The expanding roles of the ghrelin-gene derived peptide obestatin in health and disease

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Abstract

Obestatin is a 23 amino acid, ghrelin gene-derived peptide hormone produced in the stomach and a range of other tissues throughout the body. While it was initially reported that obestatin opposed the actions of ghrelin with regards to appetite and food intake, it is now clear that obestatin is not an endogenous ghrelin antagonist of ghrelin, but it is a multi-functional peptide hormone in its own right. In this review we will discuss the controversies associated with the discovery of obestatin and explore emerging central and peripheral roles of obestatin, roles in adipogenesis, pancreatic homeostasis and cancer.

Keywords: obestatin; peptide hormone; ghrelin; physiology; disease

1.0 Introduction

A little more than a decade ago, Kojima and colleagues successfully isolated the 28 amino acid growth hormone stimulating peptide, ghrelin, from the stomach (Kojima et al., 1999). As reviewed in this special issue of *Molecular and Cellular Endocrinology*, the discovery of ghrelin sparked a large number of studies investigating the role of ghrelin and its receptor in disease. In this review article, we will highlight what is known about the recently discovered ghrelin gene-derived peptide, obestatin

2.0 The controversial discovery of obestatin

Using comparative genomics analysis, Zhang and colleagues revealed that the ghrelin gene region that encodes the 66 amino acid, C-terminal region of preproghrelin contained a conserved region flanked by convertase cleavage sites, hinting that it may harbour a peptide hormone (Zhang et al., 2005). Indeed, they found that a 23 amino acid peptide, obestatin, was derived from this region (Figure 1) and it was named obestatin (derived from obese and statin) to indicate that it was anorexigenic, opposing the function of ghrelin on food intake (Zhang et al., 2005). Obestatin is thought to arise through the post-translational cleavage of the ghrelin preprohormone (Zhang et al., 2005). It is also possible that novel, specific obestatin-coding transcripts may be translated (Seim et al., 2007), although the contribution of these transcripts to obestatin peptide expression may be negligible (Volante et al., 2009). Like ghrelin, the stomach is the primary site of obestatin expression (Zhang et al., 2005), and obestatin is produced in the same endocrine cell type as ghrelin (Granata et al., 2008; Gronberg et al., 2010; Gronberg et al., 2008; Guo et al., 2008; Morash et al., 2010; Tsolakis et al., 2009; Volante et al., 2009; Zhao et al., 2008). This finding suggested that obestatin was an endocrine gut hormone, primarily acting on the brain (Figure 2).

While the initial discovery of obestatin was an exciting finding in the ghrelin field, it has since met with some controversy, particularly with regards to its effect on food intake. The original study reporting the discovery and function of obestatin demonstrated that the intraperitoneal and intracerebroventricular injections of obestatin inhibited food intake and reduced body weight in mice (Zhang et al., 2005). The effects of obestatin on feeding have been confirmed by independent investigators (Bresciani et al., 2006; Brunetti et al., 2010; Brunetti et al., 2009; Green et al., 2007; Nagaraj et al., 2008; Nagaraj et al., 2009; Zhang et al., 2008), however, the majority of studies suggest that obestatin does not have anorexigenic effects, regardless of injection site (Bassil et al., 2007; Chartrel et al., 2007; De Smet et al., 2007; Gourcerol et al., 2006; Holst et al., 2007; Kobelt et al., 2008; Lauwers et al., 2006; Mondal et al., 2008; Nogueiras et al., 2007; Samson et al., 2007; Sibilia et al., 2006; Tremblay et al., 2007; Unniappan et al., 2008; Van Dijck et al., 2009; Yamamoto et al., 2007; Zizzari et al., 2007). The initial description of obestatin also reported that it inhibited gastrointestinal motility (Zhang et al., 2005) (Figure 2), however, a number of subsequent studies failed to replicate this effect (Bassil et al., 2007; Chen et al., 2008; Chen et al., 2010; Depoortere et al., 2008; Gourcerol et al., 2006; Yamamoto et al., 2007). Recent studies by Inui and colleagues, however, demonstrated that intravenously administered obestatin could inhibit gastrointestinal motility, acting via corticotropin-releasing factor (CRF) receptors in the hypothalamic nuclei of the brain (Ataka et al., 2008; Fujimiya et al., 2008). Results in obesity have also been inconsistent, with both elevations in obestatin levels (Reinehr et al., 2008; Vicennati et al., 2007) and reductions in plasma obestatin (Fontenot et al., 2007; Gao et al., 2010; Gao et al., 2008) associated with obesity and overweight in different studies (Hassouna et al., 2010). The large number of divergent observations regarding the actions of obestatin may have arisen as a result of major, or perhaps subtle differences in the various *in vivo* and *in vitro* experimental models employed to date. Conflicting results in the obestatin

