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The Differences of c-Met Expression Between AFP-Producing Gastric Cancer and Common Gastric Cancer

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Background: Alpha-fetoprotein (AFP)–producing gastric cancer is a distinct type of gastric cancer with a high incidence of liver metastasis. c-Met is considered to play an important role in liver metastasis of gastric cancer.

Objective: The purpose of this study was to compare the expression of c-Met in AFP-producing gastric cancer and gastric cancers not producing AFP.

Methods: We evaluated 23 patients with AFP-producing gastric cancer (AFP+) and 18 patients with common gastric cancer (AFP–) were evaluated for c-Met expression using immunohistochemical analysis.

Results: The incidence of c-Met expression in 2 groups was the same ($P > 0.05$), but the AFP+ group had a higher strong positive rate of c-Met expression than the AFP– group ($P < 0.01$).

Conclusion: The higher expression of c-Met might be the reason for the high incidence of liver metastasis in AFP-producing gastric cancer.

Key words: AFP-producing gastric cancer – c-Met – Liver metastasis of gastric cancer

Alpha-fetoprotein–producing gastric cancer (AFP-GC) is a distinct histologic type of gastric adenocarcinoma, mostly characterized by positive immunoreactivity to α-fetoprotein (AFP) and hepatoid differentiation.[1–5] AFP-GC is associated with high rates of lymphatic metastasis, venous invasion of the gastric wall, and liver metastasis. The survival rate for patients with AFP-GC is significantly poorer than for patients with other types of gastric cancer (GC).[1] However, the molec-

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ular mechanisms causing the poor prognosis of AFP-GC has not been revealed, yet.

Hepatocyte growth factor (HGF) and its receptor (c-Met) are involved in the progression of cancer cells to malignant invasive phenotypes and the development of distant metastasis.[6] According to a study, AFP-GC is associated with higher expression of c-Met than AFP-negative GC.[7] We designed this study to compare the expression of c-Met in AFP-GC and common GC, and to explore whether the difference, if any, is associated with pathologic stages.

Materials and Methods

Population study

From 2010 to 2013, there were 248 patients with GC admitted for surgery at the General Surgery Department of the Huashan Hospital, Fudan University, China. A total of 28 patients had elevated preoperative serum AFP levels (AFP >10 ng/mL); in 23 of them, AFP was detected in gastric cancer cells by immunohistochemical staining: these patients composed the AFP-GC group (AFP+). For comparison, 23 patients with GC and normal levels of serum AFP were selected at random and samples of the correspondent GC surgically removed were tested for AFP immunoreactivity. Of that number, 18 were confirmed AFP-negative and were enrolled in the AFP-negative GC group (AFP–). Gastric cancer specimens from both groups were subjected to c-Met staining. The antibodies for AFP and Met were purchased from DAKO (Carpinteria, California); the anti-AFP antibody was a rabbit monoclonal antibody to human AFP (D12C1, Rabbit mAb) and the anti-Met antibody was a rabbit monoclonal antibody to human Met (D1C2, XP, Rabbit mAb). All patients were staged according to the tumor, node, metastasis (TNM) staging of GC (American Joint Committee on Cancer (AJCC), 7th edition, 2010).

Immunohistochemistry

Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase (SP) method. The sections were deparaffinized in xylene and rehydrated through graded alcohols, followed by: 3 washes in phosphate buffered saline (PBS) for 2 minutes; incubation with 3% hydrogen peroxide for 10 minutes; 3 washed with PBS (three times, for 2 minutes). Nonspecific antibody-binding sites were blocked incubating the sections with blocking serum for 15 minutes. Next the sections were incubated overnight at 20 to 25°C with a 1:50 dilution of the primary antibodies, washed in PBS, incubated at 20 to 25°C with biotinylated secondary antibodies, and washed in PBS, incubated with horseradish peroxidase (HRP)–conjugated streptavidin for 15 minutes, washed in PBS, and incubated with diaminobenzidine (DAB) for 5 to 10 minutes to visualize the antigens. The sections were counterstained with hematoxylin, dehydrated, and mounted. Negative control sections were treated with PBS instead of the primary antibodies. AFP- and Met-positive liver cancer sections were used as positive control.

