INTRODUCTION

Cancers of the gastrointestinal tract are a major cause of morbidity and mortality worldwide and elucidation of the molecular mechanisms underlying disease is critical to the development of new diagnostic tools and therapeutics. Ghrelin production mainly occurs in the gastrointestinal tract, and as such could be affected by the development of cancer. Greatly reduced expression of ghrelin mRNA and peptide in oesophagogastric adenocarcinomas, as compared to adjacent nonneoplastic gastric mucosa, suggests that ghrelin production may be compromised during disease progression by the replacement of normal ghrelin-producing mucosa with adenocarcinoma ***(Aydin et al., 2005; Mottershead et al., 2007)***.

D'Onghia et al. identified significantly lower plasma ghrelin levels in colon cancer patients compared to controls, with serum levels decreasing further during progression from early to late-stage tumours. These results also suggest a progressive loss of ghrelin production with malignancy, possibly due to damage to the normal mucosa and loss of cell differentiation ***(D'Onghia et al., 2007)***.

The functions of ghrelin include food intake regulation, gastrointestinal (GI) motility, and acid secretion by the GI tract. Many GI disorders involving infection, inflammation, and malignancy are also correlated with altered ghrelin production and secretion. Although suppressed ghrelin responses have

already been observed in various GI disorders, such as chronic gastritis, *Helicobacter pylori* infection, irritable bowel syndrome, functional dyspepsia, and cachexia, elevated ghrelin responses have also been reported in celiac disease and inflammatory bowel disease ***(Cheung and Che-Yuen, 2013).***

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R), which was discovered in the stomach. It is the first-identified orexigenic peptide of the peripheral tissues, and consists of a 28-amino acid peptide cleaved from the 117-amino acid precursor, preproghrelin ***(Yagi et al., 2012).***

Plasma ghrelin consists of two forms; the active acylated ghrelin, which is the ligand for the GH secretagogue (GHS) receptor, and the non-acylated ghrelin, which constitutes greater amounts in the blood than the acylated form. Ghrelin is involved in energy homeostasis. Ghrelin plasma concentrations are decreased in obesity, satiety and food intake and increased in states of negative energy balance such as fasting, anorexia or cachexia, as well as being inversely correlated to body mass index (BMI) and insulin secretion. Ghrelin plasma concentrations increase before meals and decrease after eating ***(Strickertsson et al., 2011).***

Acyl ghrelin induces a signal for mealtime hunger and meal initiation, it also affects body weight and adiposity. In addition, acyl ghrelin induces a protective effect in the gastric

mucosa and is involved in the regulation of gastrointestinal motility, Des-acyl ghrelin affects food intake, gut motility, body size development, adipogenesis, insulin secretion, and resistance to increased papillary muscle tension, as well as cell proliferation and survival. Des-acyl ghrelin may block the orexigenic activity of acyl ghrelin ***(Chen et al., 2009).***

Ghrelin was implicated in a variety of physiological processes that include cell proliferation, metabolism, cell protection, reproduction, etc. Of these, the effects of ghrelin on food intake and metabolism have had the biggest impact; unlike other peripheral signals associated with energy balance, ghrelin increases appetite and leads to the accumulation of body fat. Indeed, the stimulatory effects of ghrelin on food intake and its apparent opposite relation to the anorectic hormone, leptin, have been proposed as the yin-yang model for hormonal regulation of energy balance ***(Alfonso and Horvath, 2012).***

Ghrelin concentrations are reduced by 65% after gastrectomy. Currently, there is only one ghrelin receptor (GHSR) identified, and the activation of this receptor requires the presence of the acyl side chain on the ghrelin molecule ***(Kirchner, 2013).***

Ghrelin has an important role in the regulation of appetite and feeding ***(Wren et al., 2001).*** Change in ghrelin production during cancer has been hypothesised to contribute to cancer-related cachexia. However, ***Huang et al.*** measured

circulating plasma ghrelin in cachectic gastric and colorectal cancer patients, and found no change in levels as compared to controls ***(Huang et al., 2007).*** Nonetheless, the authors suggest that exogenous ghrelin therapy may still be beneficial for these patients, given the ability of ghrelin to increase energy intake in cancer patients with impaired appetite ***(Neary et al., 2004).*** Indeed ghrelin and ghrelin mimetics are in clinical trials to test their orexigenic potential in cancer-related cachexia and anorexia nervosa patients ***(Ueno et al., 2010).***

