Histol Histopathol (2004) 19: 701-706

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Aberrant expression of a fetal glycoprotein 68 in hepatocellular carcinoma: a comparative study on the expression of alpha-fetoprotein and carcinoembryonic antigen

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Summary. A rat IgG_{2a} monoclonal antibody against a stage-specific fetal glycoprotein with a molecular mass of 68 kDa (FGP68) was produced and applied to paraffin sections. This monoclonal antibody was used to compare the expression of FGP68 with that of both alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) in 75 hepatocellular carcinomas (HCCs). Seventy-five primary HCCs from patients aged 36 to 77 years were examined. Formalin-fixed, paraffin-embedded tissue sections were used for immunohistochemical analyses. Histologically, 6 cases of HCC were classified as type I according to the Edmondson and Steiner criteria, 57 cases as type II, and 12 cases as type III. The cancer tissues showed positive reactions with the antibody against FGP68. Approximately one-third of the HCCs (26/75) contained tumor cells that expressed FGP68 - (21/57 for Edmondson and Steiner type II; 4/12 for type III; and 1/6 for type I) - and positive immunoreactivity was observed in the cytoplasm of the cancer cells. Twenty-five of the 75 HCCs had tumor cells that expressed AFP and there was a significant correlation between FGP68 expression and AFP expression. Twenty-three of the 75 HCCs had tumor cells that expressed CEA and there was no significant correlation between FGP68 expression and CEA expression. No positive reactions for FGP68, AFP and CEA were observed in samples of non-neoplastic liver tissues. Based on the possibility that stage-specific FGP68 plays an important role in liver embryogenesis, FGP68-expressing tumor cells might ontogenetically revert to more primitive cells.

Key words: Fetal glycoprotein 68, Hepatocellular carcinoma, α-fetoprotein, Carcinoembryonic antigen, Immunohistochemistry

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Introduction

The rapid growth of tumor tissues whose proliferation is not under normal control may resemble a kind of normal embryogenic cell growth (Alexander, 1972). Morphologically, tumor cells derived from adult organ cells sometimes look like fetal organ cells: embryogenic characteristics reappear in some tumors. These tumor cells sometimes produce several embryonic antigens that have been designated as oncofetal antigens; for example, alpha-fetoprotein (AFP) (Abelev et al., 1963), carcinoembryonic antigen (CEA) (Gold and Freedman, 1965), fetal fibronectin (Wunderlich et al., 2001) and immature laminin receptor (Zelle-Rieser et al., 2001). These oncofetal antigens are diagnostically applied as tumor markers, and they are also analyzed to elucidate the cytobiological characteristics of the tumor. Furthermore, protein-specific genetic therapy against tumor cells which produce these proteins has been applied (Vollmer et al., 1999; Ueda et al., 2001).

A fetal development-specific glycoprotein, termed fetal glycoprotein 68 (FGP68), with a molecular mass of 68 kDa and a pI value of 5.6 was initially isolated from mouse embryos on the 11th day of gestation by using the two-dimensional gel-electrophoretic method of Noguchi et al. (1984). In mouse livers, FGP68 was expressed between the 13th day and the 17th day of gestation (Noguchi et al., 1984) or in the 12th day of gestation (Morita et al., 1998). However, the level of FGP68 was hardly detectable after these days. These findings suggest that this developmental stage-specific FGP68 might play an important role in liver embryogenesis. Although FGP68 is one of the fetal-specific proteins originating from the liver, its biological characteristics remain unknown. Moreover, it is unknown whether this FGP68 is expressed in human liver tissues and hepatocellular carcinoma (HCC) cells or not. To address these points, we have applied a monoclonal antibody against the purified mouse FGP68 to HCC cells since the

sequence of human FGP68 is still unknown. To obtain a new insight into the biological significance of the fetal-specific FGP68 in HCC cells, comparative immunohistochemical studies were employed to characterize the expression of FGP68, AFP and CEA in these cells.

