Involvement of Ghrelin-Growth Hormone Secretagogue Receptor System in Pathoclinical Profiles of Digestive System Cancer

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Abstract  Ghrelin receptor has been shown to be expressed along the human gastrointestinal tract. Recent studies showed that ghrelin and a synthetic ghrelin receptor agonist improved weight gain and lean body mass retention in a rat model of cancer cachexia by acting on ghrelin receptor, that is, growth hormone secretagogue receptor (GHS-R). This study aims to explore the expression and the distribution of ghrelin receptor in human gastrointestinal tract cancers and to investigate the possible involvement of the ghrelin-GHS-R system in human digestive cancers. Surgical human digestive cancer specimens were obtained from various portions of the gastrointestinal tract from different patients. The expression of ghrelin receptor in these tissues was detected by tissue microarray technique. Our results showed that ghrelin receptor was expressed in cancers throughout the gastrointestinal tract, mainly in the cytoplasm of mucosal layer cells. Its expression level possibly correlated with organ type, histological grade, tumor-nodes-metastases stage, and nutrition status (weight loss) of the patients. For the first time, we identified the distribution of ghrelin receptor in digestive system cancers. Our results implied that the ghrelin-GHS-R system might be involved in the pathoclinical profiles of digestive cancers.

Keywords  ghrelin; receptor; digestive system cancer; gene expression

Ghrelin, a peptide hormone originally identified as an endogenous ligand of the growth hormone secretagogue receptor (GHS-R), is secreted primarily by stomach and secondarily by small intestine and colon. Ghrelin is also expressed in the pancreatic islets, hypothalamus, pituitary, and several tissues in the periphery [1]. The wide expression of GHS-R implies its diverse physiologic roles. A growing body of evidence suggested that, in addition to its predicted effect on growth hormone secretion, ghrelin plays important roles in short-term regulation of appetite and long-term regulation of energy balance and blood glucose homeostasis [2]. Recent studies have implicated ghrelin in the regulation of gastrointestinal, cardiovascular, and immune function and suggested a role for ghrelin in bone physiology [3]. Despite this rapid progress, many questions remain unanswered, including the regulation of ghrelin secretion, the downstream pathways that mediate its effects, and its precise physiologic endocrine and paracrine roles.

GHS-R is a newly identified and ubiquitously expressed molecule that has been involved in a wide array of endocrine and non-endocrine functions, including cell proliferation [4]. Two subtypes of ghrelin receptors have been identified: the biologically active 1a subtype; and the biologically inactive 1b subtype [5]. It is reported that GHS-R-1a is able to activate different intracellular second-messenger systems depending on its agonist. The regulation of the ghrelin-activated early signaling pathways by adenosine might have unexpected implications in GHS-R-1a actions [6].

Ghrelin and its receptors are now recognized as components of the growth hormone axis and are therefore potentially involved in tissue growth and development. Recently, it has been suggested that ghrelin might be involved in the pathogenesis of many diseases...
and be a therapeutic target in these diseases [7]. Evidence is rapidly emerging to indicate that ghrelin/GHS-R might play an important autocrine/paracrine role in some cancers [8]. Recently, studies identified the expression of ghrelin and its functional receptor, GHS-R-1a, in several human hormone-dependent cancers including ovarian, testicular, breast tumors, and gastrointestinal stromal tumors (GISTs) [9,10]. Furthermore, ghrelin has also been recognized as an important endogenous regulator of gastrointestinal motor function in mammals, mediated by GHS-R [11].

Digestive tract, being the main source of ghrelin production and one of the most important target organs of ghrelin, is also an organ where high frequencies of malignancy are often noted. Therefore there is an intriguing question of whether ghrelin plays a role in digestive cancer development, progression, and metastasis. Until now, the distribution of ghrelin receptor in digestive tract and its possible relationship with digestive cancer has not been explored.