Ghrelin expression has been detected in the majority of gastric carcinoids, some intestinal neuroendocrine tumours ***(Papotti et al., 2001)*** and pancreatic neuroendocrine tumours ***(Volante et al., 2002).*** This first evidence of ghrelin-producing gastrointestinal endocrine tumours led to the hypothesis that circulating ghrelin levels may be useful diagnostically. In 2004, ***Tsolakis et al.*** described a patient with a malignant gastric ghrelinoma, displaying massively elevated levels of both total and acyl ghrelin ***(Tsolakis et al., 2004).*** However, a study by ***Corbetta et al.*** showed no statistical difference in plasma ghrelin levels in patients with gastrointestinal and pancreatic neuroendocrine tumours compared to controls, with only one patient displaying high circulating ghrelin levels due to a pancreatic neuroendocrine tumour, suggesting substantial ghrelin production is restricted to a minority of these tumours ***(Corbetta et al., 2003).***

Several studies have investigated the role of ghrelin in malignant cell proliferation in vitro, identifying a pro-proliferative effect in a variety of cancer cell lines ***(Chopin et al., 2011)***, including breast ***(Jeffery et al., 2005)***, prostate ***(Yeh et al., 2005)*** and hepatoma ***(Murata et al., 2002)***. In 2008, ***Waseem et al.*** identified excessive ghrelin secretion by malignant colorectal cancer cell lines ***(Waseem et al., 2008)***.

Colorectal cancer cell showed significant proliferation and invasion/migration even in the absence of growth factor, and this behaviour was abrogated by pre-treatment with a GHS-R1a antagonist or ghrelin-neutralizing antibody. Furthermore, ghrelin and GHS-R1b, a non-signalling splice variant of GHS-R1a, expression were elevated in malignant colorectal tissue samples, in a stage-dependent manner, whilst expression of GHS-R1a decreased. Expression of ghrelin and both receptors declined in advanced grade, poorly differentiated tumours, suggesting a role for the ghrelin axis in promoting intestinal epithelial cell growth and differentiation in low-grade tumours ***(Waseem et al., 2008).***

In contrast to the majority of evidence suggesting a proproliferative effect of ghrelin, there have been reports of an anti-proliferative effect ***(Ghe et al., 2002). D'Onghia et al.*** therefore hypothesised that decreased ghrelin levels could compromise this function, leading to uninhibited cell proliferation and thus representing a risk factor for colorectal cancer ***(D'Onghia et al., 2007).*** In summary, the role of the

ghrelin axis in gastrointestinal tract cancer is still not fully understood, and there have been conflicting reports regarding changes of expression. Such discrepancies could be the result of differences in tumour stage, or compounding factors such as H. pylori infection, dietary modifications, or changes to the acyl/total ghrelin ratio, which is altered in cachexia ***(Jeffery et al., 2005).*** Despite these discrepancies, careful risk-stratification of patients receiving ghrelin or growth hormone secretagogue treatment in the future is warranted.

AIM OF THE WORK

To identify and clarify the relation between ghrelin hormone and colorectal carcinoma, to assess its role as a marker of diagnosis.

Chapter One

GHRELIN

Discovery

Ghrelin was discovered in 1999 as the long sought ligand of what was until then known as the Growth Hormone Secretagog receptor (GHS-R). The fascinating history of the discovery of ghrelin and of the GHS-R has been reviewed many times. In relation to the effect of ghrelin on the gastrointestinal tract, it is of interest to note that 7 months before the discovery of ghrelin, a peptide with the same sequence was described, and named motilin-related peptide (MTRLP), because the authors noted a similarity with the sequence of motilin ***(Hattori et al., 2011).***

The Ghrelin Gene

The ghrelin gene (GHRL) consists of 6 exons which span 7.2 kb of genomic DNA on chromosome 3p25–26 and includes a 20 bp non-coding first exon (exon 0), encodes 2 separate mRNAs which start at position 80 and 555. The main mRNA codes for the 117 amino-acid preproghrelin, which is enzymatically cleaved into proghrelin and processed into mature ghrelin and a C-terminal polypeptide. The 28 amino acid ghrelin peptide is encoded by parts of exon1, which also includes the preproghrelin signal peptide, and exon 2. Exon 3 codes for obestatin, a peptide with anorexigenic biological activities opposing those of ghrelin ***(Waseem et al., 2008).***

Ghrelin O-acyltransferase (GOAT) is a conserved membrane-bound O-acyl transferase (MBOAT) and genetic disruption of the GOAT gene in mice leads to complete absence of acylated ghrelin in circulation. Murine GOAT, localized within the endoplasmic reticulum of the gastrointestinal tract and testes, specifically binds covalently octanoyl residues to Ser3 of ghrelin. Human GOAT, expressed in the stomach and pancreas, is also capable of acylating ghrelin with other fatty acids besides octanoate. Structure function studies showed that the octanoyl group at Ser3 and the 4–5 amino acids at the N-terminus are necessary for binding to its receptor and its GH-releasing activities. Rat and human ghrelin differ only by 2 amino acids and mouse and rat amino acid sequences are homologous, suggesting a strong evolutionary conservation across mammals. Ghrelin is the endogenous ligand of the growth hormone secretagogue receptor (GHS-R) ***(Jeffery et al., 2011).***

