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The role of leptin in gastric cancer: Clinicopathologic features and molecular mechanisms

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ABSTRACT

Obesity is associated with certain types of cancer, including gastric cancer. However, it is still unclear whether obesity-related cytokine, leptin, is implicated in gastric cancer. Therefore, we aimed to investigate the role of leptin in gastric cancer. The expression of leptin and its receptor, Ob-R, was assessed by immunohistochemical staining and was compared in patients with gastric adenoma ($n = 38$), early gastric cancer (EGC) ($n = 38$), and advanced gastric cancer (AGC) ($n = 38$), as a function of their clinicopathological characteristics. Gastric cancer cell lines were studied to investigate the effects of leptin on the signal transducer and activator of transcription-3 (STAT3) and extracellular receptor kinase 1/2 (ERK1/2) signaling pathways using MTT assays, immunoblotting, and inhibition studies. Leptin was expressed in gastric adenomas (42.1%), EGCs (47.4%), and AGCs (43.4%). Ob-R expression tended to increase from gastric adenoma (2%), through EGC (8%), to AGC (18%). Leptin induced the proliferation of gastric cancer cells by activating STAT3 and ERK1/2 and up-regulating the expression of vascular endothelial growth factor (VEGF). Blocking Ob-R with pharmacological inhibitors and by RNAi decreased both the leptin-induced activation of STAT3 and ERK1/2 and the leptin-induced expression of VEGF. Leptin plays a role in gastric cancer by stimulating the proliferation of gastric cancer cells via activating the STAT3 and ERK1/2 pathways.

1. Introduction

Obesity is known to increase the risk of certain types of cancer, including gastric cancer. The risk of cancer in obesity has been demonstrated in many epidemiological studies [1], suggesting that cancer could be managed by modifying certain obesity-related factors. Among these factors are cytokine-like peptides, one of which is a product of the obese (*ob*) gene, leptin.

Leptin is secreted mainly by adipocytes and function in controlling food intake and energy metabolism [2], however, also by non-adipose tissues including the gastric mucosa [3]. Also, it may function in cellular development, maturation, hematopoiesis, reproduction, immune responses and angiogenesis [4–6], furthermore, in stimulating the proliferation of malignant cells [7–9]. A possible link between leptin and cancer was also demonstrated by that its synthesis is influenced by factors associated with the cancerous process such as insulin, glucocorticoids, and reproductive hormones, and its secretion is induced by the hypoxic conditions, which are frequently observed in solid tumors [6,10].

The role of leptin in cancer has been investigated in various studies [6]. Circulating levels of leptin were identified to be associated with the risk of cancers by epidemiological studies. Leptin and its receptor, Ob-R, are both expressed at higher levels in breast cancer tissue than in normal tissue [11]. Similarly, higher levels of Ob-R transcripts are contained in estrogen receptor (ER)-positive cancer cells than ER-negative cells [12]. Furthermore, leptin treatment was documented to stimulate the proliferation of colon cancer cells [13], suggesting its mitogenic effect on colonic epithelial cells [8,14]. Elsewhere, leptin was observed to have an association with cancer invasiveness in pre-malignant epithelial cells of the colon and kidney [15]. These findings suggest that leptin and Ob-R may influence the proliferation of cancers that are epidemiologically related to obesity by acting on certain intracellular signaling pathways.

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Some of the well-studied intracellular signaling pathways involved in cell growth and proliferation include the extracellular signal-regulated kinase-1 and -2 (ERK1/2) pathway, the mitogen-activated protein kinase (MAPK) pathway, and the signal transducer and activator of transcription 3 (STAT3) pathway [16,17]. Alterations of ERK1/2 and STAT3 pathways have been observed in various cancers [5,18,19]. However, alterations of these pathways in gastric cancer are not clearly demonstrated in obesity. Also, findings concerning the role of leptin in the proliferation of gastric cancer have been inconsistent. A descriptive study showed that proximal gastric cancer was associated with obesity [20], and the same cancer was shown to strongly express leptin [21]. In in vitro models, leptin was found to stimulate the growth of gastric cancer cells [22], whereas another study showed that circulating levels of leptin were low in patients with advanced gastric cancer (AGC) [23]. Similar conflicting findings have been found with respect to the link between leptin and gastric cancer invasiveness [21,24,25]. Therefore, the aim of this study was to further investigate the role of leptin in gastric cancer based on patients' clinicopathological characteristics and molecular mechanisms.

