Ghrelin and cancer

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Abbreviations: NSCLC (Non-small cell lung cancer); SCLC (small cell lung cancer)

Abstract

Abstract: Ghrelin is a peptide hormone that was originally isolated from the stomach as the endogenous ligand for the growth hormone secretagogue receptor (GHSR). Ghrelin has many functions, including the regulation of appetite and gut motility, growth hormone release from the anterior pituitary and roles in the cardiovascular and immune systems. Ghrelin and its receptor are expressed in a number of cancers and cancer cell lines and may play a role in processes associated with cancer progression, including cell proliferation, apoptosis, and cell invasion and migration.

1.0 Introduction

Ghrelin is a multifunctional peptide hormone originally isolated from the stomach as the endogenous ligand for the growth hormone secretagogue receptor (GHSR) (Kojima et al., 1999). Ghrelin has a number of functions, including roles in the regulation of growth hormone release, metabolism, appetite, the cardiovascular system and insulin secretion (Kojima et al., 1999). There is also growing evidence that ghrelin plays an autocrine/paracrine role in a number of processes related to cancer progression, including cell proliferation (Jeffery et al., 2002; Jeffery et al., 2003), cell migration (Dixit et al., 2006), and apoptosis (Fung et al., 2010). This review will focus on the autocrine/paracrine role of ghrelin in cancer, and the actions of ghrelin that may promote cancer progression.

2.0 Paracrine/autocrine ghrelin

2.1 Local expression of the ghrelin-axis

While ghrelin was originally discovered in the rat and human stomach (Kojima et al., 1999), the expression of ghrelin and its receptor, the growth hormone secretagogue receptor (GHSR), has been demonstrated in a range of tissues. This includes the small and large intestine, the hypothalamus and pituitary (Korbonits et al., 2001) and a number of other peripheral tissues, including the endocrine pancreas (Volante et al., 2002) and placenta (Gualillo et al., 2001). Local production of ghrelin has been shown in a range of cancers including pituitary adenomas (Kim et al., 2001; Korbonits et al., 2001; Wasko et al., 2006; Wasko et al., 2008), and colorectal (Papotti et al., 2001; Waseem et al., 2008), gastric (An et al., 2007; Aydin et al., 2005; Ekeblad et al., 2006; Papotti et al., 2001), prostate (Cassoni et al., 2004; Jeffery et al., 2002; Yeh et al., 2005), breast (Jeffery et al., 2005), thyroid (Volante et al., 2003),

endocrine pancreatic (Ekeblad et al., 2007; Iwakura et al., 2002; Volante et al., 2002), ovarian (Gaytan et al., 2005), endometrial (Fung et al., 2010), testicular (Gaytan et al., 2004), adrenocortical (Ueberberg et al., 2008), renal (Dagli et al., 2009) and lung cancer (Cassoni et al., 2006). Ghrelin is highly expressed in the stomach and ghrelin secretion is greatly reduced in atrophic gastritis associated with *Helicobacter pylori* infection, as ghrelin-producing cells are damaged in this precancerous condition (Zub-Pokrowiecka et al., 2010). Treatment for gastric cancer, where the whole stomach is removed, therefore, greatly reduces plasma ghrelin levels (Zub-Pokrowiecka et al., 2010).

The growth hormone secretagogue receptor (GHSR) isoforms, GHSR 1a and GHSR 1b, are also widely expressed (Gnanapavan et al., 2002; Ueberberg et al., 2009). Many of the endocrine functions of ghrelin appear to be mediated by the GHSR 1a isoform, which is known as the functional ghrelin receptor. GHSR 1a expression was initially characterised in the pituitary and the hypothalamus (Howard et al., 1996) and it is also expressed in numerous peripheral tissues including the stomach, intestine, pancreas, spleen, thyroid, gonads, adrenal gland, kidney, heart, lung, liver, adipose tissue, bone and prostate (Camina, 2006; Gnanapavan et al., 2002; Jeffery et al., 2002; Kojima et al., 2005; Soares et al., 2008). The GHSR is expressed in a range of tumours, including pituitary tumours, prostate, breast and ovarian cancer and in astrocytoma (Adams et al., 1998; Dixit et al., 2006; Gaytan et al., 2005; Jeffery et al., 2002; Jeffery et al., 2005; Korbonits et al., 1998; Skinner et al., 1998). GHSR 1a expression is absent in some cases of colorectal cancer, adrenocortical tumours, non-small cell lung cancer, leukaemia and some breast cancer cell lines (Barzon et al., 2005; Cassoni et al., 2001; Ghe et al., 2002; Takahashi et al., 2006). GHSR1a expression is not required for all ghrelin functions, however, and many of the effects of ghrelin could be mediated by an

alternative receptor that has not yet been identified (for review, see Seim *et al.* in this special issue).

