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Immunohistochemical Type Distinction of α-Fetoprotein in Various α-Fetoprotein-secreting Tumors

Tsukasa Okamoto,[1] Koji Hirabayashi[2] and Tatsuya Ishiguro[3]

[1]Department of Pathology and [2]Department of Obstetrics and Gynecology, Fukuyama National Hospital, 4-14-17 Okinokami-cho, Fukuyama 720 and [3]Department of Obstetrics and Gynecology, Shiga Medical College, Tsukinowa-cho, Seta, Ohtsu 520-21

In order to clarify the histogenesis of α-fetoprotein(AFP)-secreting tumor tissues, formalin-fixed, paraffin-embedded serial sections of 148 tumors in various organs were examined by the peroxidase-antiperoxidase method for AFP, and paradoxical concanavalin A staining. Yolk sac-type AFP was found in yolk sac tumors, embryonal carcinomas, solid teratomas, (yolk sac) endodermal cell tumors, adenocarcinomas (stomach, ovary or lung) and metastatic liver cancers. Hepatic-type AFP was demonstrated in hepatocellular carcinomas, hepatoblastomas, solid teratomas and a stomach cancer. Yolk sac-type AFP was observed in the neighboring liver cells of metastatic liver cancers without relation to the type of AFP in primary cancers. The results from serum analyses of preoperative tumor-bearing patients (68 cases) were coincident with those from immunohistochemical stainings.

Key words: Immunohistochemical type distinction — Yolk sac-type AFP — Hepatic-type AFP — AFP-secreting tumor

α-Fetoprotein (AFP) is a glycoprotein with a molecular weight of 70,000 and about 4% of it is carbohydrate.[1] The binding property of serum AFP with concanavalin A (Con A) is of great help in the distinction of yolk sac-type AFP from hepatic-type AFP.[1,2] Sera from patients with yolk sac tumors (YSTs) have been found to contain a high proportion (50%) of AFP that does not bind to Con A. In contrast, Con A can bind with a very high proportion (90%) of AFP derived from patients with hepatocellular carcinomas (HCCs). This heterogeneity of AFP depends upon different degrees of glycosylation. Recently, immunohistochemical techniques such as the peroxidase-antiperoxidase (PAP) method[3] for AFP and paradoxical concanavalin A (P-Con A) staining[4] for glycoproteins, including AFP, have been developed. In previous fundamental studies,[5] serial sections of YSTs, HCCs and control materials were examined by these two staining methods. The results revealed that AFP in YSTs was unable to bind with Con A, in contrast with that in HCCs. Therefore, 148 AFP-secreting tumors in various organs, excluding tumors described in previous studies,[5] were examined by the same staining methods in order to elucidate the histogenesis of AFP-secreting tumor tissues.

Over the past 20 years, 148 AFP-secreting tumors have been collected from our own hospital and various institutions nationwide. The preoperative serum AFP level ranged from 670 ng/ml to 139,776 ng/ml. These 148 cases were classified as indicated in Table I. For light microscopy, tumor tissues were fixed in 10% formalin, embedded in paraffin, and serial sections 4 μm in thickness were prepared and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) with and without diastase digestion, Mucicarmin, Pap silver impregnation and Hall's bilirubin. The PAP method for identification of AFP and P-Con A staining for glycoprotein including AFP were performed on serial tumor sections as previously reported.[5] Observation was conducted to determine whether the distribution of specific antigen for AFP paralleled that of Con A positivity. The PAP method was also employed for identification of carcinoembryonic antigen (CEA) and human chorionic gonadotropin (hCG) as previously described.[5] Sera from preoperative tumor-bearing patients (68 cases) were examined by Con A crossed-line affinity immunoelectrophoresis.[6]

