IMPLICATIONS OF GHRELIN AXIS IN BREAST CANCER - REVIEW

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IMPLICATIONS OF GHRELIN AXIS IN BREAST CANCER - REVIEW (Abstract): Breast cancer is, by far, the most frequent cancer among women and many factors influence the physiological and pathological growth and development of the mammary gland. There is developing evidence that the hormone ghrelin, known for the growth hormone releasing effect and food intake modulator, could also play a role in the pathogenesis of breast cancer and may represent a new diagnostic marker and a potential therapeutic target. We performed a PubMed Database search of relevant studies and ten papers were included in our systematic review. Ghrelin axis seems to be definitely involved in the pathogenesis of breast cancer, although a precise role has not been yet established. In order to verify the precise role of ghrelin axis in breast cancer further studies with larger populations are necessary that should include the analysis of metabolic, genetic and environmental factors which are expected to influence the results. Keywords: GHRELIN, BREAST CANCER, NOVEL THERAPEUTIC TARGETS

Breast cancer is, by far, the most frequent cancer among women with an estimation of 1.67 million new cases diagnosed in 2012 (25% of all cancers). Also it is the most frequent cause of death in women in less developed regions, while in more developed regions it is now the second cause of death (after lung cancer), ranking as the fifth cause of death from cancer overall (522,000 deaths) (1).

There are many factors that influence physiological and pathological growth and development of the mammary gland. Endogenous hormones (sex hormones, growth hormone axis), reproductive factors (lower parity, older age at first giving birth, younger age at menarche, older age at menopause), oral contraceptives and hormone replacement therapy, family history and genetic factors (BRCA1 and BRCA2 mutations), alcohol and smoking, diet, overweight and obesity are known to be risk factors in breast cancer (2).

There is developing evidence that ghrelin, known for the growth hormone (GH) releasing effect and food intake modulator (3) could also play a role in the pathogenesis of breast cancer and may represent a new diagnostic marker and a potential therapeutic target.
THE GHR ELIN AXIS

From its discovery in 1999 (3), further studies revealed that ghrelin axis is not simple, but a complex, diverse system, regulated at several stages, which determines diverse transcripts and proteins with multiple functions, many of them still likely to be discovered.

Ghrelin is a unique 28-aminoacid (AA) peptide containing an n-octanoyl group on the serine in position 3 and it is the natural ligand for the growth hormone secretagogue (GHS) receptor (GHS-R) (3, 4, 5). Circulating ghrelin consists of more than 90% of des-acyl ghrelin and less than 10% acyl ghrelin (6).

The human ghrelin gene is localized on the chromosome 3p25-26 (7). Fig. 1 summarizes the steps which lead to the production of the different ghrelin gene products. Preproghrelin mRNA is a transcription isoform of the ghrelin gene that leads to the translation of preproghrelin (117 AA) (3). Preproghrelin contains an N-terminal signal peptide (23 AA) that is cleaved from the polypeptide to form proghrelin (94 AA). Proghrelin is further processed (mainly by prohormone convertases - PC1/3) to produce the ghrelin peptide (28 AA) (8,9).

The cleavage of the ghrelin molecule from proghrelin produces C-ghrelin (66 AA), which is then further cleaved to form obestatin (23 AA). Both C-ghrelin and obestatin circulate in human plasma (10).

Proghrelin can be posttranslationally modified with the addition of an octanoyl group to the serine 3 residue of ghrelin, through an acyl-bond, by the enzyme ghrelin O-acyltransferase (GOAT) (11,12). Not all circulating ghrelin is octanoylated and the majority of plasma ghrelin is actually the non-acylated form termed des-acyl ghrelin (27 AA). Acyl-protein thioesterase 1 (APT1) has been described to convert ghrelin to des-acyl ghrelin in the plasma (13).

Ghrelin can be also acylated with groups other than octanoic acid, depending on dietary modifications, such as decanoic acid resulting in a decanoylated form termed D-ghrelin (14).