2. Materials and methods

2.1. Tissue samples

2.1.1. Subjects

Thirty-eight patients with gastric adenomas, 38 with early gastric cancer (EGC), 38 with AGC who underwent surgical or endoscopic resection were included in the study. Expression of leptin and Ob-R was quantified in tissue samples from these patients and also in normal gastric mucosa from the specimen. The study protocol was approved by the Institutional Review Board of Hanyang University Hospital (IRB No. 2013-08-015-002).

2.1.2. Immunohistochemical staining of leptin and Ob-R

Tissue samples were cut with a Leica microtome to multiple 4 μm sections, which were transferred to adhesive-coated slides. The slides were de-waxed by heating, washed with xylene, and then rehydrated by ethanol, followed by phosphate-buffered saline (PBS). For antigen retrieval, the samples were heated in a microwave for 4 min 20 s at full power in 250 mL of 10 mM sodium citrate (pH 6.0). Primary polyclonal rabbit anti-leptin antibody (ab16227, Abcam, Cambridge, UK) was diluted 1:800, and primary monoclonal mouse anti-Ob-R (B-3) (sc-8391, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was diluted 1:20 and then incubated. After three washes with PBS, the sections were incubated with biotinylated goat anti-mouse secondary antibody for 30 min (DAKO, Carpinteria, CA, USA). After three more washes, horseradish peroxidase-streptavidin (DAKO) was added to the sections, followed by another three washes. The samples were developed with 3,3′-diaminobenzidine substrate (Vector Laboratories, Burlington, Ontario, Canada) and counterstained with Mayer's hematoxylin. The slides were then dehydrated by the standard procedure and sealed with cover slips. Negative controls were developed by omitting leptin and the Ob-R antibody.

Leptin and Ob-R expression was evaluated semi-quantitatively by two independent pathologists (Jang KS and Paik SS) without knowing the clinical data. The expression of leptin and Ob-R was classified on a 3-point scale: 0:<10% cells with positive staining; 1+:11–50% cells with positive staining; 2+:>51% cells with positive staining [26]. The staining pattern in the cancerous cells was compared with that of chief and parietal cells in normal mucosa, and were subdivided into four groups: (1) negative: no cell staining; (2) weak positive: significant but weak staining; (3) strong positive: similar or stronger staining; and (4) focal staining.

2.1.3. Statistical analysis

All statistical calculations were carried out with SPSS 17.0 software. The association between the patients' clinical characteristics and the expression of leptin and Ob-R in gastric cancer tissue was investigated using the Mann–Whitney test and Fisher's exact test. The expression of leptin and Ob-R was compared in gastric cancer tissue and normal gastric tissue using the Chi-square test and Fisher's exact test. Statistical significance was defined as $p < 0.05$.

