INTRODUCTION
Gastric carcinoma is one of the most common malignancies and has the highest mortality in China [1,2]. The most important reason causing patients’ death is the metastasis to distant important organs. It was reported that 64.2% of gastric carcinomas developed distant organ metastases, among which the rate of liver metastasis was 31.8% of all patients, and ovary metastasis rate was 43.6% of the female patients[3]. As liver is the biggest parenchymatous organ in the body, the micrometastasis of it is very difficult to be diagnosed early. The same is true in ovary metastasis because ovaries lie in the bottom of abdominal cavity. There has been no good indicator so far to objectively predict the risk of liver and ovary metastases, making the diagnosis for micrometastases of these two organs very difficult. The present study put
forward a new classification of gastric carcinoma based on the cell-function differentiation features, in an attempt to reveal the relationship between the characteristics of cell-function differentiation and the local invasion and distant organ metastases of gastric carcinomas, and to clarify the causes and molecular mechanism of metastasis to the liver or ovary.

**MATERIALS AND METHODS**

**Subjects**

Three hundred and sixty-one cases of surgically resected gastric carcinomas (from Cancer Institute, China Medical University) were involved in the study, consisting of 258 men and 103 women, including 11 without metastasis, 224 with lymph node metastases, 12 with liver metastases, and 14 with ovary metastases. At least 2 blocks were cut from each primary tumor and 1 block from each metastatic tumor of any organ involved respectively.

**Principle and standards of cell-functional differentiation classification**

Glandular epithelial cells of gastrointestinal tract can be divided into three main groups: ① absorptive cells of the small intestine, ② mucinous cells including goblet and columnar mucous cells; and ③ cells with specific secretory function, such as parietal, chief, panceath, and APUD system cells. According to the principle that cancer cells maintain more or less the functional differentiation potential of their ancestors, a group of comprehensive indicators that could objectively reflect absorptive, mucin-secreting, and specific function differentiation were selected: indicators of absorptive function differentiation were brush border enzymes, such as alkaline phosphatase (AKP) and L-aminopeptidase (LAP); indicators of mucin secreting function differentiation were sulfomucin (HID+), sialomucin (ABpH2.5+) and neutral mucin (PAS+), and indicators of specific function differentiation were antibodies to hormones produced by APUD cells, cellular keratin and others. According to the expressions of these indicators, the gastric carcinomas were divided into five cell-function differentiation types: ① gastric carcinomas, in which more than two thirds of cancer cells expressed AKP and LAP but did not secrete mucin or hormone, were defined as absorptive function differentiation type (AFDT); ② those in which more than two thirds of the cancer cells secreted only mucin but not AKP or LAP, were defined as mucin secreting function differentiation type (MSFDT); ③ those in which more than two thirds of the cancer cells not only expressed AKP and LAP but also produced mucin, were defined as absorptive and mucin-producing function differentiation type (AMPFDT); ④ those which neither expressed AKP and LAP nor produced mucin, were defined as non-function differentiation type (NFDT); and ⑤ specific function differentiation type (SFDT) was defined as that more than two thirds of cancer cells possessed some special functions, such as the tumors from APUD system and squamous cell cancer (Table 1 and Figures 1-6).

**Detection of cell-functional differentiations**

Enzyme histochemistry[4]: alkaline phosphatase (AKP) was detected by Gomori’s method, and L-aminopeptidase (LAP) by Nachal’s method. Mucin histochemistry was done by the HID/ABpH2.5 / PAS method[5]; endocrine histochemistry was performed by ABC immunostaining with antibodies to hormones produced by APUD cells. The results were evaluated using the standards given in references[6,7].

**Detection of tumor associated markers**[8-13]

The sections from all cases were stained by ABC histochemistry using antibodies to ER and CD44v6 respectively. The ploidy of DNA patterns of the cases with liver or ovary metastasis was examined by flow cytometry (FCM). p53 and laminin expressions in the gastric carcinomas with liver or ovary metastasis were also detected immunohistochemically.

**Statistics**

χ² test was employed in this study to analyze the results of histochemistry and survival data.

