

Ghrelin and its role in gastrointestinal tract tumors (Review)

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Received April 11, 2021; Accepted June 23, 2021

DOI: 10.3892/mmr.2021.12302

Abstract. Ghrelin, an orexigenic hormone, is a peptide that binds to the growth hormone secretagogue receptor; it is secreted mainly by enteroendocrine cells in the oxyntic glands of the stomach. Ghrelin serves a role in both local and systemic physiological processes, and is implicated in various pathologies, including neoplasia, with tissue expression in several types of malignancies in both *in vitro* and *in vivo* studies. However, the precise implications of the ghrelin axis in metastasis, invasion and cancer progression regulation has yet to be established. In the case of gastrointestinal (GI) tract malignancies, ghrelin has shown potential to become a prognostic factor or even a therapeutic target, although data in the literature are inconsistent and unsystematic, with reports untailored to a specific histological subtype of cancer or a particular localization. The evaluation of immunohistochemical expression shows a limited outlook owing to the low number of cases analyzed, and *in vivo* analyses have conflicting data regarding differences in ghrelin serum levels in patients with cancer. The aim of this review was to examine the relationship between ghrelin and GI tract malignancies to demonstrate the inconsistencies in current results and to highlight its clinical significance in the outcome of these patients.

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1. Introduction

Ghrelin was first discovered in 1999 and was described as an endogenous ligand of the growth hormone secretagogue receptor (GHSR) (1). Ghrelin is produced in the fundic area of the stomach, where it is secreted directly into the bloodstream (2). Initially thought to regulate growth hormone (GH) secretion, ghrelin has been shown to serve various biological functions. A main role of ghrelin is in the gut-brain interaction (3), with expression in the hypothalamus and pituitary (4). This hormone is also known to serve a key role in appetite stimulation (5), gastric motility and acid secretion (6), stress and anxiety (7), and regulation of the circadian rhythm (8). Ghrelin is typically released when the stomach is empty, suggestive of its involvement in endocrine regulation of motility-dependent interdigestive gastric secretion (9).

Ghrelin has long been considered to be involved in tumorigenic proliferation and although its precise role is still uncertain, it has received increasing attention in research regarding gastrointestinal (GI) neoplasia. The immunohistochemical (IHC) expression of ghrelin has been documented in a number of endocrine and non-endocrine tumors (10,11), but it is still unclear whether an autocrine/paracrine loop or another type of interaction is involved. At present, it has not yet been reported if the expression of ghrelin and its receptors in tumor cells are protective against neoplasia or whether they stimulate carcinogenesis. Therefore, this review provides an assessment of the current literature, presenting the emerging trends and highlighting potential gaps in current knowledge regarding the implications of ghrelin from its production to the interaction with its known receptors (known as the ghrelin axis) in digestive malignancies.

2. Ghrelin and its receptors

Ghrelin is primarily produced in the stomach (12), where X/A-like cells are found in abundance in oxyntic glands, close to parietal cells; in addition, ghrelin displays rare colocalization with other hormones (13). Ghrelin cells are closed-type cells, having no contact with the lumen (2) and release their secretions within the vascular bed (14,15). However, ghrelin and its gene transcripts have also been identified in other enteroendocrine cells, namely enterochromaffin cells, S cells, I cells, L cells and M cells (16-20). Ghrelin is also produced in variable amounts in other segments of the GI tract (duodenum, jejunum, ileum, colon) (21-24), as well as in

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Key words: ghrelin, growth hormone secretagogue receptor, carcinogenesis, cancer, pathology, prognosis

the lungs, kidneys, testes, prostate, ovaries, breasts, pituitary and hypothalamus (25-28). Ghrelin release is activated by a series of receptors, like β 1-adrenergic, calcitonin gene-related peptide, glucose-dependent insulinotropic polypeptide and secretin receptors. Moreover, ghrelin is inhibited by both short-chain and long-chain fatty acids, lactate, extracellular calcium, amino acids and somatostatin receptors (9).

Genetic mapping has placed the ghrelin gene on the short arm of chromosome 3 (3p25-26) (27), with an initial gene product known as pre-proghrelin (29,30). Pre-proghrelin is a 117 amino acid pre-protein, containing a 23-amino acid N-terminal signal peptide that is cleaved to form the 94 amino acid peptide, proghrelin (31). Proghrelin is then split by a prohormone convertase to produce the 28 amino acid ghrelin peptide and a 66 amino acid C-terminal fragment (known as C-ghrelin), which gives rise to the hormone obestatin (32).

Acylated ghrelin, desacyl ghrelin and C-ghrelin, have been found in ghrelin-secreting cells and in the circulation, and have been previously described as peripheral forms of ghrelin (33-36). Desacyl ghrelin shows a remarkable predominance in peripheral blood (35,37), mainly owing to it being the most secreted and more stable form of ghrelin (34,38). Alternative ghrelin-related peptides and mRNA splice variants were subsequently identified (31,39). With multiple variants described to date, the intron 1 (In1)-ghrelin variant has an alternative C-terminal tail but displays high areas of conservation to ghrelin that are essential in the process of acylation and allow receptor binding (40). Ghrelin must be acylated with an octanoyl group to activate its receptor, which is achieved by a post-translational enzymatic change initiated by ghrelin-o-acyltransferase (GOAT) (41). Although this process is necessary for ghrelin to be highly physiologically active through its receptor (42,43), certain studies also suggest a metabolic role for desacyl ghrelin (44,45).