The present study aims to determine the expressions of ghrelin receptor (GHS-R-1a) in a variety of digestive cancers and the relationship between GHS-R-1a and the biological characteristics of these cancers.

Materials and Methods

Patients and specimens

From January 2006 to October 2006, 50 patients who underwent resection for pathologically confirmed digestive cancer at the Department of General Surgery at Shanghai No. 6 Hospital (School of Medicine, Shanghai Jiaotong University, Shanghai, China) were enrolled into the present study, including 26 patients with gastric cancer, 12 with colorectal cancer, six with esophageal cancer, and six with liver cancer. The pathological type, histological grade, tumor-nodes-metastases (TNM) stage, and weight loss before operation were all recorded. These studies were approved by the Institutional Review Board (Shanghai Jiaotong University, Shanghai, China).

Tissue microarray and immunohistochemistry

After screening hematoxylin-eosin-stained slides for optimal tumor content, we constructed tissue microarray slides (Shanghai Biochip, Shanghai, China). Two cores were taken from each formalin-fixed, paraffin-embedded tumor sample using punch cores (0.8 mm) in greatest dimension from the center of tumor foci. Immunohistochemistry for ghrelin receptor was carried out using the avidin-biotin complex method following heat-induced antigen-retrieval procedures [12]. Incubation with polyclonal antibodies against GHS-R-1a (GHS-R 1:350 dilution; rabbit anti-human; Chemicon International, Temecula, USA) was carried out at 4 °C for 18 h. Negative controls were treated identically without the primary antibody.

Scoring of GHS-R immunoreactivity was evaluated independently by three pathologists who were blinded to the patient data. The percentage of positive tumor cells was determined by each observer, and the average score was calculated. We randomly selected 10 high-power fields (Magnification, 200×; 100 cells per high-power field) and counted 1000 cells in each core. Thus, the standard deviation (SD) does not increase with the mean. In this study, GHS-R expression was graded as the percentage of GHS-R-positive cells (defined as positive rate).

Statistical analysis

Continuous variables were expressed as the mean±SD and were compared between groups using Student’s t-test. Categorical variables were compared using the χ2-test. All statistical analysis was completed using statistical software SPSS version 12.0 (SPSS, Chicago, USA). Statistical significance was defined as P<0.05.

Results

Distribution characteristics of GHS-R

Ghrelin receptor is expressed alongside human gastrointestinal tract with distinctive distributions at both organ and tissue layers. Positive expression was mainly found in mucosa layer, but weak expression in muscle was also observed. Immunostaining signals for ghrelin receptor were uniformly stronger in cytoplasm than those in nucleus. The expression levels defined as positive rate in different cancer organs were as follows: esophagus>colorectum>stomach>liver (Table 1 and Fig. 1). Except liver, the expression levels of GHS-R were higher in cancer cells than in neighboring normal epithelial cells (P<0.05) (Table 1).

Comparison of GHS-R expression levels with histological grade and TNM stage of gastric and colorectal cancers

In terms of sample numbers, we only analyzed the relationship between GHS-R expression level and
We found that the protein expression levels of ghrelin receptors (positive rate) generally showed an inverse correlation with histological grade. We simply classified the patients into two groups, I−II group, and III−IV group, in gastric and colorectal cancer, respectively. Higher expression levels were observed in the well-differentiated (I−II) group compared with the poorly differentiated (III−undifferentiated) group, 72.3%±5.4% versus 37.5%±4.3%, and 82.8%±4.2% versus 57.2%±3.8%, in gastric cancer and colorectal cancer, respectively (Table 2 and Fig. 2).

Similarly, we classified these patients into two groups, I−II group and III−IV group, in gastric and colorectal cancer and TNM stage in gastric cancer and colorectal cancer (26 patients and 12 patients, respectively). Esophageal cancer and liver cancer were not included.