Materials and methods

Isolation of FGP68 and production of monoclonal antibody against FGP68

Pregnant BALB/c mice and three-week-old female Wistar rats were obtained from CLEA JAPAN, Inc. The rat myeloma cell line YB2/0 was purchased from Dainippon Pharmaceutical Co. (Osaka, Japan). The cerebra of BALB/c mouse embryos on the 12-14th day of gestation in these pregnant mice were removed and homogenized in ice-cold 0.01 M Tris-HCl buffer containing 2% Triton X-100, 0.15 M NaCl and 0.005% phenylmethylsulphonyl fluoride (0.01 M Tris-HCl/2% Triton X-100 buffer). After the centrifugation, the supernatant was removed and applied to an RCA-1 agarose (EY Laboratories Inc., San Mateo, USA) column. The FGP68 was eluted and its purity was confirmed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). The monoclonal antibody was prepared according to the methods of Fujita et al. (1982) and Kato et al. (2002). Each inoculation sample consisted of 300 μg of the purified FGP68 in Freund's complete adjuvant; the Wistar rats were given five injections, subcutaneously and intraperitoneally. After the final injection, the spleen cells were removed and fused to rat myeloma cells (YB2/0) according to the method of Köhler and Milstein (1975). Hybridomas were distributed into 96-well microplates and fed with growth medium in a 7% CO_2 incubator (Napco Model 6101C) (Jouan Inc., Winchester, USA) at 37 °C. Antibody activity in the supernatants of the hybridoma culture was examined by dot immunoassay (Hawkes et al., 1982). Classification of the obtained antibodies into isotypes of IgG_1, IgG_{2a}, IgG_{2b}, IgG_{2c}, IgM, or IgA was performed by Ouchterlony's immunodiffusion method employing a rat monoclonal antibody typing kit (Binding Site, Birmingham, UK). The antibody reaction with purified FGP68 or bovine serum albumin (BSA) was examined by dot immunoassay.

Western blot analysis (Burnette, 1981) was performed to confirm there was lack of cross-reactivity between the monoclonal antibody against FGP68 and human AFP (Gelco Diagnostics, Inc., Shreveport, USA). Human AFP and purified isolated FGP68 were studied by SDS-PAGE. The specific anti-FGP68 antibody reaction with purified FGP68 was tested by electroblot immunoassay.

Tissue preparations

This retrospective study was carried out on 50 surgical and 25 autopsy samples of primary HCCs that had been referred to the surgical and autopsy files at our laboratories. A total of 75 tumor specimens from patients aged 36-77 years, (62 males and 13 females) were examined. Formalin-fixed, paraffin-embedded tumor tissues were used. Histologically, 6 cases of HCC were classified according to the Köhler and Milstein (1975). Edmondson and Steiner criteria (Edmondson and Steiner, 1954) as type I, 57 cases as type II and 12 cases as type III. As a non-neoplastic disease control, we also investigated 25 surgical and autopsy liver samples from individuals aged between 32 and 75 years without HCC.

Immunohistochemical analysis

Multiple 4-μm-thick sections were prepared from each of the specimens. One section was stained with hematoxylin and eosin (H&E) and the others were used for immunohistochemical analyses. The avidin-biotin-immunoperoxidase complex (ABC) method was employed for FGP68 detection (Hsu et al., 1981). Sections were deparaffinized, endogenous peroxidase activity was quenched by incubation for 30 min with 0.3% H_2O_2 and then sections were washed with phosphate-buffered saline (PBS). Normal sera homologous with the secondary antibody were used as blocking reagents. The primary antibodies used for the immunohistochemical studies were as follows: monoclonal antibody against FGP68 (concentration: 2 mg/ml); anti-AFP monoclonal antibody (ready-to-use) (Nichirei, Tokyo, Japan); and anti-CEA polyclonal antibody (ready-to-use) (Nichirei, Tokyo, Japan). We used limiting dilution technique to set FGP68 antibody concentration for paraffin sections. Characterization and usefulness of these primary antibodies were previously reported respectively (Gold and Freedman, 1965; Tsung, 1977; Noguchi et al., 1984). The appropriate Vectastain ABC kits (Vector Laboratories, Burlingame, CA, USA) were used to detect these antibodies. As a chromogen, 3,3'-diaminobenzidine tetrahydrochloride was used. Some sections were counterstained with methyl green. FGP68 immunoreaction was considered positive when the cell cytoplasm was stained, irrespective of the percentage of positive cells. For the assessment of FGP68 immunostaining, only neoplastic areas, delineated by the histological features seen on contiguous H&E-stained serial sections, were evaluated.

The data were analyzed by the chi-square test.