The ghrelin receptor, GHSR, belongs to the G-protein coupled receptor family (46). There are two isoforms of the GHSR, GHSR1a and GHSR1b, both of which are widely expressed throughout the body. First identified in the pituitary and hypothalamus, GHSR1a is expressed in a broad range of tissues (bone; adipose tissue; lymphoid tissue) and organs, including the stomach, intestine, pancreas, spleen, thyroid gland, prostate, ovaries, testes, adrenal glands, kidneys, heart and lungs (47-50). GHSR1a is considered to be a functional receptor, as it mediates several of the effects of ghrelin, whereas GHSR1b is to be considered a non-functional receptor, devoid of signal transduction activity (51). The expression of GHSR1b is mostly similar to that of its splicing variant with regard to localization, albeit it is different in intensity, with higher detectable levels of mRNA (11,25,50).

Acylated ghrelin preferentially binds to GHSR1a, leading to GH secretion. Both the acylated and desacylated forms have been identified as functionally active in local and systemic processes, such as adipogenesis and lipid retention (52-55). However, desacyl ghrelin does not bind to GHSR1a, suggesting its effects are GHSR-independent, with its cognate receptor remaining unknown (56,57). Desacyl ghrelin mainly exerts nonendocrine activities, with involvement in vascular remodeling, cardiovascular stability, muscle atrophy, cachexia and lipolysis (38,58-62). A schematic illustration of the main steps

in the ghrelin circuit, from production to interaction with its receptor, is presented in Fig. 1.

Ghrelin is a hormone involved in a number of physiological processes outside of the GI tract; it is a key participant in the interaction between the enteric and the central nervous systems, with implications in physical, mental and behavioral changes owing to the regulation of the circadian rhythm, and stress and anxiety levels (3,7,60). In the GI tract, ghrelin interacts with all organs, stimulating gastric acid secretion, increasing GI motility (6) and modulating glucose levels through an increase of pancreatic insulin release and through insulin sensitivity (63). In the liver, ghrelin promotes gluconeogenesis and lipogenesis (64,65), the latter also being its main action in adipose tissue (66).

Even though the physiological roles of ghrelin are yet to be fully revealed, it has been demonstrated that ghrelin contributes to the pathology of a multitude of diseases. Its orexigenic role is well established (5,67,68); however, ghrelin also serves a function in inflammatory processes (69). The presence of ghrelin and its receptors on human leucocytes (T lymphocytes and macrophages) was noted as early as 2004 (70), with proven anti-inflammatory effects in both animal and human studies, in acute (71-74) and chronic settings (75-78), as well as in a neoplastic context (79). Ghrelin receptors have also been identified in cardiac tissue (80), where they modulate cardiac function and healing by influencing myocardial contraction (81), perfusion (82) and post-myocardial infarction-changes (41,72,83). In the lungs, ghrelin can attenuate pulmonary blood pressure through vascular remodeling and mitigate the changes induced by acute lung injury (84,85). Ghrelin also serves a role in the intricate pathologies of anorexia and cachexia (86,87) as well as neurodegenerative disorders, such as dementia (88), multiple sclerosis (89) and amyotrophic lateral sclerosis (90). The pleiotropic effects of the ghrelin axis are summarized in Fig. 2.

Present in both endocrine and non-endocrine tumors, such as breast, prostate, testicular, GI, pancreatic, renal, thyroid, lung and adrenal tumors (11,91-94), ghrelin and its receptors may give rise to a complex set of interactions that contribute to neoplastic development at various stages. Although the precise role of ghrelin is still uncertain, numerous studies have used different experimental approaches to document the involvement of the ghrelin axis in neoplasia.

3. Experimental studies and their role in understanding the ghrelin axis in cancer development

Experimental studies have investigated the connection between ghrelin and neoplasia, in attempts to uncover a novel prognostic factor or to find missing links in the processes of tumorigenesis, invasion and metastasis. The majority of *in vitro* studies have focused on ghrelin and GHSR splice variants expression in various tumor cell lines, investigating the effects of ghrelin on tumor development. A summary of these findings regarding GI tumor cells and highlighting the diversity of results published on this topic can be found in Table I. Ghrelin has also been reported to be expressed in experimental studies using cell lines of leukemia (95), breast carcinoma (96), pulmonary adenocarcinoma (97), pancreatic ductal adenocarcinoma (98), prostate carcinoma (99),

