The contribution of growth hormone to mammary neoplasia.

Jo K Perry, Kumarasamypet M Mohankumar, B Starling Emerald, Hichem Mertani, Peter E Lobie

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Perry Jo K [1] **, Mohankumar Kumarasamypet M** [1] **, Emerald B Starling** [1] **, Mertani Hichem C** [2] **, Lobie Peter E** [1 3 *]

[1] *The Liggins Institute and the National Research Centre for Growth and Development University of Auckland, 2-6 Park Avenue, Grafton, Private Bag 92019, Auckland 1023,NZ*

[2] *PICM, Physiologie intégrative, cellulaire et moléculaire CNRS : UMR5123, Université Claude Bernard - Lyon I, Bât. R. Dubois 43, Bvd du 11 Novembre 1918 69622 VILLEURBANNE CEDEX,FR*

[3] *Department of Molecular Medicine and Pathology University of Auckland, Faculty of Medical and Health Sciences, Private Bag 92019, Auckland, New Zealand,NZ*

* Correspondence should be adressed to: Peter E Lobie <p.lobie@auckland.ac.nz>
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Abstract

While the effects of growth hormone (GH) on longitudinal growth are well established, the observation that GH contributes to neoplastic progression is more recent. Accumulating literature implicates GH-mediated signal transduction in the development and progression of a wide range malignancies including breast cancer. Recently autocrine human GH been demonstrated to be an orthotopically expressed oncogene for the human mammary gland. This review will highlight recent evidence linking GH and mammary carcinoma and discuss GH-antagonism as a potential therapeutic approach for treatment of breast cancer.

Author Keywords Growth hormone ; breast cancer ; mammary development

Introduction

Current paradigms in oncology suggest that neoplasia may be a pathologic recapitulation of developmental processes. Reflecting this concept, malignant tissues appear to share many features in common with embryonic tissues. These similarities extend to include numerous genes implicated in both developmental and oncogenic processes [1].

The role that the endocrine system plays in development of the mammary gland has been known for many years and necessitates complex interactions between ovarian and pituitary hormones including estrogen, growth hormone (GH) and prolactin [2]. Estrogen is a key regulator of mammary development and is critical for the two major phases of development; ductal elongation during puberty and lobuloalveolar development during pregnancy. In addition, early studies using hypophysectomised and gonadectomised rats demonstrated that GH is also obligatory for mammary development during puberty and is essential for formation of the rapidly proliferating terminal end bud structures found in the developing mammary gland [2, 3].

GH is the major mediator of postnatal somatic cell growth [4]. The effects of this peptide hormone on cell growth and differentiation are mediated through interaction with a cell surface GH receptor (GHR) [4, 5]. Binding of GH to the GHR activates signal transduction pathways critical for cell growth and survival including the Janus kinase-2/signal transducers and activators of transcription (JAK-2/STAT), the c-Src/p44/42 mitogen activated protein kinase (MAPK), and the phosphoinositide 3-kinase (PI3K) pathways [4, 5]. Upregulation of components of these pathways has been observed in a wide range of malignancies.

As well as its role in mammary development, the endocrine system also contributes to mammary neoplasia, and the hormone dependency of breast cancer has been widely reported. Life-time exposure to estrogen is the major risk factor for breast cancer [6]. Furthermore, accumulating evidence has implicated the GH/insulin-like growth factor-1 (GH/IGF-1) axis in mammary tumorigenesis [7, 8].

The early observation of dramatic regression of a metastatic mammary tumour following hypophysectomy by Luft and colleagues [9] and the subsequent employment of hypophysectomy as therapy for breast cancer patients in the 1950's was an early indication of the involvement of hGH in the pathogenesis of breast cancer [10]. The pituitary hormone prolactin was presumed a likely candidate for the remission observed in some patients with anti-estrogen refractory metastatic breast cancer following hypophysectomy [11]. While the involvement of prolactin in breast cancer progression is accepted, recent publications have proposed that prolactin may not be oncogenic and may in fact have “protective” attributes in some instances [12]. hGH has been demonstrated to bind and activate both the hGHR and the prolactin receptor [13]. Much of the effects of GH on somatic cell growth are mediated through induction of hepatic IGF-1 secretion [4] and the role of IGF-1 and the IGF-1 receptor (IGF-1R) in mammary carcinoma have been extensively reported [7, 14]. However, GH has additional IGF-1 independent effects on growth and neoplasia.

Studies conducted in our laboratory have demonstrated a critical oncogenic role for autocrine hGH in mammary carcinoma [8, 15–21]. Remarkably, expression of autocrine hGH is sufficient to promote oncogenic transformation of an immortalised, but otherwise normal, human mammary epithelial cell line [19], and can increase the replicative capacity of the human mammary epithelial cell (HMEC), the evidence for which is discussed below. Thus, it is likely that the oncogenic capacity of autocrine hGH recapitulates the normal developmental function of this hormone in the mammary gland.

GH influences development of the mammary gland

The mammary gland is one of the very few organs that undergoes dramatic postnatal changes in size, shape and function. Critical stages of mammary development are initiated early in puberty and continue through pregnancy, parturition, lactation and involution [2, 3]. Ovarian and pituitary hormones play a critical role in mammary development as exemplified by the fact that estrogen can rescue mammary development in ovariectomized animals. However, estrogen replacement does not rescue mammary development in hypophysectomised animals indicating the requirement of pituitary hormones in the process [2, 3]. GH acts on both the stromal and epithelial components of the mammary gland, through induction of IGF-1 expression, to promote ductal elongation and differentiation of ductal epithelial into highly proliferative structures known as the terminal end buds (TEBS) [2, 3, 22, 23] (Fig. 1). Mammary ductal development in GHR-null mice is impaired [24]. Furthermore, antagonism of GH signal transduction delays mammary ductal development and is associated with a reduction in side branching and a decrease in the number of TEBs in the rodent mammary gland [25]. Evidence suggests that GH also influences alveolar development as the mammary glands of GH-deficient rats have substantially less alveolar development when compared with wild-type rats. Concordantly, infusion of GH induces mammary epithelial cell proliferation and alveolar development similar to that of wild-type animals [26].