**RESULTS**

The carcinomas were divided into five types: 82 cases classified as AFDT (22.7%); 54 MSFDT (15%); 180 AMPFDT (49.9%); 32 NFDT (8.9%) and 13 SFDT (3.6%). Tables 2 and 3 illustrate the relationship between the cell function classification and the patients’ age and sex. The relationship between the cell-function classification and histological types, growth patterns, clinicopathological stages, ER expression, CD44v6 expression, ability forming laminin-positive basement membrane structure, lymph node metastasis, liver and ovary metastases, and postoperative 5-year survival rates are shown in Tables 4-13 and Figures 1-12.
Figure 1  Primary stomach cancer of AFDT with liver metastasis. AKP was moderately positive and distributed along the free edge of cancerous papillary structure. Frozen section, ×20
Figure 2  The same case in Figure 1, Tp53 protein was expressed in most of primary cancer cells. Immunostain, ×16
Figure 3  The same case of Figure 1. CD44v6 was expressed in most of primary cancer cells. Immunostain, ×20
Figure 4  The same case of Figure 1. There was obvious basement membrane like structure with Laminin positive in the primary tumor. Immunostain, ×40
Figure 5  The same case of Figure 1. There was also obvious basement membrane like structure with laminin positive in the liver metastatic tumor. Immunostain, ×20
Figure 6  The same case of Figure 1. CD44v6 was also expressed in the liver metastatic cancer cells. Immunostain, ×20
Figure 7  Primary gastric carcinoma of AMPFDT with ovary metastasis. LAP was moderately positive and distributed in the membrane and cytoplasm of cancer cells. Frozen section, ×40
Figure 8  The same case of Figure 7. Sialomucin and neutral mucin were positive in the primary cancer cells. Mucin histochemistry, ×20
Figure 9  The same case of Figure 7. Most of primary cancer cells expressed ER, which was distributed in the nuclei and cytoplasm. Immunostain, ×20
Figure 10  The same case of Figure 7. LAP was moderately positive in the ovary metastatic cancer cells. Frozen section, ×20
Figure 11  The same case of Figure 7. Sulfomucin was positive in the ovary metastatic cancer cells. Mucin histochemistry, ×40
Figure 12  The same case of Figure 7. Most of the ovary metastatic cancer cells expressed ER. Immunostain, ×40
### Table 2: Relationship between cell function classification and age of patients with gastric carcinoma

| Type         | n  | Age                   | AFDT (%) | MSFDT (%) | AMPFDT (%) | SFDT (%) | NFDT (%) |
|--------------|----|-----------------------|----------|-----------|------------|----------|----------|
|              |    | <45 yrs (%)           | 29 (78.4)† | 11 (29.4) | 33 (81.8)  | 11 (28.1)|          |
|              |    | 45-65 yrs (%)         | 2 (5.4)   | 2 (5.1)   | 0          | 0        |          |
|              |    | >65 yrs (%)           | 6 (16.2)  | 0         | 0          | 0        |          |

*P<0.05 (χ² = 4.5575), †vs AFDT of the patients below 45 years old.

### Table 3: Relationship between cell-function classification and sex of patients with gastric carcinoma

| Type         | n (%) | Female (%) | Male (%) |
|--------------|-------|------------|----------|
|              |       | n (%)      | n (%)    |
| AFDT         | 82    | 16 (19.5)  | 66 (80.5)|
| MSFDT        | 54    | 15 (27.8)  | 39 (72.2)|
| AMPFDT       | 180   | 62 (34.4)  | 118 (65.6)|
| SFDT         | 13    | 3 (23.1)   | 10 (76.9)|
| NFDT         | 32    | 7 (21.9)   | 25 (78.1)|
| Total        | 361   | 103 (28.5) | 258 (71.5)|

*P<0.05 (χ² = 6.0079), †vs AFDT of female patients.