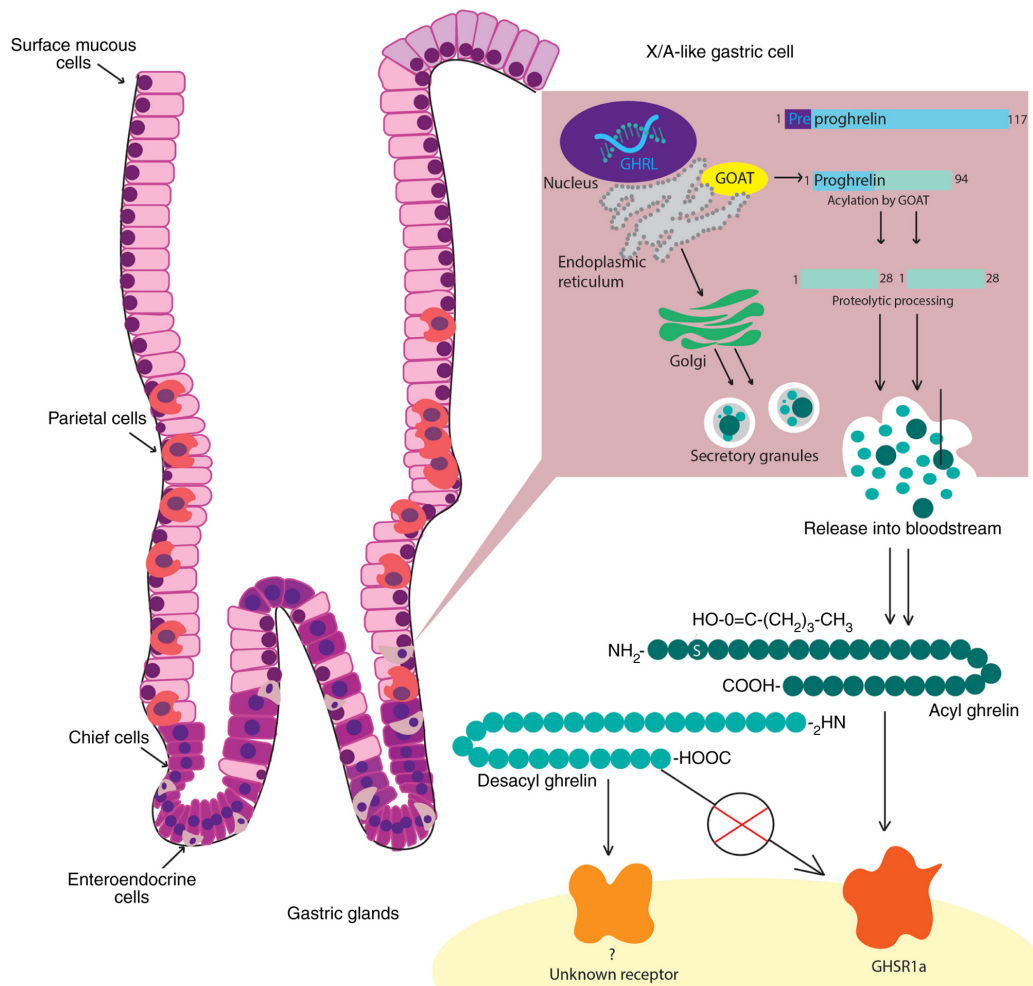


Figure 1. Synthesis of ghrelin and its mechanisms of action. Ghrelin is produced by X/A-like enteroendocrine cells located in the stomach. GOAT is an enzyme responsible for the acylation of this hormone, located within the endoplasmic reticulum. Proghrelin is cleaved in the Golgi body to form ghrelin, in its acylated and desacylated forms, which are secreted directly into the bloodstream. Circulating ghrelin can also be converted to the desacylated form, which is predominant in the peripheral blood. The known receptor for ghrelin is GHSR1a, which does not bind desacyl ghrelin, the receptor for desacyl ghrelin remains unknown. GHRL, growth hormone secretagogue receptor ligand; GHSR, growth hormone secretagogue receptor; GOAT, ghrelin-o-acyltransferase.

gastric adenocarcinoma (100) and colorectal adenocarcinoma (101,102). and it has shown pleiotropic effects in the vast majority of these tumor cell lines (11).

Ghrelin expression is less variable compared with the expression of its receptors, which is highly variable between different types of cancer. GHSR1a and GHSR1b are most often co-expressed, with GHSR1a expression levels demonstrated to be higher compared with those for GHSR1b in oral squamous cell carcinoma (SCC) and gastric cancer (GC) cell lines (100,103). For breast cancer cell lines, an initial study reported that lines MDA-MB-231, MCF-7, MDA-MB-435 and T47D have positive IHC expression of the ghrelin/GHSR axis (27), whereas GHSR1a mRNA could not be detected in lines MDA-MB231, MCF7 and T47D, in spite of specific binding of ghrelin in these cells, implying that an unidentified interaction or binding site modulates these effects (96). High GHSR1b expression was identified in breast cancer cell line MDA-MB-231 (39). A similar difference in expression was demonstrated in colon cancer cell lines (101).

The effects of ghrelin can vary according to the type of cancer. Previous studies have found ghrelin can

have a proliferative effect on a number of cancer cell lines including breast (27), prostate (28), gastric (100), colorectal (101,102,104), oral (103), pancreatic (98), adrenocortical (105) and endometrial (106). Conversely, D-Lys-growth hormone releasing peptide 6 or other ghrelin-specific antibodies can antagonize ghrelin and inhibit this proliferative effect (101). Certain studies attribute this proliferative effect to the desacyl form (27,91,95), suggesting that in these cell lines ghrelin can stimulate cell proliferation via an autocrine pathway independent of GHSR1a (95). However, ghrelin has also been shown to inhibit proliferation and exhibit an antiapoptotic effect in cell lines of the same cancer type, as is the case for breast carcinoma cell lines MCF7 and MDA-MB231 (96) and prostate carcinoma cell lines PC-3 and DU-145 (91). Both acylated and deacylated ghrelin inhibit DU-145 cell proliferation (91,99,107). A recent study on chemosensitive ovarian cells demonstrated that acylated ghrelin promotes cell proliferation and survival, and inhibit apoptosis through its interaction with GHSR1a via the PI3K/Akt signaling pathway, thereby rendering the cells resistant to chemotherapy, even at very low levels (108).

