

Localization of Placental Specific Proteins in Hepatoma Tissue, Possible Markers in Oncology

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SUMMARY

By means of the indirect immunofluorescent technique, localization of placental specific proteins (PP₅, PP₁₀, PP₁₁ and PP₁₂) were investigated in 21 patients with hepatoma. PP₅, PP₁₁ and PP₁₂ were detected in the cytoplasm of the hepatoma cells, but were absent in normal hepatic cells. Thus, ectopic production of proteins normally synthesized by trophoblasts occurs in hepatoma cells. PP₁₁ was present in 19.0% of the cases, PP₅ in 4.7%, PP₁₀ in 0% and PP₁₂ in 4.7%. PP₁₀ and PP₁₂ were also found in mononuclear cells. PP₁₁ seems to be highly specific to hepatoma. PP₁₁ was found in 23.5% of patients with a histological diagnosis of Grade II. In addition, PP₁₁ was often detected in bile duct cells. Thus, trophoblasts may possess antigens in common with bile duct cells as well as with hepatoma cells.

Key words: Placenta, Trophoblast, Placental specific proteins, Hepatoma, Primary liver carcinoma, Tumor marker, Ectopic production

INTRODUCTION

A number of placental proteins have been detected by immunohistochemical methods in extracts obtained from human placentas (1, 2). Several of these proteins have already been isolated, characterized and investigated for their usefulness as tumor markers (1, 2, 3). Placenta-specific proteins (PP₅, PP₁₀, PP₁₁, PP₁₂) are proteins specific to the trophoblast. They can not be detected in extracts of other normal human fetal or adult tissues (4, 5, 6, 7).

In the present study, localization of PP₅, PP₁₀, PP₁₁ and PP₁₂ were studied in hepatoma tissues by the immunofluorescent method using their specific antisera.

MATERIALS AND METHODS

Specimens. Thirty-one autopsy specimens were obtained from 21 patients

with hepatoma. The classification of Edmondson and Steiner was used for the histological grading of primary liver carcinoma (8). Normal human livers were obtained from patients who died of other diseases. Human placenta was also used as a positive control.

Preparation of tissue sections. Tissues were cut into small blocks and fixed in cold 95% ethanol. After dehydration with absolute alcohol, the blocks were passed through xylene and embedded in paraffin at 56°C. Microtome sections, 4 μ m thick, were cut from these blocks.

Antisera. Antisera to placenta-specific proteins (PPs) were prepared by injecting rabbits as reported previously (2).

Immunofluorescent staining. Fluorescent staining was performed by the indirect method of Coons and Kaplan (9), with a slight modification (10). Sections were deparaffinized in xylene baths after cutting, hydrated through successive ethanol baths, and washed in several changes of cold phosphate-buffered saline (PBS), pH 7.2. The sections were then incubated with antiserum to PP₅, PP₁₀, PP₁₁ or PP₁₂ diluted 1 : 20 for 30 min at 37°C. Thereafter, they were stained with FITC-labeled antiserum to rabbit IgG (Behringwerk AG) diluted 1 : 20 for 30 min at room temperature. One of the serial sections was stained with hematoxylin and eosin (H and E) for the identification of structural localization.

RESULTS

In the sections of hepatoma tissues which were exposed to anti-PP₅, PP₁₁ and PP₁₂ antisera, followed by FITC-labeled antiserum to rabbit IgG, there was a bright fluorescence of hepatoma cells, although not all the hepatoma cells on the tissue section showed fluorescence (Fig. 1a, b and c). Antiserum to PP₁₀ did not react with any hepatoma tissues (Fig. 1d). The fluorescence of hepatoma cells appear in 2 forms: (a) a bright fluorescent line of the cytoplasmic membrane (Fig. 1a) and (b) diffuse, finely granular fluorescence of the cytoplasm (Fig. 1b). The first type was the most common and mass of tumor cells showing fluorescence was usually observed.

By immunofluorescent staining with antisera to PPs, hepatic cells showed no fluorescence in normal livers obtained from patients with other diseases (Fig. 1e) and even in livers bearing hepatoma (Fig. 1b). Fig. 1f shows H and E staining of almost same area shown in Fig. 1b. No fluorescence is seen in the hepatic cells.

PP₁₀ and PP₁₂ were found to react with mononuclear cells in hepatoma tissues (Fig. 1d), but PP₅ and PP₁₁ did not (Fig. 1b and 2a). Specificity of PP₁₁ for immunofluorescent staining of the trophoblasts was shown in Fig. 2b. Strongly positive staining is observed in the cytoplasm of the syncytio trophoblastic cells.

