

The Characteristics of Immunoreactivity of Alpha-fetoprotein Producing Gastric Cancer

Alpha-Fetoprotein Producing Gastric Cancer

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Abstract

Alphafetoprotein (AFP) producing gastric cancer (AFP-GC) is very malignant and highly metastatic compared with common gastric cancer. We encountered six patients with AFP-GC. The purpose of this study was to characterize the immunoreactivity of alpha-fetoprotein producing gastric cancer, using a panel of hepatocytic markers, including alpha-fetoprotein, hepatocyte antigen, carcinoembryonic antigen, and CD10. Five of 6 cases showed cytoplasmic reactivity for alpha-fetoprotein. Immunoreactivity with a cytoplasmic, membranous, or canalicular pattern, or a mixed pattern was found for polyclonal CEA. Positive immunostaining for hepatocyte antigen was noted in only 2 of 6 cases. Negative immunostaining was found in all 6 patients for CD10. This study demonstrated that most AFP producing gastric cancer, hepatoid or non-hepatoid, were immunoreactive for AFP and p-CEA, but non-reactive for CD10. Therefore, CD-10 might be helpful to distinguish primary hepatocellular carcinoma from AFP-GC when it metastasizes to the liver. In this small series of patients with gastric cancer, AFP production indicated the poor prognosis, regardless hepatoid or non-hepatoid.

Keywords: Alpha-fetoprotein, carcinoembryonic antigen, hepatocyte antigen, hepatoid gastric carcinoma, immunohistochemistry

1. Introduction

Alpha-fetoprotein (AFP) is an albumin-like glycoprotein with a molecular weight of 70,000 daltons. AFP was first identified in the human fetus in 1956 (Bergstrand et al., 1956). It is produced by yolk sac cells, fetal hepatic cells, and some fetal gastrointestinal cells. Elevated serum levels of AFP were initially used for screening and monitoring hepatocellular carcinoma (HCC). Later, high serum levels of AFP were found in many other malignant neoplasms including gastric cancer. Since the first case of alpha-fetoprotein producing gastric cancer (AFP-GC) was reported (Bourreille et al., 1970), many cases have been reported all over the world. In China, the reported incidence of AFP-GC was 2.3% (Liu et al., 2010). In Japan, the incidence ranged from 1.5 to 3% (Kono et al., 2002; Matsunou et al., 1994). Until present, there was only one case reported in Taiwan (Huang et al., 2002). AFP-GC has been considered as having unfavorable long-term survival rate due in part to the higher incidence of liver metastasis and lymphovascular invasion. When it metastasizes to the liver, differential diagnosis between AFP-GC and HCC is important. We encountered six patients of AFP-GC. In this study, we intended to characterize the immunoreactivity of AFP-GC of these patients, using a panel of hepatocytic markers, including alpha-fetoprotein (AFP), hepatocyte antigen (Hep Par 1), polyclonal CEA (p-CEA), and CD-10. The aim of this study is to find a marker or markers to distinguish AFP-GC from HCC.

2. Materials and Methods

Six cases of AFP -GC (2 hepatoid type, 3 intestinal type and 1 signet-ring cell type) were selected from the surgical files at Sun Yat-Sen Cancer Center, from 1995 to 2002. Two cases of non-AFP producing gastric cancer (1 intestinal type and 1 signet-ring cell type) were included for the comparative study. The charts of these 8 patients were reviewed. The clinical data were summarized in Table 1. None of these patients had other primary tumor. No clinical or biochemical evidence of liver cirrhosis or hepatitis was found.

Table 1. Clinical data of eight patients with gastric carcinoma

Case No./Age/Sex	Tumor Site and Procedure	Serum Level of AFP (ng/ml)	Lymph Node and Hepatic metastasis	Follow-up One year
1.78/M	Cardia, Gastrectomy	490	No	Alive with no metastasis
2.59/M	Body, Bx	74901	Yes	DOD
3.79/M	Antrum, Bx	35350	Yes	DOD
4.61/M	Pylorus, Bx	1306	Yes	DOD
5.80/M	Body, Bx	174	Yes	DOD
6.82/M	Pylorus, Bx	23041	Yes	DOD
7.73/M	Body, subtotal gastrectomy	<20	No	Alive
8.58/M	Body, radical gastrectomy	<20	No	Alive