All 35 YSTs failed to stain for Con A, although the PAP staining pattern for AFP was predominantly positive in reticular areas with Schiller-Duval bodies as well as intracellular hyaline globules (ICHGs). AFP-positive hepatoid cells in YSTs were negative for Con A (Fig. 1). In all 18 embryonal carcinomas, yolk sac-type AFP was seen in eosinophilic cells as well as a few ICHGs of papillary, solid or tubular structures. In all 13 cases of mature and immature solid teratoma, endodermal gland suggesting enteric differentiation showed yolk sac-type AFP (Fig. 2). Luminal contents positive for AFP also failed to stain for Con A (Fig. 2). In 5 immature solid teratomas, hepatoblasts had hepatic-type AFP (Fig. 3). A few ICHGs in hepatoblasts also contained hepatic-type AFP. Therefore, 5 of 13 solid teratomas revealed both yolk sac-type AFP and hepatic-type AFP. In all 23 (yolk sac) endodermal cell tumors (YSECTs), yolk sac-type

Table I. Type Distinction of AFP in AFP-secreting Tumors

Tumor (case)	Yolk sac-type AFP (case)	Hepatic-type AFP (case)
Yolk sac tumor in ovary, testis, mediastinum (35)	35	0
Embryonal carcinoma in ovary, testis (18)	18	0
Solid teratoma[a] in testis, ovary (13)	13	5
YSECT[b] in ovary, testis, stomach, mediastinum, retroperitoneum, brain (23)	23	0
Adenocarcinoma in stomach, ovary, lung (20)	19	1
Metastatic liver cancer (14–12 cases of stomach and 2 of rectum as primary focus)	12–4[c]	2–1[c]
Hepatocellular carcinoma (18)	0	18
Hepatoblastoma (3)	0	3
Pancreatoblastoma (2)	1	1
Nephroblastoma (1)	0	1
Sertoli-Leydig cell tumor in ovary (1)	1	0

a) Five cases out of thirteen contained both yolk sac-type AFP and hepatic-type AFP.
b) (Yolk sac) endodermal cell tumor.
c) Four cases out of twelve and one case out of two showed positive localization for yolk sac-type AFP in neighboring liver cells.

