Expression pattern of leptin and leptin receptor (OB-R) in human gastric cancer

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Abstract
AIM: To examine the expression of leptin and its receptor, OB-R, in normal gastric mucosa and neoplasia.

METHODS: By immunohistochemical staining using specific antibodies, we evaluated the expression of leptin and OB-R in 207 gastric carcinomas (100 early and 107 advanced carcinomas) and analyzed their relationship with clinicopathological features.

RESULTS: Both normal gastric epithelium and carcinoma cells expressed a significant level of leptin. In cases with OB-R staining, carcinoma cells showed OB-R-positive expression, but the intensity was weaker than that in normal mucosa. The expression of OB-R showed a significant correlation with the level of leptin expression. The expression levels of both leptin and OB-R tended to increase as the depth of tumor invasion or TMN stage increased (P < 0.01). Lymph node metastasis was detected in 49.5% (47/95) of leptin-strong cases and in 50.5% (48/95) of OB-R-positive cases, and the rate was 33% (37/126) in leptin-weak cases and 17% (19/112) in OB-R-negative cases. Both venous and lymphatic invasion also tended to be observed frequently in positive tumors as compared with negative tumors. Interestingly, in the 96 leptin- or OB-R-positive tumors, hematogenous metastasis was detected preoperatively in 3 (3.1%) patients. In contrast, none of the carcinomas that lacked expression of leptin and OB-R showed hematogenous metastasis.

CONCLUSION: Overexpression of leptin and expression of OB-R may play a positive role in the process of progression in gastric cancer. Functional upregulation of leptin/OB-R may have a positive role in the development and initial phase of progression in gastric cancer.

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the brain, placenta, pancreas, adrenal gland, hematopoietic cells, liver, lung and heart\cite{21,24-26}. In addition, OB-R has been identified in malignant cells, including lung and gastric carcinoma and leukemic cells\cite{22,29-32}.

In this study, therefore, we used antibodies to leptin and OB-R, and immunohistochemically characterized the expression pattern of these two proteins in gastric carcinoma and evaluated the possible role of leptin in the tumorigenesis and spread of gastric cancer.

**MATERIALS AND METHODS**

Two hundred and seven carcinomas, which were surgically resected in the Department of Surgery, The University of Tokyo, from 1991 to 2002, were included in this study. In all cases, serial-step sections 3-mm wide were cut, fixed in 10% formalin solution, and then embedded in paraffin. All the resected primary tumors and regional lymph nodes were histologically examined by hematoxylin-eosin staining according to the Japanese Classification of Gastric Carcinoma\cite{33}. Tumors were histologically classified into two types based on the predominant features: differentiated type (well and moderately differentiated adenocarcinoma) and undifferentiated type (poorly differentiated adenocarcinoma and signet ring cell carcinoma). In addition, we examined several discrete histological parameters, including lymphatic invasion, venous invasion and lymph node metastasis.

**Immunohistochemical study of leptin and OB-R**

We investigated the expression of OB-R and leptin with immunohistochemical staining using affinity purified rabbit polyclonal antibodies against leptin (Santa Cruz, Biotech, CA, USA) and goat polyclonal antibodies against OB-R (M-18, Santa Cruz Biotech)\cite{30,32}, respectively. Sections (3-μm thick) were deparaffinized in xylene, hydrated through a graded series of ethanol, and heated in a microwave oven for two 7-min cycles (500 W). After being rinsed in phosphate buffered saline (PBS), endogenous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxide in 100% methanol for 30 min. After 3 washes in PBS, nonspecific reaction was blocked by incubation with PBS containing 5% skimmed milk for 30 min at room temperature, and then the sections were incubated with normal rabbit or goat serum for 30 min. The sections were incubated overnight at 4°C in humid chambers with the primary antibody to leptin at 1/70 dilution or the primary antibody to OB-R at 1/100. After three washes with PBS, the sections were incubated with biotinylated rabbit anti-goat or anti-rabbit immunoglobulin for 30 min. After washing again with PBS, the slides were treated with peroxidase-conjugated streptavidin for 30 min, and developed by immersion in 0.01% H2O2 and 0.05% diaminobenzidine tetrahydrochloride for 3 min. Light counterstaining with Mayer’s hematoxylin was performed.

**Statistical analysis**

All statistical calculations were carried out using Stat View-J 5.0 statistical software (SAS Institute, USA). Student’s t-test and Wilcoxon’s test were used to analyze data. Differences with a P value of less than 0.05 were considered to be statistically significant.

**RESULTS**

**Immunohistochemical detection of leptin and OB-R in normal mucosa and carcinoma**

In all cases, the lower part of the fundic glands in the normal part of the mucosa expressed a significant level of leptin, suggesting that leptin is mainly produced in chief and parietal cells (Figure 1A). Leptin could be detected in the cytoplasm as well as in the cell membrane, but not in the nucleus. However, the surface epithelium of normal gastric mucosa totally lacked expression of leptin. This staining pattern was similar to that described in the gastric epithelium in a previous report\cite{22}.

