ORIGINAL ARTICLE

Effects of Compounded Human Ghrelin in a Mouse Model of Pancreatic Carcinoma

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ABSTRACT

Ghrelin is a peptide that is secreted from the stomach and plays a role in appetite, weight gain, and skeletal muscle composition. Thus, compounded human ghrelin is a candidate drug for improving nutritional status after pancreatic surgery. However, in patients with pancreatic carcinomas, adverse influences on the occult tumor growth of ghrelin-induced secretion are a concern. The present study describes the effects of the administration of compounded human ghrelin on weight gain and pancreatic cancer cell growth in a mouse model. Changes in body weight and tumor growth in a subcutaneously transplanted pancreatic carcinoma cell line *in vivo* (5-week-old BALB/c-nu/nu mice) were examined with or without the administration of compounded human ghrelin. Compounded human ghrelin was administered at 44 days after post-transplantation" Changes in weight were not significantly different between the control and compounded human ghrelin groups 8 days after compounded human ghrelin administration, and no association between weight and concentration of compounded human ghrelin was identified. Tumor growth after the administration of compounded human ghrelin was significantly lower than that of the control group, with the magnitude of the decrease being associated with increasing compounded human ghrelin concentration ($p<0.05$). At 6 and 8 days after compounded human ghrelin administration, increases in tumor weights of the control groups (0.5±0.3 g and 0.9±0.2 g, respectively) were significantly greater than those observed for groups receiving 3, 15, and 30 nmol per kg of compounded human ghrelin (0.1 and 0.2, 0.2 and 0.3, and 0.2 and 0.3, respectively). There were no adverse effects of compounded human ghrelin administration. Plasma leptin levels were significantly lower in cancer cells compared with the control vehicle ($p<0.05$), which was decreased in mice receiving 30nmol per kg of compounded human ghrelin in comparison with those receiving vehicle ($p<0.05$). Although the administration of compounded human ghrelin did not influence weight gain, compounded human ghrelin significantly inhibited pancreatic cancer cell growth and might inhibit plasma leptin levels.

INTRODUCTION

In pancreatic cancer patients, weight loss and malnutrition are evident after pancreatic resection due to the advanced tumor stage and the invasiveness of radical surgery [1,2]. To promote early recovery after major surgery, nutritional and hormonal forms of support are necessary during the perioperative period. However, such effective supportive treatments have not been established to date.

Ghrelin was discovered as an intrinsic ligand for the growth hormone secretagogue receptor (GHSR) in 1999 by Kojima *et al*. and Kangawa *et al*. [3, 4]. Endogenous ghrelin is primarily produced in the stomach. Ghrelin has multiple functions, such as exerting orexigenic effects on the hypothalamus or gastrointestinal motility, stimulating growth hormone secretion, performing anti-inflammatory activities, and strengthening skeletal muscle, as well as various other metabolic functions [3-11]. In particular, ghrelin is a powerful gastrointestinal appetite-stimulating hormone, a function that is regulated by the circadian rhythm [12]. Recently, clinical trials of compounded human ghrelin (CHG) were undertaken to increase oral feeding and weight gain and induce early recovery and anti-inflammatory protection after invasive

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Abbreviations BALB an albino, laboratory-bred strain of the house mouse; CHG compounded human ghrelin; MIA-PaCa2 pancreatic carcinoma cell line
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surgeries, such as gastrectomy and esophagectomy, in cancer patients [13-16]. In the fields of biliary and pancreatic surgery, we also undertook a clinical trial for CHG in patients who underwent major hepatectomy and pancreatectomy (in press). However, in many of these patients, occult cancer cells may remain, even though a radical operation has been performed, leading to a higher rate of recurrence in comparison with that of patients with gastrointestinal carcinomas [17, 18]. Ghrelin has been used for the improvement of cancer- or chemotherapy-related cachexia [19, 20]; therefore, this peptide is predicted not to have adverse effects on cancer progression. However, the precise mechanism for this phenomenon has not been fully clarified to date. As ghrelin also has other functions, including stimulation of pancreatic secretion [21, 22], the influence of CHG administration on pancreatic tumor growth is concerning. We must clarify whether CHG stimulates cancer cell growth before using it in future clinical applications.

Thus, in the present study, we examined the changes in body weight and tumor growth associated with pancreatic carcinoma cell lines transplanted into mice and compared these variables between groups receiving either control vehicle or CHG in an *in vivo* mouse model.

MATERIALS AND METHODS

Tumor Xenograft and CHG Administration Protocol

Five-week-old BALB/c-nu/nu mice (body weight, 14-16 g; CLEA Japan, Inc., Tokyo, Japan) were used in this study. Mice were housed 2-3 per plastic cage at 23±2 °C under a 12-hour light-dark cycle. Water and food (CRF-1; Oriental Yeast Co., Tokyo, Japan) were freely available.

In these experiments, 1×10^7 MIA-PaCa2 (pancreatic carcinoma) cells [23] suspended in 150 μL of Dulbecco's Modified Eagle's Medium were inoculated subcutaneously into the hemi-lateral abdomen of mice (n=24). In the control group, only Dulbecco's Modified Eagle's Medium was injected (n=24). The maximum and minimum axes of the produced tumors were precisely measured by the Vernier caliper, and due to difficulties in measuring tumor volume, the tumor weight was calculated using the following formula [24] because of difficulty of measuring tumor volume itself in each group:

tumor weight (g) = [(maximum axis (cm)) × (minimum axis (cm))^2]/2

Body weight and tumor growth (size or weight) at 6 and 8 days after CHG administration were measured.