field may also partly be due to the lack of stability of the obestatin peptide and its short half-life (Vergote et al., 2008). It is clear that much research is needed to clearly define the role of obestatin as a hormone involved in body weight regulation and gastrointestinal motility.

Obestatin may also have other effects unrelated to food intake including emerging roles in memory improvement and reduction of anxiety (Carlini et al., 2007) and regulation of metabolism and sleep during torpor (mediated centrally by acting on the brain) (Szentirmai et al., 2009; Szentirmai and Krueger, 2006). Obestatin may also inhibit thirst (Samson et al., 2007; Samson et al., 2008) (Figure 2), although this effect was not observed in an independent study (Van Dijck et al., 2009). Like ghrelin, obestatin may play an immunomodulatory role and it protects against the development of pancreatitis in a curulein-induced rat model (Ceranowicz et al., 2009).

It was initially reported that GPR39 (G-protein coupled receptor 39), a member of the ghrelin receptor-family, was the obestatin receptor (Zhang et al., 2008; Zhang et al., 2005). Studies by other research groups have failed to reproduce these findings, however, and it is now appreciated that GPR39 is not a cognate receptor for obestatin (Chartrel et al., 2007; Holst et al., 2007; Lauwers et al., 2006; Yasuda et al., 2007). It was recently reported that obestatin binds the GLP-1R (glucagon-like peptide 1 receptor) in cultured β-cells (Granata et al., 2008), however, a subsequent study showed that obestatin did not bind GLP-1R, nor displace the GLP-1 ligand, in GLP-1R overexpressing cells (Unniappan et al., 2008). Nevertheless, it is clear from numerous studies that obestatin binds and activates a G-protein coupled receptor, ultimately stimulating extracellular signal-regulated kinase (ERK 1/2) (Alloatti et al., 2010; Alvarez et al., 2009; Camina et al., 2007; Dun et al., 2006; Gurriaran-Rodriguez et al., 2010; Meszarosova et al., 2008; Pazos et al., 2009; Pazos et al., 2007).

As obestatin is a gut hormone (produced in the stomach), obestatin may act as an endocrine hormone and may circulate in the blood to reach the brain through the blood brain barrier, it could signal to the brain by affecting primary afferent neurons, or both. Unexpectedly, obestatin rapidly degrades in the plasma and, unlike ghrelin, it is not able to cross the blood-brain barrier by a saturable transport system (Pan et al., 2006; Vergote et al., 2008; Zizzari et al., 2007). Since the obestatin receptor has not been identified, it is currently difficult to dissect how peripheral obestatin may control central systems, such as feeding. There is some evidence, however, that obestatin, like ghrelin, may stimulate vagal afferent (sensory) pathways from the gut. For example, vagotomy abolished the stimulation of pancreatic juice secretion from the exocrine pancreas by intravenously administered obestatin (Kapica et al., 2008; Kapica et al., 2007). The vagal afferent pathway may, therefore, play a role in gastrointestinal motility induced by obestatin (Ataka et al., 2008; Fujimiya et al., 2008).