The truncated, 5-transmembrane domain, ghrelin receptor isoform, GHSR 1b, is believed to be a non-functional isoform of the receptor (Howard et al., 1996; Kojima et al., 1999). In a number of cancers, GHSR 1a expression is downregulated or absent (Barzon et al., 2005; Cassoni et al., 2001; Ghe et al., 2002; Takahashi et al., 2006), while the non-functional, truncated form of the receptor, GHSR 1b is widely expressed in cancer and expression may be upregulated compared to normal tissues (Waseem et al., 2008).

2.2 Paracrine/autocrine actions of the ghrelin axis in cancer progression

2.2.1 Ghrelin in cell proliferation

As ghrelin is synthesised locally in many tissues, it could act as an autocrine/paracrine growth factor in normal and cancer tissues (Jeffery et al., 2002). While most studies indicate that ghrelin stimulates cell proliferation in normal cell lines (Andreis et al., 2003; Maccarinelli et al., 2005; Nanzer et al., 2004; Pettersson et al., 2002; Wang et al., 2009; Xia et al., 2004), the effect of ghrelin in cancer cell lines has proven more controversial.

Ghrelin may act as a growth factor in a range of cancers and increase cell proliferation, a hallmark of cancer (Hanahan et al., 2000; Jeffery et al., 2003; Soares et al., 2008). Ghrelin stimulates proliferation in a number of cancer cell lines, including the HepG2 human hepatoma cell line (Murata et al., 2002), human erythroleukaemic (De Vriese et al., 2005),

and leukaemic cell lines (De Vriese et al., 2008), in adrenocortical carcinoma (Barzon et al., 2005), in pancreatic adenocarcinoma cell lines (Duxbury et al., 2003), colorectal cancer (Waseem et al., 2008), the JEG-3 choriocarcinoma cell line (Rak-Mardyla et al., 2010) and in prostate (Jeffery et al., 2002; Yeh et al., 2005), breast (Jeffery et al., 2005) and endometrial cell lines (Fung et al., 2010). Ghrelin-induced proliferation is mediated by the ERK1/2 MAPK pathway in a number of cell lines, including prostate cancer cells lines (Yeh et al., 2005), the rat normal pituitary-derived GH3 cell-line (Nanzer et al., 2004), and the rat thyrocyte FRTL-5 cell line (Park et al., 2008).

While a number of studies have demonstrated that ghrelin stimulates cell proliferation, some reports indicate that ghrelin may inhibit proliferation. This includes thyroid (Volante et al., 2003), prostate (Diaz-Lezama et al., 2010) and breast cancer (Cassoni et al., 2001) and small cell lung carcinoma (Cassoni et al., 2006) cell lines. Studies in the ARO anaplastic thyroid carcinoma and the N-PAP papillary follicular thyroid carcinoma cell lines demonstrated a modest inhibition of cell growth using crystal violet staining-based assay (Volante et al., 2003). In the N-PAP cell line, a statistically significant decrease in cell proliferation was seen with 100nM and 1μM concentrations of ghrelin after 96 hours. In the ARO cell line, a significant decrease in cell number was only seen with 1μM ghrelin treatments and no effect was seen in cells treated with 10nM ghrelin (Volante et al., 2003). In contrast, no change in cell proliferation was seen in the ARO cell line treated with ghrelin, or in this cell line stimulated with thyroid stimulating hormone in another study (Park et al., 2008). It is unclear if ghrelin was replenished during the assay in these studies.

The role of ghrelin in stimulating cell proliferation in prostate cancer remains controversial. Studies performed by our research group have shown that ghrelin stimulates proliferation of the PC-3 prostate cancer cell line at levels within the physiological range (5-10nM) using a metabolic, colourimetric MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to estimate changes in cell number (Jeffery et al., 2002; Yeh et al., 2005). A similar response in the PC3 cell line has been reported, where low concentrations of ghrelin (10-100pM) stimulated cell proliferation, but higher concentrations (1μM) inhibited proliferation (Cassoni et al., 2004). In contrast, a recent study demonstrated that ghrelin treatment (10-50nM) decreased the incorporation of ^{3}H thymidine in the PC3 cell line, indicating that it decreased cell proliferation (Diaz-Lezama et al., 2010). Ghrelin treatment stimulated an increase in intracellular free calcium and the decrease in cell number was inhibited by treatment with T-type calcium channel blockers (Diaz-Lezama et al., 2010). We have also demonstrated that ghrelin stimulates cell proliferation in the LNCaP prostate cancer cell line (Yeh et al., 2005). In contrast to our findings, Cassoni *et al*., 2004 demonstrated no effect on LNCaP prostate cancer cell line proliferation. In the androgen-independent DU145 prostate cancer cell line, ghrelin and des-ghrelin alone had no effect on cell proliferation (measured by ^{3}H thymidine incorporation), but they inhibited cell proliferation stimulated by IGF-I treatment (Cassoni et al., 2004).