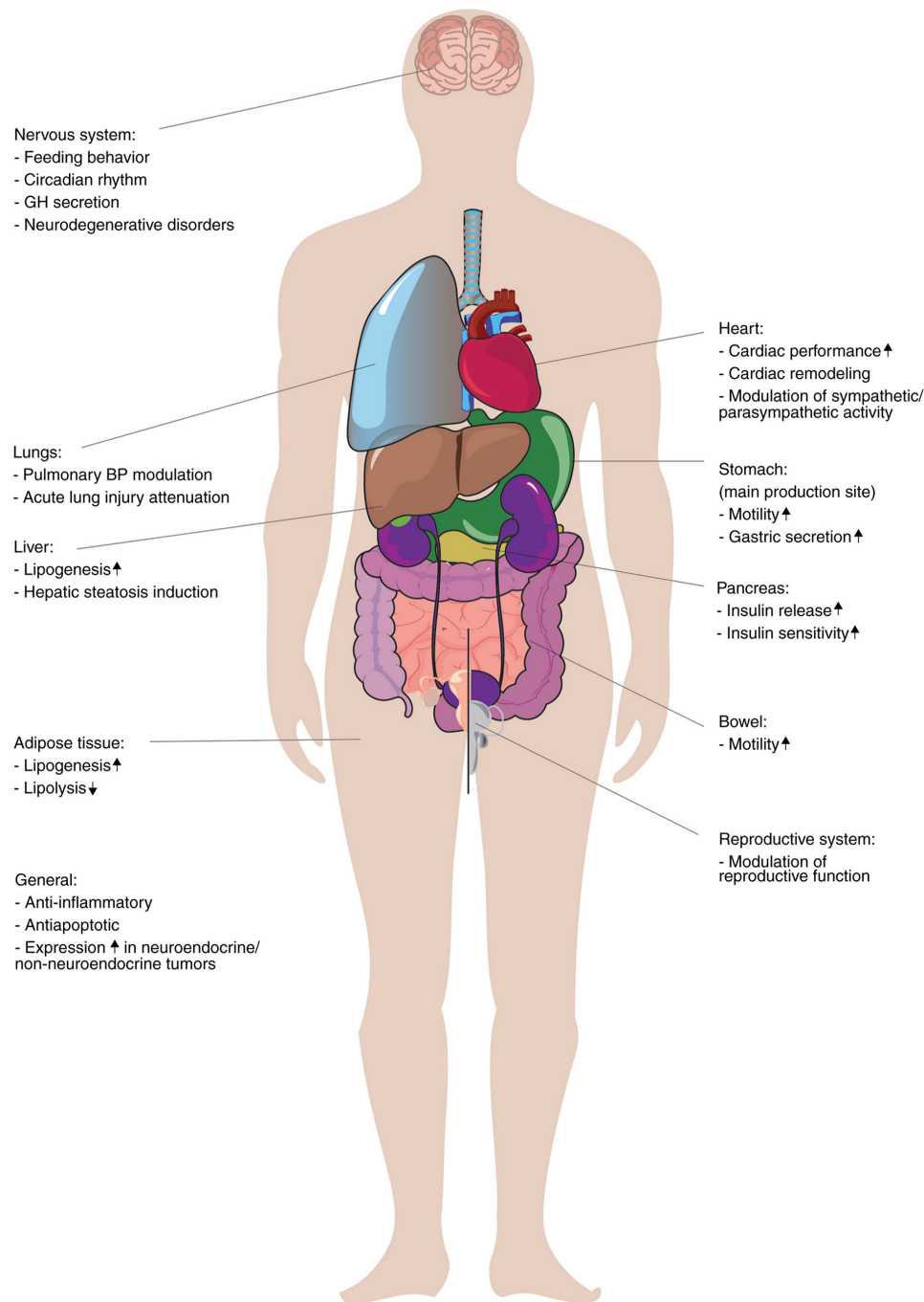


Figure 2. Overview of the implications of the ghrelin axis in pathophysiological processes in the body. The physiological functions of ghrelin extend well beyond the local influence on GI motility, spanning over a multitude of organs and systems. Ghrelin is a key element of the brain-gut axis and plays a major role in manipulating satiety, in the plasticity of adipose tissue and modulation of metabolic pathways in various organs. Furthermore, ghrelin is implicated in complex pathological processes, such as inflammation, cardiac remodeling or tumorigenesis.

The ghrelin splice variant, In1-ghrelin, can increase the proliferation rate of the MDA-MB-231 breast cancer cell line (39) and stimulate proliferation and migration of MCF-7 and MDA-MB-231 cells (109). Expression of this variant was also found in pancreatic adenocarcinoma cell lines, which do not express ghrelin, wherein it enhanced the proliferation and migration of tumor cells (110).

To better document and understand these effects, the activation pathway of ghrelin has been examined extensively, and numerous pathway activation mechanisms have been identified. Through the interaction of ghrelin and GHSR the

PI3-K/Akt/mTOR signaling pathway is able to mediate the migration and invasiveness of pancreatic adenocarcinoma cells (98). One study demonstrated that NF- κ B signaling pathway activation can contribute to ghrelin-induced cell migration in glioma cells (111). Furthermore, Lin *et al* (94) determined ghrelin has diverse implications in tumorigenesis and tumor spread, with the ability to promote metastasis in the case of renal cell carcinoma (RCC) via Snail, a transcriptional repressor of E-cadherin, and invasion through the upregulation of the kinase Aurora A, an activating mechanism which was also associated with a poor prognosis (112). Similarly,

Table I. Summary of ghrelin axis expression and effects in gastrointestinal tract cancer cell line cultures.