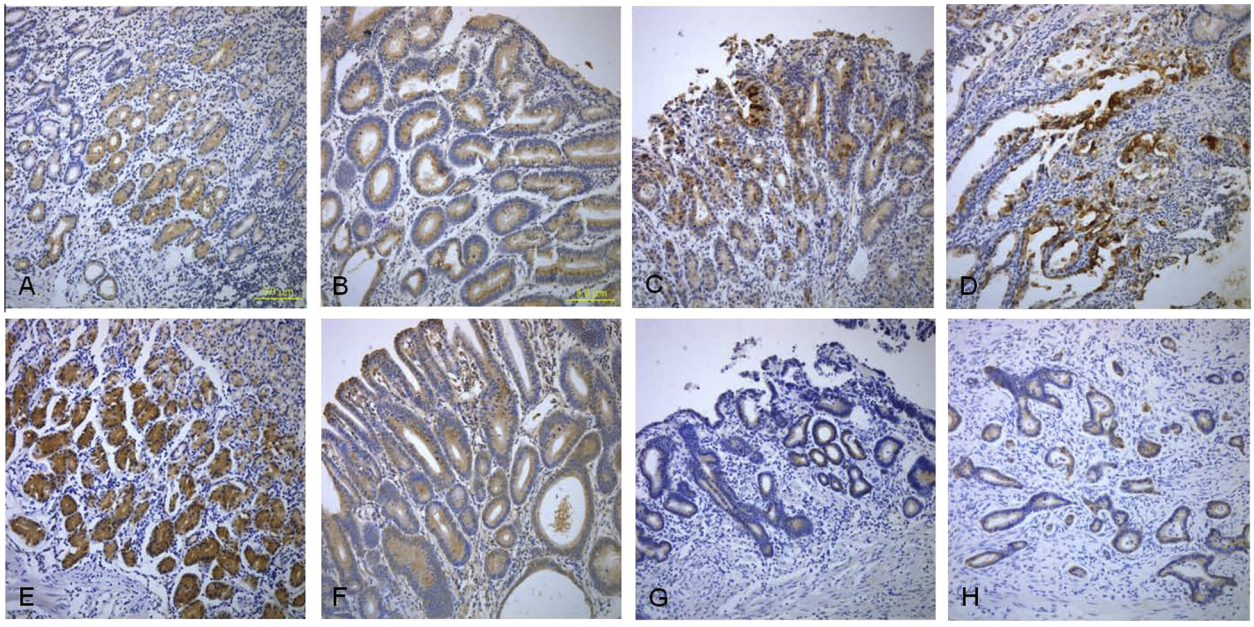

Fig. 1. Immunohistochemical staining of leptin and Ob-R in gastric mucosa, adenoma, and cancer tissue. (A) Leptin expression in chief and parietal cells of normal gastric mucosa (control); (B) Leptin expression in adenoma; (C) Leptin expression in early gastric cancer; (D) Leptin expression in advanced gastric cancer; (E) Ob-R expression in chief and parietal cells of normal mucosa (control); (F) Ob-R expression in adenoma; (G) Ob-R expression in early gastric cancer; (H) Ob-R expression in advanced gastric cancer.

Table 1
Leptin and Ob-R expression in gastric mucosa, adenoma, and cancer tissue.

Tissue sample	Number of patients	Leptin (%)		Ob-R (%)	
		Negative	Positive	Negative	Positive
Gastric mucosa	10	10 (100.0%)	0 (0%)	10 (100.0%)	0 (0%)
Gastric adenoma	38	22 (57.9%)	16 (42.1%)	37 (97.4%)	1 (2.6%)
Gastric cancer	76	41 (53.9%)	35 (46.1%)	66 (86.8)	10 (13.4)
EGC	38	20 (52.6%)	18 (47.4%)	35 (92.1%)	3 (7.9%)
AGC	38	21 (56.7%)	17 (43.3%)	31 (81.6%)	7 (18.4%)

EGC, early gastric cancer; AGC, advanced gastric cancer.

Table 2
Relationship between clinicopathological features and leptin and Ob-R expression in patients with AGC.

	Leptin expression		Ob-R expression	
	Negative (n = 21)	Positive (n = 17)	Negative (n = 21)	Positive (n = 17)
Gender (male:female)	7:14	6:11	7:24	6:1
Age (years, mean ± SD)	58.0 ± 11.3	58.9 ± 20.1	58.9 ± 13.3	62.6 ± 7.4
Tumor size (cm, mean ± SD)	6.0 ± 2.8	6.2 ± 3.9	6.1 ± 3.6	6 ± 0.8
Histological type				
Differentiated	6 (28%)	6 (35%)	7 (22.6%)*	5 (71.4%)*
Undifferentiated	15 (72%)	11 (64%)	24 (77.4%)*	2 (28.6%)*
Location				
Cardia	0*	4 (100%)*	3 (9.7%)	1 (14.3%)
Non-cardia	21 (63.6%)*	13 (36.4%)*	28 (90.3%)	6 (85.7%)
Borrmann type				
I + II	6 (28.6%)	5 (29.4%)	6 (28.6%)	5 (29.4%)
III + IV	15 (71.2%)	12 (70.6%)	25 (71.2%)	2 (70.6%)
TNM stage				
I + II	12 (57.1%)	9 (52.9%)	13 (41.9%)	6 (85.7%)
III + IV	9 (42.9%)	8 (47.1%)	18 (58.1%)	1 (14.3%)
Lymph node metastasis				
Negative	5 (23.8%)	1 (5.9%)	4 (12.9%)	2 (28.6%)
Positive	16 (76.2%)	16 (94.1%)	27 (87.1%)	5 (71.4%)
Serosal invasion				
Negative	14 (66.7%)	9 (52.9%)	17 (54.8%)	6 (85.7%)
Positive	7 (33.3%)	8 (47.1%)	14 (45.2%)	1 (14.3%)
Vascular invasion				
Negative	17 (81.0%)	12 (70.6%)	24 (77.4%)	5 (71.4%)
Positive	4 (19.0%)	5 (29.4%)	7 (22.6%)	2 (28.6%)

AGC, advanced gastric cancer.
* $p < 0.05$ in Fisher's exact test.

2.2. Cell culture experiments

2.2.1. Reagents

Recombinant human leptin was purchased from R&D Systems (Minneapolis, MN, USA), Lipofectamin™ reagent and Lipofectamin 2000, from Invitrogen (Gergy Pontoise, France), PD98059 and AG490, from Calbiochem (San Diego, CA, USA). PD98059 and AG490 were dissolved in DMSO (Sigma Chemical, St Louis, MO, USA), and stored at −20 °C: The concentration of DMSO was 0.001%, and the same concentration was used as vehicle.