### Table 4: Relationship between cell-function and WHO's histological classifications of gastric carcinoma

| Types                  | n (%) | AFDT (%) | MSFDT (%) | AMPFDT (%) | SFDT (%) | NFDT (%) | χ²       |
|------------------------|-------|----------|-----------|------------|----------|----------|----------|
| Papillary adenocarcinoma| 37    | 29 (78.4)† | 2 (5.4)   | 6 (16.2)   | 0        | 0        |<0.01(Exact test: χ² = 10.2339), †vs AFDT of female patients. |
| Tubular adenocarcinoma  | 20    | 17 (85.0) | 0         | 2 (10.0)   | 0        | 1 (5.0)  |<0.01(Exact test: χ² = 4.5575), †vs AMDFDT. |
| Well-diff. ade.        | 59    | 20 (33.9) | 5 (8.5)   | 27 (45.8)  | 1 (1.7)  | 6 (10.2) |<0.01(Exact test: χ² = 9.8900), †vs AMDFDT. |
| Poor. diff. ade.       | 126   | 15 (11.9) | 14 (11.1)| 79 (62.7)  | 2 (1.6)  | 16 (12.7)|<0.01(Exact test: χ² = 3.9245), †vs AMDFDT. |
| Undiff. car.           | 30    | 1 (3.3)   | 7 (23.3)  | 13 (43.3)  | 0        | 9 (30.0) |<0.01(Exact test: χ² = 12.2793), †vs AFDT, AMPFDT and NFDT. |
| SRC                    | 41    | 0         | 5 (12.2)  | 6 (17.8)   | 0        | 0        |<0.01(Exact test: χ² = 15.11320), †vs AMPFDT. |
| Macous ade.            | 38    | 0         | 21 (55.3)| 17 (44.7)  | 0        | 0        |<0.01(Exact test: χ² = 28.6780), †vs others of the papillary adenocarcinomas; †vs others of signetring cell carcinomas. ade: adenocarcinoma; diff.: differentiated; mode.: moderately; undiff.: undifferentiated; SRC: signet ring cell carcinoma |
| Carcinoid              | 9     | 0         | 0         | 0          | 9 (100.0)| 0        |<0.01(Exact test: χ² = 15.11320), †vs AMPFDT. |
| Squamous car.          | 1     | 0         | 0         | 1 (100.0)  | 0        | 0        |<0.01(Exact test: χ² = 15.11320), †vs AMPFDT. |
| Total                  | 361   | 82 (22.7) | 54 (15.0)| 180 (49.9) | 13 (3.6) | 32 (8.9) |<0.01(Exact test: χ² = 15.11320), †vs AMPFDT. |

### Table 5: Relationship between cell-function and Lauren’s classifications of gastric carcinoma

| Types                  | n (%) | AFDT (%) | MSFDT (%) | AMPFDT (%) | SFDT (%) | NFDT (%) | χ²       |
|------------------------|-------|----------|-----------|------------|----------|----------|----------|
| Intestinal             | 124   | 69 (54.4) | 11 (29.4)| 33 (81.8)  | 11 (28.1)|          |<0.05 (Exact test: χ² = 3.9245), †vs AFDT of female patients. |
| Diffuse                | 237   | 13 (5.5) | 43 (18.1)| 147 (61.7)| 2 (15.4) | 32 (13.5)|<0.05 (Exact test: χ² = 3.9245), †vs AMDFDT. |
| Total                  | 361   | 82 (22.7)| 54 (15.0)| 180 (49.9)| 13 (3.6) | 32 (8.9) |<0.05 (Exact test: χ² = 3.9245), †vs AMDFDT. |

### Table 6: Relationship between cell-function classification and histological growth patterns of gastric carcinomas

| Patterns               | n (%) | AFDT (%) | MSFDT (%) | AMPFDT (%) | SFDT (%) | NFDT (%) |
|------------------------|-------|----------|-----------|------------|----------|----------|
| Mass                   | 19    | 12 (63.2)| 7 (36.8)  | 0          | 0        | 7 (36.8) |
| Nest                   | 84    | 37 (44.3)| 47 (55.7)| 0          | 7 (8.3)  | 0        |
| Diffuse                | 208   | 13 (6.3)| 195 (92.8)| 0          | 0        | 3 (1.5)  |
| Total                  | 311   | 62 (19.9)| 249 (79.9)| 0          | 0        | 10 (3.3) |