A, Esophageal cancer									
Ghrelin		GHSR1a		GHSR1b		Effect		(Refs.)	
Cancer cell line	mRNA	protein expression	mRNA	protein expression	mRNA	protein expression	GOAT	Other	Effect
OK-19	-	-	n/a	n/a	n/a	n/a	n/a	n/a	No antiapoptotic effect; anti-inflammatory
B, Gastric cancer									
Ghrelin		GHSR1a		GHSR1b		Effect		(Refs.)	
Cancer cell line	mRNA	protein expression	mRNA	protein expression	mRNA	protein expression	GOAT	Other	Effect
AGS	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	Proliferative (ghrelin and des-acyl ghrelin-concentration based); migration; invasion
SGC-7901	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	Proliferative (ghrelin and des-acyl ghrelin-concentration based); migration; invasion
C, Colorectal cancer									
Ghrelin		GHSR1a		GHSR1b		Effect		(Refs.)	
Cancer cell line	mRNA	protein expression	mRNA	protein expression	mRNA	protein expression	GOAT	Other	Effect
SW-48	+	n/a	+	n/a	+	n/a	n/a	n/a	Proliferation; migration; invasion
RKO	+	n/a	+	n/a	+	n/a	n/a	n/a	Proliferation; migration; invasion
FHs74Int	+/-	+	+	+	+	+	+	n/a	Proliferation
Caco-2	+/-	+	+	+	+	+	+	n/a	Proliferation (GHSR-1a independent)
D, Pancreatic cancer									
Ghrelin		GHSR1a		GHSR1b		Effect		(Refs.)	
Cancer cell line	mRNA	protein expression	mRNA	protein expression	mRNA	protein expression	GOAT	Other	Effect
PANC1	+	-	+	+	+	+	+	n/a	Proliferation; migration; invasion
MIAPaCa2	-	-	+	+	+	+	+	n/a	Proliferation; migration; invasion
BxPC3	-	-	+	+	+	+	+	n/a	Proliferation; migration; invasion
Capan2	-	-	+	+	+	+	+	n/a	Proliferation; migration; invasion

Table I. Continued.

E, NET											
Cancer cell line	Ghrelin		GHSR1a		GHSR1b		GOAT	Other	Effect	(Refs.)	
	mRNA	protein	Tissue expression	mRNA	Protein expression	mRNA					Protein expression
BON-1	n/a	+	-	n/a	+	n/a	+	In1-ghrelin +	Proliferation; migration	(159,160)	
QCP-1	n/a	+	-	n/a	+	n/a	+	In1-ghrelin +	Proliferation; migration		
*Early studies refer to GHSR as target receptor for ghrelin, not taking into account currently known splice variants. GHSR, ghrelin hormone receptor; GOAT, Ghrelin-O-Acyltransferase; NET, neuroendocrine tumors; In1-ghrelin: Intron-1 ghrelin; +, positive expression; -, negative expression; +/-, equivocal expression.											

^aEarly studies refer to GHSR as target receptor for ghrelin, not taking into account currently known splice variants. GHSR, ghrelin hormone receptor; GOAT, Ghrelin-O-Acyltransferase; NET, neuroendocrine tumors; In1-ghrelin: Intron-1 ghrelin; +, positive expression; -, negative expression; +/-, equivocal expression.

invasion in GC was determined to be a result of GHSR/NF- κ B signaling pathway activation (100).

4. Ghrelin and GI tract tumors

Although ghrelin serves a known role in the inflammatory processes of the GI (113,114), data regarding its promoting role in GI carcinogenesis is still controversial. Studies on malignant cell lines have shown conflicting results, as aforementioned. Therefore, research has now shifted from cell line experiments to analyzing tissue expression and circulating levels of ghrelin and its isoforms. The complex dynamics of ghrelin in GI tumors is described in Table II, which provides an integrated overview of the representative results in this research field.

Esophageal cancer. In the esophagus, expression of ghrelin was first demonstrated in SCC by IHC, with tissue ghrelin levels correlating with degree of differentiation, depth of tumor invasion, lymphovascular invasion and tumor stage (115). However, ghrelin expression levels showed no correlation with patient survival (115). IHC detection of ghrelin expression in esophageal adenocarcinoma was undetectable (116). Ghrelin serum levels have an inverse relationship with the risk of developing esophageal malignancies, especially SCC (93,117), and patients with a low ghrelin level are seven-times more likely to develop this histological subtype (117). This unexpected relationship between ghrelin levels and the risk of malignancy has been validated in a larger cohort study (118).

Compared with esophageal SCC, ghrelin serum levels did not correlate with the risk of developing esophageal adenocarcinoma (93). High serum concentrations of ghrelin (>3,200 pg/ml) were associated with a lower risk of developing esophageal adenocarcinoma in overweight patients with a body mass index (BMI) >25 (119). With well-known orexigenic effects, ghrelin may prove to be the basis for the association between abdominal obesity and Barrett's esophagus (BE) (120). A case-control study involving 886 patients with a diagnosis of BE demonstrated that higher ghrelin serum concentrations were positively associated with an increased risk of BE, irrespective of *Helicobacter pylori* infection and BMI (120). A positive association between ghrelin and BE development was also supported in another study (121). Upregulated expression of GHSR in BE compared with normal esophageal mucosa has also been reported (122). However, no correlation between ghrelin levels and the risk of BE was found in a recent meta-analysis compared with other adipokines, but only two studies approaching this interaction were considered (123).

Considering the various factors that influence ghrelin production, recent studies have looked at digestive tract malignancies from a broader perspective. For example, serum levels of ghrelin are correlated with nutritional status (124), with similar levels of active (acylated) and desacyl ghrelin in a balanced (well-nourished vs. malnourished) population of patients with esophageal cancer; it has been argued that active ghrelin is positively correlated with energy metabolism. Together, with a positive correlation with IL-6 levels, these findings may suggest the existence of a form of ghrelin resistance (125). Ghrelin serum levels also correlate with inflammatory responses and high levels have been observed in

Table II. Ghrelin axis expression evaluation in patients with gastrointestinal tumors and related conditions.