2.2.2. Cell culture

The human gastric cancer cell lines used in this study were AGS, SNU-484, SNU-601, SNU-638, SNU-668 and SNU-719. We maintained them in humidified atmosphere with 5% CO_2 at 37 °C, in culture medium which was changed every 3–4 days. The culture medium was RPMI 1640 (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% (vol/vol) fetal bovine serum (FBS; Hyclone Labs, Inc., Logan, UT, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma Chemicals). After being serum-starved for 24 h, we treated them with leptin (at the indicated doses), Ob-R RNAi (100 nM), PD98059 (MEK inhibitor, 25 μM), or AG490 (STAT inhibitor, 50 μM) for the indicated times.

2.2.3. RT-PCR

We isolated total RNA with TRI reagent (Molecular Research Center, Inc., Cincinnati, OH, USA), and synthesized complementary DNA (cDNA) with 1 μg of total RNA using an RNA PCR Kit (TaKaRa Bio Inc., Otsu, Japan). Primers were designed either to detect the presence of any of the Ob-R isoforms (using a shared sequence in the extracellular domain) or to be specific for the long isoform (huOb-Rb) or the short isoform (huOb-Ra) with the aid of primer designing software using the human leptin receptor, huOb-Rb (GenBank accession No. U43168) cDNA sequence and the huOb-Ra (GenBank accession No. U52914) sequence obtained from Entrez: They are listed in Supplementary Table 1. We performed PCR for 40 cycles with a 60 s denaturation step at 94 °C, a 60 s annealing step at 55 °C, and a 90 s extension step at 72 °C with a Gene Amp PCR system 9600 (Perkin Elmer, CA, USA). Finally, 5 μL of the product was loaded onto agarose gel, and the fluorescence of the ethidium bromide stained band was recorded.

2.2.4. Cell proliferation assay

We determined the proliferative effects of leptin on human gastric cancer cells by measuring the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) dye absorbance of living cells. Briefly, gastric cancer cells (10^4 cells/well) were seeded in 96-well plates and exposed to leptin at different concentrations for 24, 48 and 72 h. Then, they were incubated with 50 μL of MTT solution (Sigma Chemicals; 2 mg/mL in PBS), for 4 h at 37 °C. After removal of the MTT solution, 200 μL of DMSO was added to the cells. Then, we shook the plates for 30 min at room temperature, and measured the absorbance at 540 nm on a scanning multi-well spectrophotometer.

2.2.5. Western blot analysis

Cells were washed with PBS, suspended in an extraction buffer (Intron Biotechnology, Korea) on ice for 15 min, and clarified by centrifugation at 13,000 rpm for 20 min. Equal amounts of cell extract (20 μg) were resolved on approximately 8–10% SDS–PAGE gels, transferred to a nitrocellulose membrane and probed with 0.1 μg/mL of goat anti-human Ob-R (Santa Cruz Biotechnology), rabbit anti-human STAT-3, phospho-STAT-3, ERK1/2, and phospho-ERK1/2 (Cell Signaling Technology, Beverly, MA, USA). After washing and incubation with the appropriate secondary antibody (1:2000 dilution) in 1% skimmed milk/Tris-based saline with 0.05% Tween-20 (TBST) for 2 h at room temperature, immunoreactive