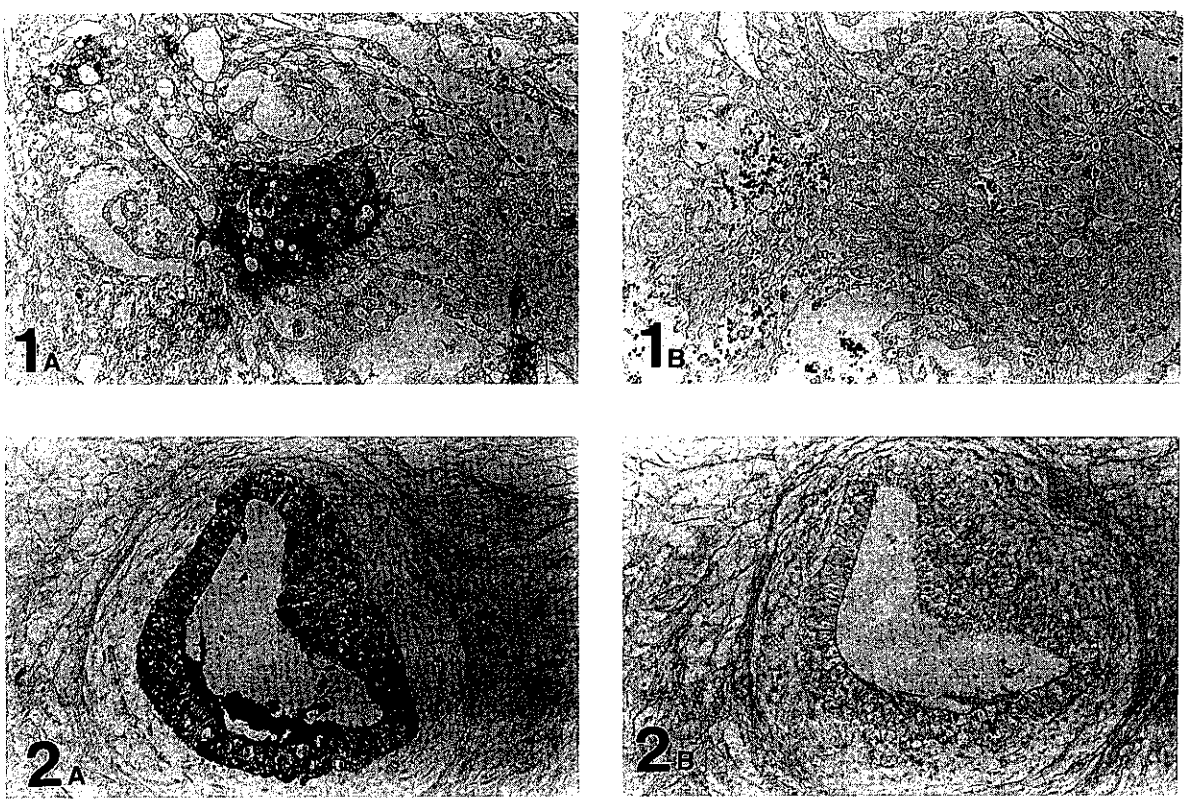

Fig. 1. Hepatoid cells in YST (case 5). Hepatoid tumor cells (center) are positive for AFP, but negative for Con A. Erythrocytes show positive reaction for Con A (A, PAP-AFP; B, P-Con A). ×210.

Fig. 2. Endodermal gland suggesting enteric differentiation in immature solid teratoma (case 88). A gland as well as luminal materials is positive for AFP, but negative for Con A (A, PAP-AFP; B, P-Con A). ×210.

AFP was demonstrated in eosinophilic or clear cells of solid, alveolar and glandular pattern (Fig. 4). A few ICHGs in these tumors showed a positive localization for yolk sac-type AFP. Twenty adenocarcinomas in stomach, ovary or lung showing tubular, papillary or solid structures disclosed yolk sac-type AFP in eosinophilic or clear cells of solid-tubular pattern. A few ICHGs were positive for yolk sac-type AFP too. In one stomach cancer showing poorly differentiated adenocarcinoma, hepatic-type AFP was seen in clear cells of papillary pattern. Five of 14 metastatic liver cancers that had originated from stomach or rectum showed a positive