A, Esophagus									
Tumor type/associated conditions	Ghrelin ^a			GHSR1a ^a		GHSR1b ^a		Other	(Refs.)
	mRNA	Tissue protein expression	Serum	mRNA	Protein expression	mRNA	Protein expression		
BE	n/a	n/a	High levels associated with an increased risk of BE		^b GHSR upregulation			n/a	(120-122)
ADK	-	IHC -	Low	n/a	IHC +/-	n/a	IHC +/-	n/a	(93,116)
SCC	n/a	IHC+ correlated with clinicopathological factors	Low, associated with increased risk of malignancy	n/a	n/a	n/a	n/a	n/a	(93,115,117)
	n/a	n/a	Low, associated with decreased risk of malignancy	n/a	n/a	n/a	n/a	n/a	(118)
B, Stomach									
Tumor type/associated conditions	Ghrelin ^a			GHSR1a ^a		GHSR1b ^a		Other	(Refs.)
	mRNA	Tissue protein expression	Serum	mRNA	Protein expression	mRNA	Protein expression		
ADK	-/+	ELISA, low; IHC, low/-	No difference		^b GHSR upregulation				(116,129)
	+	Low, associated with increased risk of malignancy	High, associated with increased risk of malignancy		^b GHSR upregulation			GHRL overexpression associated with poor outcome	(118,130,132,136)
NET	n/a	IHC+	No difference	n/a	n/a	n/a	n/a	n/a	(156)

Table II. Continued.

C, Colon									
Tumor type/associated conditions	Ghrelin ^a			GHSR1a ^a		GHSR1b ^a		GOAT ^a	Other (Refs.)
	mRNA	Tissue protein expression	Serum	mRNA	Protein expression	mRNA	Protein expression		
ADK	n/a	IHC+ (stage-dependent)	n/a	n/a	n/a	n/a	n/a	n/a	(101)
	n/a	n/a	Low, associated with increased risk of malignancy	n/a	n/a	n/a	n/a	n/a	(140,143)
	n/a	n/a	No difference	n/a	n/a	n/a	n/a	n/a	(72,142)
	n/a	n/a	High, correlated with tumor size	n/a	n/a	n/a	n/a	n/a	(124,141)
	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	(145)
D, Pancreas and other NETs									
Tumor type/associated conditions	Ghrelin ^a			GHSR1a ^a		GHSR1b ^a		GOAT ^a	Other (Refs.)
	mRNA	Tissue protein expression	Serum	mRNA	Protein expression	mRNA	Protein expression		
NET	+	+	No difference	+	+	n/a	n/a	n/a	(148,156,153)
	+	+	n/a	^b GHSR upregulation (mRNA and IHC+)		n/a	n/a	n/a	
	n/a	IHC low/-	No difference	n/a	n/a	n/a	n/a	n/a	(150,158)
	n/a	n/a	High	n/a	n/a	n/a	n/a	n/a	(157)
	+	n/a	n/a	+/-low	n/a	++	n/a	++	In1-ghrelin (splice variant) high

^aNumber of + indicate relative expression; ^bearly studies refer to GHSR as target receptor for ghrelin, not taking into account currently known splice variants. +, positive expression; -, negative expression; +/-, equivocal expression; ADK, adenocarcinoma; BE, Barrett's esophagus; GHSR, ghrelin hormone receptor; GOAT, Ghrelin-O-Acyltransferase; IHC, immunohistochemical; n/a, not applicable; NET, neuroendocrine tumors; In1-ghrelin, intron-1 ghrelin; SCC, squamous cell carcinoma.

a high-inflammation group of patients with GI neoplasms (124), a finding which could be further explored and integrated into the context of inflammation as a prognostic factor for these malignancies (126,127).

GC. Ghrelin axis expression determined by IHC examination shows undetectable levels in gastric adenocarcinoma (116). However, previous studies have a limited number of patients which may make it difficult to draw conclusions. In a study of 10 patients, none tested ghrelin-positive (116), these findings were supported by a similar study analyzing 9 patients with gastric adenocarcinoma (128).

The fluctuation of ghrelin levels over time highlights the potential role of this peptide as a biomarker in gastric malignancies (118). A study on a small cohort of Korean patients with GC showed a 10-fold increase in plasma ghrelin levels of these patients (129). However, ghrelin levels of the tumor tissue were lower compared with those of the normal gastric mucosa, suggesting that gastric tumorigenesis can inhibit ghrelin production in the adjacent mucosa (129). Furthermore, certain studies have demonstrated a significant increase in the risk of non-cardia GC and esophago-gastric junction cancer in individuals with lower baseline serum ghrelin concentrations, changes which occur early in the carcinogenic process (93,130). The risk of these diseases remains increased, irrespective of *H. pylori* infection (130).

Ghrelin levels can fluctuate depending upon lesions associated with the gastric mucosae with certain studies indicating a reduction in ghrelin plasma levels in cases of *H. pylori* infection, which steadily increase following the eradication of infection (129,131,132). In addition, a histologically higher degree of gastric atrophy seems to correlate with a lower plasma ghrelin concentration (132).

Different surgical approaches seem to radically influence ghrelin levels in patients with GC. Ghrelin levels were higher in patients who had a distal gastrectomy compared with those who had a total gastrectomy, and the physiological regulation of ghrelin secretion and plasma levels was unaffected due to preservation of the gastric fundus (133). In patients with proximal resection, postoperative ghrelin increased more slowly compared with that in patients after fundus-preserving resection (134). Takachi *et al* (135) demonstrated that there was a significant decrease in the concentration of ghrelin following total gastrectomy, with very low levels observed in the long-term follow-up. Furthermore, Zub-Pokrowiecka *et al* (133) demonstrated that plasma levels of ghrelin were lower in patients with GC and in those who formerly had GC and undergone surgery 4-5 years previously, compared with the healthy control group.