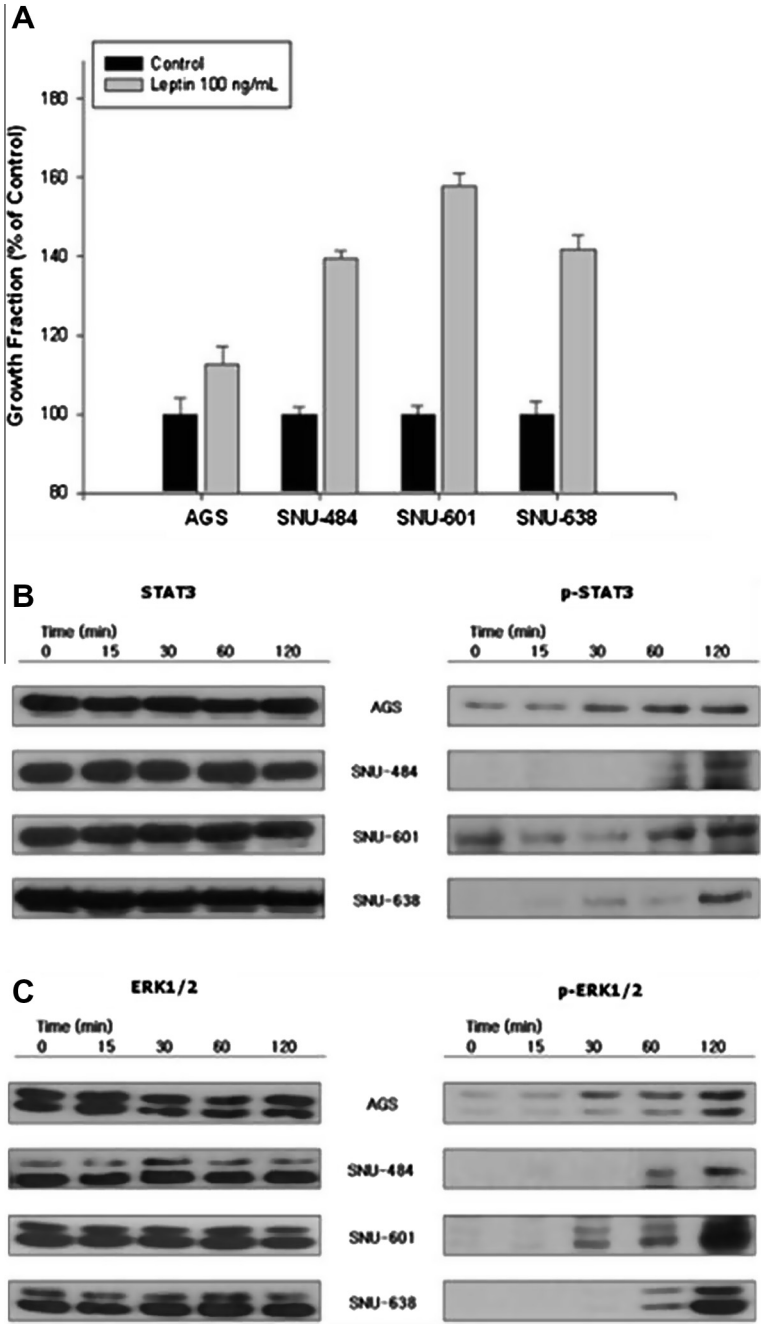

Fig. 2. Effect of leptin on the proliferation of human gastric cancer cells (A). Leptin-induced phosphorylation of STAT3 (B). Leptin-induced activation of ERK1/2 (C).

proteins were visualized by the WEST-ZOL™ Western blot detection system (Intron Biotechnology).

2.2.6. RNAi experiments

Stealth RNAi complementary to the Ob-R genes (GenBank accession Nos. U4368 and U52914; 5′-GGAGCAGCCTTTACTGTTCTG ATAT-3′) was designed using Stealth™ RNA from Invitrogen. The cells were transfected with 100 nM of RNAi, Lipofectamine™ reagent, and Lipofetamine 2000 according to the manufacturer's instructions. They were serum-deprived overnight and exposed to 100 ng/mL leptin for 72 h. Transfection efficiency was monitored by RT-PCR with Ob-R expression.

2.2.7. Statistical analysis

Data were analyzed using Student's *t*-test. p values <0.05 were considered to be statistically significant.

3. Results

3.1. Tissue samples study

Tissue specimens were obtained from 38 patients with gastric adenoma, 38 patients with EGC and 38 patients with AGC.

3.1.1. Leptin expression

In normal gastric tissue, leptin was expressed mainly in chief and parietal cells not in epithelial cells (Fig. 1A). In gastric adenoma, it was expressed more locally than in normal gastric mucosa (Fig. 1B). Leptin expression in EGC and AGC tissues is shown in Fig. 1C and D. Leptin expression was not significantly different in the three groups: 42.1% (16/38) of gastric adenomas, 47.4% (18/38) of EGC tissues, and 43.4% (17/38) of AGC tissues (Table 1). Its

expression was strongly positive in 28.9% (11/38) of gastric adenomas and in 21.1% (8/38) of both EGC and AGC tissues.

3.1.2. Ob-R expression

In normal gastric tissue, Ob-R was strongly expressed in chief and parietal cells but not expressed in epithelial cells (Fig. 1E). By comparison, in gastric adenoma, it was expressed locally (Fig. 1F). Ob-R expression in EGC and AGC tissues is shown in Fig. 1G and H. The frequency of Ob-R expression tended to increase from adenoma through EGC to AGC (2.6% (1/38), 7.8% (3/38), and 18.4% (7/38)) (Table 1).