Ghrelin Receptors

Ghrelin acts via the specific receptor (GHS-R), which belongs to G protein coupled receptor family. GHS-R has two splice variants: functional type 1a, which contains seven transmembrane domains and truncated type 1b, composed of only the first five transmembrane domains. It arises from an alternative splicing. GHS-R1a is a specific receptor for ghrelin, whereas the function of the 1b type of receptor is still unclear ***(Majchrzak et al., 2012).***

Structure of The Ghrelin Receptor

1-GHS-R1A Signaling

GHS-R1a expression has been demonstrated in wide range of tissues, including: central nervous system (mainly: hypothalamus, thalamus, hippocampus, cortex, regions of appetite control, food intake and energy homeostasis), thyroid and parathyroid glands, pancreas, spleen, myocardium, cardiovascular system, adrenal glands, kidney, ovaries, testis and prostate ***(Nikolopoulos et al., 2014).***

2- GHS-R1B Signaling

GHS-R1b is also widely distributed in various tissues but interestingly it is also over expressed in many tumors. Although this form of receptor was regarded as non-functional it may play a role in turmorigenesis. It may modulate the function of ghrelin-GHS-R axis presumably by increasing internalisation of GHS-R1a. More over it can act as a dominant-negative mutant of GHS-R1a and transform its signaling ***(Inge et al., 2013).***

3- Desacyl Ghrelin Receptors

Multiple studies have suggested the occurrence of other ghrelin receptors, such as: receptor for desacyl ghrelin or common receptor for ghrelin and desacyl ghrelin. This problem is still unclear especially in the face of fact that some researchers suggest that des-acyl ghrelin might also act through GHS-R, which was ruled out so far ***(Omoto et al., 2014).***

However, high concentrations of ghrelin demonstrated lack of specificity by elevating adrenocorticotropic hormone (ACTH), cortisol, and prolactin (PRL) levels as well as GH. Another important role of ghrelin is an energy homeostasis control and stimulation of appetite and food intake ***(DeBoer, 2011).***

Circulating Ghrelin:

In human, circulating ghrelin consists of desacyl ghrelin (>90%), acyl ghrelin, and C-ghrelin. Circulating C-ghrelin is decreased by about 80% in rat and in humans following surgical gastric mucosa removal. It presently remains unknown if ghrelin and desacyl ghrelin are both secreted into the bloodstream via similar or distinct secretory pathway(s). The high desacyl/acyl ghrelin ratio in the circulation can be explained by the shorter half life of ghrelin compared to desacyl ghrelin and plasma ghrelin deacylation. Desacyl ghrelin mostly circulates as a free peptide, while acyl ghrelin circulates bound to lipoproteins ***(De Vriese et al., 2007).***

Tissue distribution of Ghrelin

Ghrelin is produced prevalently in the stomach by the X/A- like cells within the oxyntic glands of the gastric fundus mucosa, although minor amounts are present elsewhere in the body. The placenta, testis, kidney, pituitary, small intestine, pancreas, lymphocytes, brain, lung and ovary also express

significant levels of ghrelin. At any rate, two-thirds of plasma ghrelin levels comes from the stomach, and at least one-third from the small intestine. The ubiquitous expression of ghrelin in a host of tissues is suggestive of local paracrine and/or autocrine actions. Thus, it has been found that ghrelin regulates testicular steroidogenesis and testosterone secretion by the Leydig cells ***(Murphy et al., 2011).***

Physiological Functions:

1- Growth hormone secretion.

Via its binding to GHS-R1A, present on pituitary somatotropic cells, ghrelin is a potent stimulator of growth hormone (GH) secretion. GH secretion is induced by both ghrelin-induced cyclic GMP/nitric oxide signaling pathway. Hypothalamus also appears to be involved in the ghrelin-induced GH secretion. Vagus nerve is also required for maximal ghrelin-induced GH secretion ***(Anderson and Scanes, 2012).***