Ghrelin alone may not be a useful biomarker in evaluating the risk of gastric malignancies, but when coupled with other early detection biomarkers, such as pepsinogen I and pepsinogen I/II ratio, as part of a complex panel it may provide higher accuracy (118). Recent advances in the field point towards the potential role of the ghrelin gene, growth hormone secretagogue receptor ligand (GHRL), as a poor prognosis biomarker in GC (136). A study involving 295 patients with GC and data from 4 gene expression microarrays, found 12 upregulated and 59 downregulated differentially expressed genes, with high expression of GHRL associated with poor overall survival of patients with GC (136).

Colorectal cancer (CRC). The interaction between ghrelin and its receptors, as demonstrated through IHC and molecular studies, is indicative of the presence of an autocrine/paracrine mechanism involved in colorectal carcinogenesis (101,137). In CRC, the ghrelin axis serves a role in the initial stages of carcinogenesis, with positive expression of ghrelin and its receptors in low-grade tumors, as opposed to almost complete loss of expression in high-grade tumors (101). Axis upregulation has also been demonstrated by Liu *et al* (138) in well-differentiated and moderately differentiated adenocarcinomas, whereby the role of promoting cell growth in CRC was attributed to the interaction of ghrelin with the GHSR1a receptor. However, in spite of this Waseem *et al* (101) demonstrated that the expression levels of ghrelin and its orphan GHSR1b receptor were increased in patients with CRC, contrary to the decrease of GHSR1a with advancing tumor stage. Although tissue expression of ghrelin seems to have a positive correlation with tumor stage, a study on 110 patients with CRC failed to correlate ghrelin plasma levels with any CRC clinicopathological features (101).

Increased tissue expression of ghrelin and its receptors in CRC has initiated the idea of its role as a potential biomarker. However, even though this research area has attracted the interest of numerous research groups it is not without controversy. An initial study on a small sample of 20 patients found no difference in circulating ghrelin levels in patients with CRC compared with a control group and no correlation with tumor clinicopathological characteristics (139). A similar study on a group of 29 patients with lower GI tract malignancies, found a statistically significant difference in ghrelin levels between patients with CRC and the control group and noticed a decrease in ghrelin levels with tumor progression (140). This study therefore advanced the idea of ghrelin serum levels as being inversely correlated with tumor aggressiveness and their potential use as a prognostic parameter (140).

The role of circulating ghrelin in the evaluation of patients with CRC was demonstrated by Nikolopoulos *et al* (141), indicating a significant positive correlation between ghrelin levels and tumor size. Ghrelin plasma levels were increased in end-stage disease and were correlated with the degree of differentiation, being higher in poorly differentiated CRC. However, no significance was detected in using ghrelin as a predictor of CRC survival (141). A similar study found no difference in ghrelin peripheral blood expression between patients with CRC compared with the control group (142). Perioperative serum levels were reported by Zhu *et al* (124) as being higher in patients with cancer compared with patients in the control group, a finding that contrasted with previous studies (139). The aforementioned study also noted a dramatic decrease of ghrelin levels following surgical removal of the tumors, advancing the possibility of a new early-warning marker of CRC and other GI tract malignancies (124).

A large case-control study published in 2018 demonstrated a positive correlation between decreased levels of ghrelin and the risk of developing CRC, spanning over a 10-year period (143). Furthermore, a validation study comparing plasma ghrelin levels in 60 patients with CRC in a perioperative context and a 5-year interval preceding the diagnosis of CRC prior to surgery, demonstrated that ghrelin was not

associated with an increased risk of malignancy, with ghrelin levels remaining stable over time (144).

Current data indicates that there are no significant changes in GHRL methylation levels (145). Furthermore, GHRL methylation is unlikely to become a biomarker of CRC, as no significant differences in hypermethylation of GHSR were observed in CRC compared with normal mucosa (145). This process is present in adenomas, irrespective of the degree of dysplasia, and results have not demonstrated any increase in hypermethylation correlated with tumor progression (145).

As in the case of esophageal cancer, it has been suggested that ghrelin plasma levels should not be interpreted in the absence of nutritional status and BMI (146). It is well documented that ghrelin levels increase in the case of cancer cachexia and decrease with obesity (147).

Neuroendocrine tumors (NETs) of the GI tract. Expression of ghrelin has been detected in NETs of gastropancreatic origin. Rindi *et al* (148) detected varying levels of ghrelin expression in digestive NETs, particularly in gastric tumors; these findings were supported by prior detection of ghrelin mRNA and were reported in other studies (11,149). A study investigating NETs of the GI tract in patients with multiple endocrine neoplasia type 1, determined that only 25% of cases were reported as ghrelin-positive and no clinicopathological correlation was established (150). The proportion of ghrelin-positive cells varied between 1 and 20% (148,150).

Pancreatic NETs express ghrelin in up to 95% of cases, validated by quantitative PCR (qPCR), whereas up to 67% of cases can be detected by IHC, with some tumors having only a small proportion of cells staining positively and others with >80% positive cells (148,151,152). Ghrelin expression has been documented in both functioning and non-functioning tumors of the pancreas (62 vs. 69% of cases, respectively) (153). Papotti *et al* (149) demonstrated ghrelin expression in intestinal NETs; however, others did not find any ghrelin-positive intestinal NET cells (148,153). Furthermore, the expression of ghrelin in neuroendocrine carcinoma was initially reported to be absent (148), whereas subsequent studies found ghrelin expression in scattered cells or focally in up to 10% of the tumor cells, which was associated with hyperghrelinemia (154,155).

Plasma ghrelin levels in patients with gastroenteropancreatic NETs were initially demonstrated to not be significantly different between patients with tumors and healthy controls, neither initially, nor during progression of the disease (153,156). A small subset of patients had high plasma ghrelin levels (>1280 ng/dl); however, two out of five such patients had tumors that had complete lack of ghrelin protein expression (153). In patients with metastatic disease, the levels of ghrelin were elevated, compared with the control group, in up to 85% of cases (157). A later study focusing on the active peripheral forms of ghrelin (acylated ghrelin; desacyl ghrelin; acylated ghrelin/desacyl ghrelin ratio) found no statistical difference between patients with NETs and the control group (158).