3.1.3. Association between leptin and Ob-R expression and clinicopathological features

The association between the expression of leptin and Ob-R and the clinicopathological characteristics of the patients with AGC and EGC are shown in Table 2 and Supplementary Table 2, respectively. Between leptin-positive and -negative patients with EGC and AGC, no significant correlation was identified according to demographic factors and factors indicating tumor invasiveness including tumor size and differentiation. Between Ob-R-positive and -negative AGC patients, there were no differences with respect to these variables.

However, leptin expression in patients with AGC was associated with the location of the cancer: all cases of AGC in the cardia showed leptin positivity, compared to only half of all non-cardia cases (4/4 vs. 13/34, $p = 0.032$). Furthermore, Ob-R expression in patients with AGC was higher in differentiated than undifferentiated cancer (5/12 vs. 2/26, $p = 0.022$).

3.2. Cell culture experiments

3.2.1. Ob-R is expressed in gastric cancer cells

In an analysis of total RNA extracted from six gastric cancer cell lines by RT-PCR, Ob-R and its short and long isoforms (Ob-Ra and Ob-Rb) were expressed in the AGS, SNU-484, SNU-601 and SNU-638 gastric cell lines (Supplementary Fig. 1A). Ob-R proteins were also identified by Western blotting (Supplementary Fig. 1B).

3.2.2. Leptin stimulates the proliferation of gastric cancer cells

Growth of gastric cancer cell lines (AGS, SNU-484, SNU-601 and SNU-638) significantly increased over 72 h after treatment with 100 ng/mL of leptin than in the absence of leptin (Fig. 2A).

3.2.3. Leptin activates STAT3 and ERK1/2 in gastric cancer cells

Phosphorylated but not total STAT3 and ERK1/2 increased over time after leptin treatment (Fig. 2B and C).

3.2.4. Pharmacological inhibitors decrease STAT3 and ERK1/2 in leptin-treated gastric cancer cells

When the gastric cancer cells were treated with pharmacological agents that specifically inhibit the phosphorylation of STAT3 and ERK1/2 (50 μM AG490 and 25 μM PD98059, respectively), phosphorylated but not total STAT3 and ERK1/2 and the proliferation of gastric cancer cells decreased (Fig. 3A and B).

3.2.5. Lepin-Induced proliferation of gastric cancer cells is inhibited by blockade of Ob-R using RNAi

When gastric cancer cells were transfected with 100 nM RNAi to block Ob-R, treatment with 100 ng/mL leptin failed to stimulate their growth (Fig. 4A), and phosphorylated STAT3 and ERK1/2 levels also decreased (Fig. 4B and C).

3.2.6. The effects of leptin are mediated by vascular endothelial growth factor (VEGF) in gastric cancer cells

When gastric cancer cells were treated with 100 ng/mL leptin, the level of VEGF increased and this increase was prevented by RNAi against Ob-R (Supplementary Fig. 2).

4. Discussion

In the present study to investigate the role of leptin in gastric cancer, we have shown that leptin is closely associated with gastric cancer. Our result showed that leptin receptor, Ob-R, was associated with the advanced and differentiated forms of gastric cancer: Ob-R expression was higher in EGC than in adenomas, and higher still in AGC. Furthermore, in AGC, Ob-R was detected more frequently in differentiated than in undifferentiated forms of cancer, which is consistent with previous studies reporting that the level of Ob-R was down-regulated in the less differentiated cancers [21,24,27], suggesting that Ob-R production may cease during the process of de-differentiation of gastric cancers. Also, leptin expression varied according to the cancer location: it was positive in all cancers of the cardia, but only in a third of all non-cardia cancers. This finding is consistent with that of epidemiological studies showing that proximal gastric cancer is related to obesity [21], and supports the hypothesis that gastric cardia cancers may have different tumor biology from non-cardia cancers.

