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• *RAPID COMMUNICATION* •

Possible involvement of leptin and leptin receptor in developing gastric adenocarcinoma

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Abstract

AIM: To investigate the expression of leptin and leptin receptor (ob-R) in intestinal-type gastric cancer and precancerous lesions, and to explore the possible mechanism and role of the leptin system in developing intestinal-type gastric adenocarcinoma.

METHODS: Immunohistochemistry was performed to examine the expression of leptin and leptin receptor in archival samples of gastric adenocarcinoma and preneoplastic lesions, including intestinal metaplasia and mild to severe gastric epithelial dysplasia. Positive staining was identified and percentage of positive staining was graded.

RESULTS: Dual expression of leptin and leptin receptor were detected in 80% (16/20) intestinal metaplasia, 86.3% (25/30) mild gastric epithelial dysplasia, 86.7% (26/30) moderate gastric epithelial dysplasia, 93.3% (28/30) severe gastric epithelial dysplasia, 91.3% (55/60) intestinal-type gastric adenocarcinoma and 30.0% (9/30) diffuse-type gastric carcinoma. The percentage of dual expression of leptin and leptin receptor in intestinal-type gastric adenocarcinoma was significantly higher than that in diffuse-type gastric adenocarcinoma ($\chi^2 = 37.022$, $P<0.01$).

CONCLUSION: Our results indicate the presence of an autocrine loop of leptin system in the development of intestinal-type gastric adenocarcinoma.

Key words: Leptin; Leptin receptor (ob-R); Intestinal-type gastric adenocarcinoma; Intestinal metaplasia; Gastric epithelial dysplasia

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INTRODUCTION

Leptin, the product of the obesity gene (ob gene), is a cytokine-like peptide capable of signal transduction via interaction with its specific receptor (ob-R). It was initially found to be synthesized by adipocytes and act centrally in the hypothalamus to regulate food intake and energy expenditure[1]. Subsequently, expressions of leptin or ob-R were detected in a wide variety of tissues with multiple role in hematopoiesis, reproductive control, angiogenesis, cardiovascular medication, immunomodulation and carcinogenesis[2–6]. Recently, the presence of leptin has also been demonstrated in stomach and thought to be stomach-derived. This endogenous gastric leptin acts as a gastrointestinal hormone via autocrine and/or paracrine pathway in gastrointestinal tract and plays an important role in digestive physiological activities, including short-term meal size control, gastric mucosal cytoprotection and nutrition, regulation of gastric acid and gastric hormone secretion, and modulation of intestinal transport[7].

According to Lauren, gastric cancer can be divided into two histological types: diffuse and intestinal[8]. During the carcinogenesis of intestinal-type gastric adenocarcinoma, a stepwise process proposed by Correa has been widely accepted, which suggested that prolonged *H pylori* infection leads to atrophic gastritis, then with further mutational events leads to the development of intestinal metaplasia, epithelial dysplasia and finally intestinal-type gastric adenocarcinoma[9]. Gastrointestinal hormones and cytokines, including gastrin, epithelial growth factor, vasoactive intestinal peptide and IL-6, seem to play important roles in this transformation processes[10–16]. However, whether leptin, a cytokine-like gastrointestinal hormone that share similarity with aforementioned hormones or cytokines, is involved in the development of intestinal-type gastric adenocarcinoma remains unclear. In the present study, using immunohistochemistry, we examined the expression of leptin and ob-R protein in the tissues of intestinal metaplasia, gastric epithelial dysplasia and intestinal-type gastric adenocarcinoma. Their presence may suggest an autocrine/paracrine pathway of leptin system in gastric carcinogenesis.