Luque *et al* (159) investigated the In1-ghrelin splice variant of the ghrelin gene in NETs and reported increased expression of In1-ghrelin in NETs compared with native ghrelin expression; these findings were confirmed in later years by another study (160). However, the differences in In1-ghrelin expression

between the two studies demonstrated further the increased heterogeneity in NETs (159,160). High levels of In1-ghrelin were detected in patients with metastasis (159).

Mesenchymal tumors. Although they are more rare than epithelial tumors, mesenchymal tumors of the GI tract contribute to the overall cancer burden of the region and may exhibit variable clinical behavior (161,162). Among the most frequent are GI stromal tumors (GISTs) (161). Ghrelin expression in GISTs has only been reported by a single study, which found both IHC and qPCR-detectable expression, with 77% of analyzed tumors showing immunoreactivity for ghrelin and all tumors displaying detectable levels of ghrelin mRNA, albeit this analysis was performed on only about a third of the 22 cases (151). However, the study did not find any statistical correlation between ghrelin levels and tumor location, size, morphological parameters or clinical behavior (151), the value of these results being reiterated by another recent paper recognizing the potential role of the ghrelin axis in GISTs and supporting further research on this topic (163). Studies of large patient cohorts need to be implemented to better understand the role of the ghrelin axis in the pathogenesis of such tumors. However, limitations arise as a result of their low incidence and variability.

5. Ghrelin interactions and expression in neoplasia outside the GI tract

As previously mentioned, ghrelin and its receptors are expressed in numerous endocrine and non-endocrine tumor cell types, such as pituitary, prostate, breast and lung (10,96,99,164). Since ghrelin acts not only as a local, but also as a systemic hormone, its potential role in cancer development in other organs has been investigated.

Oral SCC demonstrates the expression of the ghrelin axis, with ghrelin levels being negatively correlated with tumor invasiveness (165). Furthermore, decreasing ghrelin levels appear to be correlated with lower degrees of differentiation, indicating the potential value of tissue ghrelin expression as a prognostic marker (165). Another study demonstrated that expression of ghrelin and GHSR were gradually increased in oral tumors as benign cells developed cytological and architectural features of dysplasia and further progressed to becoming malignant (166).

An initial study on kidney tumors reported low to absent expression of ghrelin in RCC, with all 21 cases of conventional-subtype analyzed having no IHC ghrelin expression and partial-to-total loss of ghrelin expression when assessed through radioimmunoassays (167). However, certain kidney tumors, such as oncocytomas, did express ghrelin, albeit less than normal kidney tissue (167). Furthermore, Lin *et al* (94) assessed ghrelin expression levels in a cohort of 562 clear-cell type RCC cases and demonstrated that ghrelin levels were high and indicated a poor prognosis, with high ghrelin expression being associated with lymph node and distant metastases.

Ghrelin is expressed in both normal and tumoral breast tissue (96,168). While ghrelin expression was investigated in normal mammary epithelium (168), Grönberg *et al* (169) also investigated ghrelin expression and the potential role of this hormone in male breast carcinogenesis, revealing that

ghrelin expression was associated with a lower risk of breast cancer death. A study on 144 female breast cancer specimens determined ghrelin to be moderately to strongly expressed, which was positively correlated with survival and disease-free interval (92).

Cassoni *et al* (91) demonstrated prostatic adenocarcinomas were positive for ghrelin expression when assessed by reverse transcription-PCR but showed no immunoreactivity when assessed by IHC (91). Serum levels of ghrelin are higher in patients with prostate cancer compared with patients with benign prostate hyperplasia (170). Increased levels of In1-ghrelin and GOAT were also observed in patients with prostate cancer compared with a healthy control group (109).

Ghrelin may also serve a role in endometrial carcinogenesis through coupling with its GHSR1a receptor, with hormone and receptor expression detectable in benign and malignant tissue specimens (171). Expression has also been identified in various histological subtypes of lung cancer, accompanied by the expression of GHSR1a (172,173).

6. Conclusions

The role of ghrelin in GI tract malignancies is still incompletely characterized. The complexity of the ghrelin axis and the interaction between this hormone and cancer cells is still unclear. Studies on cancer cell lines show a spectrum of ghrelin and ghrelin receptor expression, and new splice variants of ghrelin involved in carcinogenesis are emerging. The role of IHC to detect expression is unclear, as most papers do not focus on tumor histological subtype or other factors such as tumor heterogeneity. The use of ghrelin alone as a potential serological biomarker is debatable, as it is regulated by various metabolic factors and is linked with cancer-associated inflammation. Although certain of the effects of ghrelin can be quantified, there are still large discrepancies in this field owing to the variety of peripheral, circulating forms of ghrelin incompletely matched with a corresponding receptor. Further investigation is required to identify a potential desacyl ghrelin receptor that will facilitate the accurate identification of sites of action for this peptide and to elucidate its underlying mechanisms of action. The present review has demonstrated that our current understanding of the ghrelin system does not provide sufficient evidence to justify its role as a useful biomarker in GI malignancies. Therefore, large cohort studies with well-defined exclusion criteria and a complex framework, factoring in plasma levels, tissue expression and genetic alterations, are needed to establish direct correlations between ghrelin levels and GI malignancies.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

IAS conceived the paper, designed the overall concept and created the figures. IDC supervised the project. IAS, IDC, DGAC and SEG contributed equally to collecting the data and in editing and shaping the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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