Results

Specificity of FGP68 and monoclonal antibody against FGP68

The purified FGP68 isolated from the mouse embryos on the 12-14th days of gestation revealed a single band of 68kDa by SDS-PAGE analysis. Four clones that were obtained by cell fusion and cloning produced antibodies of the IgG_{2a} type. These antibodies recognized FGP68, but did not react with BSA. In

Western blot analysis, a single band was detected in the isolated FGP68 lane, but no positive reaction was observed in the human AFP lane (Fig. 1).

Immunohistochemistry

Histologically, the majority of cancer cells exhibited a trabecular growth pattern (Fig. 2A) and had abundant cytoplasm and prominent nucleoli. Tissue sections of HCC were classified according to the Edmondson and Steiner criteria (Edmondson and Steiner, 1954) as type II (57 cases), type III (12 cases) and type I (6 cases). When control sections were incubated with PBS and non-immunized rat IgG, no staining was detected. Furthermore, the anti-FGP68 antibody pretreated with an excess of the purified FGP68 antigen did not stain sections. The immunohistochemical results for the 75 HCCs are summarized in Tables 1 and 2. We have succeeded in applying this anti-FGP68 monoclonal antibody for use on paraffin sections. The staining intensity and proportion of positively-stained tumor cells varied. Cytoplasmic staining with anti-FGP68 antibody was observed in many tumors, and the staining pattern was heterogeneous; patchy staining was seen in some specimens (Fig. 2B). No significant immunoreactivity was found in vascular or mesenchymal cells in any of the cancers examined. A positive reaction with anti-FGP68 antibody was observed in 26 out of the 75 HCCs (35%). FGP68 expression was seen in 21 out of the 57 HCCs (37%) classified as Edmondson type II, 4 out of the 12 HCCs (33%) classified as type III, and 1 out of the 6 HCCs classified as type I (17%) (Table 1). When the tumors were classified as having 0%, <5%, 5%-0%, or >20% of cells positive for FGP68, more than 20% of the cancer cells were stained in 1 out of the 6 Edmondson type I HCCs. In 4 out of the 21 Edmondson type II HCCs, more than 20% of the cancer cells were stained, and in 10 out of the 21 specimens, the proportion of immunostained cells ranged between 5% and 20%; in 7 specimens, less than 5% of the cancer cells were positively stained (Table 1). Although there were no statistically significant differences in FGP68 expression among the histologically different specimens, the number of moderately differentiated HCCs with FGP68-positive tumor cells was higher than that of well or poorly differentiated HCCs. The staining of the surrounding non-tumor liver tissues was negative. Furthermore, the 25 samples of non-neoplastic liver tissue which did not contain HCC were also negative for FGP68.

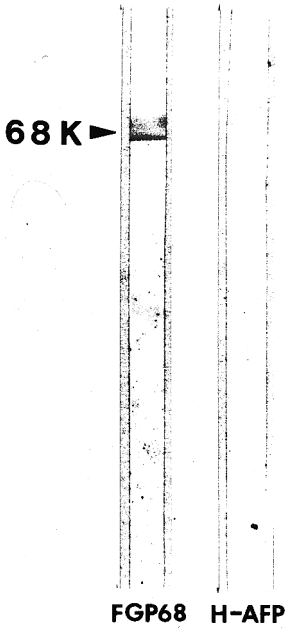

Fig. 1. Western blot analysis using monoclonal antibodies against fetal glycoprotein 68 (FGP68) and alpha-fetoprotein (AFP). Although a single band is detected in the lane containing isolated FGP68, no positive reaction is observed in the lane containing human alpha-fetoprotein (H-AFP).

The results of comparison between FGP68 expression and AFP expression as well as between FGP68 expression and CEA expression are summarized in Table 2. A positive reaction with anti-AFP antibody was found in 25 out of the 75 HCCs. AFP expression was seen in 21 out of the 57 Edmondson type II HCCs, 3 out of the 12 type III HCCs, and 1 out of the 6 type I HCCs. Positive staining for AFP was found throughout the cytoplasm (Fig. 2C), and showed a staining pattern similar to that of FGP68 (Fig. 2B,C). In 23 out of the 75 HCCs, a surface membranous pattern was observed with the anti-CEA antibody. CEA expression was seen in 16 out of the 57 Edmondson type II HCCs, 5 out of the 12 type III HCCs and 2 out of the 6 type I HCCs. There was a significant relationship between FGP68 expression and AFP expression ($P<0.01$), but no significant relationship between FGP68 expression and CEA expression (Table

Table 1. Immunohistochemical expression of fetal glycoprotein 68 in hepatocellular carcinoma.