Gastric carcinoma cells mostly showed positive immunoreactivity, although the staining intensity varied among the samples. According to the staining pattern, tumors were subdivided into two groups. When investigators agreed that the staining intensity of carcinoma cells was significantly weaker than that of chief and parietal cells in corresponding normal mucosa, those tumors were categorized as having weak expression (Figure 1B). In contrast, when carcinoma cells stained to a similar degree or more strongly than normal gastric mucosa, those tumors were categorized as having strong expression (Figure 1C).

OB-R was also detected in normal mucosa, and the immunostaining pattern was mostly consistent with that of leptin staining (Figure 1D). In cancer tissue, however, some carcinoma cells showed significant expression while
was significantly higher than 31.3% (47/150) of OB-R-negative cases. Interestingly, in the 74 tumors with high leptin expression, hematogenous metastasis was detected preoperatively in 3 (4.1%) patients, and peritoneal dissemination was detected intraoperatively in 5 (6.8%) patients. However, in 133 tumors with low leptin expression, only one case showed peritoneal dissemination and none was associated with hematogenous metastasis (P < 0.05).

**DISCUSSION**

Leptin is well known to play a major role in the regulation of weight and adiposity. Recently, many studies have shown that increased body weight is associated with increased risk of cancer and cancer-related mortality, suggesting a possible role of leptin in the pathogenesis of cancer. Leptin is reported to be abundantly produced in the stomach[34,35]. In gastric carcinoma, some reports have shown that obesity is one of the main risk factors[36,42]. These findings suggest that leptin may be critically involved in the development and progression of gastric cancer. This idea encouraged us to evaluate the expression of leptin and its receptor in gastric cancer tissues.

In our series, leptin and OB-R were predominantly expressed in chief and parietal cells but not in the surface epithelium in normal parts of the gastric mucosa that were adjacent to cancer tissue, which is mostly consistent with data of previous studies[22,43,44]. However, carcinoma cells showed a variety of staining patterns for leptin or OB-R. Leptin was detected in all carcinoma cells, although the level of expression could be divided into two categories according to the staining intensity, whereas OB-R was detected in some tumors but not in others, and the level of expression of leptin showed a positive correlation with OB-R expression. This suggests the existence of a common regulatory mechanism in the expression of leptin and its receptor in the gastric epithelium.

The main finding in our study was that the expression levels of both leptin and OB-R tended to increase as the depth of tumor invasion or TMN stage increased (P < 0.01). Moreover, nodal and distant metastasis, as well as pathological lymphatic or vascular invasion, was frequently detected in leptin-strong and OB-R-positive tumors as compared with leptin-weak and OB-R-negative tumors. Shunieder et al[43] reported that leptin led to significantly increased proliferation in AGS cells, and the MAP-kinase-1 specific inhibitor U0126 blocked leptin-induced cell proliferation in a dose-dependent manner. Tessitore reported that in colorectal cancer patients, plasma leptin level in stage IV patients was significantly higher than that in stage I patients. In addition to stimulating proliferation, leptin has been shown to promote invasiveness of renal and colonic epithelial cells via PI3-kinase-, rho- and rac-dependent cascades[35]. All these findings support that leptin may have a promoting effect on cancer invasion and metastasis. Our findings were consistent with these results and suggest that leptin and OB-R might function as an autocrine growth factor during the development and progression of gastric cancer.

Another interesting finding was that the expression of

### Table 1  Relationship between expression of Ob-R and leptin

| Leptin expression | Ob-R expression | Positive (67) | Negative (140) | P |
|-------------------|-----------------|---------------|---------------|---|
| Strong (74)       | OB-R expression | 45            | 29            | < 0.001 |
| Weak (133)        |                 | 22            | 111           |     |
leptin/OB-R was correlated with the differentiation of gastric cancer. In our series, cancers of undifferentiated type tended to have weak expression of leptin as well as negative OB-R expression as compared with differentiated cancers. In each type, expression of leptin/OB-R showed a positive association with stage and metastasis (data not shown). This suggests that the different expression of leptin/OB-R was determined at the early stage of carcinogenesis. The carcinogenic pathway of differentiated type carcinoma is considered to begin with _H pylori_ infection, followed by atrophic gastritis and intestinal metaplasia, and inappropriate activation of gut specific transcription factor CDX2 has an important role in the early stage of carcinogenesis. In contrast, dysfunction