### Table 7: Relationship between cell-function classification and clinicopathological stages of gastric carcinomas

| Stages                 | n (%) | AFDT (%) | MSFDT (%) | AMPFDT (%) | SFDT (%) | NFDT (%) |
|------------------------|-------|----------|-----------|------------|----------|----------|
| EGC                    | 50    | 20 (40.0)| 30 (60.0)| 0          | 0        | 7 (14.0) |
| AGC                    | 42    | 8 (19.0) | 34 (81.0)| 0          | 0        | 0        |
| Through serosa         | 269   | 54 (20.2)| 215 (79.8)| 127 (47.3)| 122 (45.8)| 10 (3.7) |
| Total                  | 361   | 82 (22.7)| 249 (79.9)| 0          | 0        | 10 (3.3) |

*P<0.01 (Exact test; χ² = 12.7293), †vs MSFDT, AMPFDT and NFDT. |<0.01 (Exact test: χ² = 10.1203, 8.9078 and 6.7487 respectively), †vs AFDT, AMPFDT and NFDT. |

### Table 8: Relationship between cell-function classification and ER expression of gastric carcinomas

| ER expression | AFDT (%) | MSFDT (%) | AMPFDT (%) | SFDT (%) | NFDT (%) | Total |
|---------------|----------|-----------|------------|----------|----------|-------|
| Positive/examined | 7/82    | 12/54     | 129/180    | 0/13     | 7/32     | 155/361|
| Positive rate (%) | 8.5    | 22.2      | 71.7       | 21.9     | 42.9     |       |

*P<0.01 (χ² = 4.56418), †vs others. |<0.01 (Exact test: χ² = 3.9245), †vs AMPFDT. |

### Table 9: Relationship between cell-function classification and CD44v6 expression in gastric carcinoma tissues

| CD44v6     | n (%)  | AFDT (%) | MSFDT (%) | AMPFDT (%) | SFDT (%) | NFDT (%) |
|------------|--------|----------|-----------|------------|----------|----------|
| - (%)      | 241    | 44       | 32        | 132        | 9        | 24       |
| + (%)      | 120    | 38 (68.3)| 22 (31.7)| 48 (26.7)  | 4 (30.8) | 8 (25.0) |
| Total      | 361    | 82       | 54        | 180        | 13       | 32       |

*P<0.01 (χ² = 9.8900), †vs AMPFDT; †P<0.05 (χ² = 3.9245), †vs AMPFDT.
Table 10  Relationship between cell-function classification and metastases of gastric carcinomas

| Types          | n(%) | AFDT(%) | MSFDT(%) | AMPFDT(%) | SFDT(%) | NFDT(%) |
|----------------|------|---------|----------|-----------|---------|---------|
| No mets        | 111  | 28(34.1)| 13(24.1) | 51(28.3)  | 64(62)  | 13(40.6)|
| LN mets        | 224  | 45(34.9)| 36(70.4) | 116(64.4)| 64(62)  | 19(59.4)|
| Liver mets     | 12(3.3)| 9(11.0) | 1(1.9)   | 1(0.6)   | 1(7.7)  | 0       |
| Ovary mets     | 143  | 3(2.1)  | 2(3.7)   | 12(6.7)  | 0       | 0       |
| Total          | 361  | 82(22.7)| 54(15.0) | 180(49.9)| 13(3.6) | 32(8.9) |

Table 11  Relationship among cell-function classification, metastasis and sex of patients with gastric carcinoma

| Types          | Liver metastasis | Ovary metastasis | Male (%) | Female (%) | Male (%) | Female (%)|
|----------------|------------------|------------------|----------|------------|----------|------------|
| AFDT           | 9/ 66 (13.6)     | 0/ 16            | 0/ 16    | 0/ 16      | 0/ 16    | 0/ 16      |
| MSFDT          | 0/ 39            | 1/ 15 (6.7)      | 2/ 15 (13.3)|          |
| AMPFDT         | 1/ 118 (0.8)     | 0/ 62            | 12/ 62 (19.4) |      |
| SFDT           | 1/ 10 (10.0)     | 0/ 3             | 0/ 3     |            |          |
| NFDT           | 0/ 25            | 0/ 7             | 0/ 7     |            |          |
| Total          | 11/258 (4.3)     | 1/103 (1.0)      | 14/103 (13.6)|         |