Although the reasons for these discrepancies are not immediately apparent, these studies varied in the concentrations of ghrelin used and in the assay method applied. The application of supraphysiological doses of ghrelin could have an inhibitory effect, while physiological levels could stimulate cell proliferation (Lanfranco et al., 2008; Nikolopoulos et al., 2010). In our assays, ghrelin was replenished every 24 hours, while Cassoni *et al*., treated the cells with

ghrelin every 48 hours. Ghrelin has been reported to have a short half- life and it is rapidly de-acetylated and also protelytically cleaved (De Vriese et al., 2004; Hosoda et al., 2004) and therefore, chronic ghrelin treatments require the frequent addition of ghrelin to the media.

There is also conflicting data regarding the effect of ghrelin on breast cancer cell proliferation, with studies demonstrating either proliferative or anti-proliferative effects. Cassoni *et al*., (2001) demonstrated that ghrelin treatment (1μM) significantly decreased cell number after 48 or 96 hours of treatment in the oestrogen-dependent MCF7 breast cancer cell line and the oestrogen-independent MDA-MB 231 cell line. This study was performed using cell counting after cells were stained with crystal violet (Cassoni et al., 2001). They also demonstrated that ghrelin and desacyl ghrelin inhibited serum-stimulated ^{3}H thymidine incorporation after 20 hours of treatment with a range of concentrations of ghrelin (Cassoni et al., 2001). This effect was not seen with the MDA-MB231 cell line or in oestrogen-stimulated MCF7 cells, unless concentrations of more than 1-2μM ghrelin were applied (Cassoni et al., 2001). In contrast, we have demonstrated that ghrelin stimulates cell proliferation in the MDA-MB-231, and the MDA-MB-435 breast cancer cell lines, but ghrelin had no significant effect on cell proliferation in the MCF7 or the normal-derived MCF10A cell line (Jeffery et al., 2005). The origin of the MDA-MB435 cell line is currently controversial, however, with suggestions that it is actually derived from a melanoma rather than breast cancer (Chambers, 2009). Cassoni *et al*., (2001) found that GHSR 1a was not expressed in the MDA-MB231 and MCF7 breast cancer cell lines using RT-PCR, suggesting that the anti-proliferative effects of ghrelin could be mediated by an alternative receptor. The inhibitory effects of ghrelin in breast cancer cell lines occurs at high ghrelin concentrations, as reported in studies in thyroid and prostate cancer cell lines (Cassoni et al., 2001; Volante et al., 2003). In pancreatic cancer cell lines, an

increase in proliferation was seen at lower ghrelin concentrations and this effect decreased at higher concentrations (Duxbury et al., 2003).

Both ghrelin and des-ghrelin inhibited cell proliferation in the H345 small cell lung cancer cell line (Cassoni et al., 2006). In contrast, in the Calu-1 lung epidermoid carcinoma cell line, ghrelin treatment (1nM-2μM) had no significant effect on cell proliferation after 24 hours (Ghe et al., 2002). In this cell line, however, synthetic peptide analogues of ghrelin, hexarelin and EP-80317, inhibited both basal proliferation and insulin like growth factor (IGF)-II stimulated proliferation in a dose-dependent manner (Ghe et al., 2002). As these lung cell lines do not express GHSR 1a, these effects are likely to be mediated by an alternative ghrelin receptor (Cassoni et al., 2006; Ghe et al., 2002).

Desacyl ghrelin also stimulates cell proliferation in some cell lines. As desacyl ghrelin does not activate the GHSR 1a at physiological doses, it presumably acts through an unidentified alternative ghrelin receptor. Desacyl ghrelin and ghrelin have similar proliferative effects in the rat GH3 somatotroph cell line, and in the SW-13 and NCI-H295R adrenocortical tumour cell lines, and this suggests that these effects are not mediated by GHSR 1a (Delhanty et al., 2007; Nanzer et al., 2004).

2.2.2 Ghrelin and apoptosis

Ghrelin protects against basal or induced apoptosis in a number of normal cell types, including cardiomyocytes, vascular smooth muscle cells and endothelial cells (Baldanzi et al., 2002; Kui et al., 2009), adrenocortical cells (Mazzocchi et al., 2004), adipocytes (Kim et al.,

2004), mouse osteoblastic MC3T3-E1 cells (Kim et al., 2005), β-pancreatic islet cells (Granata et al., 2007; Zhang et al., 2007) and hypothalamic neuronal cells (Chung et al., 2007). In the H9c2 cardiomyocyte and PAE (porcine aortic) endothelial cell lines, ghrelin and desacyl ghrelin protect against apoptosis, with ghrelin signaling through the ERK1/2 and PI3K/Akt pathways (Baldanzi et al., 2002). These effects are likely to be mediated through the alternative ghrelin receptor, as H9c2 cells do not express GHSR 1a (Baldanzi et al., 2002).