EDMONSON CLASSIFICATION	NUMBER OF CASES	FGP68-POSITIVE CASES	PERCENTAGE OF FGP68-POSITIVE TUMOR CELLS		
			<5%	5%-20%	>20%
type I	6	1(17)			1
type II	57	21(37)	7	10	4
type III	12	4(33)	1	3	0
type IV	0	0(0)			

The percentages of tumors expressing fetal glycoprotein 68 (FGP68) are in parentheses. No staining was negative.

2, Fig. 2B,D). Most individual HCC cells that expressed FGP68 also expressed AFP (Fig. 2B,C).

Discussion

In addition to AFP (Abelev et al., 1963) and CEA (Gold and Freedman, 1965), several oncofetal proteins such as fetal fibronectin (Wunderlich et al., 2001), immature laminin receptor (Zelle-Rieser et al., 2001), M2A (Marks et al., 2001) and 5T4 oncofetal antigen (Starzynska et al., 1998) have been reported and applied to tissue diagnosis. We have newly purified a developmental stage-specific protein, FGP68 from fetal mouse embryos and in addition have successfully produced a rat IgG_{2a} monoclonal antibody against it. FGP68 was derived from a fetal development-specific glycoprotein with a molecular mass of 68 kDa. Although oncofetal proteins are produced in some tumor tissues in large amounts, they are detected only in very small quantities in normal adult tissues (Ritchie 1990; Rosai

Table 2. Comparison with the expression of fetal glycoprotein 68, alpha-fetoprotein and carcinoembryonic antigen in hepatocellular carcinoma.

	ALPHA-FETOPROTEIN[a]		CARCINOEMBRYONEIC ANTIGEN	
	+	-	+	-
FGP68 positive	14	12	11	15
negative	11	38	12	37

Numbers represent case numbers. [a]: There is a significant relationship between fetal glycoprotein 68 (FGP68) expression and alpha-fetoprotein expression (P<0.01, chi-square test)