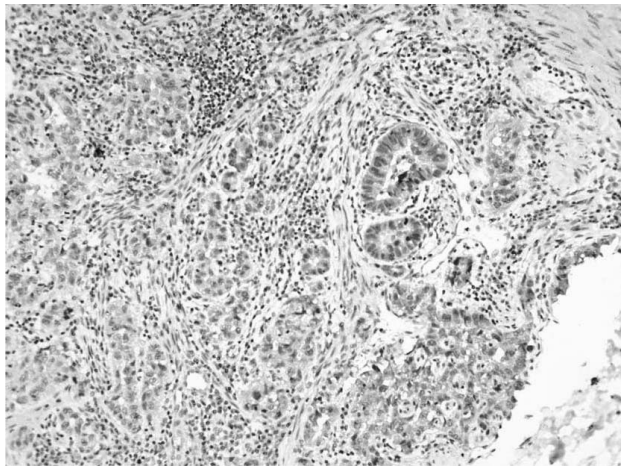

Fig. 1 AFP positive staining (×40).

Analysis

All sections were classified according to the grade of immunostaining in the carcinoma cells, as follows: negative (–), no carcinoma cells were stained; moderate positive (+), fewer than two-thirds of the cells were stained; strong positive (++), more than two-thirds of the cells were stained. Significance was calculated according to the χ^2 test.

Results

The cancer sections from patients of the AFP+ and AFP– groups were stained for AFP and c-Met; AFP and Met were mostly observed in the cytoplasm and on the cell membrane, respectively, as indicated in Figs. 1 and 2.

The data obtained are summarized in Table 1. The incidence of c-Met-positive expression in the AFP (+) group was 82.5% (19/23), and the strong positive rate was 52.2% (12/23). The incidence of c-Met-positive expression in the AFP– group was 77.7%

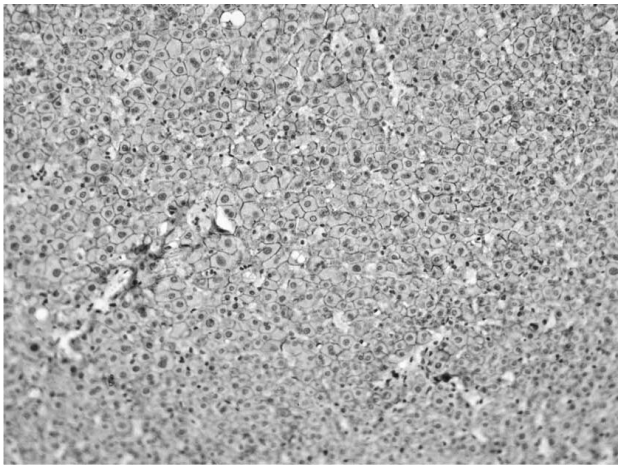

Fig. 2 Met positive staining (×40).

(14/18), and the strong positive rate was 11.1% (2/18). The rate of strong positives was higher in the AFP+ ($P < 0.01$); however, the incidence in c-Met expression (positive versus negative samples) was not significantly different among the two groups ($P > 0.05$).

The staging of the cancer samples was also taken into consideration. In samples from patients with stage II GC, the incidences of c-Met-positive expression in the AFP+ and AFP– groups were 100% (6/6) and 83.3% (5/6), respectively; and, the rates of strong positive samples were 66.6% (4/6) and 16.6% (1/6), respectively. In samples from patients with stage III GC, the incidences of c-Met-positive expression in the AFP+ and AFP– groups were 73.3% (11/15) and 80.0% (8/10), respectively, while the strong positive rates were 46.6% (7/15) and 10.0% (1/10), respectively. Therefore, the expression of c-Met in AFP-GC was not related to the pathologic stage of the tumor.