In addition to hGH secretion from the pituitary, hGH is also produced locally in the mammary gland [27, 28] and influences mammary development (Fig. 1). In mice, autocrine GH mRNA and protein expression is primarily observed in the epithelium of the mammary gland and maximal expression is observed during puberty [23]. Expression of a hGH transgene in mice results in precocious development of the mammary gland [29] while experimentally engineered functional GH deficiency results in severely impaired mammary gland development [30]. In humans, autocrine hGH and hGHR mRNA and protein are predominantly expressed in the luminal epithelial and myoepithelial ductal cells of normal mammary tissue [27, 28, 31, 32].

Animal studies implicating GH in mammary gland neoplasia

Animal models which disrupt GH and IGF-1 signalling clearly demonstrate the role of the GH/IGF-1 axis in mammary neoplasia [7, 8]. One early study linking GH to mammary neoplasia demonstrated that suppression of GH secretion through somatostatin (SS) administration reduced mammary carcinogenesis in rats when accompanied by a reduction in serum prolactin [33]. In addition, experimentally engineered functional GH deficiency resulted in virtual resistance to the spontaneous development of hyperplastic alveolar nodules [30]. Subsequently Mol et al. described an acromegaly-like phenotype associated with an increase in plasma GH levels in dogs following administration of progestins [34, 35]. The progestin-induced GH excess was demonstrated to originate from foci of the hyperplastic ductular epithelium of the mammary gland by immunohistochemical analysis. Interestingly, increased plasma GH was associated with the development of benign mammary tumours in these animals [34, 35]. GH mRNA expression has also been identified in hyperplastic and neoplastic canine mammary tissue [36]. Further evidence has come from studies in aging monkeys where systemic GH treatment was demonstrated to induce hyperplasia of the mammary gland, an effect which was independent of IGF-1 [37].

In lit/lit mice, which have reduced levels of GH and IGF-1 due to a point mutation in the GHRH gene, growth of a transplanted human mammary carcinoma cell line (MCF-7) is significantly reduced when compared with control mice [38]. The Spontaneous Dwarf rat (SDR) which lacks a functional GH allele due to a point mutation in the GH gene has been demonstrated to be almost completely refractory to chemically induced mammary carcinogenesis in several studies [26, 39–41]. This effect is abrogated by re-introduction of circulating GH which restores mammary tumorigenesis to that of the wild-type animals [39–41]. A recent study by Shen et al. elegantly illustrates the absolute requirement of GH in chemically induced mammary carcinogenesis [41]. Treatment of GH-deficient SDR rats, resistant to the development of N-methyl-N-nitrosourea-induced mammary tumours, with rat or bovine GH restored the tumour incidence to that of wild-type Sprague Dawley rats. Remarkably, the advanced mammary tumours which had developed in these rats complete regressed within four weeks following cessation of GH treatment and tumour growth subsequently resumed on the continuation of GH treatment [41].

Further studies from Swanson and colleagues addressed the potential utility of disruption of GH signaling for the treatment of estrogen-independent mammary tumours [42]. Transgenic female C3(1)/Tag mice are prone to developing estrogen receptor-negative mammary carcinoma due to expression of the SV40 large T-antigen [43]. Zhang et al. observed delayed mammary tumour development in C3(1)/Tag mice crossed with the Laron mouse in which the GHR gene had been disrupted. Disruption of GH signaling decreased mammary tumour number and

volume in these mice [42]. In addition, tumours from GHR-null, C3(1)/Tag mice displayed a more differentiated morphology and were associated with a more benign phenotype.

In transgenic studies, mice overexpressing the GHR antagonist, hGH-G120R, are smaller in terms of size and body weight, have reduced IGF-1 levels and are resistant to dimethylbenz[a]anthracene (DMBA)-induced mammary tumours [44]. Mice transgenic for the hGH gene have increased serum IGF-1, mammary gland hyperplasia and spontaneous mammary tumorigenesis [45]. It should be noted that transgenic mouse studies may represent both endocrine and autocrine/paracrine actions of hGH as while increased plasma concentrations of hGH may be observed, hGH may also be expressed in multiple tissues thus having both paracrine and autocrine effects.

The role of hGH in human mammary neoplasia

Recently an increasing number of studies have emerged which confirm a pivotal role for hGH in human mammary gland neoplasia [7, 8, 11]. These include an altered risk of cancer associated with polymorphisms in genes within the hGH/IGF-1 axis [46, 47], altered risk of breast cancer in pathological conditions resulting from abnormal hGH levels [48, 49], and increased hGH expression in human mammary proliferative disorders [28, 31]. These will be discussed in the following sections.

Childhood and adolescent anthropometric factors associated with breast cancer risk

hGH plays a central role in longitudinal growth and development. Numerous epidemiological studies have demonstrated that increased birth weight is independently associated with increased pre- and postmenopausal breast cancer risk [50, 51]. An association between height at various stages of development and risk of developing breast cancer has also been noted [51–53]. An extensive review conducted by Gunnell et al., investigating associations of height with breast cancer, observed increased risk of mammary carcinoma of approximately 10–60% over multiple cohort studies, in the highest height category when compared with the lowest [53]. Height at 8 years of age [51] and height velocity between the ages of 4 to 7 correlates positively with risk of developing breast cancer [52]. In addition, the increase in height at puberty is associated with breast cancer risk suggesting that women who grow faster during childhood and reach an above average height adult height are at particularly increased risk of mammary carcinoma [51, 52]. In a recent study of breast cancer risk in two prospective twin cohorts from Sweden and Finland, which controlled for genetic and early shared environmental factors, the tallest women had a significantly increased risk of breast cancer [54].

Likely mechanisms contributing to the relationship between height and breast cancer risk have been proposed [53]. These include genetic factors, the role of energy intake, infections and circulating levels of growth promoting hormones, during childhood and early adulthood [53]. However, the twin cohort study by Lundqvist et al. indicates that genetic or shared early environmental factors are unlikely to explain the association between height and breast cancer [54]. One candidate for the observed relationship is therefore the GH/IGF-1 axis. The GH/IGF-1 axis is the main mediator of somatic growth during childhood and plays an essential role in the development of the mammary gland by regulating cell proliferation, differentiation and apoptosis [3]. As will be discussed below, several polymorphisms located associated with genes within the GH1/IGF-1 axis have also been linked with risk of developing breast cancer.

