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Review

Tumour-Derived Human Growth Hormone As a Therapeutic Target in Oncology

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The growth hormone (GH) and insulin-like growth factor-1 (IGF1) axis is the key regulator of longitudinal growth, promoting postnatal bone and muscle growth. The available data suggest that GH expression by tumour cells is associated with the aetiology and progression of various cancers such as endometrial, breast, liver, prostate, and colon cancer. Accordingly there has been increased interest in targeting GH-mediated signal transduction in a therapeutic setting. Because GH has endocrine, autocrine, and paracrine actions, therapeutic strategies will need to take into account systemic and local functions. Activation of related hormone receptors and crosstalk with other signalling pathways are also key considerations.

Introduction

GH is a classical pituitary hormone, with endocrine functions and additional autocrine and paracrine actions. GH is secreted from the anterior pituitary in a pulsatile fashion, while a variant placental GH is secreted tonically from the placenta during pregnancy. In addition to essential and well-described roles in growth and metabolism, the GH and IGF1 axis has wide-reaching functions in numerous physiological systems, for example by promoting lipolysis, neurogenesis, folliculogenesis, and increasing muscle mass by promoting myoblast fusion [1,2].

Increasing evidence in animals and humans also supports a role for GH in the aetiology and progression of cancer. The evidence points not only to the involvement of systemic GH but also to autocrine/paracrine effects through local tumour expression. The purpose of this review is to highlight recent advances and challenges in the field with specific reference to these autocrine and paracrine functions. Owing to space considerations we have focused on the recent literature, and reviews have been cited instead of original works in many instances. We refer readers to reviews for further background reading regarding the actions of GH and IGF1 in cancer [1,3–9].

Epidemiology and Cancer Risk Factors

The somatotrophic (GH and IGF1) axis is the key regulator of longitudinal growth, and promotes postnatal bone and muscle growth. Interestingly, a clear and consistent association between adult height and cancer risk has been observed across numerous cancer sites in both large cohort and ecological studies [10–13]. The association is stronger for particular cancer sites, such as melanoma, colon, and breast cancer, while for others the relative risk is small. In a recent meta-analysis of data from more than five million women it was estimated that a 10 cm

Trends

The evidence indicates that GH exerts not only endocrine effects but also autocrine/paracrine effects through local tumour expression.

Expression of GH by tumour cells is associated with reduced survival and local metastasis in patients with breast, endometrial, and liver cancer.

Autocrine GH enhances multiple aspects of cancer progression and promotes a cancer stem cell-like phenotype.

Antagonism of GH signal transduction reduces tumour growth and sensitises to radiotherapy in preclinical studies.

Therapeutic use of GH antagonism in cancer need not be restricted to GH-positive tumours because GH-negative cancer cells may be more sensitive to systemic GH stimulation.

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increase in height was associated with a 17% increased risk of hormone-dependent breast cancer [14]. For some cancers, adult height may be causally related to risk and not merely represent a surrogate marker of environmental and lifestyle risk factors; Mendelian randomisation studies using genetic determinants of height indicate a potential causal role in breast, colorectal, and lung cancer [14–16], but not in prostate cancer. For breast cancer, an increase in genetically predicted height of 10 cm was associated with a 21–22% increased risk of breast cancer [14]. This suggests that particular genetic factors and biological pathways which impact on adult height have an important role in the aetiology of specific cancers.

The GH/IGF1 axis is certainly important for longitudinal growth, but height variation in humans is a complex trait with multiple genetic and environmental determinants. Hundreds of genetic variants across 180 loci have been linked to human height through genome-wide association studies (GWAS), and this contributes to ~10% of the phenotypic variation in height [17]. Furthermore, many genes associated with adult height are also associated with cancer [18]. While it is unclear how much the GH/IGF axis contributes to the association between height and cancer risk, components of the GH/IGF1 pathway are generally well represented in these studies. Further GWAS studies have identified the GH signal transduction pathway as one of three key signalling pathways that are highly associated with breast cancer susceptibility from a total of 421 pathways investigated [19].

If increased systemic GH contributes to cancer progression, one would expect increased cancer incidence in conditions associated with elevated serum GH. Acromegaly is a rare debilitating disorder typically associated with chronic hypersecretion of GH from a benign pituitary adenoma and is characterized by elevated IGF1 levels. The disease is associated with a higher mortality rate, mainly attributed to cardiovascular and respiratory disease [20]. The risk of malignant neoplasms in acromegalic patients remains a topic of debate [21–23]. Some studies report an increased risk of thyroid and colon cancer in patients with acromegaly. This is consistent with studies in transgenic animals, or studies involving systemic administration of GH, which have reported an increased incidence of hyperplasia and cancer [9,24]. However, other studies have observed limited or no association between acromegaly and cancer risk [21,25,26]. However, this may not be a clear representation because in some large-population studies acromegalic patients with pre-existing comorbidities were excluded, including patients with a previous diagnosis of cancer. Furthermore, as discussed, there are many issues and limitations in terms of quantifying the risk of cancer in patients harbouring a rare disease such as acromegaly, and with improvements in management the risk may be reduced in patients with controlled disease and reduced levels of GH and IGF1 [21,23]. Further prospective studies in this area may help to clarify the association. By comparison, the evidence for other neoplasms in acromegaly is weak. However, in their case–control study, Wolinski *et al*. found an increased risk of breast cancer in acromegalics which may warrant further attention [23].