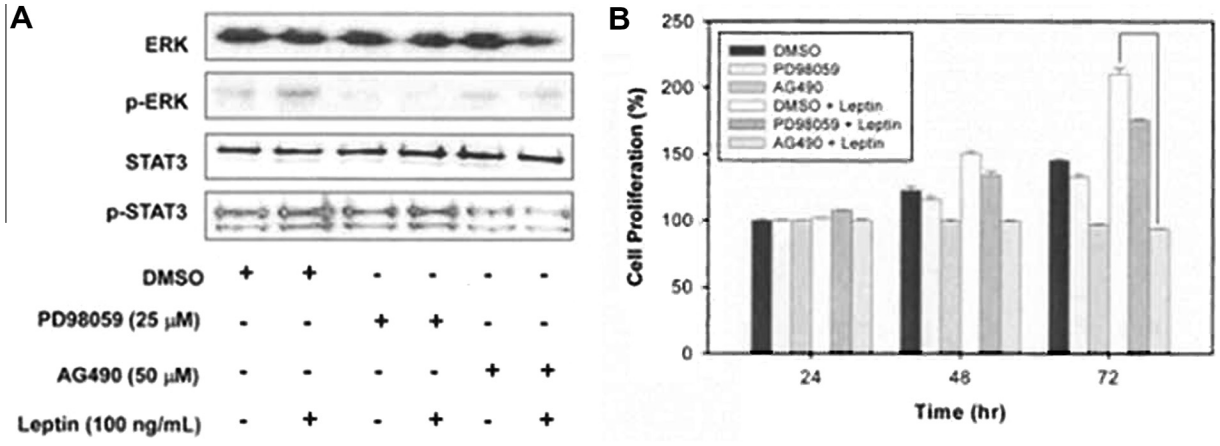

Fig. 3. Effect of pharmacological inhibitors on leptin-induced proliferation of human gastric cancer cells. Gastric cancer cells were pre-treated with PD98059 or AG490, incubated with leptin, and lysates were immunoblotted with antibodies against total and phosphorylated STAT3 and ERK1/2 (A). MTT assays were performed and showed pharmacologic inhibition of the proliferation of gastric cancer cells ($p < 0.05$) (B).

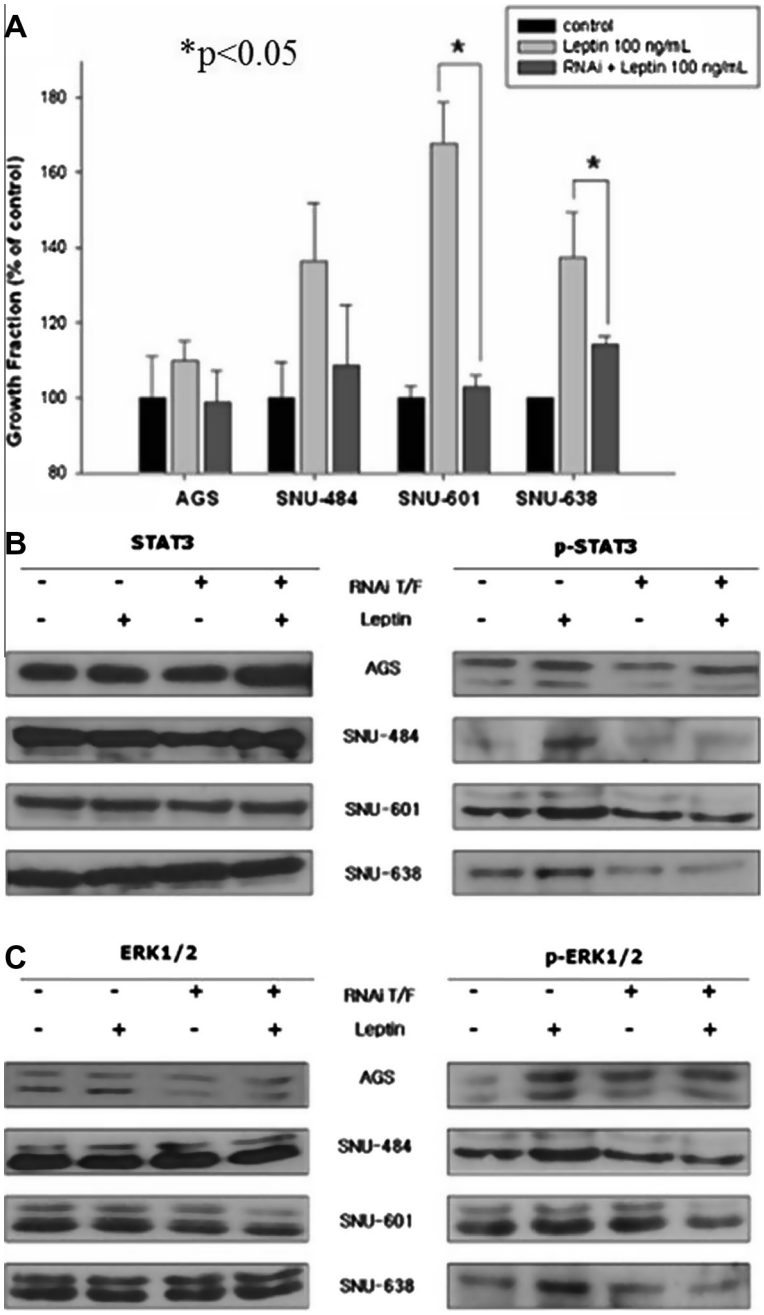

Fig. 4. RNAi inhibition of leptin-induced proliferation of gastric cancer cells (A). RNAi inhibition of leptin-induced STAT3 phosphorylation in gastric cancer cells (B). RNAi inhibition of leptin-induced ERK1/2 phosphorylation in gastric cancer cells (C).