Reproductive history and breast cancer risk

Besides genetic factors and age, reproductive history is the strongest and most consistent risk factor for breast cancer, and the protective effect of parturition has been well documented [55]. Women who have an early first pregnancy which is carried to term reduce their lifetime risk of developing breast cancer by approximately 2-fold compared with nulliparous women [55]. Several theories have been proposed to explain this protective effect [55, 56]. Firstly, decreased levels of circulating hGH following parturition results in an altered hormonal environment [56]. Such an effect has been demonstrated in rats, where parity-induced protection against mammary tumorigenesis has been linked to a decrease in circulating GH levels while levels of several other hormones investigated remained unchanged [57]. In addition, there is evidence to suggest that there is reduced proliferation in the mammary gland post parturition and that an alteration in cell fate occurs, mediated by specific molecular changes induced by estrogen and progesterone [56]. Finally, it has been hypothesised that the mammary gland cell population undergoes differentiation which may provide a less responsive environment to oncogenic stimuli [56, 58].

Cancer stem cells

There is a growing body of evidence that supports the idea that cancer originates in progenitor or stem cells and this is known as the cancer stem cell hypothesis [59]. The majority of breast cancer originates in the rapidly dividing undifferentiated TEBs which are densely populated with mammary stem cells. As discussed, this region is one of the primary targets of GH in the developing mammary gland [2, 3]. An association between the presence of TEBs and the development of experimental mammary tumours has been observed [3]. In addition, Russo and colleagues have suggested that parturition-induced protection against breast cancer results from mammary stem cell differentiation with an

associated shift in molecular profile conferring resistance to carcinogenesis [58]. In this regard it is interesting that the GH/IGF-1 axis has been suggested to influence mammary stem cell number, another risk factor for breast cancer [59, 60]. In addition, the hGHR gene is downregulated post parturition in parous rats [61] and is highly expressed in a population of mammary stem cells associated with TEBs which are hypothesised to be responsible for the development of mammary carcinoma [58] Furthermore, the expression of the hGHR is increased in primary HMEC cultured as mammospheres when compared adherent HMEC cultures [59] suggestive that the hGHR may be a so called “stem cell factor”.

Clinical correlations with disease

Epidemiological studies linking levels of serum hGH and breast cancer have been limited possibly due to the difficulties in obtaining reliable measures of patient serum hGH levels. hGH is secreted from the pituitary in a pulsatile fashion and serum levels can vary significantly over a 24 hour period. In addition, there is a large number of assays available which vary in their ability to detect hGH at lower concentrations [62]. However, in one study 40% of breast cancer patients were demonstrated to have elevated serum hGH [63]. Increased levels of circulating hGH is known to increase IGF-1 expression which has also been linked to risk of breast malignancy in numerous studies [7, 64, 65]. Another study failed to observe a change in hGH serum levels in a group of premenopausal women with breast cancer [66]. Recently increased human growth hormone binding protein (hGHBP) which forms a complex with hGH in serum, has been associated with a three-fold increased risk of breast cancer [67]. hGHBP is derived from the extracellular portion of the hGHR and serum levels of this protein may serve as an indicator of the tissue hGHR status [68].

Increases in serum hGH may be consequent to tumour formation or play a more fundamental role in tumour development. Recent studies have demonstrated an association of low-penetrant polymorphic variations in the hGH gene (GH1) with breast malignancies suggesting a causal role for hGH in breast oncogenesis [8, 47]. Haplotypes from the highly polymorphic proximal promoter of the hGH gene have been demonstrated to increase breast cancer risk when in combination with haplotypes from within the hGH locus control region (LCR) which is 14.5 kb upstream of the hGH gene [69]. Interestingly, specific haplotypes from the LCR have previously been demonstrated to affect tissue-specific expression of the hGH promoter [70]. Further analyses have identified polymorphisms in members of the GH/IGF-1 axis involved in hGH synthesis and secretion including the hGHR, SS, the SS receptor 2 (SSTR2) and the hGHRH receptor as having a protective influence on breast cancer risk [71–73]. Mammographic density has also been demonstrated to be a risk factor for breast cancer and is influenced by circulating levels of IGF-1 and GH. A recent study has demonstrated that polymorphisms in the hGH gene known to affect hGH expression levels, are associated with mammographic density [74].

Pathological conditions which affect hGH signalling in humans have been associated with cancer. It is documented that acromegalics have an increased risk of colorectal cancer [75]. An increased risk of breast cancer has also been observed in these patients; however these associations have mostly been based on small epidemiological surveys and circumstantial evidence and requires further large scale investigation for confirmation [75]. One striking observation comes from a study of cancer incidence in 222 patients with a non-functional hGHR (Laron Syndrome) or with hGH deficiency [49]. While 338 first and second-degree relatives had what could be considered a normal rate of cancer incidence, there were no reports of any malignancy detected in any of GH deficient or nonresponsive patients [49].

5 GH replacement therapy and cancer risk

Given the pivotal role of autocrine hGH in human neoplastic progression it is perhaps not surprising that attention has focused on the large number of patients receiving recombinant hGH (rhGH) as treatment for a variety of growth related disorders. Multicenter, observational surveillance of a large number of patients receiving hGH replacement therapy during childhood has revealed that rhGH administration does not result in an increased risk of carcinoma [75–80]. This may reflect the clinical application of rhGH administration in these patients which has the primary aim of correcting hGH deficiency thus presumably returning the patient to a state of normality in regards to hGH serum levels [75]. An increased risk of mortality from Hodgkin's disease and an increased risk of incidence and mortality from colorectal cancer has been observed in one study of patients treated with pituitary hGH in childhood or early adulthood, although the cohort studied was relatively small [81]. In several recent studies it has been observed that Childhood Cancer Survivor Study patients treated with rhGH to maximise growth have an elevated risk of secondary malignant neoplasms including breast [77, 80, 82, 83]. No evidence of an increase of disease recurrence was detected in these studies. Overall, the risk of secondary neoplasms associated with hGH treatment remains small and needs to be weighed against the potential benefits of hGH replacement therapy. However, longterm surveillance of children receiving rhGH therapy needs to be maintained.