Fewer studies have investigated the role of ghrelin in regulating apoptosis in cancer, however. Recently, ghrelin treatment has been demonstrated to protect against apoptosis in endometrial cancer cell lines in response to treatment with the apoptosis-inducing agent doxorubicin (Fung et al., 2010). Ghrelin inhibits apoptosis through a range of mechanisms. In the rat PC-12 phaeochromocytoma cell line (derived from an adrenal medullary tumour) ghrelin protects against apoptosis by inhibiting the activation of ASK1 (apoptosis signal regulating kinase 1) by inducing Hsp70 (heat shock protein) (Yang et al., 2007). ASK1 is a component of the MAPK signalling pathway and plays a role in stimulating apoptosis in response to a range of apoptosis-inducing agents (Yang et al., 2007). In one study in the SW-13 adrenocortical carcinoma cell line, ghrelin treatment reduced basal apoptosis by 15-20% and caspase 3/7 activity was also downregulated (Delhanty et al., 2007). In this study, desacyl ghrelin had a more pronounced protective effect than acylated ghrelin. In contrast, Belloni *et al*., (2004) described a marked increase in the basal apoptotic rate in the SW-13 and NCI-H295 adrenocortical carcinoma cell lines and in primary aldosteroma cells (Belloni et al., 2004). These authors suggested that by promoting apoptosis, ghrelin may have useful anti-tumour actions (Belloni et al., 2004). Pro-apoptotic effects have also been described in the H345 small cell lung carcinoma cell line (Cassoni et al., 2006), and in the PC3 prostate cancer cell

line, where ghrelin also decreased the rate of cell proliferation (Diaz-Lezama et al., 2010). This pro-apoptotic effect was thought to be mediated by signalling through T-type calcium channels (Diaz-Lezama et al., 2010). Ghrelin was not pro-apoptotic in oesophageal adenocarcinoma cells treated with a number of apoptosis-inducing agents, nor did it protect against apoptosis (Konturek et al., 2008). As the majority of studies have reported that ghrelin has an anti-apoptotic effect, ghrelin may, therefore, protect against apoptosis in some cell types and be pro-apoptotic in others. The effects of ghrelin on apoptosis could also vary with the apoptosis-inducing agents and assay methods used in these studies.

2.2.3 Ghrelin in cell migration and invasion

Ghrelin may also have a role in promoting cell motility and invasion in a number of cancers. Human astrocytoma cell lines secrete ghrelin, and ghrelin treatment increases their rate of cell migration in scratch assays and Transwell migration assays (Dixit et al., 2006). Ghrelin also stimulates cell invasion in a dose-dependent manner (Dixit et al., 2006).

Finally, ghrelin increases cell proliferation, migration and invasion in pancreatic cancer cell lines (Duxbury et al., 2003). Invasion and migration in response to ghrelin treatment occurred through the activation of the PI3K/Akt pathway, a pathway which is often associated with an increase in cell motility and invasion (Duxbury et al., 2003). The effects of ghrelin on cell motility may also be mediated through an alternative ghrelin receptor. In colorectal cell lines, autocrine ghrelin appears to stimulate cell invasion and migration, and these processes can be

almost abolished in assays where cells are treated with the ghrelin antagonist, D(Lys-3)-GHRP-6, or by a ghrelin neutralising antibody (Waseem et al., 2008).

3.0 Conclusion

Ghrelin is expressed in a wide range of cancer tissues and plays a role in a number of key processes in cancer progression, including cell proliferation, cell migration and invasion, and apoptosis. As there have been a number of conflicting reports, it is currently unclear whether ghrelin promotes cancer or inhibit its development and it is possible that it could have both stimulatory and inhibitory effects. In most cell lines, ghrelin stimulates cell proliferation and is anti-apoptotic, and these effects could combine to promote cancer progression. Although there have been few studies, ghrelin appears to increase cell migration and invasion, processes which are important in cancer metastasis and spread. While further studies are required to establish the role of ghrelin in cancer development, there is evidence to suggest that ghrelin should be used with caution as a therapeutic to stimulate growth hormone release or for the treatment of cancer cachexia.

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References

Adams, E.F., Huang, B., Buchfelder, M., Howard, A., Smith, R.G., Feighner, S.D., van der Ploeg, L.H., Bowers, C.Y. and Fahlbusch, R., 1998. Presence of growth hormone secretagogue receptor messenger ribonucleic acid in human pituitary tumors and rat GH3 cells. J Clin Endocrinol Metab 83, 638-42.

An, J.Y., Choi, M.G., Noh, J.H., Sohn, T.S., Jin, D.K. and Kim, S., 2007. Clinical significance of ghrelin concentration of plasma and tumor tissue in patients with gastric cancer. J Surg Res 143, 344-9.

Andreis, P., Malendowicz, L., Trejter, M., Neri, G., Spinazzi, R., Rossi, G. and Nussdorfer, G., 2003. Ghrelin and growth hormone secretagogue receptor are expressed in the rat adrenal cortex: Evidence that ghrelin stimulates the growth, but not the secretory activity of adrenal cells. FEBS Letters 536, 173-9.

Aydin, S., Ozercan, I.H., Dagli, F., Dogru, O., Celebi, S., Akin, O. and Guzel, S.P., 2005. Ghrelin immunohistochemistry of gastric adenocarcinoma and mucoepidermoid carcinoma of salivary gland. Biotech Histochem 80, 163-8.