Table 12  Comparison of cell-function differentiation classification and molecular biological features of gastric carcinoma with liver or ovary metastases

| Comparison          | GC with liver mets | GC with ovary mets |
|---------------------|--------------------|--------------------|
| Positive/12 cases(%)| Positive/14 cases(%)|
|                      |                    |                    |
| AFDT                | 9 (75.0)^d         | 0                  |
| MSFDT               | 1 (8.3)            | 2 (14.3)           |
| AMPFDT              | 1 (8.3)            | 12 (85.7)^d        |
| SFDT                | 1 (8.3)            | 0                  |
| NFDT                | 0                  | 0                  |
| Comparison of molecular biological features |
| Estrogen-dependent ER(+) | 0 (12.5) (5.7)  | 0 |
| LN(+) BM structure (+) | 12 (100.0)         | 0 |
| Mutant p53 protein (+) | 10 (83.3)         | 3 (21.4) |
| DNA ploidy-Diploid | 3 (25.0)           | 10 (71.4)          |
| Tetraploid        | 0                  | 2 (14.3)           |
| Aneuploid         | 9 (75.0)^e         | 2 (14.3)           |
| CD44v6 expression (+) | 10 (83.3)^f        | 0 |

*LN: laminin; BM: basement membrane; Mets: metastasis

Discussion

In the light of differentiation degree, malignant tumors from epithelial cells of gastrointestinal tract may retain more or less the functional differentiation potential of their ancestors, which has effects on their biological behavior. Previous studies revealed the relationship between the morphological differentiation and the pathobiological behavior of gastric cancer[14-22], but so far there have been very few studies on the phenotypes of functional differentiation of gastric cancer cells[23-25].

We have studied simple morphological and the functional differentiation indicators of gastric carcinoma to infer its biological behavior during the last fifteen years, and found that there was a correlation between the functional differentiation of gastric carcinoma cells and their biological behavior[20-32]. In this study, we investigated more cases and compared the degree of functional differentiation and morphological differentiation features of gastric carcinomas, and found no definite correlation between them. Although most well-differentiated papillary and tubular adenocarcinomas possessed absorptive functions, all signet ring cell cancers were classified as AMPFDT and carcinoid tumors as SFDT, the direction of functional differentiation of most moderately and poorly differentiated adenocarcinomas were undetermined. Most so-called undifferentiated cancers determined histologically displayed some functional differentiation, and were mostly MSFDT or AMPFDT gastric carcinomas; truly undifferentiated cancers made up only 8.3% in our study. Interestingly, 55.3% of mucinous adenocarcinomas classified by histology had mucin-secreting function (MSFDT) but the other 44.7% had absorptive and mucin-producing function (AMPFDT). Furthermore, Tumors of MSFDT were significantly different from those of AMPFDT in patient age, the serosa involvement, estrogen dependence and the CD44v6 expression. The results indicated that the cancer cells of mucinous adenocarcinoma possessed obvious heterogenicity in cell function differentiation and biological behavior, which should be paid special attention to predict the invasive and metastatic features.