CHG (Peptide Institute, Osaka, Japan) was injected intraperitoneally when the mean body weight of the mice began to decrease. Animals of the same weight were allocated to either a control group or one of four groups for CHG administration (at the time of CHG administration; 44 days after inoculation in experimental models). For the CHG groups, 0, 3, 15, or 30 nmol/kg CHG dissolved in 100 μL of saline was injected twice daily (at 10:00 h and 18:00 h) for 6 days. Forty-eight hours after the final injection, blood was taken from the vena cava under general anesthesia, and the heparinized blood was centrifuged at 3000 rpm for 15 minutes to obtain plasma. Plasma leptin concentrations were measured using an ELISA kit (Morinaga Institute of Biological Science Inc., Yokohama, Japan).

Statistical Analysis

Data are expressed as the mean ± standard deviation (SD). Statistical significance was determined by two-way repeated-measures ANOVA, one-way factorial ANOVA, unpaired t-test, and multiple comparison Tukey's test using the statistical package SPSS Statistics 19 (IBM, NY, NY, USA). A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

During the *in vivo* experiments, 5 vehicle mice and 3 mice transplanted with MIA-PaCa2 cells exhibited dermatitis and were therefore excluded from the study. In total, 19 vehicle and 21 MIA-PaCa2 mice were used for the present study.

Figure 1 shows the changes in body weight over the course of 44 days for the groups with and without inoculation of MIA-Paca2 cell lines. There were no significant differences between the groups. Variability of calculated tumor weight was 0.4-4.3g in non-CHG group, 0.7-1.7g in the 3nmol/kg group, 0.2-2.5g in the 15nmol/kg group and 0.3-2.6g in the 30 nmol/kg group. There were no significant differences of tumor weight before CHG administration between groups. **Figure 2** shows the changes in body weight for 8 days after CHG injection; no changes in body weight were identified between the groups. No side effects of CHG were observed in any mice. **Figure 3** shows that the group that did not receive CHG had increasing tumor growth (size); in contrast, in the CHG-administered groups, this growth was significantly lower ($p<0.05$). Furthermore, the inhibition of tumor growth was more significant with increasing concentrations of CHG. The increase in tumor growth at days 6 and 8 was inhibited by all doses of CHG administered, as shown in **Figure 4**. Compared with the control (no treatment), the tumor weight for MIA-PaCa2 cells was significantly lower upon administration of 3, 15, and 30 nmol/kg CHG: for 3 nmol/kg (0.5±0.15 g), 15 nmol/kg (0.2±0.1 g), and 30 nmol/kg (0.2±0.1%) at 6 days; and for 3 nmol/kg (0.9±0.2 g), 15 nmol/kg (0.3±0.15), and 30 nmol/kg (0.3±0.1%) at 8 days. There were no significant differences between the doses of CHG that were administered. In **Figure 5**, plasma leptin concentrations at day 8 are shown. With 0 nmol/kg CHG, leptin concentrations were significantly lower in vehicle mice than in mice inoculated with MIA-PaCa2 cells ($p<0.05$). Although leptin concentrations did not change with administration of CHG doses up to 15 nmol/kg, leptin concentrations were significantly decreased in tumor-bearing mice after administration of 30 nmol/kg CGH in comparison with mice receiving 0 nmol/kg CGH ($p<0.05$).