The ghrelin receptor belongs to G-protein coupled receptors (GPCR) superfamily. The GHSR gene is located on chromosome 3q26.2 (15). There are documented two splice receptor variants, type 1a (GHSR1a) and type 1b (GHSR1b). GHSR1a is known as the functional form of the receptor that mediates many of the ghrelin effects. GHSR1b is a truncated form and has been considered as a non-functional receptor because ghrelin does not bind or activate this receptor (16).

A range of alternative splice variants arise from the ghrelin gene, many of which may encode novel peptides. Although their receptors have not yet been identified, they have already proven to be active, having intriguingly subtle but opposite physiological actions to ghrelin (17, 18, 19, 20).
PHYSIOPATHOLOGICAL ROLES OF GHRELIN WITH IMPLICATIONS IN BREAST CANCER PATHOGENESIS

Ghrelin and ghrelin-receptors are expressed in a wide range of normal and tumor tissues suggesting it has many physiological functions and pathological implications. Ghrelin can exert its effects through endocrine or autocrine / paracrine actions.

The major role of ghrelin is to stimulate GH secretion, via its binding to GHSR1a on the pituitary somatotropic cells, acting in synergy with GH-releasing hormone (GHRH), thus activating GH/IGF-1 axis with major proliferative effect on both normal and tumor cell types (21, 22). Ghrelin is also able to stimulate the pituitary secretion prolactin (PRL) and adrenocorticotropin hormone (ACTH) inducing further cortisol release (23).

Ghrelin is also a major food intake modulator stimulating appetite by central and peripheral pathways and via the vagus nerve. Ghrelin is secreted in a pulsatile manner increasing before the onset of meal, during fasting, and decreasing after feeding (24). The clinical application of these effects have been investigated in both eating disorders and muscle wasting conditions, including obesity, cachexia and sarcopenia, conditions that are also encountered in the tumoral genesis and evolution of breast cancer (25).

Besides these two major effects, ghrelin has a multiplicity of other physiological roles affecting energy and glucose homeostasis, gastrointestinal, cardiovascular, pulmonary systems, immune function and bone physiology (25).

Ghrelin is involved in long-term weight regulation fluctuating in a compensatory manner to body weight modifications, thus decreasing with weight gain (overfeeding, high fat diet) and conversely increasing with weight loss (food restriction, cachectic conditions like in advanced cancers) (25).

In connection with body weight and body fat weight it was shown that ghrelin and des-acyl ghrelin may inhibit aromatase expression and activity in human adipose cells. This is a very important effect because aromatase converts androgens into estrogens and its expression within adipose stromal cells is believed to be the major driver of estrogen-dependent cancers in older women (26).

Concerning glucose homeostasis (with implications in insulin/IGF-1 axis), ghrelin was shown to decrease insulin sensitivity depending on the acyl ghrelin/des-acyl ghrelin ratio as acyl ghrelin determines insulin resistance, while des-acyl ghrelin neutralizes this effect (27).

Ghrelin also controls several aspects of female reproductive system inhibiting hypothalamic gonadotropin-releasing hormone (GnRH) and of both LH and FSH secretion (28, 29, 30).

More direct effects in cancer pathogenesis were studied and showed that ghrelin is involved in a number of processes associated with cancer progression, including cell proliferation (probably via MAPK / ERK1/2 pathway), cell migration and invasion, the inhibition of apoptosis and ghrelin could play a role in angiogenesis and cancer-related inflammation (31).

GHRELIN AXIS AND BREAST CANCER

Although it is known that many tumors express members of the ghrelin axis, their role in cancer has not been extensively studied. Searching PubMed database on the implications of ghrelin axis in breast cancer...
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(excluding articles on orexigenic effect of ghrelin), ten original articles were found concerning this subject. A similar search was performed in 2012 by Stefanaki et al (32) and our systematic review is updated with the latest studies results.