DOD=Died of disease Bx=Biopsy

The tissue had been routinely fixed in 10 % neutral formalin and embedded in paraffin. The paraffin tissue block with tumor was selected from each case. Sections were cut at 4 micron thick, deparaffinized in xylene, and rehydrated in graded ethanol. Routine hematoxylin and eosin stains were performed. Immunohistochemical stains were performed in a Dako Auto-Stainer. Appropriate positive and negative tissue controls were used throughout. Antibodies used in this study were listed in Table 2. Immunoreactivity was evaluated according to the intensity of the tumor cells staining (0-3+), as well as the percentage of tumor cells that were stained. The tumor cells were considered unequivocally positive if >10% of the tumor cells reacted with any intensity.

Table 2. The list of antibodies used

Antibody	Type	Dilution	Source
Hep Par 1	monoclonal	1:100	DAKO
AFP	polyclonal	1:500	DAKO
CD 10	monoclonal	1:5	Novocastra
p-CEA	polyclonal	1:60	DAKO

3. Results

A spectrum of histologic patterns and cytologic features was observed in 6 AFP-GC; two cases showed typical features of hepatoid adenocarcinoma. The malignant cells were arranged in cords or trabecular patterns, with abundant eosinophilic cytoplasm, large nuclei, prominent cherry red nucleoli (Ishikura et al., 1987).

One case showed diffusely infiltrated malignant cells containing cytoplasmic vacuoles pushing the nuclei to the side, producing a signet ring appearance. The remaining 3 cases were typical intestinal type adenocarcinoma, composed of malignant glands lined by cuboid or columnar cells.

The results of immunoreactivity were summarized in Table 3. Five cases (83%), except for the signet-ring cell type showed cytoplasmic positivity for AFP (Figure 1); all of them were focally positive, ranging from 15 to 80%. Only two of 6 cases (33%) were positive for Hep Par 1; one intestinal type with 3+, diffuse cytoplasmic granular pattern (Figure 2), and one signet-ring cell type with diffuse, weak cytoplasmic granular pattern (Figure 3). None of two cases of hepatoid adenocarcinoma showed reactivity for Hep Par 1.

Table 3. Results of immunoreactivity

Pt. No.	Type of tumor	AFP	p-CEA	Hep Par 1	CD10
1.	Intestinal Type	15%, 3+ Cytoplasmic	Diffuse, 3+, Cytoplasmic	negative	negative
2.	Intestinal Type	80%, 3+ Cytoplasmic Cytoplasmic Granular	Diffuse, 3+ Cytoplasmic	80%, 3+	negative
3.	Intestinal Type	40%, 2+ Cytoplasmic	15%, 3+ Membraneous	negative	negative
4.	Hepatoid type	15%, 2+ Cytoplasmic	Diffuse, 3+ Cytoplasmic Membraneous	negative	negative
5.	Hepatoid type	20%, 2+ Cytoplasmic	20%, 3+ Canalicular Cytoplasmic	negative	negative
6.	Signet ring type	Negative	Diffuse 3+ Membranous	70%, 1+ Cytoplasmic Granular	negative
7.	Signet ring type	Negative	Diffuse 3+ Membranous	70%, 1+ Cytoplasmic Granular	negative
8.	Intestinal type	negative	Diffuse 3+ Membraneous	negative	negative

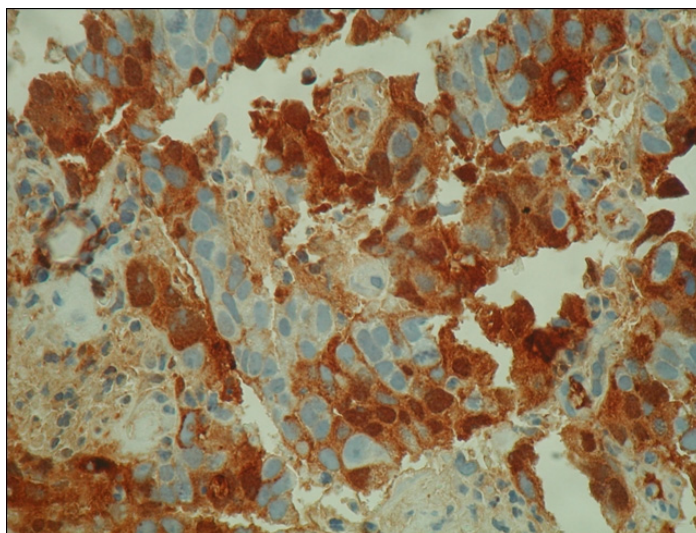


Figure 1. Strong cytoplasmic immunoreactivity in gastric carcinoma, intestinal type. (AFP, x400). Case 2