The role of autocrine hGH in breast cancer

Recent literature has demonstrated a clear association between localised autocrine hGH expression and breast cancer [8, 11]. Studies from our laboratory have demonstrated that autocrine hGH is an orthotopically expressed oncogene for the human mammary epithelial cell [19]. Furthermore, recent studies have demonstrated that hGH expression can increase telomerase activity [21] and extend the replicative capacity of

a primary mammary epithelial cell line (Fig. 2). Thus autocrine hGH is the first example of a human gene that can both potentially immortalise and oncogenically transform the human epithelial cell and the evidence for this is discussed (Fig. 3).

Correlation of hGH expression with cancer

Historically, a number of sporadic cases of ectopic hGH secretion associated with malignancy have been noted in the literature, including breast and ovarian cancer [84]. hGH mRNA and protein expression has been identified in mammary tumour tissue [27, 28]. In situ RT-PCR has demonstrated that increased epithelial expression of the hGH gene and de novo stromal expression is associated with the acquisition of pathological proliferation of the mammary gland [28]. Furthermore, increased expression of the hGH gene was observed to be associated with metastatic mammary carcinoma cells [28]. In another study hGH expression was detected by RT-PCR in RNA extracted from the normal human mammary gland in addition to mammary carcinoma [27].

Localised expression of hGHR mRNA and/or protein has been detected in the normal mammary tissue of several species including rabbit [85], murine, bovine and human mammary gland epithelia [31] and refs therein). hGHR mRNA and protein expression has also been identified in human breast cancer [31, 86]. hGHR transcript and protein is expressed in the epithelial cells of normal, proliferative and neoplastic lesions of the breast. In addition, stromal components of the mammary gland express the hGHR gene [31]. While increased levels of hGH mRNA and protein are associated with proliferative disorders of the mammary gland, hGHR mRNA and protein levels per cell remain constant throughout the process of neoplastic progression [31]. However, a second study has observed increased hGHR mRNA and protein in human mammary carcinoma samples when compared to adjacent normal tissue [87] although the samples were not controlled for epithelial content and may simply reflect increased cellularity of the neoplastic mass.

Proteins that regulate the secretion of hGH from the pituitary have also been implicated in mammary neoplasia. RT-PCR and Western analysis have demonstrated expression of hGHRH, which induces hGH secretion from the anterior pituitary, and its receptor in breast cancer tissue [88, 89]. Antagonists for GHRH inhibit the proliferation of a breast cancer cell line in vitro and inhibit growth of experimental mammary tumours demonstrating potential clinical utility [90]. Expression of Pit–1 mRNA and protein, which regulates hGH expression, has also been detected in normal and malignant breast tissue [91]. Forced expression of Pit-1 increases expression of hGH mRNA and protein in human mammary carcinoma cells [91]. However, stimulation of autocrine hGH expression has also been demonstrated to occur through Pit-1 independent mechanisms in the dog [92] suggesting the involvement of different regulatory mechanisms.

Cell survival and proliferation

The acquired ability to overcome proliferative arrest and resist apoptosis is a key step in the oncogenic process [93, 94]. Using an in vitro cell-based model of mammary carcinoma we have examined the role of autocrine hGH in mammary carcinoma development and progression. [15, 17, 19]. Autocrine hGH expression promotes cell proliferation [15] and cell survival [95] in both mammary carcinoma cells and in an immortalised human mammary epithelial cell line [19] (Fig. 3). These effects are mediated solely through the hGH receptor as evidenced by their reversal following treatment with the hGHR antagonist, B2036 [16].

Immortalisation

Human mammary epithelial cells (HMECs) have a limited replication capacity and eventually enter into a period of senescence. A critical step in tumorigenesis involves the loss of senescence checkpoints and subsequent unrestricted cell proliferation in a phenomenon known as immortalisation. The key regulator of this process is the telomere [96, 97]. Autocrine hGH has been demonstrated to increase telomerase activity [21] through increasing mRNA and protein levels of the of catalytic subunit of telomerase, hTERT (Fig. 3). The increase in hTERT gene expression is the result of increased hTERT mRNA stability and is not due to increased transcriptional activation of the hTERT promoter [21]. This occurs through autocrine hGH mediated upregulation of two poly(C)-binding proteins, αCP1 and αCP2, which bind to cis-regulatory elements within the hTERT mRNA [21]. Another study has demonstrated PI3-Kinase-dependant upregulation of telomerase activity in CHO cells by exogenously added GH with consequential increases in telomere length [98]. Increased telomerase activity and telomere length has the potential to contribute to cell immortalisation. Accordingly, we have demonstrated that autocrine hGH increases the replicative life span of the human primary mammary epithelial cell (Fig. 2).

Oncogenic transformation

Resistance to anoikis, or anchorage independent growth, is a characteristic of oncogenically transformed cells [94]. Autocrine hGH promotes anchorage independent cell growth in mammary carcinoma cells and tumour growth in vitro [15, 17] (Fig. 3). Remarkably, autocrine hGH expression can also oncogenically transform the human mammary epithelial cell. Studies conducted in the immortalised, but otherwise normal human mammary epithelial cell line, MCF-10A have demonstrated that expression of the hGH gene increases cell proliferation and

survival [19] and promotes anchorage independent growth [19]. When MCF-10A cells are cultured in Matrigel, which resembles the complex extracellular environment found in many tissues, acinar structures resembling the in vivo morphology of the mammary gland are formed [99, 100]. Autocrine hGH expression in MCF-10A cells disrupts normal mammary acinar architecture in three-dimensional epithelial cell culture and results in luminal filling and de-regulated cell proliferation [19]. This is significant as filling of the luminal space is a hallmark of early epithelial tumours, such as atypical hyperplasia and ductal carcinoma in situ [101–103]. Autocrine hGH expression in MCF-10A cells also results in tumour formation in a xenograft mouse model whereas control cells do not form tumours [19]. Autocrine hGH therefore fulfils the criteria to be displayed as a human mammary epithelial oncogene.

Phenotypic conversion

The phenotypic conversion of cells from an epithelial to a mesenchymal morphology associated with acquisition of a migratory and invasive phenotype during carcinoma progression is referred to as epitheliomesenchymal transition (EMT). This process is accompanied by concomitant changes in gene expression [104, 105]. Autocrine hGH promotes EMT in mammary carcinoma cells with epithelial morphology, thus resulting in a mesenchymal cell characteristics [17] (Fig. 3). This is achieved through down regulation of plakoglobin, relocalisation of E-cadherin to the cytoplasm and increased activity of the matrix metalloproteases (MMP) 2 and 9. Such molecular alterations result in dissolution of cell to cell contacts and decreased cell height and are accompanied by increased cell migration and cell invasion [17]. Thus autocrine hGH may increase the metastatic potential of human mammary carcinoma cells.