The first study to show that ghrelin has a potential clinical application in breast cancer was performed by Cassoni et al in 2001 (33). The study demonstrated that there are specific receptors present in human breast carcinomas, in both estrogen-dependent and estrogen-independent breast cancer cell lines, which bind natural and synthetic GH secretagogues, thus proving they may have other biological activities, independent of their GH releasing effect. The same study concluded that these receptors were also having an inhibitory action on breast cancer cell proliferation in vitro, probably via activation of binding sites, different from GSHR1. Additionally, the entity of the binding was independent from the histological type of the tumor, stage, estrogen receptor status, proliferative index and whether patients were in pre- or post-menopause. However, it was positively correlated to the grade of tumor differentiation, in a way that well-differentiated carcinomas showed a higher GHS binding than moderately and poorly differentiated carcinomas (33).

The following study was performed by Jeffery et al in 2005 (34). The aim of this study was to investigate ghrelin expression in breast cancer tissues and cell lines and to examine the effect ghrelin has on breast cancer cells proliferation in vitro. According to their results, ghrelin plays a role in breast cancer by stimulating cell proliferation in both estrogen-dependent and estrogen-independent tumors. They reported that the truncated isoform GHSR1b is highly expressed in breast cancer tissue with negligible expression in normal breast specimens. Taking into account that GHSR1 is expressed in both normal and cancer tissues and cell lines, this may suggest that GHSR1b could represent a new marker for breast cancer. In their study, Jeffery et al also described the expression of exon 3-deleted preproghrelin (a human proghrelin isoform), which encodes mature ghrelin and a novel C-terminal peptide. This peptide was found to be highly expressed in the estrogen-independent cell lines, but also in the glandular epithelium of high-grade cancer tissue, exhibiting the highest immunoreactivity and thus indicating it may play a functional role in breast pathogenesis (34).

In 2006, Wagner et al (35) investigated the effect of a total 12 single-nucleotide polymorphisms and 3 dinucleotide repeats in the genes encoding GHRH, GHRL and somatostatin (SST) and their receptors (GHRHR, GHSR, SSTR2) on the risk of breast cancer. The results demonstrated that the GHRL, GHRHR, SST and SSTR2 gene polymorphisms may contribute to a decreased breast cancer risk and this would be consistent with a decreased activity of the IGF-1 pathway (35).

Another study was performed by Dossus et al in 2008 (36), which investigated the association of 15 genetic variants in the GHSR and GHRL genes with breast cancer risk, circulating IGF-1 and IGFBP-3 levels, and anthropometric measures. In their study, GHRL SNP rs171407 was found to be associated with an increased breast cancer risk (20%), with a greater height (+0.4%) and with a lower BMI (-1.9%). Similarly, GHSR SNP rs572169 was also associated with increased breast cancer risk (20%) and greater height (+0.4%), but not
with BMI. Another GHRL SNP - r3755777 - was associated with higher circulating IGF-1 levels. The findings of Dossus et al study might reflect an effect of these polymorphisms in GHSR and GHRL genes on breast cancer through the GH axis, independently of BMI or obesity and consequently independently of the ghrelin action on appetite and food intake (36).

In the same year, Feigelson et al (37) conducted a case-control study among postmenopausal women to establish whether genes associated with obesity increase the risk for breast cancer. Among these genes was also GHRL gene and the study found no association between the gene and breast cancer risk (37), contrary to previous study of Dossus et al (36).

Also in 2008, Ordway et al (38) conducted a study on DNA methylation alterations in breast cancer that identified a single locus within the promoter region of GHSR gene that is hypermethylated, which is capable of distinguishing infiltrating ductal breast carcinoma from cancer-free breast tissue with a sensitivity and specificity of 90% and respectively 96% (38).