The detection of obestatin in biological samples has proven problematic and reproducible and consistent data has been lacking (Hassouna et al., 2010). Measurements of obestatin levels in plasma and tissues should, therefore, be interpreted with caution. There is some doubt regarding the results of current antibody-based detection methods that may not be specific for obestatin (Bang et al., 2007; Mondal et al., 2008) and immunohistochemical methods are unable to distinguish between obestatin and its precursor form, proghrelin. There is also a lack of international assay standards for obestatin, putative obestatin carrier proteins could interfere with obestatin antisera, and sampling and pulsatile variations could lead to differences in levels of obestatin measured (Hassouna et al., 2010). Some studies have failed to detect significant amounts of obestatin in the serum and its role as an endocrine hormone has been questioned (Bang et al., 2007; Mondal et al., 2008; Pemberton and Richards, 2008).

The physiological significance of plasma obestatin levels and the effects on food intake are currently controversial (Gao et al., 2008). While it is recognised that there are reservations regarding obestatin measurements, the plasma ratio of ghrelin to obestatin may be a more informative measure than levels of obestatin alone and this ratio could prove a useful biomarker for some disease states. The ghrelin to obestatin ratio is reduced in a number of diseases, including inflammatory bowel disease (Alexandridis et al., 2009), chronic atrophic gastritis (not associated with *Helicobacter* infection) compared to controls (Gao et al., 2008) and in Chinese patients infected with *Helicobacter pylori* (Gao et al., 2009), indicating that obestatin may play a role in the pathogenesis of these diseases. Obestatin may also play a role in the pathogenesis of metabolic diseases, including anorexia nervosa, where obestatin blood levels are divergent (Germain et al., 2010; Monteleone et al., 2008; Sedlackova et al., 2010). The ghrelin to obestatin ratio has also been reported as elevated in studies of obese patients (Guo et al., 2007) and this ratio could prove to be important in the regulation of energy metabolism (Hassouna et al., 2010).

3.0 Emerging peripheral effects of obestatin

Although the majority of obestatin is produced by the stomach, the obestatin peptide has been reported to be expressed in a range of peripheral tissues, including the pancreas (Granata et al., 2008; Gronberg et al., 2008; Guo et al., 2008; Volante et al., 2009; Zhao et al., 2008), liver (Guo et al., 2008), testis (Dun et al., 2006), mammary gland (Gronberg et al., 2010; Gronberg et al., 2008), thyroid (Karaoglu et al., 2009), and lung (Volante et al., 2009). This may indicate that obestatin has local autocrine/paracrine roles in addition to its actions as an endocrine hormone. As noted, obestatin is an orphan ligand and the search for the obestatin receptor is still ongoing, however, binding sites for the obestatin peptide have been identified

in the pancreas, heart, white adipose tissue and other tissues (Alloatti et al., 2010; Granata et al., 2008; Iglesias et al., 2007; Zhang et al., 2008).

3.1 The endocrine pancreas

Obestatin may be an important regulator of endocrine pancreatic function and β-cell survival. In the endocrine pancreas, obestatin is co-expressed in the same cell type as ghrelin, suggesting a role for obestatin in pancreatic function (Granata et al., 2008; Granata et al., 2010; Gronberg et al., 2008; Volante et al., 2009; Walia et al., 2009; Zhao et al., 2008). Obestatin is secreted by pancreatic cell lines and human pancreatic islet cells where it binds to high affinity binding sites (Granata et al., 2008). Obestatin may play a role as an autocrine/paracrine growth and survival factor in the pancreatic islets (Granata et al., 2010; Granata et al., 2008) and the maintenance of pancreatic β-cell mass is important for pancreatic endocrine function and glucose balance. Obestatin increases cell proliferation and promotes cell survival in human isolated pancreatic islet cells and in the HIT-T15 and INS-IE pancreatic β-cell lines (Granata et al., 2008). Chronic obestatin treatment, like ghrelin, promotes the maintenance of islet size and β-cell mass *in vivo*, in a streptozotocin (STZ)-induced neonatal rat model of diabetes (Granata et al., 2010).