Baldanzi, G., Filigheddu, N., Cutrupi, S., Catapano, F., Bonissoni, S., Fubini, A., Malan, D., Baj, G., Granata, R., Broglio, F., Papotti, M., Surico, N., Bussolino, F., Isgaard, J., Deghenghi, R., Sinigaglia, F., Prat, M., Muccioli, G., Ghigo, E. and Graziani, A., 2002. Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. J Cell Biol 159, 1029-37.

Barzon, L., Pacenti, M., Masi, G., Stefani, A.L., Fincati, K. and Palu, G., 2005. Loss of growth hormone secretagogue receptor 1a and overexpression of type 1b receptor transcripts in human adrenocortical tumors. Oncology 68, 414-21.

Belloni, A.S., Macchi, C., Rebuffat, P., Conconi, M.T., Malendowicz, L.K., Parnigotto, P.P. and Nussdorfer, G.G., 2004. Effect of ghrelin on the apoptotic deletion rate of different types of cells cultured in vitro. Int J Mol Med 14, 165-7.

Camina, J.P., 2006. Cell biology of the ghrelin receptor. J Neuroendocrinol 18, 65-76.

Cassoni, P., Allia, E., Marrocco, T., Ghe, C., Ghigo, E., Muccioli, G. and Papotti, M., 2006. Ghrelin and cortistatin in lung cancer: expression of peptides and related receptors in human primary tumors and in vitro effect on the H345 small cell carcinoma cell line. J Endocrinol Invest 29, 781-90.

Cassoni, P., Ghe, C., Marrocco, T., Tarabra, E., Allia, E., Catapano, F., Deghenghi, R., Ghigo, E., Papotti, M. and Muccioli, G., 2004. Expression of ghrelin and biological activity of specific receptors for ghrelin and des-acyl ghrelin in human prostate neoplasms and related cell lines. Eur J Endocrinol 150, 173-84.

Cassoni, P., Papotti, M., Ghe, C., Catapano, F., Sapino, A., Graziani, A., Deghenghi, R., Reissmann, T., Ghigo, E. and Muccioli, G., 2001. Identification, characterization, and biological activity of specific receptors for natural (ghrelin) and synthetic growth hormone secretagogues and analogs in human breast carcinomas and cell lines. J Clin Endocrinol Metab 86, 1738-45.

Chambers, A.F., 2009. MDA-MB-435 and M14 cell lines: identical but not M14 melanoma? Cancer Res 69, 5292-3.

Chung, H., Kim, E., Lee, D.H., Seo, S., Ju, S., Lee, D., Kim, H. and Park, S., 2007. Ghrelin inhibits apoptosis in hypothalamic neuronal cells during oxygen-glucose deprivation. Endocrinology 148, 148-59.

Dagli, A.F., Aydin, S., Karaoglu, A., Akpolat, N., Ozercan, I.H. and Ozercan, M.R., 2009. Ghrelin expression in normal kidney tissue and renal carcinomas. Pathol Res Pract 205, 165-73.

De Vriese, C. and Delporte, C., 2008. Ghrelin: a new peptide regulating growth hormone release and food intake. Int J Biochem Cell Biol 40, 1420-4.

De Vriese, C., Gregoire, F., De Neef, P., Robberecht, P. and Delporte, C., 2005. Ghrelin is produced by the human erythroleukemic HEL cell line and involved in an autocrine pathway leading to cell proliferation. Endocrinology 146, 1514-22.

De Vriese, C., Gregoire, F., Lema-Kisoka, R., Waelbroeck, M., Robberecht, P. and Delporte, C., 2004. Ghrelin degradation by serum and tissue homogenates: identification of the cleavage sites. Endocrinology 145, 4997-5005.

Delhanty, P.J., van Koetsveld, P.M., Gauna, C., van de Zande, B., Vitale, G., Hofland, L.J. and van der Lely, A.J., 2007. Ghrelin and its unacylated isoform stimulate the growth of adrenocortical tumor cells via an anti-apoptotic pathway. Am J Physiol Endocrinol Metab 293, E302-9.

Diaz-Lezama, N., Hernandez-Elvira, M., Sandoval, A., Monroy, A., Felix, R. and Monjaraz, E., 2010. Ghrelin inhibits proliferation and increases T-type Ca2+ channel expression in PC-3 human prostate carcinoma cells. Biochem Biophys Res Commun 403, 24-9.

Dixit VD., Weeraratna AT., Yang H., Bertak D., Cooper-Jenkins A., Riggins GJ., Eberhart CG., Taub DD., 2006. Ghrelin and the growth hormone secretagogue receptor constitute a novel autocrine pathway in astrocytoma motility. J Biol Chem 281, 16681-90.

Duxbury, M. and al., e., 2003. Ghrelin promotes pancreatic adenocarcinoma cellular proliferation and invasiveness. Biochem Biophys Res Commun. 309, 464-468.

Ekeblad, S., Lejonklou, M.H., Grimfjard, P., Johansson, T., Eriksson, B., Grimelius, L., Stridsberg, M., Stalberg, P. and Skogseid, B., 2007. Co-expression of ghrelin and its receptor in pancreatic endocrine tumours. Clin Endocrinol (Oxf) 66, 115-22.