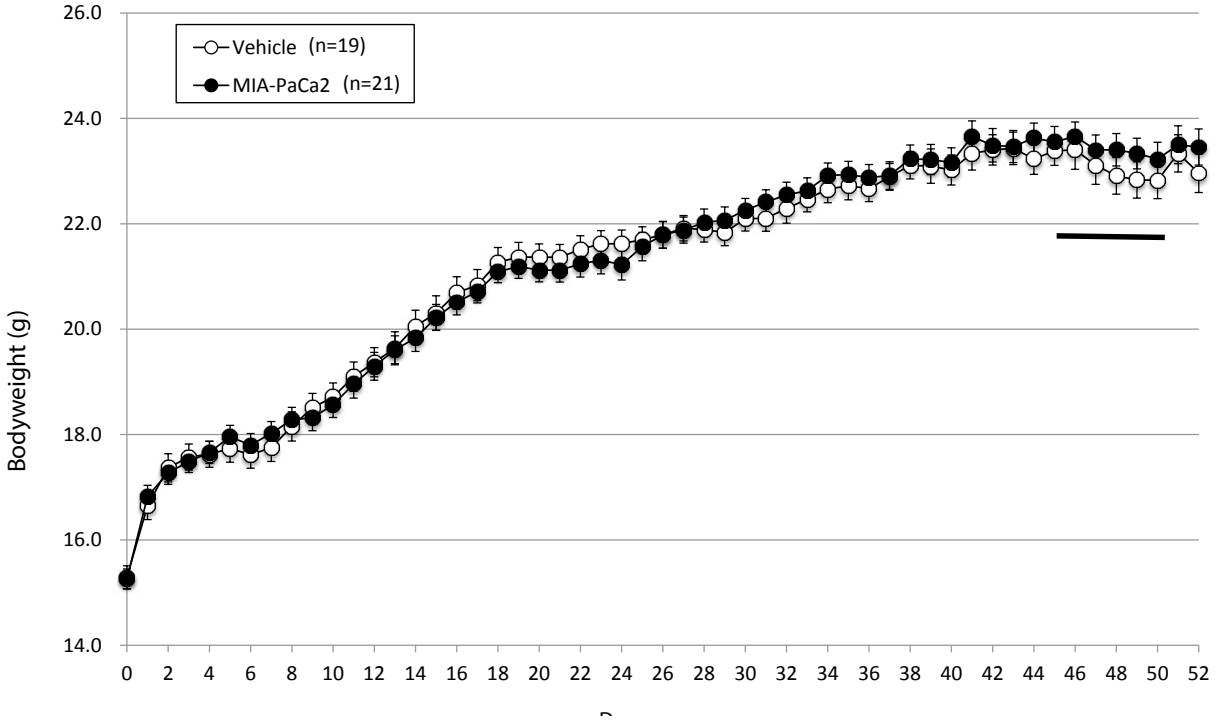

Figure 1. *In vivo* model. Five-week-old BALB/c-nu/nu mice were transplanted subcutaneously with MIA-PaCa2 cells leading to the formation of pancreatic tumors in the back. Comparison of changes in body weight after cell transplantation between the control group (n=19) (open circles) and transplanted mice (n=21) (closed circles).

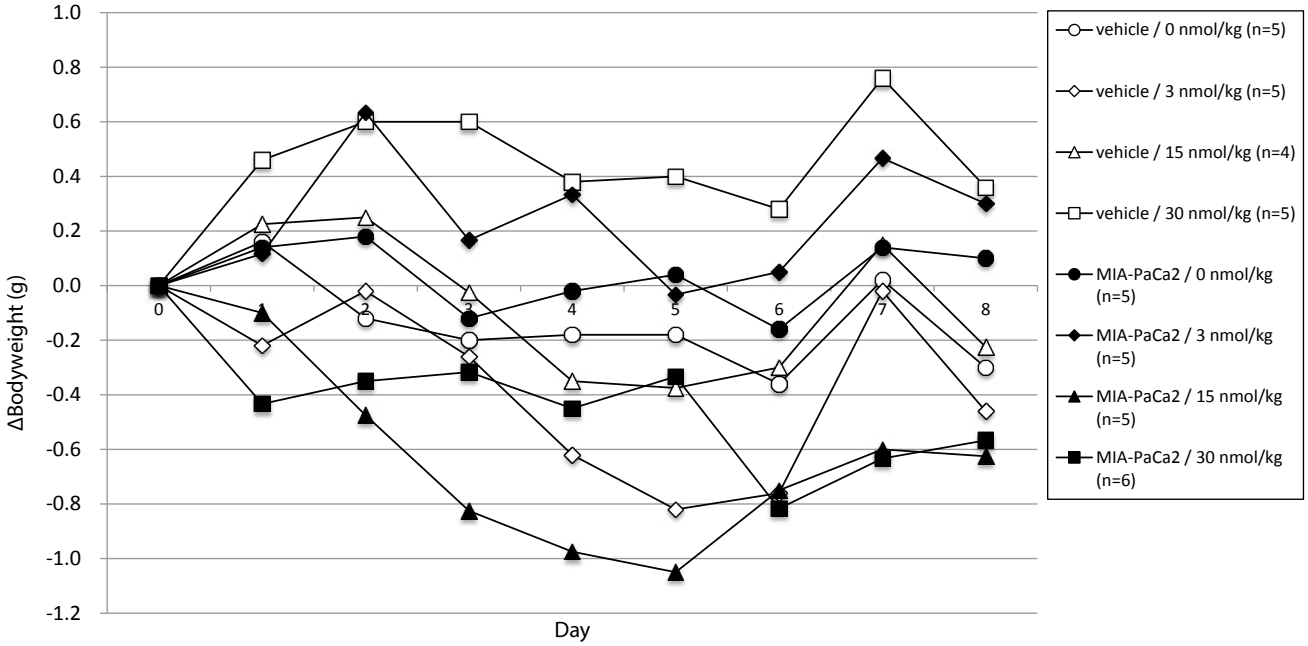

Figure 2. Changes in body weight of the control and transplanted groups receiving various concentrations of CHG for 8 days after the final injection.

DISCUSSION

Although the role of ghrelin as an orexigenic hormone has long been documented, its mechanism is still debated. The role of ghrelin in cancer induction has not been disputed, but its characterization as a selective inhibitor of tumor growth is controversial. This study attempted to solve the mystery behind this hormone. Ghrelin induces pancreatic endocrine and exocrine secretion via the brain-gut axis system [21, 22, 25], as well as pancreatic cellular proliferation and growth hormone secretion [3-11, 25]. Thus, the stimulation of occult cancer cell proliferation by ghrelin administration is a clinical concern. Before using ghrelin to enhance a patient's nutritional recovery after pancreatic resection for malignant diseases, in the present study, we undertook to examine the adverse effects of CHG on pancreatic cancer cells. Previous studies have reported that ghrelin was used to improve appetite loss in patients with cachexia who underwent treatment with anti-cancer drugs; however, its influences on tumor progression or tumor inhibition have not been discussed to date.

