels of serum transaminases and γ -globulin and was admitted in February 1975 for further evaluation. On admission, significant physical findings were 3 spider angiomas at the chest wall and a 2 finger breadth-palpable nodular liver below the xiphoid process and serum GOT and GPT levels were 143 and 110 K. U., respectively, ZTT 14 U. and total protein 7.4 g/dl with 29% γ -globulin. HBs antigen was positive and K_{100} index was decreased to 0.12. Selective angiography of the celiac artery and peritoneoscopic examination suggested the presence of a tumor in the left lobe of the liver. Left lateral segmentectomy of liver was performed successfully in March 1975. Other clinical features and histological findings of these cases are summarized in Table 1 and Figure 2.

Case 4. A 70 year old male was admitted because of 4 month history of fullness in the upper abdomen and the abdominal pain. Endoscopic examination of the stomach showed a cancer in the lesser curvature of the stomach. Physical findings and laboratory data were unremarkable. Exploratory laparotomy disclosed multiple metastases in the liver from the stomach cancer. Histological diagnosis of the tumor was adenocarcinoma tubulale. There was no sign of cirrhosis in the liver.

Tumor tissues obtained at the time of operation were processed as described in our previous paper (2), and stained for FDPase by applying the method developed for histochemical demonstration of rat liver FDPase (1). Hematoxyline and eosin

TABLE 1. *Clinical Findings and Histological Findings*

	Case 1	Case 2	Case 3
Onset of illness to operation	7 months	9 months	7 months
Serum α -fetoprotein	—	+	—
Tumor-bearing liver	not cirrhotic	not cirrhotic	cirrhotic
Tumor tissue			
Gross appearance	single nodular	multinodular	single nodular
Site of tumor	right lobe	left lobe	left lobe
Tumor size	whole right lobe	whole left lobe	tennis ball
Metastasis	—	—	—
Arrangement of tumor cells	trabecular	trabecular	trabecular
Nucleus	large	large	large
Bile secretion	+	—	—
Storage of fat	+	—	—
Vascular lake	+	+	+

staining was obtained for each sample. Serum α -fetoprotein was analyzed by a micro-Ouchterony method.

RESULTS

The results of FDPase staining in the control liver, hepatoma and stomach cancer tissues are shown in Fig. 1. The activity of FDPase in the control liver was demonstrated as dark blue deposits of formazan in the cytoplasm of hepatic parenchymal cells (Fig. 1, a) with a homogeneous intralobular distribution. In hepatoma Case 1, formazan deposition as representing FDPase activity was clearly seen in the tumor cells (Fig. 1, c *vs.* d). Slightly less intense but readily recognizable color development was found in Case 2, also being confined to the tumor cells (Fig. 1, e *vs.* f). In Case 3, the tumor cells were stained faint but significant for FDPase (Fig. 1, g) as compared with the blank staining (Fig. 1, h). FDPase activity of the metastatic liver cancer from stomach cancer was negative for staining and there was no

TABLE 2. *Fructose 1,6-Diphosphatase Activities*

	Tumor (mU/mg protein)	Tumor-bearing and control liver
Case 1	71.0	129.1
Case 2	18.5	89.3
Case 3	7.5	57.0
Stomach carcinoma	(not detectable)	78.5
Control liver		122.0

difference in the staining between the complete system (Fig. 1, b) and a blank staining (not shown). The degree of color development in those cases paralleled with that of FDPase activity, which was determined by a spectrophotometric method on tissue extracts as may be seen in Table 2. The histological findings of the three hepatocellular carcinoma cases are shown in Fig. 2. In each case the cellular arrangements are trabecular, and the nucleus are large and the vascular lake are seen occasionally. In the Case 1, the bile secretion and storage of fat are recognized. Hepatocellular carcinomas of the above three cases were all well differentiated as revealed by clinical features as well as histological findings (Table 1) and they were classified histologically as Grade II according to Edmondson and Stainer (4). In comparison of these results with the above FDPase activities of hepatocellular carcinoma cells, it was apparent that the FDPase activity varied widely even with similar degree of histological differentiation.

DISCUSSION

FDPase is one of the rate limiting enzymes of gluconeogenesis and considered to be an adult type liver enzyme. The present report might be the first one to demonstrate the existence of FDPase, as a marker enzyme of adult liver, in hepatocellular carcinoma cells in man. The intensity of histochemically demonstrated FDPase activities in hepatoma cells correlated well with the activities demonstrated on extracts of tumor tissues provided that liver cells were not included in the tumor tissues as in the present cases. These results provide some evidences to assume that the reported FDPase activities on extracts of hepatoma tissues (7) do represent the FDPase activities of tumor cells in the tissue in so far as the possibility of co-existence of intact liver cells in tumor tissues is included for some reasons.

The wide distribution of FDPase activity was observed among hepatocellular carcinomas with similar degrees of differentiation. It still requires a further work before generalizing this observation to other human hepatocellular carcinomas, because the number of cases studied was limited in the present work. However, there are some evidences in favor of the above view; *i. e.* a much wider distribution of FDPase activity in tumor extract has been formed even with an increased number of human hepatocellular carcinomas (6).

It becomes more apparent that no strict correlation exists between the extents of tumor differentiation and the activities of differentiated marker enzymes, when the activities of other enzymes of adult liver are taken into consideration together with FDPase. For example, the tumor tissue of Case 3 had nearly three times as high pyruvate kinase-L activity as normal control liver (5), although the FDPase activity in this case relatively low as compared with those of Case 1 and intact liver.

The above findings and considerations may give a clue for not only histochemical

Fig. 1. Staining of FDPase in control liver, hepatocellular carcinoma and stomach cancer tissues. a, control liver, complete system; b, stomach cancer case, complete system; c, Case 1, complete system; d, Case 1, complete system minus substrate; e, Case 2, complete system; f, Case 2, complete system minus substrate; g, Case 3, complete system; and h, Case 3, complete system minus substrate. c, and d, $\times 400$; a, b, e, f, g and h, $\times 100$

Fig. 2. Hematoxyline and eosin staining of hepatocellular and stomach carcinoma tissues. a, Case 1; b, Case 2; c, Case 3 and d, stomach cancer, $\times 100$

classification of human hepatomas but also for fundamental treatment of well differentiated hepatocellular carcinomas.

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