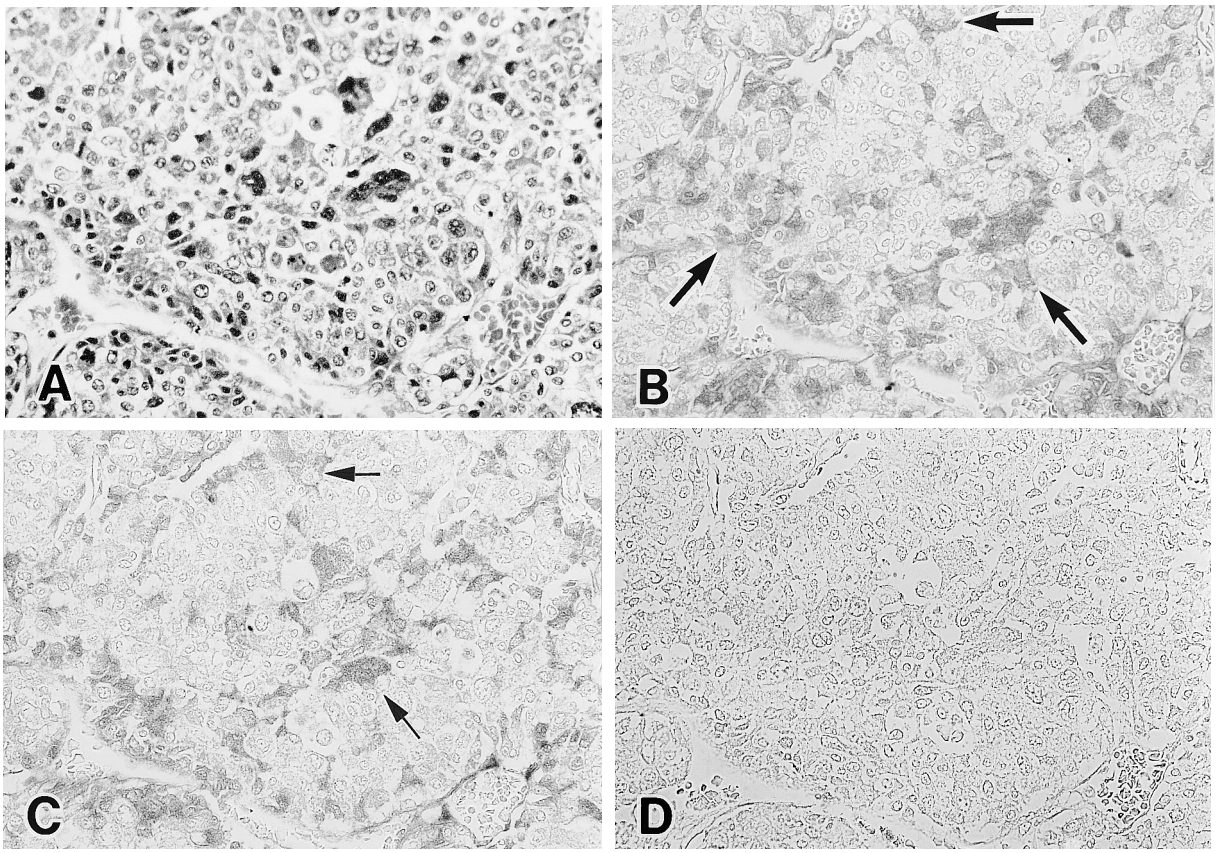

Fig. 2. Serial sections of hepatocellular carcinoma (HCC) stained with hematoxylin and eosin (H&E) **(A)** and immunostained by the antibodies against fetal glycoprotein 68 (FGP68) **(B)**, alpha-fetoprotein (AFP) **(C)** and carcinoembryonic antigen (CEA) **(D)**. A. This tumor presents a trabecular pattern, and shows moderate variation in cell and nuclear size. **B.** The cytoplasm of some tumor cells is strongly stained by the anti-FGP68 antibody (large arrows). **C.** The cytoplasm of some tumor cells is strongly stained by the anti-AFP antibody (small arrows). **D.** No immunoreaction products to CEA are seen in the tumor cells. A-D x 200

1996). Therefore, immunohistochemical analyses using optimally diluted antibody against each of these oncofetal proteins have shown that only oncofetal antigen-producing tumor cells can be easily visualized in association with greatly-reduced background staining. We have successfully applied our monoclonal antibody against FGP68 for staining of paraffin sections. Using this antibody, FGP68 was not detected immunohistochemically in normal liver tissue from control individuals, but overexpression of FGP68 was found in approximately one third of the HCCs we examined. This is the first report of aberrant expression of FGP68 in HCCs, although the biological significance of this and its possible relationship to tumor behavior is still unclear.

AFP and CEA as oncofetal proteins are reported to be frequently overexpressed in HCCs (Ishak et al., 2001). The frequency of AFP expression in HCCs is reportedly to be from 35-73% (Thung et al., 1979; Kojiro et al., 1981), and its expression is more frequent in Edmondson type II or III than in other types. AFP is expressed in the cytoplasm of tumor cells. Regarding CEA expression, between 30% and 60% of HCCs express CEA, and positive staining is found in the surface membranes of the tumor cells (Imoto et al., 1985; Balaton et al., 1988). The expression of FGP68 in HCCs was similar to that of AFP in terms of both frequency and expression pattern, and there was a significant relationship between the expression of the two proteins. Based on the findings FGP68 could be another marker for HCC, in addition to several others.

It has been reported that AFP might play some type of biological role such as suppression of the immune response (Murgita and Tomasi, 1975a,b), estrogen-binding (Ureil et al., 1972), as a carrier protein (Aoyagi et al., 1978, 1979), for inhibition of protein synthesis (Aoyagi et al., 1982) or stimulation of tumor-cell growth (Wang and Xie, 1999), and CEA might play a role in cell-adhesion activity in vitro (Oikawa et al., 1989) and in the production of cytokines (Minami et al., 2001). Although the definitive biological significance of these oncofetal proteins has yet to be determined, FGP68 expression in certain brain tumors was reported to be related to proliferation potential (Kato et al., 2002). In the present study, FGP68 appeared to be expressed in certain HCCs. Taken together with the fact that FGP68 expression is similar to that of AFP, our data in this report may lead to a new insight, which is that FGP68 is also an oncofetal protein in HCCs. To ultimately elucidate the relationship between FGP68 expression and AFP expression, a further complete understanding of the molecular mechanism of FGP68 expression in HCCs cells will be necessary.