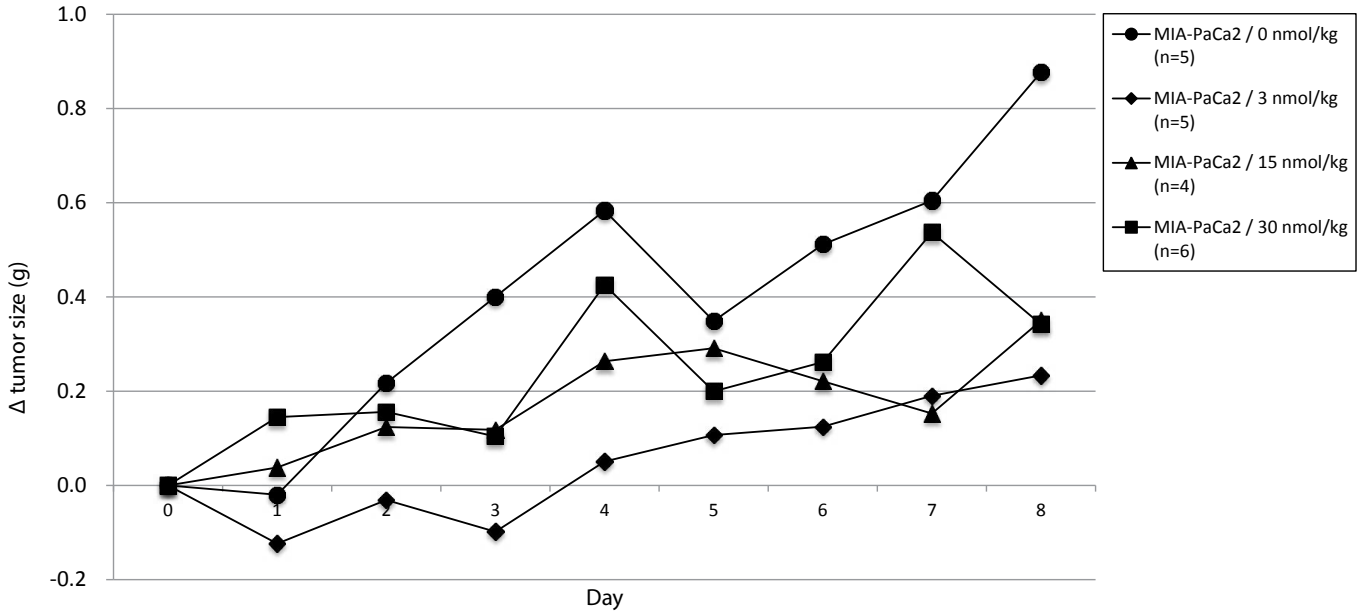

Figure 3. Changes in tumor weight of only the transplanted groups and transplanted groups receiving various concentrations of CHG for 8 days after the final injection.

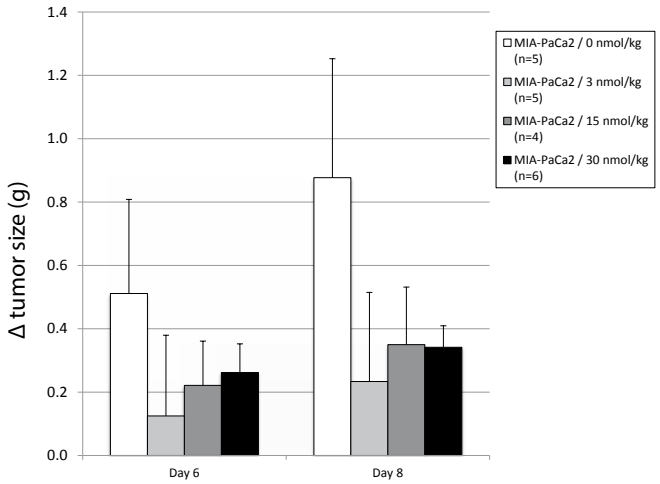

Figure 4. Changes in tumor size of only the transplanted groups and transplanted groups receiving various concentrations of CHG at 6 and 8 days after the final injection.

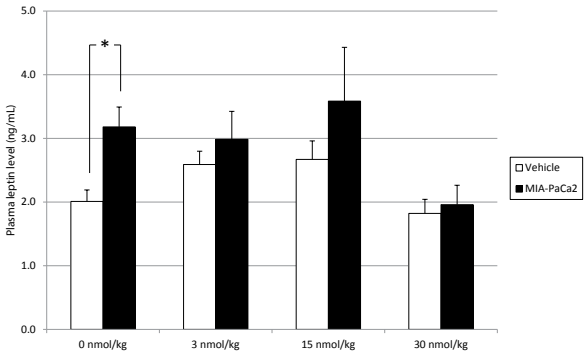

Figure 5. Relationship between plasma leptin levels and the administered CHG concentration of the control and transplanted groups.