Apoptosis is a key process causing β-cell death in diabetes mellitus and is often associated with pro-inflammatory cytokines (Granata et al., 2010). In pancreatic islet cell lines, obestatin protected against apoptosis stimulated by pro-inflammatory cytokines, and apoptosis stimulated by serum widthdrawal (Granata et al., 2008). Interestingly, D-(lys-3) GHRP-6 (a ghrelin receptor antagonist) and treatment with an anti-ghrelin antibody inhibited these

effects in response to obestatin treatment, suggesting that there may be cross-talk between the actions of obestatin and ghrelin in the endocrine pancreas (Granata et al., 2008).

The role of obestatin in stimulating insulin secretion is currently somewhat controversial, with some studies demonstrating that obestatin inhibits insulin secretion, stimulates secretion or has no effect on insulin secretion (Granata et al., 2008). Obestatin may play a role in glucose metabolism in humans and an inverse relationship has been demonstrated between obestatin and insulin levels (Gao et al., 2008; Li et al., 2010; Lippl et al., 2008). In isolated human pancreatic islets, obestatin has been demonstrated to increase insulin secretion and activate genes associated with insulin synthesis (Granata et al., 2008). In contrast, in isolated mouse and rat islets, obestatin inhibited insulin release in a dose dependent manner (Qader et al., 2008) and in an *in vivo* rat model, obestatin inhibited glucose-stimulated insulin release (Ren et al., 2008). Obestatin was reported to have no effect on glucose or insulin levels under basal or fasting conditions in rats and mice (Green et al., 2007; Ren et al., 2008). Another study indicated that obestatin may have dual effects on insulin release in response to glucose in perfused rodent islets, enhancing insulin release at low obestatin concentrations and inhibiting insulin release at higher concentrations when glucose levels were maintained at a constant level (Egido et al., 2009). In this study, β-cells were more responsive to obestatin when glucose levels were high than when glucose levels were low (Egido et al., 2009). In the STZ-induced rat model of diabetes, obestatin prevented the development of diabetes mellitus symptoms and improved glucose metabolism (Granata et al., 2010).

In isolated human pancreatic islets, obestatin upregulates the expression of a range of genes associated with cell survival, β-cell mass, growth and differentiation and glucose metabolism (Granata et al., 2008). The expression of the transcript for the transcription factor Pdx1 is

upregulated in isolated human pancreatic islets and in STZ neonatal rat diabetic islets after obestatin treatment (Granata et al., 2008; Granata et al., 2010). Pdx1 plays an important role in maintaining β-cell mass and in stimulating the differentiation of β-cells in the pancreas (Granata et al., 2010). The ability of obestatin to regulate genes that are important in β-cell function, increasing β-cell mass by stimulating cell proliferation and protecting against apoptosis mediated by pro-inflammatory cytokines, indicates that it could be useful in the future development of therapeutics for diabetes mellitus (Granata et al., 2008). Studies into the role of obestatin in the pancreas are sure to reveal important insights into the pathobiology of diabetes, pancreatitis and pancreatic cancer and obestatin shows promise as a future target for the development of novel therapeutics for diabetes mellitus (Granata et al., 2010).

3.2 Adipose tissue

While the role of obestatin in obesity and related pathologies that affect adipose homeostasis remains controversial, preliminary studies indicate that obestatin may indeed be a functional peptide in adipose tissue. It is now well-established that adipose tissue is an endocrine organ that can secrete peptides and express receptors for a range of peptide hormones (Galic et al., 2010). Peripheral administration of obestatin induced expression of the gene *c-fos*, an early response gene that undergoes rapid transcriptional activation by growth factors and mitogens, in white adipose tissue and cultured mouse adipocytes (Zhang et al., 2008). Moreover, injection of obestatin significantly decreased the levels of the gene *ABCA1* (ATP-binding cassette A1), a key cholesterol transporter, in white adipose tissue in cows. While this suggests a role for obestatin in cholesterol transport (Grala et al., 2010), this hypothesis remains to be investigated. A recent report employing transgenic mice

expressing the full-length preproghrelin peptide indicates that adipose tissue does not secrete obestatin into the blood (Zhang et al., 2008). Very recently, it was reported that adipocytes secrete obestatin and that it promotes adipogenesis in an autocrine/paracrine manner (Gurriaran-Rodriguez et al., 2010). In an elegant set of experiments, obestatin secreting 3T3-L1 preadipocytes were subjected to anti-obestatin antibody and preproghrelin gene silencing treatments, which demonstrated that obestatin directly regulates adipocyte differentiation (Gurriaran-Rodriguez et al., 2010). These findings indicate that autocrine/paracrine obestatin, as well as circulating obestatin from the stomach, or other tissues, may act on obestatin receptors in adipose tissue to regulate growth and differentiation.