Ekeblad, S., Nilsson, B., Lejonklou, M.H., Johansson, T., Stalberg, P., Nilsson, O., Ahlman, H. and Skogseid, B., 2006. Gastrointestinal stromal tumors express the orexigen ghrelin. Endocr Relat Cancer 13, 963-70.

Fung, J., Seim, I., Wang, D., Obermair, A., Chopin, L.K. and Chen, C., 2010. Expression and in vitro functions of the ghrelin axis in endometrial cancer. Hormones and Cancer 1, 245-255.

Gaytan, F., Barreiro, M.L., Caminos, J.E., Chopin, L.K., Herington, A.C., Morales, C., Pinilla, L., Paniagua, R., Nistal, M., Casanueva, F.F., Aguilar, E., Dieguez, C. and Tena-Sempere, M., 2004. Expression of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in normal human testis and testicular tumors. J Clin Endocrinol Metab 89, 400-9.

Gaytan, F., Morales, C., Barreiro, M.L., Jeffery, P., Chopin, L.K., Herington, A.C., Casanueva, F.F., Aguilar, E., Dieguez, C. and Tena-Sempere, M., 2005. Expression of growth hormone secretagogue receptor type 1a, the functional ghrelin receptor, in human ovarian surface epithelium, mullerian duct derivatives, and ovarian tumors. J Clin Endocrinol Metab 90, 1798-804.

Ghe, C., Cassoni, P., Catapano, F., Marrocco, T., Deghenghi, R., Ghigo, E., Muccioli, G. and Papotti, M., 2002. The antiproliferative effect of synthetic peptidyl GH secretagogues in human CALU-1 lung carcinoma cells. Endocrinology 143, 484-91.

Gnanapavan, S., Kola, B., Bustin, S., Morris, D., McGee, P., Fairclough, P., Bhattacharya, S., Carpenter, R., Grossman, A. and Korbonits, M., 2002. The Tissue Distribution of the mRNA of Ghrelin and Subtypes of Its Receptor, GHS-R, in Humans. Journal of Clinical Endocrinology and Metabolism 87, 2988.

Granata, R., Settanni, F., Biancone, L., Trovato, L., Nano, R., Bertuzzi, F., Destefanis, S., Annunziata, M., Martinetti, M., Catapano, F., Ghe, C., Isgaard, J., Papotti, M., Ghigo,

E. and Muccioli, G., 2007. Acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 3',5'-cyclic adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphatidyl inositol 3-Kinase/Akt signaling. Endocrinology 148, 512-29.

Gualillo, O., Caminos, J., Blanco, M., Garcia-Caballero, T., Kojima, M., Kangawa, K., Dieguez, C. and Casanueva, F., 2001. Ghrelin, a novel placental-derived hormone. Endocrinology 142, 788-94.

Hanahan, D. and Weinberg, R., 2000. The hallmarks of cancer. Cell 100, 57-70.

Hosoda, H., Doi, K., Nagaya, N., Okumura, H., Nakagawa, E., Enomoto, M., Ono, F. and Kangawa, K., 2004. Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. Clin Chem 50, 1077-80.

Howard, A., Feighner, S., Cully, D., Arena, J., Liberator, P., Rosenblum, C., Hamelin, M., Hreniuk, D., Palyha, O., Anderson, J., Paress, P., Diaz, C., Chou, M., Liu, K., McKee, K., Pong, S., Chuang, L., Elbrecht, A., Dashkevicz, M., Heavens, R., Rigby, M., Sirinathsinghji, D., Dean, D., Melillo, D., Patchett, A., Nargund, R., Grifin, P., DeMartino, J., Gupta, S., Schaefer, J., Smith, R. and Van der Ploeg, L., 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 12, 137-145.

Iwakura, H., Hosoda, K., Doi, R., Komoto, I., Nishimura, H., Son, C., Fujikura, J., Tomita, T., Takaya, K., Ogawa, Y., Hayashi, T., Inoue, G., Akamizu, T., Hosoda, H., Kojima, M., Kangawa, K., Imamura, M. and Nakao, K., 2002. Ghrelin expression in islet cell tumors: augmented expression of ghrelin in a case of glucagonoma with multiple endocrine neoplasm type I. J Clin Endocrinol Metab 87, 4885-8.

Jeffery, P., Herington, A. and Chopin, L., 2003. The potential autocrine/paracrine roles of ghrelin and its receptor in hormone dependent cancer. Cytokine and Growth Factor Reviews 14, 113-122.

Jeffery, P., Murray, R., Yeh, A., McNamara, J., Duncan, R.P., Francis, G., Herington, A. and Chopin, L., 2005. Expression and function of the ghrelin axis, including a novel preproghrelin isoform, in human breast cancer tissues and cell lines. Endocrine Related Cancer 12, 839-850.

Jeffery, P.L., Herington, A.C. and Chopin, L.K., 2002. Expression and action of the growth hormone releasing peptide ghrelin and its receptor in prostate cancer cell lines. J Endocrinol 172, R7-11.