With respect to tumor progression, investigators have reported that ghrelin increased cancer progression or affected cancer invasiveness in neuroendocrine tumors or carcinomas of the kidney, colorectum, esophagus, stomach, prostate, mammary gland, uterus, ovary, pancreas, and thyroid [26-42]. Among these reports, Duxbury *et al*. described that ghrelin promoted cellular proliferation and invasion of a pancreatic carcinoma cell line [39]. This cell line expresses the ghrelin receptor. This manuscript indicated that pancreatic carcinoma is a ghrelin-responsive malignancy. Conversely, other investigators demonstrated that ghrelin inhibited tumor progression or prevented carcinogenesis in the colorectum, breast, ovary, esophagus, stomach, and kidney [43-54] or did not affect cancer progression [55-57]. Therefore, the mechanism by which ghrelin administration influences cancer progression or invasion remains controversial. The effects of growth hormone and its modulation by ghrelin might be associated with tumor growth [58], while on the other hand, ghrelin might produce anti-inflammatory cytokine responses [59]. Kawaguchi *et al*. clarified the effect of tumor suppression by anti-inflammatory responses in inflammation-based colon carcinoma [43]. Therefore, the effect of CHG in various carcinomas is most likely different. Our results in the present study show that CHG significantly suppressed the growth of pancreatic cancer cells (MIA-PaCa2) in a concentration-dependent manner. Therefore, in the case of pancreatic cancer patients undergoing chemotherapy or upon the appearance of occult pancreatic cancer cells after surgery, the administration of CHG may not produce adverse effects that induce cancer progression. Therefore, CHG is a clinically useful drug that not only improves cachexia and nutrition but also inhibits cancer growth. To clarify such effects, the model used for this study needs to be examined further with respect to additional variables.

Leptin is a regulator peptide that controls appetite and its effects are opposite to those of ghrelin [60]. In the present study, the plasma leptin levels in the cancer model were significantly lower in comparison with those in the control. Previous reports suggest that leptin might

be increased in some cancers [61-63]. Although each peptide may influence assimilation or catabolism [64], direct ghrelin-induced effects on leptin have not been reported to date. Administration of low levels of CHG did not change plasma leptin levels. However, leptin levels were decreased in the cancer model by high concentration of CHG. Potential reasons for this include the following: 1) Ghrelin might affect adipose cells, leading to inhibition of leptin secretion. 2) Ghrelin also may affect cancer cells, leading to inhibition of tumor progression, which results in blocking of leptin production in cancer cells. 3) Ghrelin may also affect the immune system by decreasing the production of TNF-α TNF- α, which inhibits leptin section [65-68]. Thus, ghrelin might decrease leptin levels, as previous reports suggest [69]. The relationship between these peptides remains controversial; therefore, further study is necessary to clarify the mechanisms of these peptides' effects on cancer growth.

In addition, we expected to observe an increase in body weight upon CHG administration in the pancreatic cancer model in the present study. As described above, previous reports suggested that ghrelin improved appetite or weight gain in patients with advanced-stage cancers [3-11, 21, 22, 25]. In the present model, differences in weight change between the control group and the pancreatic cancer model were not significant. Thus, the use of a more advanced cachexia model is necessary. In addition, the effect of CHG on body weight was also not significant. To optimize the effect of CHG on body weight, a severe cachexia model of pancreatic cancer will be a necessary next step. Controlled study models need to elucidate the cause-effect relationship in the future step.

In conclusion, tumor progression of pancreatic cancer was significantly inhibited by ghrelin *in vivo*. The use of ghrelin as treatment for nutritional improvement in patients with malnutrition is thus expected not to promote pancreatic cancer progression.

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Conflict of Interest

The authors have no conflict of interest to declare

References

1. Cooper C, Burden ST, and Molassiotis A. An explorative study of the views and experiences of food and weight loss in patients with operable pancreatic cancer perioperatively and following surgical intervention. Support Care Cancer 2015; 23:1025-1033. [PMID: 25277960]

2. Tisdale MJ. Metabolic abnormalities in cachexia and anorexia. 2000; Nutrition 16:1013-1014. [PMID: 11054609]

3. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402:656-660. [PMID: 10604470]

4. Kojima M, Hosoda H, Matsuo H, Kangawa K. Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 2001; 12:118-122. [PMID: 11306336]

5. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, et al. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology 2001; 120:337-345. [PMID: 11159873]

6. Muccioli G, Tschöp M, Papotti M, Deghenghi R, Heiman M, Ghigo E. Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. Eur J Pharmacol 2002; 440:235-254. [PMID: 12007539]

7. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology 2000; 141:4797-4800. [PMID: 11108296]

8. Sibilia V, Lattuada N, Rapetti D, Pagani F, Vincenza D, Bulgarelli I, Locatelli V, Guidobono F. Ghrelin inhibits inflammatory pain in rats: involvement of the opioid system. Neuropharmacology 2006; 51:497-505. [PMID: 16759671]

9. Pamukcu O, Kumral ZN, Ercan F, Yegen BC, Ertem D. Anti-inflammatory effect of obestatin and ghrelin in dextran sulfate sodium-induced colitis in rats. J Pediatr Gastroenterol Nutr 2013; 57:211-218. [PMID: 23549326]