Leptin expression in malignant tissues has been demonstrated previously. In colorectal cancer, leptin expression increased progressively from normal mucosa to adenoma, and then to cancer [26]. Although such a progressive relationship was not found in our study, we found a high rate of leptin expression in gastric adenomas and cancers. Previously, leptin was found to be associated with gastric cancers with a more aggressive biology [21]. Leptin was expressed more strongly in more advanced tumors with poorer survival [25]. However, this was not the case in our study, where factors related to cancer invasiveness were not significantly associated with leptin expression. Conversely, although previous studies reported a strong correlation between leptin and Ob-R expression, our data showed only a weak correlation [21]. While this may be due to the lower rate of Ob-R positivity in gastric cancer tissues in the present study, it can also suggest that there are other pathways that stimulate Ob-R in gastric cancer. Taken together, our findings suggest that leptin may affect gastric cancer through leptin signaling and further support the link between leptin and gastric cancer identified in vivo.

Leptin acts via its specific receptor, Ob-R. Among its six isoforms, the long isoform of Ob-R is responsible for activating intracellular signaling pathways. In the present study, expression of both isoforms, Ob-Ra and Ob-Rb, was observed in the gastric cancer cell lines. Additionally, leptin treatment increased the proliferation of gastric cancer cells, an effect that was associated with phosphorylation of STAT3 and ERK1/2. Moreover, the leptin-induced proliferation decreased, when phosphorylation of STAT3 and ERK1/2 was inhibited by the appropriate pharmacological

inhibitors and Ob-R production was blocked by transfection of RNAi, as shown by reduced phosphorylation of STAT3 and ERK1/2. Jointly, these results indicate that the activation of the STAT3 and ERK1/2 pathways by leptin is involved in the proliferation of gastric cancer cells.

Activation of the STAT3 and ERK1/2 pathways has been reported to promote the proliferation of various cancers [18,19]. By activating these pathways, leptin stimulated the growth and invasiveness of endometrial cancers [28], and STAT3-mediated leptin signaling was demonstrated to be involved in the adenoma-carcinoma sequence in colorectal cancers [29]. Leptin-STAT3 signaling pathways also mediated the proliferative and survival effects on colon cancer in leptin- and Ob-R-deficient animal models. Similarly, Ob-R expression was shown to decrease or increase in response to β-catenin knockdown or Wnt stimulation, two well-known factors associated with colon cancer development [14]. Leptin binding to STAT3 plays a critical role in liver cancer development by inducing telomerase reverse transcriptase [30]. Furthermore, leptin-induced STAT3 phosphorylation developed in a time- and dose-dependent manner in ovarian cancer cells [31]. Recent studies to investigate leptin-signaling mechanisms using a model of gastric tumorigenesis concluded that activation of STAT3 plays a crucial role in the development of gastric cancer [32]. Moreover, the STAT3 and ERK2 pathways were found to independently stimulate the growth of gastric cancer cells [33].

We obtained some evidence that VEGF mediates the effects of leptin on gastric cancer cells. VEGF expression was not only higher in leptin-treated than in leptin-untreated cancer cells, but it was also reduced when leptin was blocked with RNAi. VEGF are known to mediate angiogenesis, which facilitates cancer progression, invasion, and metastasis. In the presence of VEGF, leptin-mediated angiogenesis played an important role in liver cancer [34]. Leptin-induced angiogenesis seems to be related to Ob-R in vascular endothelial cells [4], which induce angiogenesis through matrix metalloproteinases [35]. Additionally in colon cancer cells, leptin and VEGF expression was stimulated by the STAT3 pathways [36]. Taken together, our results imply that leptin may affect gastric cancer cells via VEGF and therefore, that Ob-R blockade prevents angiogenesis in leptin-mediated carcinogenesis by suppressing VEGF.

In summary, our study suggests that leptin and Ob-R play a role in gastric cancer by the STAT3 and ERK1/2 signaling pathways responsible for the leptin-induced proliferation of gastric cancer cells. Consequently, treatments targeting leptin-associated signaling pathways could be potential strategies for managing gastric cancer, although larger studies are necessary to confirm it.

Conflict of interest

None of the authors have any conflict of interest to report.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.02.072.

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