MATERIALS AND METHODS

Samples

Formalin-fixed samples of 30 cases of intestinal

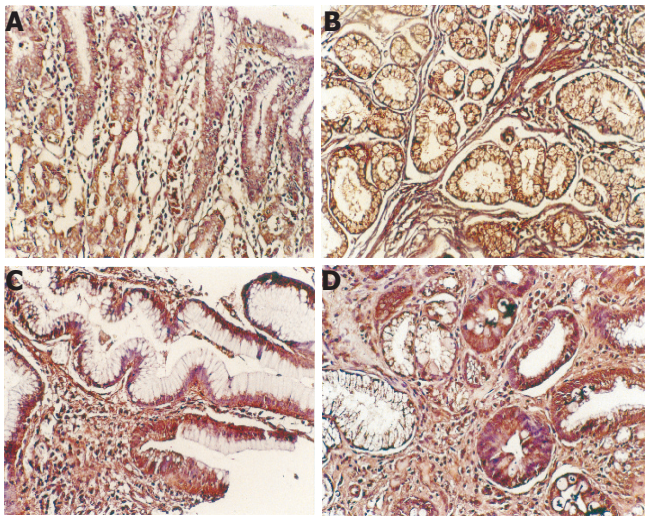

Figure 1 Immunohistochemical stainings of leptin located in cytoplasm (×200). **A:** Normal gastric mucosa; **B:** intestinal metaplasia; **C:** gastric dysplasia; **D:** intestinal-type gastric adenocarcinoma.

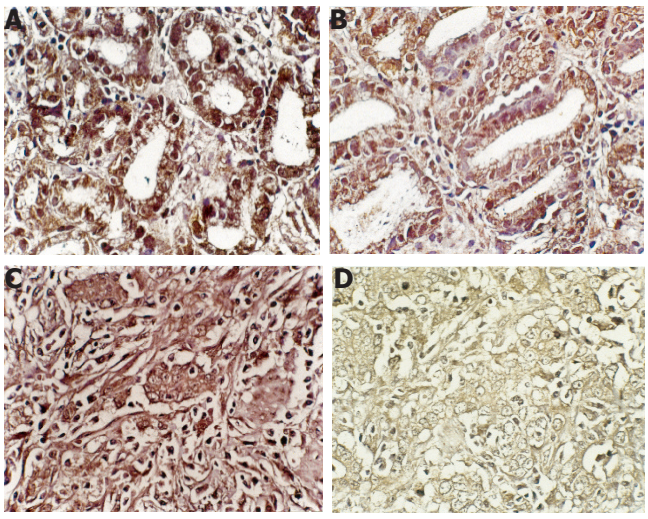

Figure 2 Immunohistochemical stainings of leptin receptor located at cell membrane (×200). **A:** Normal gastric mucosa; **B:** intestinal metaplasia; **C:** gastric dysplasia; **D:** intestinal-type gastric adenocarcinoma.

metaplasia, 90 cases of epithelial dysplasia (mild dysplasia, moderate dysplasia and severe dysplasia, each 30 cases), and 90 cases of gastric adenocarcinoma (60 intestinal-type, 30 diffuse-type) were obtained from pathology archives during 1995-2004 from Renmin Hospital, Wuhan University, Wuhan, China. Those patients had never received chemotherapy, radiation therapy or *H pylori* eradiation. For each case, a paraffin-embedded section with hematoxylin-eosin-safran staining was subjected to pathological identification by a consultant histopathologist on the basis of the Lauren's classification and WHO classification.

Immunohistochemistry

For immunohistochemistry analysis, paraffin-embedded tissue blocks were serially sectioned (4-μm thickness) and mounted onto histostick-coated slides and kept in an oven at 72 °C for 2 h. Sections were deparaffinized in xylene and rehydrated before analysis. Endogenous peroxides was quenched with 30 mL/L hydrogen peroxide in methanol for 10 min. Antigen retrieval was performed by means of microwave irradiation for 15 min and blocked for 15 min with normal goat serum, followed by incubation overnight at 4 °C with a rabbit polyclonal antibody (SC-842) against human leptin (Santa Cruz Biotechnology) and a rabbit polyclonal antibody (SC-1834) against human leptin receptor (Santa Cruz Biotechnology) both at a dilution of 1:70. Replacement of the primary antibodies by PBS solution was used as a negative control. Sections of normal gastric fundus mucosa that had previously shown expression of leptin and ob-R were used as positive

Table 1 Expression of leptin and ob-R in gastric cancer and precancerous lesions

	n	Leptin						Ob-R						Dual expression of leptinand ob-R	
		–	+	+ +	++ +	++ ++		–	+	++	++ +	+++ +		*n*	(%)
Intestinal metaplasia	30	5	4	5	17	0		4	5	7	14	0		25	(83.3)
Gastric dysolasia	90	6	12	28	39	0		9	15	25	35	4		79	(87.7)
Gastric cancer	90	25	9	16	16	24		24	9	22	12	25		75	(83.3)

For comparison of positive percentage for dusl expression of loptin and ob-R among (1) intestinal metaplsia (2) gastric dysplasia (3) gastric carcinoma, (1) *vs* (2) $\chi^2 = 0.3864$, P=0.5351; (1) *vs* (3) $\chi^2 = 0.3864$, $P = 1.000$; (2) *vs* (3) $\chi^2 = 0.7193$, $P = 0.3964$