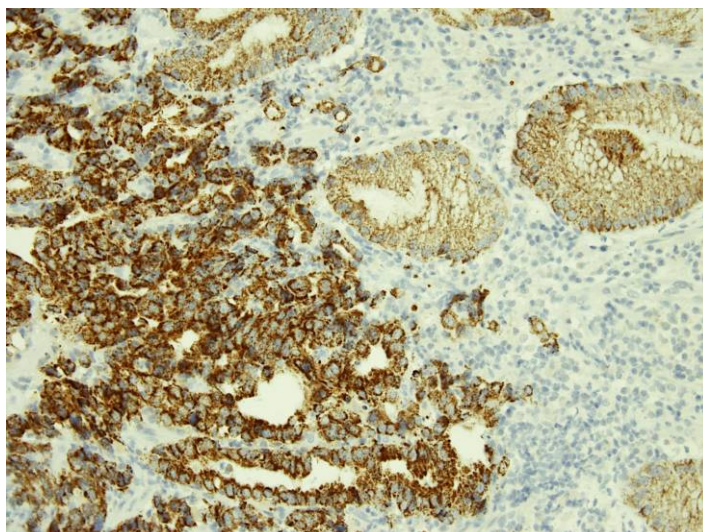


Figure 2. Strong cytoplasmic, granular immunoreactivity in gastric carcinoma, intestinal type (Hep Par 1. x200). Case 2

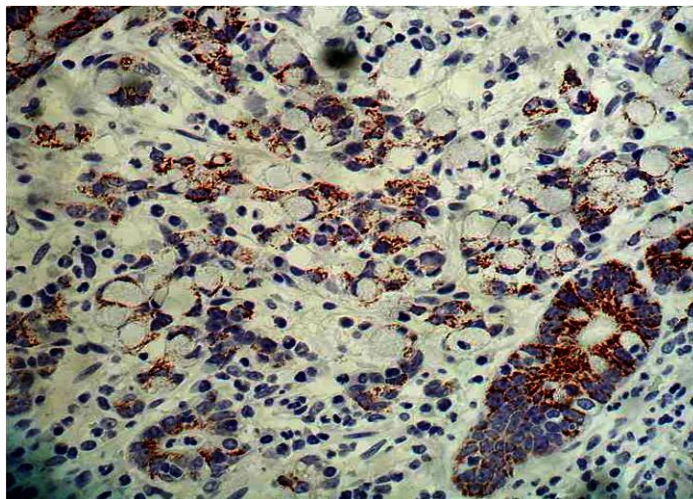


Figure 3. Weak cytoplasmic, granular immunoreactivity in gastric carcinoma (Hep Par 1. x200). Case 6, Signet-ring cell type

Polyclonal CEA (p-CEA) expression was noted in all cases examined. The staining patterns of p-CEA were diffuse, 3+ cytoplasmic; mixed cytoplasmic and membraneous; mixed cytoplasmic and canalicular (Figure 4), and membraneous (Figure 5). None of 6 cases showed immunoreactivity for CD10.