Based on the possibility that fetal stage-specific expression of FGP68 plays an important role in liver embryogenesis, FGP68-overexpressing tumor cells might ontogenetically revert to more primitive or regenerating cells. Whether these considerations apply to HCCs, whose cells lack normal proliferation control mechanisms, remains to be determined.

References

Abelev G.I., Perova S.D., Khramkova N.I., Postnikova Z.A. and Irlin I.S. (1963). Production of embryonal alpha-globulin by transplantable mouse hepatomas. Transplantation 1, 174-180.

Alexander P. (1972). Foetal "antigens" in cancer. Nature 235, 137-140.

Aoyagi Y., Ikenaka T. and Ichida F. (1978). Copper(II)-binding ability of human α-fetoprotein. Cancer Res. 38, 3483-3486.

Aoyagi Y., Ikenaka T. and Ichida F. (1979). α -fetoprotein as a carrier protein in plasma and its bilirubin-binding ability. Cancer Res. 39, 3571-3574.

Aoyagi Y., Takahashi Y., Odani S., Ogata K., Ono T. and Ichida F. (1982). Inhibitory effect of α-fetoprotein on protein synthesis in a reticulocyte lysate cell-free system. J. Biol. Chem. 257, 9566-9569.

Balaton A.J., Nehama-Sibony M., Gotheil C., Callard P. and Baviera E.E. (1988). Distinction between hepatocellular carcinoma, cholangiocarcinoma, and metastatic carcinoma based on immunohistochemical staining for carcinoembryonic antigen and for cytokeratin 19 on paraffin sections. J. Pathol. 156, 305-310.

Burnette W.N. (1981). "Western blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. 112, 195-203.

Edmondson H.A. and Steiner P.E. (1954). Primary carcinoma of the liver. A study of 100 cases among 48900 necropsies. Cancer 7, 462-503.

Fujita S.C., Zipursky S.L., Benzer S., Ferrüs A. and Shotwell S.L. (1982) Monoclonal antibodies against the Drosophila nervous system. Proc. Natl. Acad. Sci. USA 79, 7929-7933.

Gold P. and Freedman S.O. (1965). Specific carcinoembryonic antigens of the human digestive system. J. Exp. Med.122, 467-481.

Hawkes R., Niday E. and Gordon J. (1982). A dot-immunobinding assay for monoclonal and other antibodies. Anal. Biochem. 119, 142-147.

Hsu S.M., Raine L. and Fanger H. (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 577-580.

Imoto M., Nishimura D., Fukuda Y., Sugiyama K., Kumada T. and Nakano S. (1985). Immunohistochemical detection of a-fetoprotein, carcinoembryonic antigen, and ferritin in formalin-paraffin sections from hepatocellular carcinoma. Am. J. Gastroenterol. 80, 902-906.

Ishak K.G., Goodman Z.D. and Stocker J.T. (2001). Tumors of the liver and intrahepatic bile ducts. Rosai J. and Sobin L,H. (eds). Atlas of tumor pathology. Third series, Washington DC, Armed Forces Institute of Pathology 2001, 199-230.

Kato S., Shinozawa T.,Nagashige H., Nakamura M., Asano Y., Takikawa M., Kato M., Awaya A., Hirano A. and Ohama E. (2002). Monoclonal antibody to stage-specific fetal brain 68-kDa glycoprotein (FGP68) revealed increased FGP68 expression in human primary brain tumors. Acta Neuropathol. 104, 57-66.

Köhler G. and Milstein C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256, 495-497.

Kojiro M., Kawano Y., Isomura T. and Nakashima T. (1981). Distribution of albumin- and/or α-fetoprotein-positive cells in hepatocellular carcinoma. Lab. Invest . 44, 221-226.

Marks A., Sutherland D.R., Bailey D., Iglesias J., Law J., Lei M., Yeger H., Banerjee D. and Baumal R. (1999). Characterization and

distribution of an oncofetal antigen (M2A antigen) expressed on testicular germ cell tumours. Br. J. Cancer, 80, 569-578.

Minami S., Furui J. and Kanematsu T. (2001). Role of carcinoembryonic antigen in the progression of colon cancer cells that express carbohydrate antigen. Cancer Res. 61, 2732-2735.