Kim, K., Arai, K., Sanno, N., Osamura, R.Y., Teramoto, A. and Shibasaki, T., 2001. Ghrelin and growth hormone (GH) secretagogue receptor (GHSR) mRNA expression in human pituitary adenomas. Clin Endocrinol (Oxf) 54, 759-68.

Kim, M.S., Yoon, C.Y., Jang, P.G., Park, Y.J., Shin, C.S., Park, H.S., Ryu, J.W., Pak, Y.K., Park, J.Y., Lee, K.U., Kim, S.Y., Lee, H.K., Kim, Y.B. and Park, K.S., 2004. The mitogenic and antiapoptotic actions of ghrelin in 3T3-L1 adipocytes. Mol Endocrinol 18, 2291-301.

Kim, S.W., Her, S.J., Park, S.J., Kim, D., Park, K.S., Lee, H.K., Han, B.H., Kim, M.S., Shin, C.S. and Kim, S.Y., 2005. Ghrelin stimulates proliferation and differentiation and inhibits apoptosis in osteoblastic MC3T3-E1 cells. Bone 37, 359-69.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K., 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402, 656-60.

Kojima, M. and Kangawa, K., 2005. Ghrelin: structure and function. Physiol Rev 85, 495-522.

Konturek, P.C., Burnat, G., Rau, T., Hahn, E.G. and Konturek, S., 2008. Effect of adiponectin and ghrelin on apoptosis of Barrett adenocarcinoma cell line. Dig Dis Sci 53, 597-605.

Korbonits, M., Bustin, S., Kojima, M., Jordan, S., Adams, E., Lowe, D., Kangawa, K. and Grossman, A., 2001. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumours. The Journal of Clinical Endocrinology and Metabolism 86, 881-887.

Korbonits, M., Jacobs, R.A., Aylwin, S.J., Burrin, J.M., Dahia, P.L., Monson, J.P., Honegger, J., Fahlbush, R., Trainer, P.J., Chew, S.L., Besser, G.M. and Grossman, A.B., 1998. Expression of the growth hormone secretagogue receptor in pituitary adenomas and other neuroendocrine tumors. J Clin Endocrinol Metab 83, 3624-30.

Kui, L., Weiwei, Z., Ling, L., Daikun, H., Guoming, Z., Linuo, Z. and Renming, H., 2009. Ghrelin inhibits apoptosis induced by high glucose and sodium palmitate in adult rat cardiomyocytes through the PI3K-Akt signaling pathway. Regul Pept 155, 62-9.

Lanfranco, F., Baldi, M., Cassoni, P., Bosco, M., Ghe, C. and Muccioli, G., 2008. Ghrelin and prostate cancer. Vitam Horm 77, 301-24.

Maccarinelli, G., Sibilia, V., Torsello, A., Raimondo, F., Pitto, M., Giustina, A., Netti, C. and Cocchi, D., 2005. Ghrelin regulates proliferation and differentiation of osteoblastic cells. J Endocrinol 184, 249-56.

Mazzocchi, G., Neri, G., Rucinski, M., Rebuffat, P., Spinazzi, R., Malendowicz, L.K. and Nussdorfer, G.G., 2004. Ghrelin enhances the growth of cultured human adrenal zona glomerulosa cells by exerting MAPK-mediated proliferogenic and antiapoptotic effects. Peptides 25, 1269-77.

Murata, M., Okimura, Y., Iida, K., Matsumoto, M., Sowa, H., Kaji, H., Kojima, M., Kangawa, K. and Chihara, K., 2002. Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. J Biol Chem 277, 5667-74.

Nanzer, A., Khalaf, S., Mozid, A., Fowkes, R., Patel, M., Burrin, J., Grossman, A. and Korbonits, M., 2004. Ghrelin exerts a proliferative effect on a rat pituitary somatotroph cell line via the mitogen-activated protein kinase pathway. European Journal of Endocrinology 151, 233-240.

Nikolopoulos, D., Theocharis, S. and Kouraklis, G., 2010. Ghrelin: a potential therapeutic target for cancer. Regul Pept 163, 7-17.

Papotti, M., Cassoni, P., Volante, M., Deghenghi, R., Muccioli, G. and Ghigo, E., 2001. Ghrelin-producing endocrine tumors of the stomach and intestine. J Clin Endocrinol Metab 86, 5052-9.

Park, Y.J., Lee, Y.J., Kim, S.H., Joung, D.S., Kim, B.J., So, I., Park do, J. and Cho, B.Y., 2008. Ghrelin enhances the proliferating effect of thyroid stimulating hormone in FRTL-5 thyroid cells. Mol Cell Endocrinol 285, 19-25.

Pettersson, I., Muccioli, G., Granata, R., Deghenghi, R., Ghigo, E., Ohlsson, C. and Isgaard, J., 2002. Natural (ghrelin) and synthetic (hexarelin) GH secretagogues stimulate H9c2 cardiomyocyte cell proliferation. J Endocrinol 175, 201-9.