Tumour angiogenesis

Tumour cells secrete soluble factors such as vascular endothelial growth factor-A (VEGF-A) which attract neighbouring blood vessels to grow towards the tumour in a process known as angiogenesis and is a fundamental step in tumour progression [106]. This de novo angiogenesis is requisite for the establishment, growth and dissemination of cancer. Consequently, inhibition of angiogenesis has become the focus of a number of novel targeted cancer therapeutics [106].

We have demonstrated that autocrine hGH expression in a mammary carcinoma cell line promotes endothelial cell migration and tube formation in vitro and tumour angiogenesis in a mouse xenograft model. In addition autocrine hGH increases VEGF-A mRNA and protein levels in this cell line (S Brunet-Dunand, P Lobie, J Perry; unpublished observation) (Fig. 3). In an earlier microarray study we also identified thrombospondin 1 (Tsp1) as being one of 305 genes regulated by autocrine hGH [18]. The downregulation of Tsp1 by autocrine hGH is of particular interest given the well established role of Tsp1 repression in tumour progression and acquisition of an angiogenic phenotype [107].

Corroborating evidence comes from a number of animal and clinical studies which demonstrate a role of GH in angiogenesis [108–114]. Recently it has also been demonstrated that rAAV-mediated expression of hGH can improve cardiac function through promoting angiogenesis [114]. hGH transduction resulted in a significant induction of several angiogenic genes such as endothelial nitric oxide synthase (eNOS), VEGF and basic fibroblast growth factor (bFGF) in rat hearts while immunohistochemistry analysis revealed an increase in capillary density and cell proliferation [114].

Chemoresistance and radioprotection

Intrinsic or acquired resistance to chemotherapeutic drugs is a major factor influencing the efficacy of cancer therapy. Numerous factors can contribute to a chemoresistant tumour phenotype including alterations in the rate of drug efflux; genetic factors and altered gene expression influencing drug metabolism and drug targets; repair of drug-induced damage; and evasion of apoptosis. Given the capacity of autocrine hGH to stimulate mammary carcinoma cell survival, it is not surprising to find that autocrine hGH contributes to chemoresistance in mammary carcinoma cells. Studies from our laboratory suggests that autocrine hGH regulates a key enzyme in estrogen biosynthesis, P450 aromatase, and is sufficient to confer resistance to an aromatase inhibitor in mammary carcinoma cells (Yang, Perry, Lobie; unpublished). Furthermore autocrine hGH reduces sensitivity to treatment with mitomycin C in several mammary carcinoma cell lines (N Bougen, P Lobie, J Perry; unpublished observation).

Radiotherapy is a recognised treatment strategy for the management of breast cancer. Again however, resistance to ionising radiation is still a major obstacle to effective treatment. Radioresistance can result from several factors, in particular: increased DNA repair, telomere length and resolution of radical oxygen species resulting from treatment ionising radiation [115, 116]. In addition, certain growth factors, including IGF-1 [115] have been shown to possess a protective effect against radiation-induced programmed cell death. hGH has also been demonstrated to be radioprotective in several studies [8]. In addition, we have observed that autocrine hGH is radioprotective in several mammary carcinoma cell lines (N Bougen, P Lobie, J Perry; unpublished observation). Furthermore, overexpression of hGHR mRNA and protein predicts response to radiotherapy in rectal carcinoma [117].

Other proteins known to be regulated by autocrine hGH that may contribute to a chemo- or radioresistant cell phenotype include gadd153 (growth arrest and DNA damage-inducible protein 153)/CHOP (C/EBP homologous protein) [95], and several genes involved in the oxidative stress response such as catalase, superoxide dismutase 1 (SOD1), glutathione peroxidase and glutamylcysteine synthetase, thereby protecting tumour cells from oxidative stress-induced apoptosis [20]. Autocrine production of hGH by human mammary carcinoma cells confers resistance to oxidative stressinduced apoptosis, including that generated by daunorubicin through upregulation of catalase mRNA and protein, in a p44/42 MAPK-dependant manner [20].

Mechanisms of autocrine hGH-mediated oncogenesis

The oncogenic effects of autocrine hGH are mediated through regulation of gene expression. This is achieved through modulation of signal transduction pathways, transcriptional upregulation of genes, stabilisation of specific mRNA species, and the epigenetic modification of specific gene promoters and regulatory regions and the evidence for this is presented.

Autocrine hGH regulation of gene expression

While autocrine hGH has a clear role in oncogenesis, exogenous (thus mimicking endocrine) hGH does not result in oncogenic transformation in vitro [15, 17, 19]. In addition, microarray studies indicate that autocrine and exogenous hGH differentially regulate gene expression [18]. Microarray analysis of 19,000 genes identified a subset of 305 genes in MCF-7 cells that are exclusively regulated by autocrine hGH and 167 genes jointly regulated by both autocrine and exogenous hGH [18]. One explanation for the differential effects of autocrine-produced hGH as opposed to exogenously administered hGH may be the differential mode of presentation of hGH to the cell [118]. Endocrine hGH derived from the pituitary is secreted at high concentrations and in a pulsatile fashion [119] while autocrine hGH is secreted continuously at low levels. This contrasting secretion profile may lead to differential gene expression; analogously different secretory patterns of pituitary GH possess disparate effects [120]. One effect of the different mode of presentation of hGH, may be the observed transient activation of p44/42 mitogen-activated protein (MAP) kinase by exogenous hGH in comparison to the sustained activation of p44/42 MAP kinase activity by autocrine hGH. We have observed in mammary carcinoma cells that administration of exogenous hGH transiently increases p44/42 MAP kinase compared with autocrine hGH which may maintain activation of p44/42 MAP kinase for at least 48 hours [20]. It has been previously reported that transcription of certain genes requires sustained activation of p44/42 MAP kinase [121, 122] and sustained activation of signal transduction pathways by autocrine hGH may produce its oncogenic effects.