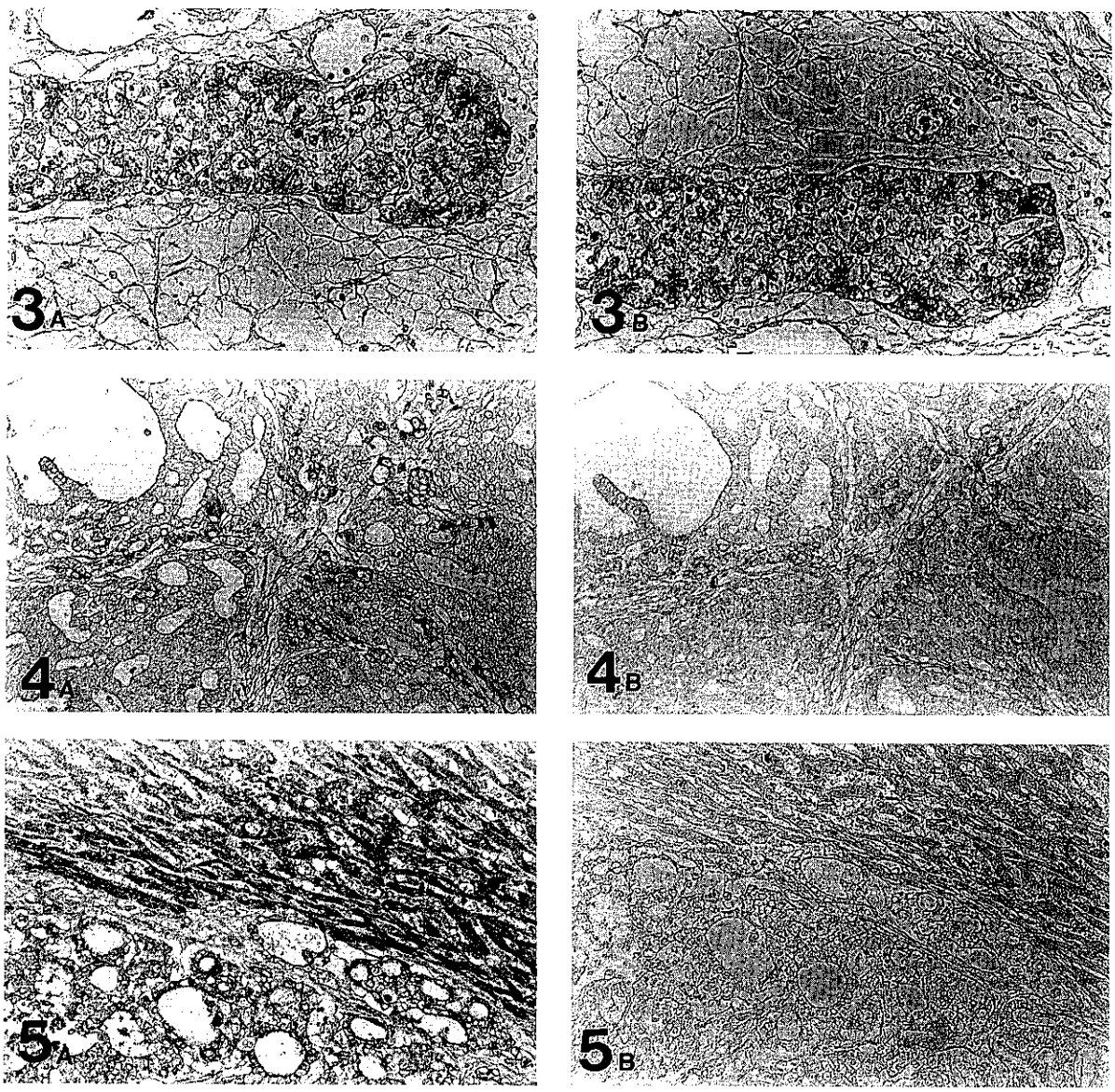

Fig. 3. Hepatoblasts in immature solid teratoma (case 88). Hepatoblasts show a positive reaction for both AFP and Con A. Erythrocytes are positive for Con A (A, PAP-AFP; B, P-Con A). ×210.

Fig. 4. (Yolk sac) endodermal cell tumor (case 32). Many tumor cells and ICHGs are positive for AFP, but negative for Con A (A, PAP-AFP; B, P-Con A). ×210.

Fig. 5. Metastatic liver cancer (case 67). Metastatic cancer cells from stomach (tubular adenocarcinoma) are positive for AFP (lower part). Neighboring liver cells are also positive for AFP (upper side). Metastatic cancer cells are negative for Con A. Neighboring liver cells show markedly diminished staining for Con A (A, PAP-AFP; B, P-Con A). ×210.