### Table 1 Relationship between growth hormone secretagogue receptor positive rate and organ types

<table>
<thead>
<tr>
<th>Organ</th>
<th>No. of cases (n)</th>
<th>GHS-R positive rate (%)</th>
<th>Cancer epithelium</th>
<th>Normal epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td>6</td>
<td>80.3±5.2*</td>
<td></td>
<td>39.7±3.7</td>
</tr>
<tr>
<td>Colorectum</td>
<td>12</td>
<td>78.4±3.8*</td>
<td></td>
<td>21.9±4.1</td>
</tr>
<tr>
<td>Stomach</td>
<td>26</td>
<td>62.3±2.5*</td>
<td></td>
<td>42.4±3.6</td>
</tr>
<tr>
<td>Liver</td>
<td>6</td>
<td>51.2±6.7</td>
<td></td>
<td>48.7±5.4</td>
</tr>
</tbody>
</table>

*Significant difference of expression positive rate in cancer cells compared with normal epithelial cells within the same organ (P<0.05).

### Fig. 1 Photomicrographic images of the immunohistochemistry of growth hormone secretagogue receptor in cancers from different digestive organs

(A) Overview of the tissue array. (B) Negative staining in gastric cancer. (C) Positive staining in esophageal cancer. (D) Positive staining in gastric cancer. (E) Positive staining in colon cancer. (F) Positive staining in liver cancer. Magnification, 200×.
Higher ghrelin expression levels were found in the early TNM staged (I–II) group than in the late staged (III–IV) group, in both gastric cancer and colorectal cancer, 68.3%±3.0% versus 45.5%±4.6%, and 78.2%±3.8% and 52.7%±4.7%, respectively (P<0.05) (Table 3).

Comparison of GHS-R expression levels with weight loss

We analyzed the relationship between ghrelin-GHS-R expression levels and the weight loss of all patients before operation, which was considered as a clinical sign of energy balance or nutrition status. Higher expression levels of GHS-R were found in groups with lower weight loss (<5 kg and 5–10 kg) than in groups with higher weight loss (>10 kg), 72.4%±2.0% versus 68.3%±3.5%, in all digestive system cancer patients (P<0.05) (Table 4).

Discussion

Ghrelin is a novel gastric hormone identified in 1999 as a mediator of growth hormone release [13]. Because growth hormone is anabolic, an important function of

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**Table 2**  
Relationship between histological grade and growth hormone secretagogue receptor (GHS-R) positive rate in gastric and colorectal cancer

<table>
<thead>
<tr>
<th>Histological grade</th>
<th>Organ and no. of cases</th>
<th>GHS-R positive rate (%)</th>
<th>GHS-R positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastric cancer (n)</td>
<td>Colorectal cancer (n)</td>
<td>GHS-R positive rate (%)</td>
</tr>
<tr>
<td>I–II</td>
<td>13</td>
<td>8</td>
<td>72.3±5.4*</td>
</tr>
<tr>
<td>III–undifferentiated</td>
<td>13</td>
<td>4</td>
<td>37.5±4.3</td>
</tr>
</tbody>
</table>

*Significant difference compared with group III–undifferentiated (P<0.05).

**Table 3**  
Relationship between tumor-nodes-metastases (TNM) stage and growth hormone secretagogue receptor (GHS-R) positive rate in gastric and colorectal cancers

<table>
<thead>
<tr>
<th>TNM stages</th>
<th>Organ and no. of cases</th>
<th>GHS-R positive rate (%)</th>
<th>GHS-R positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastric cancer (n)</td>
<td>Colorectal cancer (n)</td>
<td>GHS-R positive rate (%)</td>
</tr>
<tr>
<td>I–II</td>
<td>14</td>
<td>7</td>
<td>68.3±3.0*</td>
</tr>
<tr>
<td>III–IV</td>
<td>12</td>
<td>5</td>
<td>45.5±4.6</td>
</tr>
</tbody>
</table>

*Significant difference compared with group III–IV (P<0.05).