Discussion

AFP-GC is a distinct type of GC, in which AFP can be tested in serum of the patients and/or in their cancer cells. Hepatoid differentiation can be observed in most of the AFP-GC. AFP-GC was first reported by Bourreille *et al*[1] in 1970. In 1985, Ishikura *et al*[8] described for the first time a case of AFP-GC with hepatoid differentiation (named at the time, "hepatoid gastric cancer").[8] Hepatoid GC and AFP-GC exhibit a high frequency of vascular invasion, lymph node metastasis, liver metastasis and a poor outcome. Specifically, liver metastasis is the first cause of death in patients with hepatoid GC and AFP-GC. A study has found that the liver metastasis rate of hepatoid GC is 75.6%, and the 1-, 3-, and 5-year survival rates of patients with hepatoid GC are 30%, 13%, 9%, respectively. The liver metastasis rate of AFP-GC without hepatoid differentiation is 49.2%, and the 1-, 3-, and 5-year survival rates of patients are 64%, 47%, and 41%, respectively. The liver metastasis rate of common GC is 11.5%, and the 1-, 3-, and 5-year survival rates of patients are 95%, 57%, and 38%, respectively.[9] Therefore, AFP-GC, even without hepatoid differentiation, is associated with a higher incidence of liver metastasis and a lower survival rate than common GC.

The high frequency of liver metastases in AFP-GC is thought to be linked to the overexpression of c-Met, which is the receptor of HGF and is encoded by the *c-Met* proto-oncogene. Gardner *et al*[10] have shown that the expression of c-Met can enhance the liver metastatic ability of melanoma. Krause *et al*[11] proved that the c-Met pathway is related to liver metastasis of colon cancer, and the higher frequency of c-Met expression leads to a higher recurrence rate after resection of liver metastatic cancer. The positive rate of c-Met in common gastric cancers ranges from 18% to 71.1%. The gene amplification of *c-Met* is correlated with cancer stage, and *c-Met* is found to be overexpressed in gastric cancers with deeper invasion and distant metastasis.[12] Lee *et* al[13] indicated that the level of c-Met expression in liver metastatic GC is much higher than in the primary focus. Another study has found a higher c-Met expression in AFP-GC than in GC which do not

Table 1 Comparison of c-Met expression in gastric carcinomas of the AFP+ and AFP– groups

Stage	AFP+ N = 23			AFP– N = 18			*P* value
	−	+	++	−	+	++	
I	0	0	0	1	1	0	>0.995
II	0	2	4	1	4	1	0.1
III	4	4	7	2	7	1	0.005
IV	0	1	1	0	0	0	>0.995
	4	7	12	4	12	2	0.01

All patients were staged according to TNM staging of gastric cancer (AJCC, 7th edition, 2010). Significance according to the χ^2 test.

express AFP.[7] Nevertheless, the mechanism linking AFP and c-Met expression remains uncertain. Our research indicates that there is no significant difference between the incidence of c-Met expression in AFP-positive and -negative GC. Importantly, though, the strong positive rate of c-Met expression is much higher in the AFP-positive GC than in the negative ones, reinforcing previous studies on this topic. Our results might indicate that AFP regulates the expression of c-Met: this effect might be direct, and would require the translocation of AFP to the nucleus, or indirect, through the action of AFP on one or more transcription factors that directly regulate c-Met. These assumptions need further exploration. The in-depth understanding of the mechanism through which AFP regulates c-Met, if any, may be essential for the future development of therapeutics to treat AFP-GC. We are aware this paper does not provide the detailed mechanism through which AFP affects c-Met expression: this is one of the limitations of this study. At this stage, we can make some hypotheses. Recent reports have demonstrated that AFP may function as a regulator of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in human hepatocellular carcinoma cells (HCC)[14]: specifically, the authors found that transfection of AFP-cDNA into hepatoma HLE cells (originally AFP negative) led to a significant activation of the Akt signaling. Another study indicates that the transcription factors specificity protein 1 (Sp1) and mothers against decapentaplegic 3 (Smad3) participate in mediating c-Met expression in renal epithelial cells.[15] Increased expression of c-Met is associated with the up-regulation of hypoxia inducible factor-1 (HIF-1) in tumor cells, in papillary carcinoma of the thyroid.[16] The regulation of Sp1 and HIF-1 expression and Smad3 phosphorylation by the Akt signaling pathway has been reported.[17–19] Based on these studies, we suggest that AFP might regulate the expression of c-Met through the activation of the Akt pathway. We plan to investigate this hypothesis in the future.

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