10. Nass R, Pezzoli SS, Oliveri MC, Patrie JT, Harrell FE Jr, Clasey JL, Heymsfield SB, Bach MA, et al. Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. Ann Intern Med 2008; 149:601-611. [PMID: 18981485]

11. Sirotkin AV, Grossmann R. Interrelationship between feeding level and the metabolic hormones leptin, ghrelin and obestatin in control of chicken egg laying and release of ovarian hormones. Comp Biochem Physiol A Mol Integr Physiol 2015; 184:1-5. [PMID: 25645297]

12. Patton DF, Mistlberger RE. Circadian adaptations to meal timing: neuroendocrine mechanisms. Front Neurosci 2013; 7:185. [PMID: 24133410]

13. Takachi K, Doki Y, Ishikawa O, Miyashiro I, Sasaki Y, Ohigashi H, Murata K, Nakajima H, et al. Postoperative ghrelin levels and delayed recovery from body weight loss after distal or total gastrectomy. J Surg Res 2006; 130:1-7. [PMID: 16182310]

14. Doki Y, Takachi K, Ishikawa O, Miyashiro I, Sasaki Y, Ohigashi H, Nakajima H, Hosoda H, et al. Ghrelin reduction after esophageal substitution and its correlation to postoperative body weight loss in esophageal cancer patients. Surgery 2006; 139:797-805. [PMID: 16782437]

15. Takiguchi S, Takata A, Murakami K, Miyazaki Y, Yanagimoto Y, Kurokawa Y, Takahashi T, et al. Clinical application of ghrelin administration for gastric cancer patients undergoing gastrectomy. Gastric Cancer 2014; 17:200-205. [PMID: 24253567]

16. Takiguchi S, Murakami K, Yanagimoto Y, Takata A, Miyazaki Y, Mori M, Doki Y. Clinical application of ghrelin in the field of surgery. Surg Today 2015; 45:801-807. [PMID: 25366350]

17. Seicean A, Petrusel L, Seicean R: New targeted therapies in pancreatic cancer. World J Gastroenterol 2015; 21:6127-6145. [PMID: 26034349]

18. He XY, Yuan YZ. Advances in pancreatic cancer research: moving towards early detection. World J Gastroenterol 2014; 20:11241-11248. [PMID: 25170208]

19. Molfino A, Formiconi A, Rossi Fanelli F, Muscaritoli M. Ghrelin: from discovery to cancer cachexia therapy. Curr Opin Clin Nutr Metab Care 2014; 17:471-476. [PMID: 24905862]

20. Argilés JM, Stemmler B. The potential of ghrelin in the treatment of cancer cachexia. Expert Opin Biol Ther 2013; 13:67-76. [PMID: 23078025]

21. Yada T, Damdindorj B, Rita RS, Kurashina T, Ando A, Taguchi M, Koizumi M, Sone H, et al. Ghrelin signaling in β-cells regulates insulin secretion and blood glucose. Diabetes Obes Metab. 16 :111-117, 2014. [PMID: 25200304]

22. Chandra R, Liddle RA. Modulation of pancreatic exocrine and endocrine secretion. Curr Opin Gastroenterol 2013; 29:517-522. [PMID: 23817137]

23. Clerc P, Bensaadi N, Pradel P, Estival A, Clemente F, Vaysse N. Lipid-dependent proliferation of pancreatic cancer cell lines. Cancer Res 1991; 51:3633-3638. [PMID: 2065320]

24. Iseki H, Kajimura N, Ohue C, Tanaka R, Akiyama Y, Yamaguchi K. Cytokine production in five tumor cell lines with activity to induce cancer cachexia syndrome in nude mice. Jpn J Cancer Res 1995; 86:562–567. [PMID: 7622421]

25. Müller TD, Nogueiras R, Andermann ML, Andrews ZB, Anker SD, Argente J, Batterham RL, et al. Ghrelin. Mol Metab 2015; 4:437-460. [PMID: 26042199]

26. Lin TC, Liu YP, Chan YC, Su CY, Lin YF, Hsu SL, Yang CS, Hsiao M. Ghrelin promotes renal cell carcinoma metastasis via Snail activation and is associated with poor prognosis. J Pathol 2015 237:50-61. [PMID: 25925728]

27. Bułdak RJ, Pilc-Gumuła K, Bułdak Ł, Witkowska D, Kukla M, Polaniak R, Zwirska-Korczala K. Effects of ghrelin, leptin and melatonin on the levels of reactive oxygen species, antioxidant enzyme activity and viability of the HCT 116 human colorectal carcinoma cell line. Mol Med Rep 2015; 12:2275-2282. [PMID: 25873273]

28. Nikolopoulos D, Theocharis S, Moutsios-Rentzos A, Kouraklis G, Kostakis A. The role of serum total ghrelin level elevation in colon cancer patients. J BUON 2014; 19:388-393. [PMID: 24965396]

29. Omoto I, Matsumoto M, Uchikado Y, Kita Y, Sakurai T, Sasaki K, Setoyama T, Okumura H, et al. Immunohistochemical evidence of association between ghrelin expression and tumor growth in esophageal carcinoma. Anticancer Res 2014; 34:2727-2733. [PMID: 24922633]