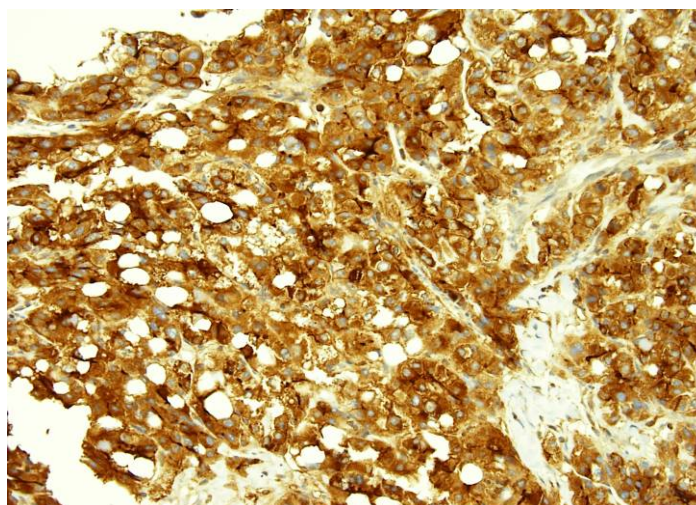


Figure 4. Strong cytoplasmic staining with canalicular pattern (p-CEA. X400) Case 5. Hepatoid type

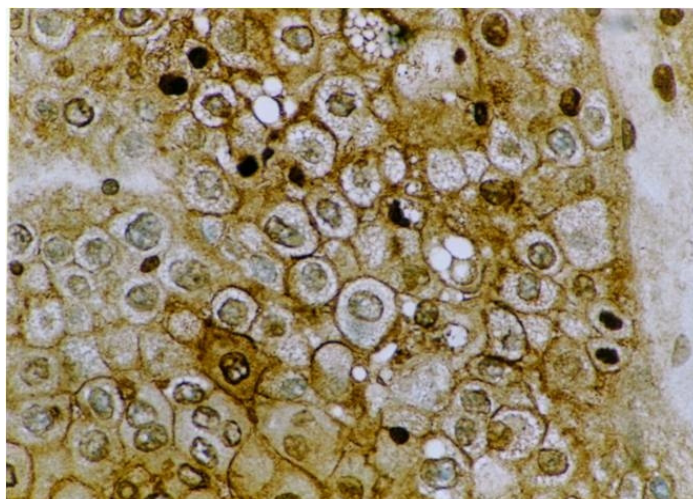


Figure 5. Strong membranous pattern in gastric carcinoma, intestinal type (p-CEA. x400). Case 3

While in the control cases, immunoreactivity for AFP, CD10, were all negative. In both cases, the malignant cells were strongly immunoreactive for p-CEA, with the membranous pattern. In the control cases, weak positive Hep Par 1 staining was observed in the gastric cancer of signet–ring cell type, and negative in the intestinal type. In the non-cancerous gastric mucosa with intestinal metaplasia, Hep Par 1 was strongly positive (Figure 6).

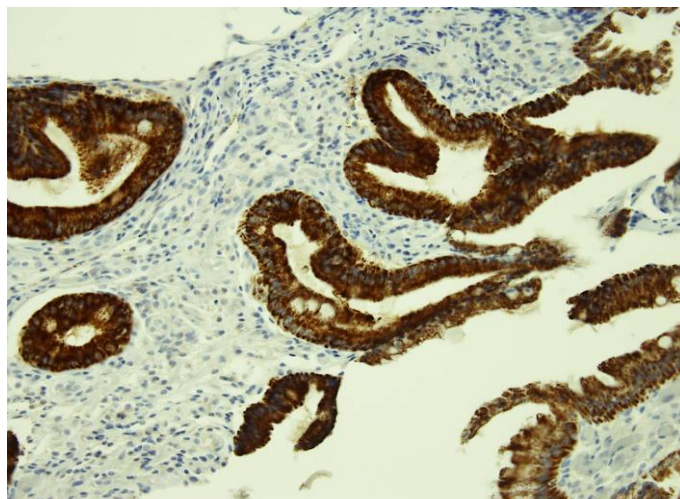


Figure 6. Hep Par 1 immunoreactivity in metaplastic gastric glands. x400. Case 6

4. Discussion

AFP-GC phenotypes can be classified into hepatoid adenocarcinoma, intestinal and signet ring type. AFP producing activity was mostly found in the hepatoid type, but also could be found in the intestinal type, as well as in the signet ring type. The hepatoid adenocarcinoma is a special type gastric carcinoma which has a striking morphologic similarity to HCC (Ishikura et al., 1987)

In the present study, immunoreactivity for AFP was demonstrated in 5 of 6 AFP -GC. The patient with signet-ring cell type had serum AFP level of 23,041ng/ml. The reason that immunoreactivity for AFP was negative might be due to the limited sampling (Fan et al., 2003). Another explanation could be related to its sensitivity. In the literature, only about 25-40% of cases of HCC were positive for AFP by immunohistochemistry (Brumm et al., 1989; Johnson et al., 1992). However, this patient had a very high serum level of AFP. Sensitivity should not have been the issue.