Morita T., Shinozawa T., Nakamura M., Awaya A., Sato N., Ishiwata I. and Kami K. (1998). Expression of a 68kDa-glycoprotein (GP68) and laminin in the mesodermal tissue of the developing mouse embryo. Okajima Folia Anat. Jpn. 75, 185-196.

Murgita R.A. and Tomasi T.B. Jr. (1975a). Suppression of the immune response by α-fetoprotein. I. The effect of mouse a-fetoprotein on the primary and secondary antibody response. J. Exp. Med. 141, 269-286.

Murgita R.A. and Tomasi T.B. Jr. (1975b). Suppression of the immune response by a-fetoprotein. II. The effect of mouse α-fetoprotein on mixed lymphocyte reactivity and mitogen-induced lymphocyte transformation. J. Exp. Med. 141, 440-452.

Noguchi S., Ogata S., Sato M. and Muramatsu T. (1984). Stage-specific polypeptides in the developing mouse brain as demonstrated by two-dimensional gel electrophoresis. J. Biochem. 96, 881-886.

Oikawa S., Inuzuka C., Kuroki M., Matsuoka Y., Kosaki G. and Nakazato H. (1989). Cell adhesion activity of non-specific cross-reacting antigen (NCA) and carcinoembryonic antigen (CEA) expressed on CHO cell surface: homophilic and heterophilic adhesion. Biochem. Biophys. Res. Commun. 164, 39-45.

Ritchie A.C. (1990). Neoplasia. In: Boyd's Textbook of Pathology. Ritchie A.C. (ed). 9th ed. Vol. I. London, Lea&Febiger, 1990: 234-278.

Rosai J. (1996). Special techniques in surgical pathology. Ackerman's Surgical Pathology. 8th ed. Vol 1. Ritchie A.C. (ed). Mosby-Year Book, Inc. St Louis, MO.. p 37.

Starzynska T., Wiechowska-Kozlowska A., Marlicz K., Bromley M., Robert S.A., Lawniczak M., Kolodziej B., Zyluk A. and Stern P.L. (1998). 5T4 oncofetal antigen in gastric carcinoma and its clinical significance. Eur. J. Gastroenterol. Hepatol. 10, 479-484.

Thung S.N., Gerber M.A., Sarno E. and Popper H. (1979). Distribution of five antigens in hepatocellular carcinoma. Lab. Invest, 41, 101-105.

Tsung S.H. (1977). Localization of α-fetoprotein synthesis in malignancies other than hepatoma. Arch. Pathol. Lab. Med. 101, 572-574.

Ueda K., Iwahashi M., Nakamori M., Nakamura M., Matsuura I., Yamaue H. and Tanimura H. (2001). Carcinoembryonic antigen-specific suicide gene therapy of cytosine deaminase/5-fluorocytosine enhanced by the cre/loxP system in the orthotopic gastric carcinoma model. Cancer Res. 61, 6158-6162.

Ureil J., de Nechaud B. and Dupiers M. (1972). Estrogen-binding properties of rat, mouse and man fetospecific serum protein. Demonstration by immunoautoradiographic methods. Biochem. Biophy. Res. Commun. 46, 1175-1180.

Vollmer C.M., Eilber F.C., Butterfield L.H., Ribas A., Dissette V.B., Koh A., Montejo L.D., Lee M.C., Andrews K.J., McBride W.H., Glaspy J.A. and Economou J.S. (1999). Alpha-fetoprotein-specific genetic immunotherapy for hepatocellular carcinoma. Cancer Res. 59, 3064-3067.

Wang X.W. and Xie H. (1999). Alpha-fetoprotein enhances the proliferation of human hepatoma cells in vitro. Life Sci. 64, 17-23.

Wunderlich H., Reichelt O., Zermann D.H., Schubert J., Berndt A. and Kosmehl H. (2001). Fetal fibronectin: a new screening-marker for bladder cancer? Oncol. Rep. 8, 669-672.

Zelle-Rieser C., Barsoum A.L., Sallusto F., Ramoner R., Rohrer J.W., Holtl L., Bartsch G., Coggin J.H. Jr. and Thurnher M. (2001). Expression and immunogenicity of oncofetal antigen-immature laminin receptor in human renal cell carcinoma. J. Urol. 165, 1705-1709.

Accepted January 7, 2004