30. Sadjadi A, Yazdanbod A, Lee YY, Boreiri M, Samadi F, Alizadeh BZ, Islami F, Fyfe V,et al. Serum ghrelin; a new surrogate marker of gastric mucosal alterations in upper gastrointestinal carcinogenesis. PLoS One 2013; 8:e74440. [PMID: 24098650]

31. Tian C, Zhang L, Hu D, Ji J. Ghrelin induces gastric cancer cell proliferation, migration, and invasion through GHS-R/NF-κB signaling pathway. Mol Cell Biochem 2013; 382:163-172. [PMID: 23807739]

32. Seim I, Lubik AA, Lehman ML, Tomlinson N, Whiteside EJ, Herington AC, Nelson CC, Chopin LK. Cloning of a novel insulin-regulated ghrelin transcript in prostate cancer. J Mol Endocrinol 2013; 50:179-191. [PMID: 23267039]

33. Majchrzak K, Pawłowski KM, Orzechowska EJ, Dolka I, Mucha J, Motyl T, Król M. A role of ghrelin in canine mammary carcinoma cells proliferation, apoptosis and migration. BMC Vet Res 2012; 8:170. [PMID: 22999388]

34. Majchrzak K, Szyszko K, Pawłowski KM, Motyl T, Król M. A role of ghrelin in cancerogenesis. Pol J Vet Sci 2012; 15: 189-197. [PMID: 22708377]

35. Chopin L, Walpole C, Seim I, Cunningham P, Murray R, Whiteside E, Josh P, Herington A. Ghrelin and cancer. Mol Cell Endocrinol 2011; 40:65-69. [PMID: 21616120]

36. He XT, Fan XM, Zha XL. Ghrelin inhibits 5-fluorouracil-induced apoptosis in colonic cancer cells. J Gastroenterol Hepatol 2011; 26:1169-1173. [PMID: 21375586]

37. Dagli AF, Aydin S, Kocdor H, Gurates B, Sahin I, Catak Z, Ozercan MR, Ozercan IH. Ghrelin expression of endometrium hyperplasia and endometrioid carcinoma. Gynecol Endocrinol 2011; 27:199-204. [PMID: 20712427]

38. Markowska A, Ziółkowska A, Jaszczyńska-Nowinka K, Madry R, Malendowicz LK. Elevated blood plasma concentrations of active ghrelin and obestatin in benign ovarian neoplasms and ovarian cancers. Eur J Gynaecol Oncol 2009; 30:518-522. [PMID: 19899406]

39. Duxbury MS, Waseem T, Ito H, Robinson MK, Zinner MJ, Ashley SW, Whang EE. Ghrelin promotes pancreatic adenocarcinoma cellular proliferation and invasiveness. Biochem Biophys Res Commun 2003; 309: 464-468. [PMID: 12951072]

40. Volante M, Allìa E, Gugliotta P, Funaro A, Broglio F, Deghenghi R, Muccioli G, Ghigo E. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. J Clin Endocrinol Metab 2002; 87:1300-1308. [PMID: 11889202]

41. Papotti M, Cassoni P, Volante M, Deghenghi R, Muccioli G, Ghigo E. Ghrelin-producing endocrine tumors of the stomach and intestine. J Clin Endocrinol Metab 2001; 86:5052-5059. [PMID: 11600584]

42. Kanamoto N, Akamizu T, Hosoda H, Hataya Y, Ariyasu H, Takaya K, Hosoda K, Saijo M, et al. Substantial production of ghrelin by a human medullary thyroid carcinoma cell line. J Clin Endocrinol Metab 2001; 86:4984-4990. [PMID: 11600575]

43. Kawaguchi M, Kanemaru A, Fukushima T, Yamamoto K, Tanaka H, Haruyama Y, Itoh H, Matsumoto N, et al. Ghrelin administration suppresses inflammation-associated colorectal carcinogenesis in mice. Cancer Sci 2015; 106: 1130-1136. [PMID: 26094822]

44. Garcia JM, Chen JA, Guillory B, Donehower LA, Smith RG, Lamb DJ. Ghrelin Prevents Cisplatin-Induced Testicular Damage by Facilitating Repair of DNA Double Strand Breaks Through Activation of p53. Biol Reprod 2015; 93: 24. [PMID: 26019260]

45. Docanto MM, Yang F, Callaghan B, Au CC, Ragavan R, Wang X, Furness JB, Andrews ZB, et al. Ghrelin and des-acyl ghrelin inhibit aromatase expression and activity in human adipose stromal cells: suppression of cAMP as a possible mechanism. Breast Cancer Res Treat 2014; 147:193-201. [PMID: 25056185]

46. Xu Y, Pang X, Dong M, Wen F, Zhang Y. Ghrelin inhibits ovarian epithelial carcinoma cell proliferation in vitro. Oncol Rep 2013; 30:2063-2070. [PMID:]

47. Bonfili L, Cuccioloni M, Cecarini V, Mozzicafreddo M, Palermo FA, Cocci P, Angeletti M, Eleuteri AM. Ghrelin induces apoptosis in colon adenocarcinoma cells via proteasome inhibition and autophagy induction. Apoptosis 2013; 18:1188-1200. [PMID: 23632965]

48. Murphy G, Kamangar F, Albanes D, Stanczyk FZ, Weinstein SJ, Taylor PR, Virtamo J, Abnet CC, et al. Serum ghrelin is inversely associated with risk of subsequent oesophageal squamous cell carcinoma. Gut 2012; 61:1533-1537. [PMID: 22180062]