A recent study (Kinjo et al., 2012) suggested that the gastric carcinoma starts on the mucosa, which differentiated into enteroblastic type and hepatoid type. During the process of tumor invasion and proliferation, the tumor cells acquire the AFP production ability. Therefore, the tumor cells from the surface may be negative for AFP reactivity.

In a study (Maitra et al., 2001), p-CEA expression was found in 5 of 5 cases of gastric carcinoma examined. In 2 cases, a focal canalicular pattern of p-CEA expression, recapitulating bile canaliculi, was seen. In the present study, 6 of 6 cases showed immunoreactivity for p-CEA with 4 staining patterns recognized including cytoplasmic; mixed membranous and cytoplasmic; mixed cytoplasmic and canalicular pattern; and membranous. A thick, waxy linear staining pattern with branching was typical for a true canalicular pattern which was sometimes difficult to assess because of the presence of a strong membranous stain.

In the same study (Maitra et al., 2001), 5 of 6 cases of hepatoid gastric cancers focally expressed Hep Par 1, while Chu et al found 4 of 13 cases of gastric cancer positive for Hep par 1, including one signet-ring cell type; one intestinal type and two cases of hepatoid carcinoma (Chu et al., 2002). In another study by Kakar et al, positive Hep Par 1 results were reported in 7 of 10 cases (Kakar et al., 2003). They did not distinguish hepatoid from nonhepatoid carcinoma. In a recent study by Terracciano et al, Hep Par 1 staining was negative in all 8 cases of hepatoid carcinoma (Terracciano et al., 2003). In the present study, 2 of 6 cases expressed Hep Par 1, including one signet-ring cell type and one intestinal type. Two cases of hepatoid carcinoma were non-reactive for Hep Par 1. The contradicting published results of Hep Par 1 immunostaining on hepatoid carcinoma could be due to different methods or different antibodies used.

In the control case, immunoreactivity for Hep Par 1 was also observed for the gastric cancer with the signet-ring cell type, while it was negative with the intestinal type. It is of interest to mention that marked intestinal metaplasia was found in the non-neoplastic gastric mucosa in the control case of the signet-ring cell type gastric cancer. Strong Hep Par 1 immunostaining was observed in the metaplastic glands (Figure 6). In a previous study (Chu et al., 2003), Hep Par 1 was specifically expressed in both complete and incomplete forms of intestinal metaplasia (IM), but not in normal gastric or esophageal mucosa. Hep Par 1 immunostaining may be helpful in difficult cases or small biopsy specimens to confirm the diagnosis of IM. Positive Hep Par 1 staining in two cases of signet-ring cell type gastric cancer suggested that this type of cancer derived from the metaplastic cells.

It was shown recently that CD10 is expressed in both normal and hepatic liver tissue and HCC tumor tissue. Thus, CD10 may serve as an additional marker for hepatic differentiation (Borscheri et al., 2001). In the literature, only 2 cases of gastric carcinoma were studied for CD10 expression which were negative (Borscheri et al., 2001). In the present study, none of 6 cases showed CD10 expression.

In summary, AFP-GC is a subtype of gastric cancer which has a more aggressive behavior, and has a poor prognosis regardless hepatoid or nonhepatoid. The cellular and molecular mechanisms responsible for the poor prognosis are not clearly understood. Early report indicated that AFP has a suppressive effect on lymphocyte transformation (Yachnin, 1983). Another report indicated that AFP can enhance the proliferative activity and increases angiogenesis (Koide et al., 1999). When AFP-GC metastasizes to the liver, it is important to differentiate it from primary hepatocellular carcinoma. In this study, we demonstrated that the malignant cells of AFP-GC were non-reactive to CD-10 using immunohistochemical staining. Therefore, it might be helpful to distinguish metastatic AFP-GC from primary hepatocellular carcinoma. However, study of a larger number of patients is needed to confirm this observation.

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