| Table 2 Expression of leptin and clinicopathologic characteristics of patients |
|---------------------------------|------------------|------------------|------------------|------------------|
|                                 | Leptin expression |                   | OB-R expression  |                   |
|                                 | High (74)         | Low (133)        | P                | Positive (67)    | Negative (140)  | P                |
| Age (yr)                        | 62.7 ± 8.9        | 61.3 ± 11.2      | 0.98             | 64.2 ± 9.9       | 61.6 ± 10.8     | 0.167            |
| Sex                             |                   |                  |                  |                  |                  |                  |
| Male                            | 64                | 91               |                  | 53               | 102              |                  |
| Female                          | 10                | 42               | 0.003            | 14               | 38               | 0.33             |
| BMI                             | 22.4 ± 3.1        | 22.7 ± 2.8       | 0.48             | 22.4 ± 3.2       | 22.7 ± 2.8      | 0.49             |
| Tumor markers                   |                   |                  |                  |                  |                  |                  |
| CEA                             | 8.6 ± 17.7        | 14.7 ± 111.3     | 0.65             | 9.9 ± 19.6       | 13.8 ± 108.3    | 0.79             |
| CA19-9                          | 89.8 ± 453.5      | 75.4 ± 347.6     | 0.81             | 138.3 ± 561.4    | 53.8 ± 270.8    | 0.16             |
| Size (cm)                       | 6.0 ± 3.2         | 5.1 ± 3.6        | 0.13             | 5.8 ± 3.2        | 5.2 ± 3.6       | 0.25             |
| Location                        |                   |                  |                  |                  |                  |                  |
| Upper part (51)                 | 23                | 28               |                  | 19               | 32               |                  |
| Middle part (94)                | 36                | 58               |                  | 29               | 65               |                  |
| Lower part (62)                 | 15                | 47               | 0.03             | 19               | 43               | 0.51             |
| Depth of tumor invasion         |                   |                  |                  |                  |                  |                  |
| T1                              | 24                | 76               |                  | 20               | 80               |                  |
| T2                              | 28                | 29               |                  | 27               | 37               |                  |
| T3                              | 18                | 28               |                  | 16               | 30               |                  |
| T4                              | 4                 | 0                | 0.001            | 4                | 0                | < 0.001          |
| Macroscopic type                |                   |                  |                  |                  |                  |                  |
| Elevated                        | 57                | 62               |                  | 48               | 71               |                  |
| Depressed/flat                  | 17                | 71               | < 0.001          | 19               | 69               | 0.005            |
| Histological type               |                   |                  |                  |                  |                  |                  |
| Differentiated                  | 45                | 43               |                  | 43               | 45               |                  |
| Undifferentiated                | 29                | 90               | < 0.001          | 24               | 95               | < 0.001          |
| TNM stage                       |                   |                  |                  |                  |                  |                  |
| IA                              | 20                | 67               |                  | 15               | 72               |                  |
| IB                              | 17                | 23               |                  | 15               | 25               |                  |
| II                              | 8                 | 12               |                  | 9                | 11               |                  |
| IIIA                            | 10                | 17               |                  | 11               | 16               |                  |
| IIIB                            | 5                 | 7                |                  | 7                | 5                |                  |
| IV                              | 14                | 7                | 0.001            | 10               | 11               | < 0.001          |
| Lymphatic invasion              |                   |                  |                  |                  |                  |                  |
| Positive                        | 39                | 45               |                  | 39               | 46               |                  |
| Negative                        | 34                | 87               | 0.002            | 28               | 94               | < 0.001          |
| Venous invasion                 |                   |                  |                  |                  |                  |                  |
| Positive                        | 46                | 49               |                  | 45               | 50               |                  |
| Negative                        | 28                | 84               | < 0.001          | 22               | 90               | < 0.001          |
| Lymph node metastasis           |                   |                  |                  |                  |                  |                  |
| Positive                        | 47                | 48               |                  | 48               | 47               |                  |
| Negative                        | 37                | 85               | 0.052            | 19               | 93               | 0.001            |
| Hematogeneous metastasis        |                   |                  |                  |                  |                  |                  |
| Positive                        | 3                 | 0                |                  | 2                | 1                |                  |
| Negative                        | 71                | 134              | 0.02             | 65               | 139              | 0.19             |
| Peritoneal dissemination        |                   |                  |                  |                  |                  |                  |
| Positive                        | 5                 | 1                |                  | 2                | 4                |                  |
| Negative                        | 69                | 133              | 0.01             | 65               | 136              | 0.95             |
of E-cadherin is considered to have critical roles in the development of undifferentiated carcinoma. The molecular interaction between leptin/OB-R and CDX2 or E-cadherin is an interesting subject for future research.[48]

In conclusion, we confirmed that the expression level of leptin/OB-R showed a positive correlation with the depth of tumor invasion, stage, and metastasis as well as tumor differentiation. Our findings suggest that coexpression of leptin and OB-R may have a positive role in the progression in gastric cancer in an autocrine or paracrine manner. Functional inhibition of leptin/leptin receptor mRNAs may effectively suppress the growth and metastasis of gastric cancer.

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