49. Grönberg M, Fjällskog ML, Jirström K, Janson ET. Expression of ghrelin is correlated to a favorable outcome in invasive breast cancer. Acta Oncol 2012; 51:386-393. [PMID: 22067021]

50. Murphy G, Kamangar F, Dawsey SM, Stanczyk FZ, Weinstein SJ, Taylor PR, Virtamo J, Abnet CC, et al. The relationship between serum ghrelin and the risk of gastric and esophagogastric junctional adenocarcinomas. J Natl Cancer Inst 2011; 103:1123-1129. [PMID: 21693726]

51. Díaz-Lezama N, Hernández-Elvira M, Sandoval A, Monroy A, Felix R, Monjaraz E. Ghrelin inhibits proliferation and increases T-type Ca2+ channel expression in PC-3 human prostate carcinoma cells. Biochem Biophys Res Commun 2010; 403:24-29. [PMID: 21040709]

52. Dagli AF, Aydin S, Karaoglu A, Akpolat N, Ozercan IH, Ozercan MR. Ghrelin expression in normal kidney tissue and renal carcinomas. Pathol Res Pract 2009; 205:165-173. [PMID: 19054628]

53. Mottershead M, Karteris E, Barclay JY, Suortamo S, Newbold M, Randeva H, Nwokolo CU. Immunohistochemical and quantitative mRNA assessment of ghrelin expression in gastric and oesophageal adenocarcinoma. J Clin Pathol 2007; 60:405-409. [PMID: 16751299]

54. Murata M, Okimura Y, Iida K, Matsumoto M, Sowa H, Kaji H, Kojima M, Kangawa K, et al. Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. J Biol Chem 2002; 277:5667-5674. [PMID:]

55. Northrup R, Kuroda K, Duus EM, Barnes SR, Cheatham L, Wiley T, Pietra C. Effect of ghrelin and anamorelin (ONO-7643), a selective ghrelin receptor agonist, on tumor growth in a lung cancer mouse xenograft model. Support Care Cancer 2013; 21:2409-2415. [PMID: 23579947]

56. Chopin LK, Seim I, Walpole CM, Herington AC. The ghrelin axis--does it have an appetite for cancer progression? Endocr Rev 2012; 33:849-891. [PMID: 22826465]

57. Nikolopoulos D, Theocharis S, Kouraklis G. Ghrelin's role on gastrointestinal tract cancer. Surg Oncol 2010; 19:e2-e10. [PMID: 19328680]

58. Mol JA, Selman PJ, Sprang EP, van Neck JW, Oosterlaken-Dijksterhuis MA. The role of progestins, insulin-like growth factor (IGF) and IGF-binding proteins in the normal and neoplastic mammary gland of the bitch: a review. J Reprod Fertil Suppl 1997; 51:339-344. [PMID: 9404304]

59. Baatar D, Patel K, Taub DD. The effects of ghrelin on inflammation and the immune system. Mol Cell Endocrinol 2011; 340:44-58. [PMID: 21565248]

60. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004; 50:1511-1525. [PMID: 15265818]

61. Noda T, Kikugawa T, Tanji N, Miura N, Asai S, Higashiyama S, Yokoyama M. Long-term exposure to leptin enhances the growth of prostate cancer cells. Int J Oncol 2015; 46:1535-1542. [PMID: 25625287]

62. Higurashi T, Endo H, Uchiyama T, Uchiyama S, Yamada E, Ohkubo H, Sakai E, Takahashi H, et al. Conditional knockout of the leptin receptor in the colonic epithelium revealed the local effects of leptin receptor signaling in the progression of colonic tumors in mice. Carcinogenesis 2014; 35:2134-2141. [PMID: 24958593]

63. Yuan HJ, Sun KW, Yu K: Leptin promotes the proliferation and migration of human breast cancer through the extracellular-signal regulated kinase pathway. Mol Med Rep 2014; 9:350-354. [PMID: 24213635]

64. Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obesity reviews 2007; 8: 21–34. [PMID: 17212793]

65. Jiang N, Sun R, Sun Q. Leptin signaling molecular actions and drug target in hepatocellular carcinoma. Drug Des Devel Ther 2014; 8:2295-2302. [PMID: 25484575]

66. Fazolini NP, Cruz AL, Werneck MB, Viola JP, Maya-Monteiro CM, Bozza PT. Leptin activation of mTOR pathway in intestinal epithelial cell triggers lipid droplet formation, cytokine production and increased cell proliferation. Cell Cycle 2015; 14:2667-76. [PMID: 26017929]

67. Uddin S, Hussain AR, Khan OS, Al-Kuraya KS. Role of dysregulated expression of leptin and leptin receptors in colorectal carcinogenesis. Tumour Biol 2014; 35:871-879. [PMID: 24014051]

68. Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, McCormic ML, Sigmund CD, et al. Ghrelin inhibits proinflammatory responses and nuclear factor-jB activation in human endothelial cells. Circulation 2004; 109:2221–2226. [PMID: 15117840]

69. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard JW Jr, Taub DD. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. 2004; J Clin Invest 114:57-66. [PMID: 15232612]