3.3 The renal system

The role of obestatin (and indeed ghrelin) in the renal system is not well-established. Interestingly, it has been reported that normal kidney tissue expresses comparable levels of ghrelin to the stomach (Dagli et al., 2009; Mori et al., 2000), suggesting that the kidney can produce high levels of ghrelin gene derived peptides, such as obestatin. The potential role of obestatin in energy balance indicates that it may play a role in chronic kidney disease, which is characterised by anorexia and muscle wasting (or cachexia) (Cheung and Mak, 2010; Mafra et al., 2010). Obestatin levels are elevated in plasma of patients with chronic kidney disease and in patients with kidney failure (end stage renal disease) (Aygen et al., 2009; Buscher et al., 2010). Haemodialysis of chronic kidney disease patients resulted in lower plasma obestatin levels (Mafra et al., 2009). No substantiated reports on obestatin function in the renal system are available to our knowledge, however the current data suggests that normal renal function is necessary for clearance of the approximately 2.5 kDa obestatin

peptide from the circulation.

3.4 Obestatin in the cardiovascular system

Elevated obestatin levels have been reported in cachexic patients with congestive heart failure (Xin et al., 2009), but not in non-cachexic congestive heart failure patients (Xin et al., 2009), or patients with ischaemic heart disease (Ozbay et al., 2008). Moreover, binding sites for obestatin have been identified in the heart (Alloatti et al., 2010) and obestatin-induced contractions of *ex vivo* hearts from frogs are blocked by a protein kinase A inhibitor, indicating that obestatin influences β-adrenergic receptors of cardiac muscle cells (Xin et al., 2009). Exogenous obestatin has no effect on cell cycle or viability in the HL-1 cardiac muscle cell line (Iglesias et al., 2007), however, a recent study showed that obestatin plays a role in protecting myocardial function following reperfusion injury of cardiac muscle cells (Alloatti et al., 2010).

Obestatin may also play a role in regulating blood pressure. The ghrelin to obestatin ratio and obestatin levels were reduced in patients with mild to moderate high blood pressure in one study (Li et al., 2010) and obestatin has also been shown to have a negative correlation with systolic blood pressure in humans (Anderwald-Stadler et al., 2007). In contrast, in the spontaneously hypertensive rat, there is a positive correlation between obestatin levels and systolic blood pressure and obestatin levels are significantly higher in these rats compared to normal Wistar-Kyoto rats (Li et al., 2010). Similarly, obestatin levels were elevated in pregnant women with pregnancy-induced hypertension compared to women with normal pregnancies (Ren et al., 2009). While it appears that obestatin may play a role in the

regulation of blood pressure, intravenous injection of obestatin did not alter blood pressure in spontaneously hypertensive rats (Li et al., 2009).

3.5 Obestatin as a growth factor of peripheral tissues

Evidence is rapidly emerging that obestatin (acting through an unknown receptor), may play a similar autocrine/paracrine role to ghrelin (Seim et al., 2010) in a variety of cellular systems. As discussed, exogenous obestatin stimulates proliferation of pancreatic β-cells and isolated human pancreatic islet cells (Granata et al., 2008) (section 3.1) and promotes adipogenesis (Gurriaran-Rodriguez et al., 2010) (section 3.2). Exogenous obestatin also stimulates cell proliferation in retinal pigment epithelial cells (Camina et al., 2007), but inhibits proliferation in the regenerating adrenal cortex (Rucinski et al., 2010). Immunoblot-based assays show that obestatin also alters the levels of proteins involved in proliferation and apoptosis in ovarian granulosa cells, suggesting that the peptide may regulate turnover and remodelling of ovarian follicles (Meszarosova et al., 2008).