localization for yolk sac-type AFP in neighboring liver cells (Fig. 5) without relation to the type of AFP in the primary cancers. Distant liver cells from metastatic cancer showed negative for AFP but gave a positive staining for Con A. All 18 HCCs were immunostained for AFP and positive for Con A. P-Con A staining showed a rather more intense reaction for Con A than the PAP for AFP. All 3 hepatoblastomas revealed stronger positivity for AFP as well as Con A than that in HCCs. In 1 of 2 pancreatoblastomas, hepatic-type AFP was seen in eosinophilic cells of solid nests. The other case showed positive for yolk sac-type AFP in similar cells. A nephroblastoma disclosed hepatic-type AFP in the eosinophilic cells of tubular structure. A Sertoli-Leydig cell tumor (SLCT) revealed yolk sac-type AFP in the Leydig cells. These results are summarized in Table I. Nine cases of 35 YSTs, 7 of 23 YSECTs and all cases of solid teratoma were immunostained for CEA. Both apical cytoplasm of enteric-type glands and luminal contents were stained for CEA. But, no positive area for CEA was coincident with positive localization for AFP in a given tumor. All cases of adenocarcinoma showed a positive stain for CEA in the tubular structures with mucous contents. In 10 of 18 cases of HCC, a positive reaction for CEA was seen in the tubular pattern. A pancreatoblastoma also disclosed a positive localization for CEA in the tubules. In 10 of 13 solid teratomas and 8 of 18 embryonal carcinomas, hCG was found in multinucleated giant cells. Serum AFP in patients with YST (17 cases), embryonal carcinoma (6 cases), solid teratoma (7 cases), YSECT (16 cases), adenocarcinoma (2 cases), and metastatic liver cancer (5 cases) was divided into two peaks suggestive of yolk sac type. Serum AFP in patients with HCC (7 cases), hepatoblastoma (2 cases), solid teratoma (5 cases), and adenocarcinoma (1 case of stomach) disclosed one subfraction, indicating a hepatic type. Serum AFP in patients with immature solid teratoma (5 cases) contained two types of subfraction which were suggestive of both yolk sac-type and hepatic-type. These results from serum analyses by immunoelectrophoresis were coincident with those by immunohistochemical stainings.

AFP is one of the oncofetal proteins that can be detected in sera of patients with YSTs, HCCs and other kinds of tumor.[7] The lectin-binding properties of serum AFP are very useful in the distinction of YST from HCC.[8] In previous studies,[5] it was clarified that YSTs failed to stain for Con A in the areas positive for AFP and, in contrast, HCCs that were positively immunostained for AFP had intense cytoplasmic staining for Con A. The mechanism involved is uncertain. It may be related to formalin fixation. P-Con A staining was rather more sensitive than the PAP method. In all YSTs and HCCs, the present studies confirmed the staining results previously reported.[5] Although hepatoid cells in YSTs were positively stained for AFP, parallel staining by P-Con A was not positive. This new finding supports the conclusion that hepatoid cells within YSTs are not hepatocytes, but human yolk sac endodermal cells[9] (HYSECs). In all embryonal carcinomas, the results suggest AFP-secreting embryonal carcinomas had cells with a differentiation to HYSECs.[10] In immature solid teratomas, two types of AFP were detected. Serum analyses of patients with the same tumor disclosed two types of subfraction suggestive of both yolk sac-type AFP and hepatic-type AFP. These facts support the findings[6,8] obtained from serum analyses of patients with similar teratomas. In all YSECTs, yolk sac-type AFP in the cells with differentiation to HYSEC was confirmed. Adenocarcinomas of stomach, ovary or lung revealed yolk sac-type AFP in the cells with the same differentiation. However, only one stomach cancer showed hepatic-type AFP. In some cases of metastatic liver cancer, neighboring liver cells were positive for yolk sac-type AFP unrelatedly to the type of AFP in the primary cancers. The reason for this is not obvious, but one possibility is fetal retrodifferentiation of the affected liver cells. This finding is supported by the fact that serum AFP derived from patients bearing metastatic liver cancers shows the same yolk sac-type subfraction as that of YSTs.[6] In all hepatoblastomas, hepatic-type AFP was more intensely seen than that in HCCs. The finding that liver cell tumors such as HCCs and hepatoblastomas yield hepatic-type AFP is easily understandable. In a pancreatoblastoma, hepatic-type AFP was demonstrated. AFP-secreting pancreatoblastoma has already been reported.[11] In a nephroblastoma, hepatic-type AFP was found in tubules. Only one AFP-secreting nephroblastoma has been described.[12] In an ovarian SLCT, yolk sac-type AFP was seen in Leydig cells. AFP-secreting ovarian SLCT has been reported.[13,14] AFP was found not only in Leydig cells but also in Sertoli cells. But, in these reports, there was no description of the type of AFP in the tumor tissues. It is concluded that the PAP method for AFP and P-Con A staining of serial sections of various AFP-secreting tumors may facilitate immunohistochemical type distinction of AFP, which will be very useful for elucidating the histogenesis of the AFP-secreting tumor tissues.

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