**Table 4**  
Relationship between weight loss (kg, before operation) and growth hormone secretagogue receptor (GHS-R) positive rate in all digestive system cancers

<table>
<thead>
<tr>
<th>Weight loss (kg)</th>
<th>Cases (n)</th>
<th>GHS-R positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>30</td>
<td>72.4±2.0*</td>
</tr>
<tr>
<td>5–10</td>
<td>14</td>
<td>68.3±3.5*</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>39.8±6.1</td>
</tr>
</tbody>
</table>

*Significant difference compared with group of >10 kg (P<0.05).
Ghrelin might be to coordinate energy need with the growth process. Newly discovered biologic roles of ghrelin imply that it might have other important physiological functions as well [14]. Interestingly, more recent studies have implied possible involvement of ghrelin and its receptor in several types of tumors, especially hormone-dependent tumors. For example, an in vitro study showed that the proliferation of pancreatic adenocarcinoma cells was suppressed dose-dependently by high concentrations of ghrelin [15]. Ekeblad et al. reported the first immunohistochemical data on the expression of ghrelin receptor in pancreatic endocrine tumors [16]. All GISTs expressed ghrelin and ghrelin receptor mRNA, implying a possible presence of ghrelin autocrine/paracrine regulation loops during the pathogenesis of GISTs [10]. A recent study further indicated that the ovarian surface epithelium and its related tumors were potential targets for systemic or local production of ghrelin, leading to the elevated expression of the functional GHS-R-1a in these tumors [4]. Similar cellular expression patterns of ghrelin and its functional receptor were also observed in human and rat testis, highly suggestive of a conserved role for this molecule in the regulation of mammalian testicular function [17].

Ghrelin is synthesized in a distinct endocrine cell type in the gastrointestinal tract of human [18]. Ghrelin receptor was reported to be expressed equally in all parts of the gastrointestinal tract, with similar expression levels in mucosal and muscle layers. Therefore, digestive tract is considered as the main organ that secretes ghrelin and an important target of ghrelin through its receptors. To our knowledge, the distribution of ghrelin receptor in the context of gastrointestinal tract malignancy has not been well established, and the possible relationship between ghrelin/GHS-R expression and patho-clinical features of digestive cancers has not been addressed. In the present study, we reported, for the first time, that ghrelin–GHS-R might be involved in digestive cancers. It is necessary to point out that we also used six cases of liver cancer in our study to investigate the distribution of GHS-R in stromal digestive organs. This is why we named our group of patients as having “digestive cancer” rather than “cancer of digestive tract”.

In this study, we confirmed that GHS-R was expressed throughout the digestive tract. We further showed that GHS-R was mainly localized in the cytoplasm of epithelial cells. Tumors in esophagus and stomach showed higher GHS-R expression levels than those in colorectum and liver. Cancer cells had higher expression levels of GHS-R than normal epithelial cells in all digestive organs except the liver. It is well established that ghrelin plays an important role in energy homeostasis, body weight control, and food intake [19]. It has profound orexigenic, adipogenic, and somatotrophic properties [14]. Malignant tumors have high metabolic characteristics, and often cause cachexia, a debilitating syndrome of anorexia and loss of lean body mass that is often accompanied with advanced cancers [20]. Therefore, we are cautious to speculate that high expression of GHS-R in cancer cells might be a pathophysiological response to cancer-induced increase of metabolism in the target cells of ghrelin. Of course, this speculation needs further data from mRNA examinations and Western blot analyses in a bigger sample.