Chondrocytes have binding sites for obestatin and secrete obestatin, which inhibits proliferation in the human C28-I2 rib chondrocyte cell line and in the ATDC5 mouse embryonal carcinoma-derived cell line after it has been differentiated into chondrocytes (Lago et al., 2007). In contrast to ghrelin, however, obestatin did not modulate known markers of chondrocyte metabolism (Caminos et al., 2005; Lago et al., 2007). It would be most interesting to examine the role of obestatin in chondrocyte disease states, such as chondrosarcoma, in future studies.

3.6 Obestatin and cancer

Finally, obestatin may have a pathophysiological role in cancer. Immunoassay-based studies have reported that obestatin levels are higher in the blood of patients with ovarian cancer than in normal controls (Markowska et al., 2009). There was no correlation between blood levels of obestatin and disease status, however, in prostate cancer (Malendowicz et al., 2009) and uterine leiomyoma patients (Markowska et al., 2009). It is likely that obestatin functions solely at the autocrine and paracrine level in cancer and systemic levels of the peptide are unlikely to reflect levels in the cancer microenvironment. Exogenous obestatin stimulates proliferation in the KATO-III gastric cancer cell line (Pazos et al., 2007), but not the AGS gastric cancer cell line (where it, on the other hand, stimulates growth hormone release) (Pazos et al., 2009). Obestatin also inhibits proliferation of TT-human medullary thyroid carcinoma and the endocrine BON-1 pancreatic tumor cell line (Volante et al., 2009). Interestingly, immunohistochemical studies suggests that obestatin expression is reduced in thyroid and pancreatic tumour tissue and that the obestatin may be specifically downregulated by these tumours types in order to circumvent the inhibition of proliferation demonstrated *in vitro* (Volante et al., 2009). Finally, in our laboratory, we have recently demonstrated that exogenous obestatin induces cell proliferation by phosphorylating Akt, PKC and ERK1/2 in the PC3 prostate cancer cell line (Amorim *et al.*, unpublished observations). We have also demonstrated that obestatin is synthesised and secreted by ovarian cancer cell lines *in vitro* (Walpole *et al*,. unpublished observations). Exogenous obestatin treatment significantly increases the migration of ovarian cancer cell lines in culture (Walpole *et al*,. unpublished observations). Taken together, the available evidence shows that obestatin is an autocrine/paracrine growth factor in peripheral tissues and may play a role in cancer progression.

4.0 Conclusion

In contrast to early suggestions, obestatin is not an endogenous antagonist of ghrelin, but recent studies indicate that obestatin is a multi-functional peptide hormone in its own right. Obestatin may have important endocrine, autocrine or paracrine roles in a number of tissues including pancreas, and adipose tissue and it may play a role in cancer progression. The obestatin receptor is yet to be discovered, and this currently limits *in vivo* and *in vitro* studies and our understanding of obestatin function. The development of more reliable asssays for obestatin will also allow this field of research to progress more rapidly and may clarify many of the current discrepancies in results. Despite early controversies, we propose that the obestatin research field will be as fruitful as the last decade of ghrelin research, and that the next few years will identify the obestatin receptor and reveal the potential of obestatin as a hormone in its own right with potential as a diagnostic and therapeutic target. In particular, we predict that obestatin may play essential roles in cancer progression.

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Figure captions

Figure 1. Schematic genomic organisation of the human ghrelin gene and the preproghrelin coding exons.

The exons shaded in white indicate extended exons. Exon sizes (bp) are shown above each exon. The regions of preproghrelin are indicated by different colours.

Figure 2. Known and putative functions of obestatin

The effects of obestatin may be endocrine, paracrine, or autocrine. The biological effects may be direct, resulting from obestatin binding its receptor on target cells, or indirect. Indirect effects can results by obestatin conveying vagal afferent information by interacting with its cognate receptor on vagal neurons, or by secretion of other hormones that are able to reach the target tissue.

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