Table 2 Expression of leptin and ob-R in mild, moderate, and severe gastric

	n	Leptin						Ob-R						Dual expression of leptinand ob-R	
		–	+	+ +	++ +	++ ++		–	+	++	++ +	+++ +		*n*	(%)
Mild dysplasia	30	3	6	9	12	0		4	8	9	9	0		25	(83.3)
Moderate dysplasia	30	3	2	12	10	3		3	3	9	13	2		26	(86.7)
Severe dysplasia	30	0	4	7	17	2		2	4	7	15	2		28	(93.3)

For comparison of positive percentage for dusl expression of loptin and ob-R among (1) mild gastric dysplasia (2) moderate gastric dysplasia (3) severe gastric dysplasia, (1) *vs* (2) $\chi^2 = 0.1307$, $P = 0.7177$; (1) *vs* (3) $\chi^2 = 1.456$, $P = 0.2276$; (2) *vs* (3) $\chi^2 = 0.7407$, $P = 0.3894$

Table 3 Expression of leptin and ob-R in intestinal-type and diffuse-type gastric cancer

	n	Leptin						Ob-R						Dual expression of leptinand ob-R	
		-	+	++	++	++		-	+	++	++	+++		*n*	(%)
				+	+	++					+	+			
Intestinal-type GC[1]	60	4	6	11	15	24		5	7	15	10	25		55	(91.7)
Diffuse-type GC[1]	30	21	3	5	1	0		19	2	7	2	0		9	(30)

[1]GC-gastric cancer for comparison of positive percentage for dual expression of leptin and ob-R between intestinal-type gastric carcinoma and diffuse-type gastric carcinoma, $\chi^2 = 37.022$, $P<0.001$.

controls. Sections were washed thrice with PBS for 2 min each and incubated with biotin-labeled anti-rabbit IgG at room temperature for 1 h. After washing thrice with PBS for 2 min each, sections were stained by streptavidin-peroxidase detection system. Antibody binding was visualized using the diaminobenzidine as chromogen and counterstained with hematoxylin. The positive cells were counted in ten randomly selected fields under ×200 or × 400 microscopic magnification for each specimen and the mean positive percentage was calculated as the density of positive cells. Immunoreactivity was performed by two experienced histopathologists without knowledge of the background features. The abundance of positive stains was graded as follows: -, no cell stained; +, <25% of cells stained; ++, 25–50% of cells stained; +++, 50–75% of cells stained; and ++++, >75% of cells stained.

Statistical analysis

Data were analyzed using SPSS 10.0 software. The χ^2 test was used to compare binomial proportions. A P value less than 0.05 was considered statistically significant.

RESULTS

Immunostainings of leptin and ob-R in normal gastric mucosa, intestinal metaplasia, gastric epithelial dysplasia, intestinal-type gastric adenocarcinoma, diffuse-type gastric carcinoma are shown in Figures 1 and 2. Positive staining of leptin was identified as brownish-yellow granules in cytoplasm. Positive staining of ob-R was identified as brownish-yellow granule at cell membrane.

DISCUSSION

In the present study, the expressions of leptin and ob-R in intestinal metaplasia, gastric epithelial dysplasia, intestinal-type gastric adenocarcinoma and diffuse-type gastric cancer were determined using immunohistochemistry. Our results demonstrated that dual expressions of leptin and leptin receptor were detected in 83.3% (25/30) intestinal metaplasia, 87.7% (79/90) gastric epithelial dysplasia, and 83.3% (75/90) intestinal-type gastric adenocarcinoma without statistically significant difference among those groups. Moreover, no obvious difference among mild gastric dysplasia (83.3%, 25/30), moderate gastric dysplasia (86.7%, 26/30) and severe gastric dysplasia (93.3%, 28/30) was observed. The percentage of dual expression in intestinal-type gastric adenocarcinoma (91.7%, 55/60) was significantly higher than that in diffuse-type gastric carcinoma (30.0%, 9/30). These results indicated that dual expression of leptin and ob-R might be a tumor-specific phenomenon in the transformation from intestinal metaplasia to intestinal-type gastric adenocarcinoma. To the best of our knowledge, our study may be the first study demonstrating dual expression of leptin and ob-R in intestinal metaplasia, gastric epithelial dysplasia and intestinal-type gastric adenocarcinoma, which may play important roles in this transformation process.