Later, in 2011, Gahete et al (39) showed than In1-ghrelin - a variant transcript processed from the GHRL gene - was expressed about 8 times higher in breast cancer samples compared to normal mammary tissue. The levels of In1-ghrelin expression was strongly correlated in breast tumors with GOAT, GHSR-1b, cyclin D3 (a proliferation marker) and Ki67, but not with native ghrelin or GHSR-1a, thus suggesting a possible relevant role in the pathology of breast cancer. Further, the In1-ghrelin over expression in estrogen-dependent breast cancer cell lines caused increased proliferation rate suggests that the blockade of In1-ghrelin variant may have some therapeutic benefit (39).

In the same year, Grönberg et al (40) conducted a study to evaluate the potential biological and prognostic role of ghrelin and obestatin expression in invasive breast cancer. The results showed that ghrelin and obestatin were positively correlated to ER expression and negatively to proliferation rate, tumor size and to the Nottingham histologic grade (NHG) status. Also ghrelin expression was associated with a prolonged survival, which is consistent with the negative correlation to tumor grade, size and proliferation rate. Further, the results showed that tumors expressing ghrelin had 2.5-3 times lower risk for recurrence or breast cancer-specific death than those lacking ghrelin expression, proving a potential role of ghrelin as a prognostic marker in breast cancer. Even though ghrelin and obestatin were significantly correlated to each other, obestatin did not provide any prognostic information (40).

Botla et al (41) performed in 2012 a study aimed to identify methylation variation that could differentiate between breast cancers and other breast tissue. They have established a signature associated with two genes - GHSR and SFRP2 (associated with poor patient survival) - which exhibited significant hypermethylation in cancers suggesting an epigenetic defect. Concerning diagnosis, the GHSR pattern demonstrated a very high sensitivity and specificity of 89.3% and 100%, respectively. To date, this represents the highest combined sensitivity and specificity for a DNA-based biomarker in breast cancer reported (41).

**CONCLUSIONS**

In this systematic review we tried to identify the implications of ghrelin axis in breast cancer. The ghrelin axis seems to be
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definitely involved in the pathogenesis of breast cancer, but a precise role is not yet established. Studies performed up to date are not sufficient to support that ghrelin itself should be used in current management strategies, but they show much promise concerning early detection, prognostic and therapy in breast cancer.

Further studies with larger populations are necessary that should include the analysis of factors (metabolic, genetic and environmental) which are expected to influence the results, in order to verify the precise role of ghrelin axis in breast cancer.

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REFERENCES


**NEWS**

**SUDDEN DEATH OF AN INFANT WITH CORONARY INVOLVEMENT DUE TO TAKAYASU ARTERITIS**

Takayasu arteritis is an inflammatory disease that involves large vessels, predominantly the aorta and its main branches. This disease is much more common in females than in males, and the age of onset usually ranges between 10 and 30 years old, but it has been reported in children as young as 2 years of age. Coronary artery lesions are occasionally found in adult patients with Takayasu arteritis and rarely in younger children. Myocardial infarction or heart failure due to coronary involvement can be one of the potentially fatal complications in this disease. Usually, the involved arteries, aorta and its main branches, were thickened and stenotic. In the reported case, the ascending aorta was thickened and showed ostial stenosis in the coronary arteries bilaterally. The proximal segment of the left and right coronary arteries showed approximately 60% and over 90% occlusion, respectively. Microscopically, the intima was thickened due to an increase of intimal cells and fibers, and the adventitia showed thickening with fibrosis. In addition, remarkable infiltration of inflammatory cells, including multinuclear giant cells phagocytosing fragmented elastic fibers, and destruction of elastic fibers were observed in the media. However, Takayasu arteritis has not been recognized as a cause of sudden coronary death in infants. This report presents a case of sudden infant death due to severe stenosis of the left and right coronary arteries caused by Takayasu arteritis, which had not been diagnosed before death. In conclusion, Takayasu arteritis can be a life-threatening condition even in infants. There may be other similar cases that were unrecognized at autopsy in children with sudden unexpected death that are needed to be diagnosed (Wang EL, Sato Y, Kitamura O, et al. Sudden death of an infant with coronary involvement due to Takayasu arteritis. Cardiovascular Pathology 2013, 22: 109–111).

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