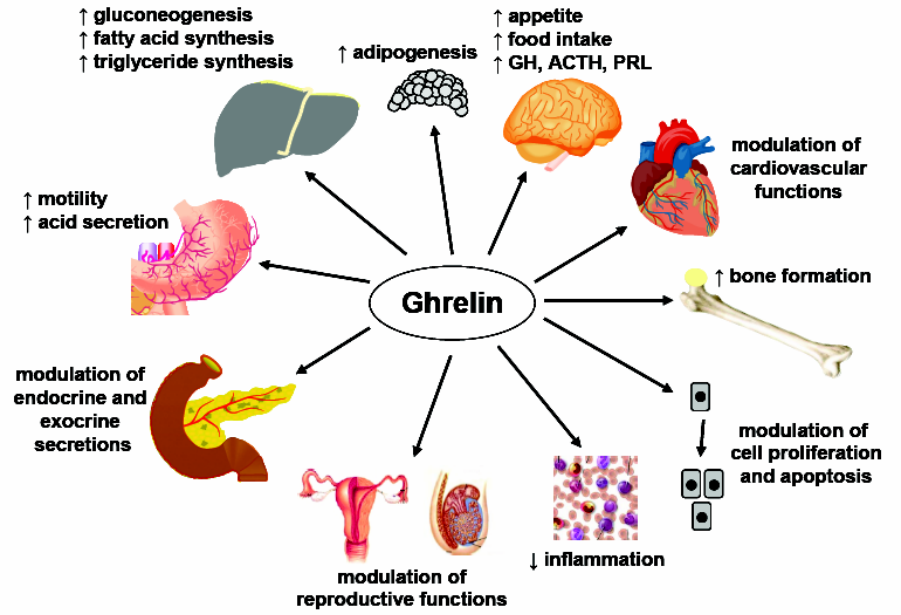

Fig. (1): Summarizes the different physiological actions of ghrelin.

The potential beneficial effects of ghrelin analogues for the treatment of GH-deficiency disorders have been investigated. Desacyl ghrelin is also able to induce GH secretion, possibly by modulating the GH-insulin growth factor axis. Ghrelin is also able to stimulate the pituitary secretion of adrenocorticotropic hormone (ACTH), cortisol, and prolactin (PRL) ***(Korrea-Silva et al., 2006).***

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Ghrelin has strong GH-releasing activity in both human and rats. The maximum stimulation of GH affected by ghrelin is greater than that of GHRH, which peaks about 5–15 min after intravenous ghrelin injection. Ghrelin stimulates GH

release from primary pituitary cells. In addition to the direct action on pituitary, ghrelin-mediated stimulation of GH release has been shown to involve the hypothalamus and the vagus nerve ***(Waseem et al., 2008).***

2- Appetite and body weight regulation

Ghrelin stimulates appetite by central and peripheral pathways and via the vagus nerve. Indeed, ghrelin is locally synthesized in the hypothalamus, ghrelin secreted by the stomach reaches the brain by crossing the blood-brain barrier, and ghrelin also transmits its signal through the vagal nerve. In hypothalamus, ghrelin activates the arcuate nucleus (ARC), paraventricular nucleus (PVN), dorsomedial region, central nucleus of amygdala, and the nucleus of solitary tract. Neurons expressing ghrelin send efferents to ARC neurons producing NPY, AGRP, POMC, and CRH. By stimulating the activity of NPY/AGRP neurons and decreasing the activity of POMC and CART neurons, ghrelin increases appetite and food intake. Ghrelin can directly inhibit PVN neurons or activate NPY/AGRP neurons and inhibit POMC neurons that are in contact with PVN. Hypothalamic 5′ AMP-activated protein kinase (AMPK) has been proposed to play a pivotal role in ghrelin's effects on appetite and food intake ***(Date, 2012).***

Ghrelin has been shown to stimulate AMPK by phosphorylation via calmodulin kinase-kinase 2 (CaMKK2) activated in response to rise in intracellular calcium concentration

induced by GHS-R1A signaling. However, a recent study demonstrated that the effect of ghrelin on AMPK signaling pathway occurs independently from GHS-R1A, thereby suggesting that the AMPK signaling pathway does not play a major role in the orexigenic effect of ghrelin ***(Verhulst et al., 2012).***

The intact cannabinoids signaling pathway is required for the effect of ghrelin on appetite and AMPK. Furthermore, control of AMPK signaling pathway by cannabinoids requires an intact ghrelin signaling pathway ***(Lim et al., 2013).***

Ghrelin also stimulates appetite via the vagus nerve. Human nodose ganglion from the vagus nerve expressing GHS-R1A are likely to be involved in the ghrelin-induced signal transmission from the stomach to the brain. Indeed, rats submitted to vagotomy or perivagal application of an afferent neurotoxin or patients with vagotomy and esophageal or gastric surgery are responding to the appetite stimulatory effect of ghrelin. Thus, through the activation of GHS-R on vagal afferent to the stomach, the signal induced by ghrelin may reach the nucleus of tractus solitarius, which communicates with the hypothalamus to increase food intake ***(Arnold et al., 2006).***