Rak-Mardyla, A. and Gregoraszczuk, E., 2010. Effect of ghrelin on proliferation, apoptosis and secretion of progesterone and hCG in the placental JEG-3 cell line. Reprod Biol 10, 159-65.

Skinner, M., Nass, R., Lopes, B., Laws, E. and Thorner, M., 1998. Growth hormone secretagogue receptor expression in human pituitary tumors. Journal of Clinical Endocrinology and Metabolism 83, 4314-20.

Soares, J.B. and Leite-Moreira, A.F., 2008. Ghrelin, des-acyl ghrelin and obestatin: three pieces of the same puzzle. Peptides 29, 1255-70.

Takahashi, K., Furukawa, C., Takano, A., Ishikawa, N., Kato, T., Hayama, S., Suzuki, C., Yasui, W., Inai, K., Sone, S., Ito, T., Nishimura, H., Tsuchiya, E., Nakamura, Y. and Daigo, Y., 2006. The neuromedin U-growth hormone secretagogue receptor 1b/neurotensin receptor 1 oncogenic signaling pathway as a therapeutic target for lung cancer. Cancer Res 66, 9408-19.

Ueberberg, B., Unger, N., Sheu, S.Y., Walz, M.K., Schmid, K.W., Saeger, W., Mann, K. and Petersenn, S., 2008. Differential expression of ghrelin and its receptor (GHS-R1a) in various adrenal tumors and normal adrenal gland. Horm Metab Res 40, 181-8.

Ueberberg, B., Unger, N., Saeger, W., Mann, K. and Petersenn, S. 2009. Expression of ghrelin and its receptor in human tissues. Horm Metab Res 41, 814-821.

Volante, M., Allia, E., Fulcheri, E., Cassoni, P., Ghigo, E., Muccioli, G. and Papotti, M., 2003. Ghrelin in fetal thyroid and follicular tumors and cell lines: expression and effects on tumor growth. Am J Pathol 162, 645-54.

Volante, M., Allia, E., Gugliotta, P., Funaro, A., Broglio, F., Deghenghi, R., Muccioli, G., Ghigo, E. and Papotti, M., 2002. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. J Clin Endocrinol Metab 87, 1300-8.

Wang, D.H., Hu, Y.S., Du, J.J., Hu, Y.Y., Zhong, W.D. and Qin, W.J., 2009. Ghrelin stimulates proliferation of human osteoblastic TE85 cells via NO/cGMP signaling pathway. Endocrine 35, 112-7.

Waseem, T., Javaid Ur, R., Ahmad, F., Azam, M. and Qureshi, M.A., 2008. Role of ghrelin axis in colorectal cancer: a novel association. Peptides 29, 1369-76.

Wasko, R., Jaskula, M., Kotwicka, M., Andrusiewicz, M., Jankowska, A., Liebert, W. and Sowinski, J., 2008. The expression of ghrelin in somatotroph and other types of pituitary adenomas. Neuro Endocrinol Lett 29, 929-38.

Wasko, R., Jaskula, M., Plewa, R., Komarowska, H., Poreba, E., Gozdzicka-Jozefiak, A., Liebert, W., Bednarek, J. and Sowinski, J., 2006. The evaluation of ghrelin mRNA expression in human somatotroph adenomas. Neuro Endocrinol Lett 27, 169-73.

Xia, Q., Pang, W., Pan, H., Zheng, Y., Kang, J.S. and Zhu, S.G., 2004. Effects of ghrelin on the proliferation and secretion of splenic T lymphocytes in mice. Regul Pept 122, 173-8.

Yang, M., Hu, S., Wu, B., Miao, Y., Pan, H. and Zhu, S., 2007. Ghrelin inhibits apoptosis signal-regulating kinase 1 activity via upregulating heat-shock protein 70. Biochem Biophys Res Commun 359, 373-8.

Yeh, A., Jeffery, P.L., Duncan, R.P., Herington, A. and Chopin, L., 2005. Ghrelin and a novel preproghrelin isoform are highly expressed in prostate cancer and ghrelin activates mitogen-activated protein kinase in prostate cancer. Clinical Cancer Research 11, 8295-8303.

Zhang, Y., Ying, B., Shi, L., Fan, H., Yang, D., Xu, D., Wei, Y., Hu, X., Zhang, X., Wang, T., Liu, D., Dou, L., Chen, G., Jiang, F. and Wen, F., 2007. Ghrelin inhibit cell apoptosis in pancreatic beta cell line HIT-T15 via mitogen-activated protein kinase/phosphoinositide 3-kinase pathways. Toxicology 237, 194-202.

Zub-Pokrowiecka, A., Rembiasz, K., Konturek, SJ., Budzynski, A., Konturek, PC., Budzynski P. 2010. Ghrelin in diseases of the gastric mucosa associated with Helicobacter pylori infection. Medical Science Monitor 16, CR493-500.

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Figure legend

Figure 1: Ghrelin plays roles in a number of processes related to cancer progression, including proliferation, apoptosis, and cell invasion and migration