In a few cases, positive staining for PP₁₁ was observed in some of hepatic cells

Table 1 Occurrence of placenta-specific proteins in hepatoma

	No. of cases	No. positive	%
PP ₅	21	1	4.7
PP ₁₀	21	0	0
PP ₁₁	21	4	19.0
PP ₁₂	21	1	4.7

Table 2 Relationship between location of P₁₁ and histological grading in hepatoma

Histological grading	No. of cases	No. positive	%
I	0	0	0
II	17	4	23.5
III	2	0	0
IV	2	0	0

within cirrhotic nodules (Fig. 2c). It was very difficult to find any difference between the histological features of the hepatic cells showing fluorescence and those not showing fluorescence. In addition, PP₁₁ was often detected in the bile duct cells, particularly in the site of bile duct proliferation (Fig. 2d). PP₅, PP₁₀ and PP₁₂ were not reactive with bile duct cells.

The occurrence of PPs in hepatoma varied with the cases (Table 1). PP₁₁ was present in 19% of the cases, PP₅ in 4.7%, PP₁₂ in 4.7% and PP₁₀ in 0%. PP₁₁ was highly specific for hepatoma. The relationship between the histological gradings of primary liver carcinoma and the presence of PP₁₁ in hepatoma was studied (Table 2). Seventeen patients had a histological diagnosis of Grade II carcinoma, 2 patients had Grade III and 2 patients had Grade IV. PP₁₁ was found only in the cases of Grade II carcinoma.

DISCUSSION

By means of the indirect immunofluorescent technique with antisera to PPs, we have demonstrated fluorescence in hepatoma cells. This reaction is specific for PPs because of the specificity of antisera, and the failure to stain normal hepatic cells. PPs were localized in the cytoplasm and the cytoplasmic membrane of hepatoma cells. It is suggested that hepatoma cells can produce PPs, although whether or not the localization represents the site of active synthesis is not yet known. Inaba *et al.* (3) have already shown that not only trophoblastic but also

non-trophoblastic carcinomas can produce PPs. The antigenic similarities between trophoblastic and neoplastic cells are an interesting phenomenon. We suggest that ectopic production of proteins normally produced by the trophoblast occurs in hepatoma cells as well as in other tumors. Determination of PPs may become a useful screening test for cancer patients.

PP₁₁ was detected in hepatoma cells from 4 of 21 patients with hepatoma. In the Grade II carcinoma, PP₁₁ was found in 23% of the cases. PP₁₁ seems to be highly specific for hepatoma, especially for Grade II primary liver carcinoma. PPs other than PP₁₁ were found in a few hepatomas. However, PP₁₀ and PP₁₂ are observed not only in hepatoma cells but also in mononuclear cells in tissues. Thus, they are unsuitable as hepatoma markers, because of the low frequency of positive cases and positive staining in mononuclear cells.

Specific localization of PP₁₁ was rarely observed in the hepatic cells of cirrhotic nodules. This may be an important finding in relation to hepatic premalignant lesions. Anthony *et al.* (11) suggested that liver cell dysplasia represents a precursor state for hepatocellular carcinoma because of the high occurrence of liver cell dysplasia in cirrhotic livers complicated by hepatocellular carcinoma. In the present study, it is difficult to find any morphological difference between the hepatic cells showing fluorescence and those not showing fluorescence. Whether or not PP₁₁ serves as a marker for hepatic premalignant diseases remains to be clarified.

In the present study, it has been shown that PP₁₁ is present in bile duct cells. This indicates that the trophoblast possesses an antigen in common with bile duct cells as well as with hepatoma cells. Thus, bile duct cells seem to possess oncofetal characteristics.

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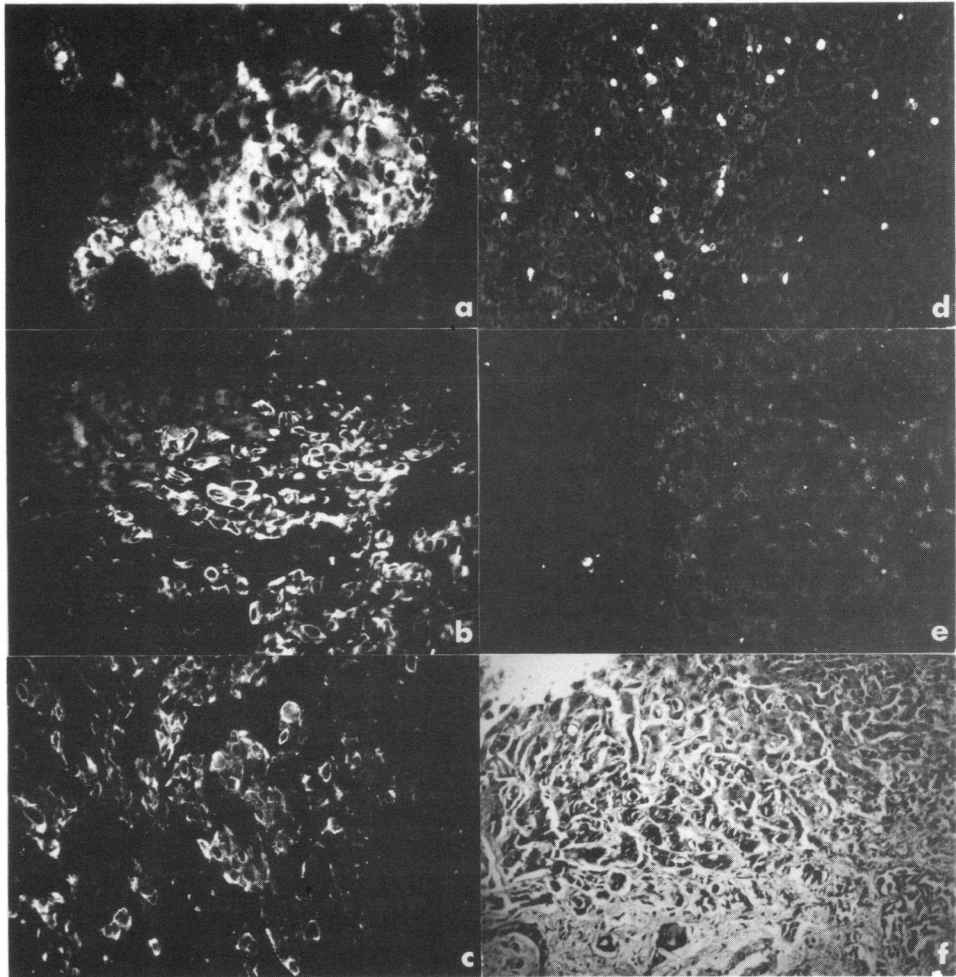