As a growth factor, leptin exhibited simulative effect on the proliferation of a variety of malignant cell lines, including leukemia, breast cancer, esophagus adenocarcinoma, prostate cancer, colon cancer, and pituitary adenomas[6,17–21]. In addition, there had been increasing evidence that leptin plays an important role in tumor invasion, metastases, angiogenesis and resistance to chemotherapy[22–26]. Based on these results, leptin is considered to serve as a multi-functional growth factor in tumorigenesis and is capable of promoting an aggressive cancer phenotype. Recently, it has been shown that leptin induced the proliferation of human gastric adenocarcinoma AGS cell line in a dose-dependent fashion[27]. Therefore, it is reasonable to speculate that dual expression of leptin and ob-R may form an autocrine/paracrine stimulatory loop based on intestinal-type gastric adenocarcinoma and precancerous lesions, and play an important role in gastric carcinogenesis.

As a member of the class I cytokine receptor family, ob-R is identified as a single membrane-spanning protein with multiple isoforms (ob-Ra, ob-Rb, ob-Rc, ob-Rd). ob-Rb contains sequence motifs of janus-kinase (JAK)/signal transduction and activation of transcription signal transduction pathways. In addition, leptin activates mitogen-activated protein kinase (MAPK) signal transduction pathways through the ob-Rb receptor and also through the ob-Ra receptor in some cases[18,28]. Those downstream signal pathways of leptin system were verified to be implicated in the formation and progression of tumor. Proliferation of prostate cancer cells appeared to be working through the PI3K and MAPK leptin receptor-activated pathways, depending on cell type[21]. In colon cancer cell line, leptin was demonstrated to stimulate proliferation via P42/44 MAPK signal pathway and induce invasion via JAK/PI3K signal pathway[18,23]. Recently, Schneilder *et al*[27]. have also reported that leptin caused a moderate, significant proliferative effect on gastric adenocarcinoma cell line AGS proliferation through MAPK signal pathways.

On the other hand, the expression of leptin protein in intestinal-type gastric adenocarcinoma and precancerous lesions in our study can be viewed as "ectopic secretion"

of gastric leptin in those tissues. Similar "ectopic secretion" of gastrointestinal hormone such as gastrin, epithelial growth factor, cholecystokinin, vasoactive intestinal peptide in gastrointestinal tumors has been well documented in previous studies[13–16]. Those "ectopic secretion" of gastrointestinal hormone acted as a growth factor and promoter of angiogenesis in gastrointestinal tumors via interaction with its specific receptor in tumors. Taking into account that those ectopic-secreting gastrointestinal hormones exert similar function in gastrointestinal tumors to their physiological functions to normal gastrointestinal tract, we hypothesized that the ectopic-secreting leptin in intestinal-type gastric adenocarcinoma and precancerous lesions may have similar function with gastric leptin[13–16]. As gastric leptin has been found to mediate gastric cytoprotection and increase mucosal blood flow via overexpression of growth factors, such as EGF, TGF-α and VEGF[29,30], it can be anticipated that leptin may enhance growth and angiogenesis of intestinal-type gastric cancer via similar mechanism mentioned above.

What activates leptin expression in gastric adenocarcinoma remains unclear. Recently, it has been reported that *H pylori* infection significantly increases gastric leptin expression[31]. In addition, leptin expression and secretion were induced by chronic inflammation of inflammatory bowel disease in colonic epithelial cells that were unable to express leptin physiologically[32]. Therefore, we postulated that leptin might be induced as a pro-inflammatory mediator in chronic gastrointestinal inflammation, including *H pylori*-associated chronic gastritis. In addition, previous reports demonstrated that K-ras mutation or amplification of chromosome 17q12-q21 induced the amplification of gastrin gene in colon or gastric cancer[33,34]. Whether *H pylori* infection and chronic gastritis might induce some special biomolecular alterations to amplify leptin expression and its downstream signal pathways during gastric carcinogenesis needs further investigation.

In conclusion, our study demonstrates constitutive dual expression of leptin and ob-R in intestinal-type gastric adenocarcinoma and precancerous lesions. Based on these results, we suggest that leptin system might act as a growth factor and angiogenesis promoter via autocrine and/or paracrine pathway in gastric carcinogenesis. Further investigations are warranted to identify the exact role of leptin system and exact mechanism of its activation in gastric carcinogenesis.

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