The role of IGF-1

Although the effects of endocrine hGH are primarily mediated by IGF-1, the reported effects of autocrine hGH detailed here in are IGF-1 independent as hGH expressing MCF-7 cells do not produce detectable levels of IGF-1 [15]. However, it is likely that in a physiological setting hGH secretion from mammary carcinoma cells will have both autocrine effects, and paracrine effects on neighbouring cells. The resultant IGF-1 expression would enhance the oncogenic potential of autocrine hGH (Fig. 1). Analogously, addition of exogenous IGF-1 to hGH producing MCF-7 cells greatly enhances proliferation of these cells when compared with a control cell line [15]. It has also been reported that systemic administration of hGH and IGF-1 concomitantly increases the proliferation of mammary epithelial cells in rhesus monkeys more than either hormone administered alone [37].

Secreted soluble factors

Autocrine hGH regulates numerous other soluble secreted peptide factors, that mediate the effects of autocrine hGH on mammary carcinoma cell function. It is likely that these soluble peptide factors, in addition to IGF-1 also contribute to the autocrine/paracrine loop generated by autocrine hGH expression [8]. Soluble secreted factors upregulated by autocrine hGH include trefoil factor 1 and 3 (TFF1 and TFF3), bone morphogenic protein-7 (BMP7), laminin 3, the postulated interleukin 27 (IL-27), osteomodulin (OMD), and thyrotropin-releasing hormone (TRH) among potential others. In addition, autocrine hGH down-regulates several secreted factors including thymosin, laminin 5, the p53-regulated placental transforming growth factor (PTGF-β) and thrombospondin-1 (Tsp1). PTGF-β is known to induce cell cycle arrest and apoptosis through inhibition of autocrine hGH-stimulation of cyclinD1 [123].

Significantly, autocrine hGH increases gene expression of two trefoil factor (TFF) proteins, TFF1 and 3 in human mammary carcinoma cells [18]. The TFF family are involved in mucosal healing processes and are expressed at abnormally elevated levels in neoplastic diseases [124, 125]. Recent compelling evidence, both from experimental and clinical studies, has emerged to indicate a pivotal role of TFFs in oncogenic transformation, growth and metastatic extension of common human solid tumours [126]. We have demonstrated that TFF3 mediates autocrine hGH oncogenic transformation of immortalised human mammary epithelial cells [18, 126]. The regulation of these potentially oncogenic soluble secreted factors, such as TFF1 and 3, by autocrine hGH, creates an autocrine/paracrine loop in neighbouring cells thus potentiating the oncogenic actions of autocrine hGH [8].

HOXA1

Either consequent to regulation of secreted factors or as a primary signaling event autocrine hGH regulates molecules involved in oncogenic transformation. Noteworthy is upregulation of the homeobox containing gene HOXA1 by autocrine hGH [127]. Homeobox A1 (HOXA1) is a member of the family of homeodomain containing transcription factors which play an important role in segmental development. HOXA1 is itself a potent human mammary oncogene [127] and increased mRNA expression is observed in mammary ductal carcinoma (Perou breast study, Oncomine data base: www.oncomine.org). HOXA1 promotes proliferation through transcriptional upregulation of cyclinD1 and c-myc and reduces apoptotic cell death though transcriptional activation of Bcl2 [127]. Forced expression of HOXA1 also promotes oncogenic transformation and aggressive tumour formation in vivo [127]. Recent studies have demonstrated that HOXA1 modulates two pathways, the MAP kinase and JAK/STAT pathways, involved in hGH-mediated signal transduction, both of which have been implicated in mammary carcinoma progression ([128], Mohankumar, B. Emerald, P. Lobie unpublished). HOXA1 upregulates multiple components of the p44/42 MAP kinase pathway resulting in increased p44/42 MAP kinase activity [128]. In addition, HOXA1 upregulates STAT3 and STAT5B mRNA and protein expression again resulting in increased transcriptional activation (Mohankumar, unpublished). Combined modulation of p44/42 MAP kinase and STAT3 and 5B pathways mediates HOXA1 stimulated oncogenicity of human breast carcinoma cells and oncogenic transformation of immortalised human mammary epithelial cells [128]. Thus autocrine hGH utilises HOXA1 to transcriptionally regulate genes involved in oncogenic transformation such as c-myc, cyclinD1 and Bcl2.

Epigenetic regulation of gene expression

Changes in gene expression can be induced by epigenetic modification which is a major contributor to neoplastic transformation and an area of intense research [129]. Aberrations in DNA methylation frequently occurs in cancer and can result in chromosome instability through global hypomethylation or in silencing of tumour suppressor genes through hypermethylation occuring at CpG islands within gene promoters [129]. We have demonstrated that autocrine hGH mediates specific changes in DNA methylation at promoter-related CpG islands, through increased expression and activity of the de novo DNA methyltransferases 3A and 3B (DNMT3A and 3B) thereby directly influencing the expression levels of genes [130]. Inhibition of methylation with 5′-Aza-2′-deoxycytidine (AZA) abrogated autocrine hGH-stimulated cellular proliferation, survival and anchorage independent growth. Furthermore, treatment with AZA was demonstrated to reverse the EMT of mammary carcinoma cells and the acquisition of an invasive phenotype induced by autocrine hGH. Autocrine hGH was demonstrated to repress PLAKOGLOBIN gene transcription though hypermethylation of the first exon located within the PLAKOGLOBIN gene promoter. siRNA-mediated depletion of DNMT3A and 3B abrogated the migratory effects of autocrine hGH on mammary carcinoma cells and released autocrine hGH-mediated suppression of the PLAKOGLOBIN gene expression [130]. Thus epigenetic modification of specific genes through regulation of DNMTs is another mechanism through which autocrine hGH exerts its oncogenic effects.

Is autocrine GH a one-step human oncogene?