Our results also identified a possible correlation between GHS-R expression levels and several patho-clinical profiles of gastric cancer and colorectal cancer. In general, GHS-R expression level correlates inversely with histological grade and TNM stage; patients with more weight loss showed down-regulated receptor expression. Taken together, we carefully summarized that decreased ghrelin receptor expression seemed to always associate with poor prognostic factors of digestive cancer patients, such as poor differentiation, advanced stage, and malnutrition. This intriguing finding, which has never been reported previously, raises new questions such as the underlying mechanisms by which ghrelin mediates the surrounding tissue’s physiological responses to malignant changes. Cancer cachexia is a complex metabolic state characterized by loss of muscle mass and adipose tissue together with anorexia [21]. We hypothesized that the expression status of ghrelin receptor might represent the response to the ghrelin-GHS-R system during important pathophysiological processes such as energy balance and hormone homeostasis, and responses to ghrelin could be attenuated or resisted in cancer cachexia. This hypothesis could also, to some extent, explain why patients with the above factors might cause ataxia and poor prognosis. Further study of tissue or serum ghrelin levels in the same clinical and pathological status as ghrelin receptor might help to explain the possible relationship between the ghrelin–GHS-R system and cancer cachexia.

Quite recently, a few studies have focused on the involvement of the ghrelin system in cancer-induced cachexia. Wang et al. found that tumor-bearing mice (MCG101) characterized by anorexia, fat loss, and muscle wasting showed improved food intake and body mass composition following treatment with high doses of exogenous ghrelin (40 mg/d i.p.) [22]. The other researchers gave either human ghrelin or a synthetic ghrelin analog BIM-28131 to a rat model of cancer cachexia, and showed that either treatment on the tumor-implanted rats...
resulted in a significant increase in food consumption and weight gain compared with saline-treated animals. They concluded that ghrelin and ghrelin receptor agonist improved weight gain and lean body mass retention through effects involving orexigenic neuropeptides and anti-inflammatory changes [20]. Our study reported here is consistent with the findings of the studies mentioned above. Moreover, we focused on the ghrelin receptor distribution and its relationship with pathochlonical profiles in digestive cancer, which has not been reported before.

The biologic roles of the ghrelin system in gastrointestinal cancer development remains unclear, yet our findings that the ghrelin system is associated with gastrointestinal cancer raise many new questions. Can the ghrelin-GHS-R system explain the occurrence of cancer-induced cachexia? Does ghrelin have antitumor effects in addition to nutritional homeostasis and metabolic processes? Wang et al. showed that tumor growth in mice was not altered by exogenous ghrelin. They proposed that factors downstream of the ghrelin-GHS-R system appeared to be more important than ghrelin to explain cancer-induced anorexia [22]. This in vivo result is different from an in vitro study from Duxbury et al. that showed that ghrelin promoted pancreatic adenocarcinoma cellular proliferation and invasiveness [15]. Consistent with Duxbury’s study, an independent study by Kim et al. also showed that ghrelin stimulated proliferation and differentiation and inhibited apoptosis of osteoblastic MC3T3-E1 cells [23]. These seemingly contradictory results of different studies imply that the involvement of ghrelin in cancer development is a complex process that awaits both further in vitro and in vivo studies.

As well as the roles mentioned above, ghrelin also has a prokinetic effect on the gastrointestinal system [24, 25]. Thus, poor response to ghrelin might cause dysfunction of gastrointestinal motility, which could be one of the underlying causes for many clinical symptoms in late-stage digestive cancer patients, such as bloating, bulging, vomiting, and loss of appetite. Therefore, the mechanism of the involvement of the ghrelin-GHS-R system in digestive cancer might involve multiple pathways. Hence, further investigation on the mechanism of the ghrelin-GHS-R system in digestive cancer is necessary. It is important to mention the limitations of this study: it included relatively few samples involving only 50 patients; we did not follow up the patients to study the relationship of the ghrelin-GHS-R system with their recovery and prognosis; and analysis of mRNA expression status of GHS-R is necessary to provide further proof.

In summary, we used tissue microarray, for the first time, to identify the distribution of ghrelin receptor in digestive system cancers. Also for the first time, our results implied possible involvement of the ghrelin system in the pathophysiological process of digestive cancers. If confirmed by further study, GHS-R might become a potential therapeutic target of digestive cancer and a valuable prognostic marker.

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