The results also indicated that stomach cancers with different functional characteristics often possessed different pathobiological behavior. For example, the MSFDT tumors, constantly growing invasively, were mostly accompanied by the...
serosa involvement, and were not obviously dependent on estrogen. NFDT independent of estrogen invades weakly, of which about 60% metastasized to lymph node. Among different functionally differentiated gastric carcinomas, AFDT and AMPFDT had specific clinicopathologically biological features as follows. Gastric carcinomas of ATDT were most common in the middle aged and senile (92.7%), only 7.3% were found in the young; with men (80.5%) surpassing women (19.5%), and intestinal type gastric carcinoma (84.1%) exceeding diffuse ones; and exhibited mainly “mass” or “nest” styles of growth. The invasion of this type of tumor was often beneath the serosa, which resisted local infiltration as a barrier. AFDT, whose growth was not very much dependent on estrogen, expressed metastasis-associated cell adhesion molecule CD44v6 at a higher rate than AMPFDT, and its postoperative 5-year survival rate was 58.5% revealed its best prognosis. Liver metastasis was frequently observed in patients with AFDT tumor whose biological behavior was as follows: (1) mutant p53 protein positively expressed at a rate of 83.3%; (2) among 75.0% of cases, DNA showed aneuploid; (3) 83.3% of this type presented positive expression of CD44v6; and (4) the thread-like basement membranous structure containing LN was often formed. Special attention should be paid clinically to the characteristics of AFDT stomach cancer mentioned above. AMPFDT gastric carcinoma often occurred in the young aged below 45 years (17.2%) with more female patients (34.3%) than in other types, and histologically, 81.7% were diffusing invasive type and 71.7% were estrogen-dependent type, and there were more ovary metastases (19.4%) than in the other types. The 5-year survival rate of 24.7% embodied the worst prognosis. AMPFDT gastric carcinoma that grew in women with ovary metastasis had the following biological behavior: 85.7% of cases had high ER expression; mutant P53 protein displayed low expression at a rate of 21.4%; no expression of metastasis-associated adhesion molecule CD44v6; and diploid DNA occurred in 71.4% of tumors. Although the molecular mechanism of liver and ovary metastases was not clear, we should pay much more attention to the cell-function differentiation and biological behavior of gastric carcinomas mentioned above in order to help with the early diagnosis and treatment of the micrometastasis in the liver and ovary.

Much attention has been paid to the mechanism of cancer invasion and metastasis[33-42], especially to the mechanism of the liver and ovary metastases from gastric carcinomas. Several hypotheses have been proposed but cannot explain the specific organic affinity satisfactorily[43-50]. Our study found that the gastric carcinomas with liver metastasis possessed different cellular biological behaviors from those with ovary metastasis (Tables 10-12). It is thus possible to consider a new explanation of the mechanisms of the organic affinity of liver and ovary metastases: the fact that the gastric carcinoma with liver metastasis displayed absorptive function differentiation and expressed LN positive basement membrane-like structure suggested that these cancer cells may have increased the number of exposed laminin receptors. When the cells with exposed laminin receptors (LR) encounter the basement membrane of capillaries, the cancer cells can combine with the basement membrane by the specific affinity between LN and LR, making it possible for the cancer cells to invade the blood vessels. Therefore, we can hypothesize that the presence of exposed LR on the surface of cancer cells is an important precondition for the metastasis through the blood vessels. Most gastric cancers with ovary metastasis displayed disordered functional differentiation in the directions of absorption and mucin-production, and all these cancers expressed estrogen receptors (ER). This finding suggests that the specific organic affinity between the ovary and the specific type of gastric carcinomas may be related to an estrogen-estrogen receptor (E-ER) link.

In addition, our study found that gastric carcinomas exhibiting “mass” or “nest” growth pattern, all expressed thread-like structure containing LN. The reason for this phenomenon is probably that LR is polarized on the basal side of the cancer cells which grow in masses or nests, so these cancer cells can arrange in a row along basement membranes containing LN. On the contrary, the laminin receptors were absent or disordered on those cancer cells which grow diffusely, so that they cannot form a regular structure[51-53].

In summary, the findings of the study indicate that there was some correlation between the cell-function differentiation and invasion and metastasis of gastric carcinomas. A further study is necessary to make clear the genotypes of different functional phenotypes of gastric carcinomas and find out new molecular biological markers for the early diagnosis and treatment of the metastasis. Additionally, the remarkable difference between liver and ovary metastasis of gastric carcinoma in cell-function differentiation, P53 gene mutation, ploidy of DNA, CD44v6 expression and laminin expression indicates their different pathways of gene regulation.

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