In their 2000 review, Hanahan and Weinberg have summarised the fundamental characteristics, or hallmarks, of cancer development [94]. The authors describe tumour development as a process analogous to Darwinian evolution, in which a stepwise accumulation of genetic changes, each conferring one or another type of growth advantage, liberates neoplastic cells from the homeostatic mechanisms that govern normal cell growth [94]. A subsequent publication described a contrasting but complementary hypothesis and proposes that deregulation of proliferation, together with a reduction in apoptosis, creates a platform that is both necessary and sufficient for development of cancer [93]. These hallmarks of cancer described by Hanahan and Weinberg include self-sufficiency in growth signals, insensitivity to anti-growth signals, limitless replicative potential, evasion of apoptosis, sustained angiogenesis, and tissue invasion and metastasis [94], characteristics that can all be attributed to expression of autocrine hGH in our mammary carcinoma cell models.

The creation of a tumour cell from a normal cell is known to require both immortalisation and oncogenic transformation [94]. While primary rodent cells can be easily transformed into tumour cells by concomitant introduction of two oncogenes, this process has previously only been achieved in human cells through a combination of the hTERT gene, required for immortalisation, with an oncogenic allele of the Hras gene (H-rasV12) and the genomic version of SV40 large T-antigen, both of which are required for oncogenic transformation [94]. Significantly, we have demonstrated that simple forced expression of the hGH gene is sufficient to oncogenically transform the immortalised human mammary epithelial cell line, MCF-10A [19]. Furthermore, forced expression of hGH stimulates oncogenic transformation in the primary HMEC as demonstrated by colony formation in soft agar (Fig 2). In addition, autocrine hGH has been demonstrated to increase telomerase activity in mammary carcinoma cells [21, 98] and extend the replicative life span of a human primary mammary epithelial cell line (Fig. 2). Thus, it is plausible that autocrine hGH is sufficient to both immortalise and oncogenically transform the human mammary epithelial cell.

The therapeutic potential of hGH antagonism

The plethora of clinical and experimental evidence supporting the role of autocrine hGH in mammary neoplasia, make antagonism of hGH signal transduction an attractive prospect in the treatment of breast cancer. While the clinical relevance of hGH antagonism in the treatment of cancer has yet to be determined, a number of studies have demonstrated the therapeutic potential of targeting GH in vitro and in xenograft models of mammary carcinoma [8]. Several strategies to reduce the detrimental effects of elevated levels of serum hGH are available or in development for the treatment of acromegaly and include hGHR antagonism, antisense oligonucleotides directed against the hGHR, as well as inhibition of hGH secretion with SS analogues [8, 131, 132].

One such agent, which has the potential to reduce both endocrine hGH and paracrine/autocrine hGH effects, is Pegvisomant, a hGHR receptor antagonist which prevents hGHR dimerisation and therefore hGH-mediated signal transduction [133]. Pegvisomant is currently FDA approved for, and has been demonstrated effective in, the treatment of acromegaly normalising the IGF-1 levels in nearly the entirety of patients [134]. Clinical trials investigating the utility of this drug as a cancer therapeutic have yet to be initiated. However, results obtained from cell culture and xenograft studies in a variety of cancer models have been promising [8, 11, 25, 135–137]. Pegvisomant or the protein component of Pegvisomant, B2036, has been demonstrated to be effective in reducing meningioma cell growth in vitro [138] and in vivo [137] and inhibited growth of a human colorectal carcinoma cell line in a xenograft mouse model [135]. A recent xenograft study has investigated the therapeutic potential of hGH antagonism in breast cancer. Divisova et al. demonstrated that Pegvisomant, was effective in both suppressing proliferation and inducing apoptosis in a mammary carcinoma cell line transplanted into immunosuppressed mice [25]. In addition, we have demonstrated that hGHR antagonism using the protein component of Pegvisomant, B2036, abrogates autocrine hGH-mediated transcriptional activation, protection from apoptosis and carcinoma cell spreading on a collagen matrix thereby demonstrating the potential therapeutic utility of such an antagonist in the treatment of breast cancer [16].

These studies suggest that Pegvisomant, either alone, or in combination with other chemotherapy and endocrine-based therapies, maybe useful for the prevention and/or treatment of breast cancer. Also, of potential interest therapeutically, is the G120R GHR antagonist. G120R was an earlier stage of Pegvisomant development and contains a single engineered amino acid substitution in the hGH gene which impairs receptor binding site 2. In contrast to Pegvisomant, G120R can bind and inactivate both the GHR and PRL receptors [139] and may therefore be more effective through inhibition of both hGH and potential prolactin stimulated oncogenic effects.

Antagonists for GHRH have also been demonstrated to be effective in inhibiting the growth of various cancer cell lines in vitro and in reducing tumour growth in xenograft models [90, 140–143]. GHRH antagonism appears to work in part indirectly through reduction of GH secretion and thus circulating IGF-1 levels. However, in xenograft models of human mammary carcinoma the effects of GHRH antagonists appear to be through inhibition of tumoral GHRH activity [90].

Concluding remarks

Clinical data for the treatment of cancer clearly demonstrates the superiority of combinatorial therapy over a single agent approach. Current approaches for treatment of breast cancer include combinations of anti-estrogen, chemotherapeutic, radiotherapeutic, targeted therapy and anti-angiogenic strategies, and clinical and preclinical trials consistently demonstrate enhanced efficacy when chemotherapy or radiotherapy is combined with anti-angiogenic agents [144]. Autocrine hGH promotes mammary carcinoma cell growth and survival; migration and invasion; induces tumour angiogenesis; chemoresistance and radioresistance; protects against oxidative cell stress; and is sufficient to oncogenically transform the human mammary epithelial cell. Thus, functional antagonism of hGH mediated-signal transduction either alone or as an adjuvant therapy, is a promising approach for both the prevention and treatment of human mammary carcinoma.