Fig. 1

- a. Immunofluorescent staining of hepatoma tissue with antiserum to PP₅. Diffuse, fine granular fluorescence of hepatoma cells is seen in tumor nests. ($\times 200$)
- b. Staining with antiserum to PP₁₁. A brightly fluorescent line of the hepatoma cell membrane is found. Hepatic cells, nuclei, and connective tissues are not reactive. ($\times 200$)
- c. Staining with antiserum to PP₁₂. Cytoplasm and cytoplasmic membranes display equivocal fluorescence. ($\times 200$)
- d. Staining with antiserum to PP₁₀. Mononuclear cells are fluorescent. Hepatoma cells and hepatic cells are not reactive. ($\times 100$)
- e. Staining of normal liver with antiserum to PP₁₁. Hepatic cells appear dark with no fluorescence. ($\times 100$)
- f. H and E staining of almost same area in Fig. 1b. Hepatic cells and hepatoma cells with Grade II are seen. ($\times 200$)

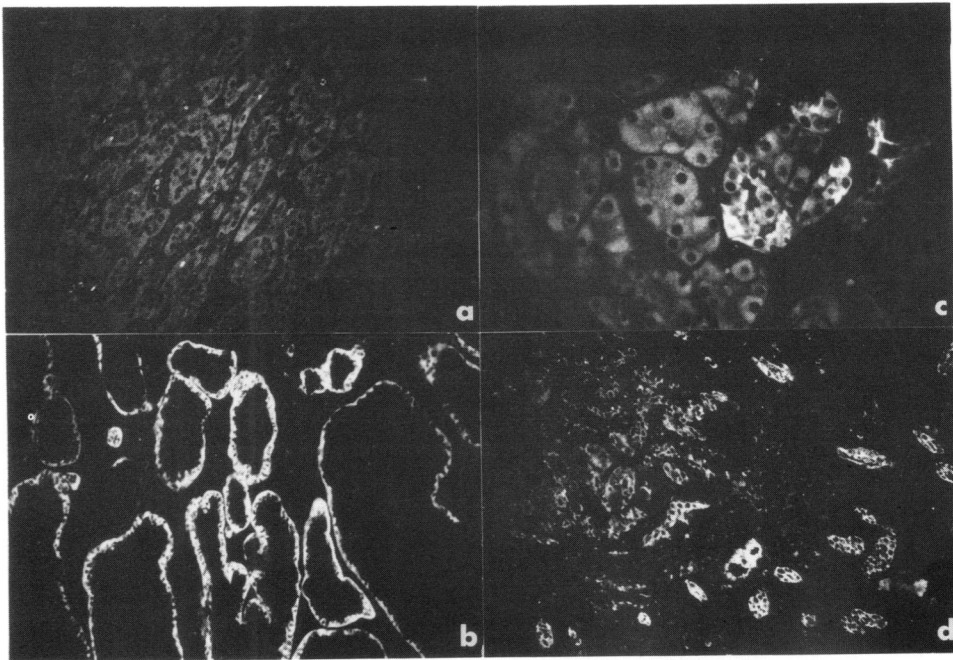


Fig. 2

- a. Staining of hepatoma with antiserum to PP₅. No fluorescence is found. ($\times 100$)
- b. Staining of placenta with antiserum to PP₁₁. The cytoplasm of the trophoblast is strongly fluorescent. ($\times 100$)
- c. Staining of a cirrhotic nodule with antiserum to PP₁₁. Cytoplasmic fluorescence of hepatic cells is seen.
- d. Staining of hepatoma with PP₁₁. Small bile duct cells are reactive. ($\times 100$)