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Abbreviations

AZA: 5′-Aza-2′-deoxycytidine
Bcl2: B-cell lymphoma protein 2
DNMT3A and 3B: DNA methyltransferases 3A and 3B
EMT: epitheliomesenchymal transition
eNOS: endothelial nitric oxide synthase
bFGF: basic fibroblast growth factor

GH: growth hormone
hGH: human growth hormone
GHR: growth hormone receptor
hGHBP: human growth hormone binding protein
HMEC: human mammary epithelial cell
HOXA1: Homeobox A1
IGF-1: insulin-like growth factor-1
IGF-1R: insulin-like growth factor-1 receptor
JAK-2: Janus kinase-2
LCR: locus control region
MAPK: mitogen activated protein kinase
MMP2 & 9: metalloproteases 2 & 9
PD: population doubling
PI3K: phosphoinositide 3-kinase
rhGH: recombinant human growth hormone
SDR: Spontaneous Dwarf rat
SS: somatostatin
SSTR2: SS receptor 2
TEB: terminal end bud
TFF1 & 3: Trefoil Factor 1 & 3
VEGF-A: Vascular endothelial growth factor A
VEGFR1: Vascular endothelial growth factor receptor 1
SDR: spontaneous dwarf rat
SOD: superoxide dismutase 1
SS: somatostatin
Tsp1: Thrombospondin 1
TERT: telomerase reverse transcriptase

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Figure 1

Endocrine, paracrine and autocrine effects of hGH in the mammary gland

The endocrine effects of hGH secreted from the anterior pituitary impact on numerous tissues resulting in postnatal somatic growth. One of the main effects of increased circulating hGH is the induction of hepatic IGF-1 secretion. Both hGH and IGF-1 are essential for mammary gland development. hGH in the pubertal mammary gland influences TEB formation and effects epithelial (orange) stromal and (blue) endothelial (red) cell characteristics through autocrine and paracrine effects.

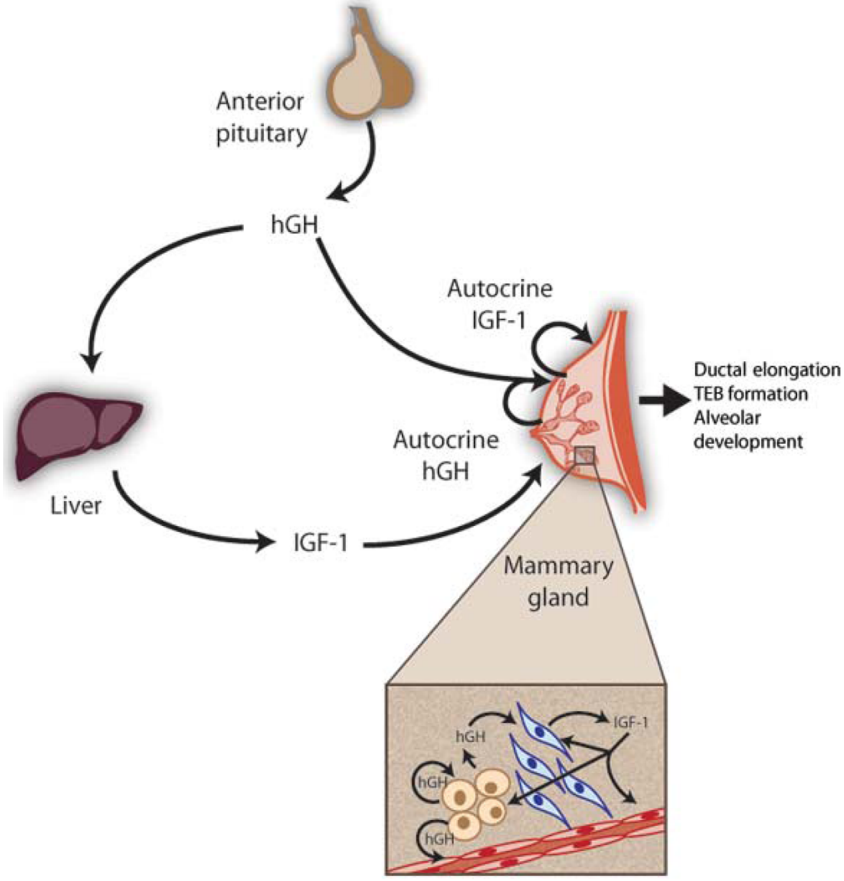

Figure 2

Autocrine hGH extends the replicative capacity of human mammary epithelial cells (HMECs) in vitro

Stable forced expression of hGH in human primary HMECs (Clonetics) was established (designated HMEC-hGH) while a control cell line was generated by stable transfection with vector alone (designated HMEC-VEC). a) The population doubling (PD) time of these two cell lines in mammary epithelial cell growth medium was calculated from PD27. b) HMEC colony formation in soft agar. HMEC-hGH and - VEC cells were embedded in 0.35% agarose as previously described [19] and allowed to form colonies over 14 days culture in mammary epithelial cell growth medium. HMEC-VEC cells did not form colonies in soft agar.

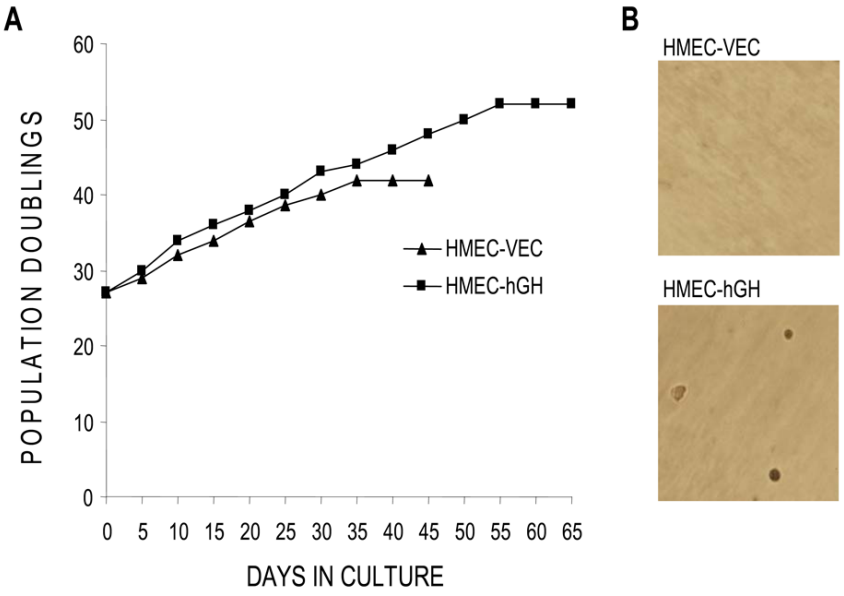

Figure 3

The role of autocrine hGH in mammary neoplasia

Autocrine hGH influences cell growth and survival, migration and invasion, epitheliomesenchymal transition (EMT), replicative potential and oncogenic transformation through differential regulation of gene expression. Genes known to be upregulated or downregulated by autocrine hGH implicated in oncogenesis are listed.