Conversely, humans born with deficient GH receptor (GHR)-mediated signal transduction, and animals with reduced circulating GH or a GHR deletion, have a reduced risk of developing cancer [9,27–29]. Laron syndrome is a rare genetic condition resulting from an inactivating mutation in the GHR. Individuals with Laron syndrome have a markedly reduced risk of developing cancer compared with unaffected relatives who develop cancer at rates similar to those in the general population, albeit with the caveat that the average age of the Laron syndrome cohort was younger than the control group [27,28].

Clinical Correlations

A small number of studies have found that elevated circulating GH correlates with an increased risk of breast, gastric, colon, or lung cancer [4,30–33]. Owing to the pulsatile nature of pituitary GH secretion, studies investigating serum GH concentrations in cancer can be problematic.

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One source of elevated serum GH may in fact arise from GH secretion from tumour cells – studies of canine mammary tumours have demonstrated a strong link between high serum and intratumoural GH and IGF1 concentrations [34]. Local/autocrine GH and GHR expression has also been detected in a wide range of different human cancers, including breast, colon, liver, prostate, endometrial, and lung cancer (reviewed in [5,9,35]). More recent studies have identified potential roles for GH and the GHR in melanoma, glioma, craniopharyngioma, hepatocellular, gastric, pancreatic, and triple-negative breast cancer [36–43]. Metastatic melanoma cell lines express relatively high levels of *GHR* mRNA, indicating the GHR signal transduction may support the growth this type of cancer [38].

Polymorphisms in GH/IGF axis genes are associated with changes in circulating serum IGF1 and GH concentrations, and are also associated with the risk of developing osteosarcoma, breast, or colon cancer [44–48]. However, the outcomes of these studies have not been straightforward. A good example is the T1663A polymorphism located within intron 4 of the growth hormone 1 (*GH1*) gene. Two studies reported that the AA genotype was associated with a decreased risk of colorectal cancer [45,49], while a third study found that the TT genotype was associated with reduced risk of colorectal adenomas and hyperplastic polyps [48]. A fourth study found no association with genotype and colon cancer risk. However, an interaction with exercise was observed; only individuals with the A allele who did not exercise had a significantly reduced colorectal cancer [50]. Other studies suggest that body mass index (BMI), physical activity, and ethnicity may modify the association between this *GH1* polymorphism and risk of breast or colorectal cancer [45,46,50,51].

With respect to target validation, one area which requires further research is the extent to which tumour GH expression correlates with clinical outcome. Low levels of GH expression in tumour cells can have a profound effect on phenotype, but low expression in tumours can be difficult to detect by immunohistochemistry or *in situ* hybridisation . Difficulties in detection are compounded by the fact that GH is a secreted protein. In addition, GH family members are encoded by a cluster of five highly related genes located on chromosome 17q23.3 (*GH1*, *GH2*, *CSH1*, *CSH2*, and *CSHL1*) which share >90% sequence identity, and furthermore share substantial sequence similarity with prolactin (PRL). As a result, discrimination between these genes and their proteins is problematic because of the high degree of sequence homology. However, more recent studies are beginning to address this. Wu *et al*. [52] observed *GH* mRNA and protein expression in over half of 159 breast carcinoma and 70 endometrial carcinoma samples. GH expression was associated with HER2 positivity and lymph node metastasis in breast cancer, and with myometrial invasion in endometrial cancer, and predicted a significant difference in survival outcome for patients with these tumours [52]. More recently, increased mRNA and protein expression of GH and the evolutionarily related hormone, PRL, has been observed in 148 hepatocellular carcinoma samples relative to non-neoplastic liver tissues [41]. GH expression was significantly associated with tumour size and tumour grade, and both GH and PRL were associated with worse relapse-free and overall survival in patients. Genomic amplification of both the *GH* and *PRL* genes was also observed in hepatocellular carcinoma samples [41]. However, further studies in larger cohorts will be necessary to confirm clinicopathological associations and prognostic significance of GH. GH1-specific ELISAs are now commercially available, and we have recently described a GH2-specific ELISA assay which does not cross-react with GH1, PRL, CSH1, or CSH2 [53]. As further antibodies specific for GH1, GH2 and CSH1/2 are utilised, determining clinical correlations with specific family members will become more practical.

Autocrine, Paracrine, and Systemic Functions in Cancer

GH acts in an endocrine and autocrine/paracrine fashion, impacting on both cancer cells and the tumour microenvironment, and contributes to multiple aspects of cancer progression

(Figure 1). In addition to secretion from tumour cells, GH is also secreted from cells in the microenvironment such as endothelial cells [54]. Functional effects of tumour-expressed GH include oncogenic transformation, cell proliferation, cell survival, epithelial to mesenchymal transition, cell migration, and invasion of melanoma, breast, liver, and endometrial cancer cells [24,38,41,55–59]. Autocrine or exogenously added GH also enhances angiogenic and lymphangiogenic endothelial cell characteristics such as cell migration or tube formation and sprouting on matrigel [54,60]. Compared to exogenously added GH, autocrine expression of GH from tumour cells promotes a more aggressive cellular phenotype; autocrine GH is sufficient to promote oncogenic transformation in immortalised mammary epithelial cells, whereas GH administration mimicking endocrine secretion is not [24,57].

In vivo, autocrine or administered GH increases tumour growth, tumour angiogenesis and vascularisation, and promotes metastatic dissemination of tumour cells [39,40,42,43,54,61,41] (Figure 1). Expression of GH by tumour cells is also associated with resistance to particular chemotherapeutic drugs [62–65] and to radiation *in vitro* and *in vivo*

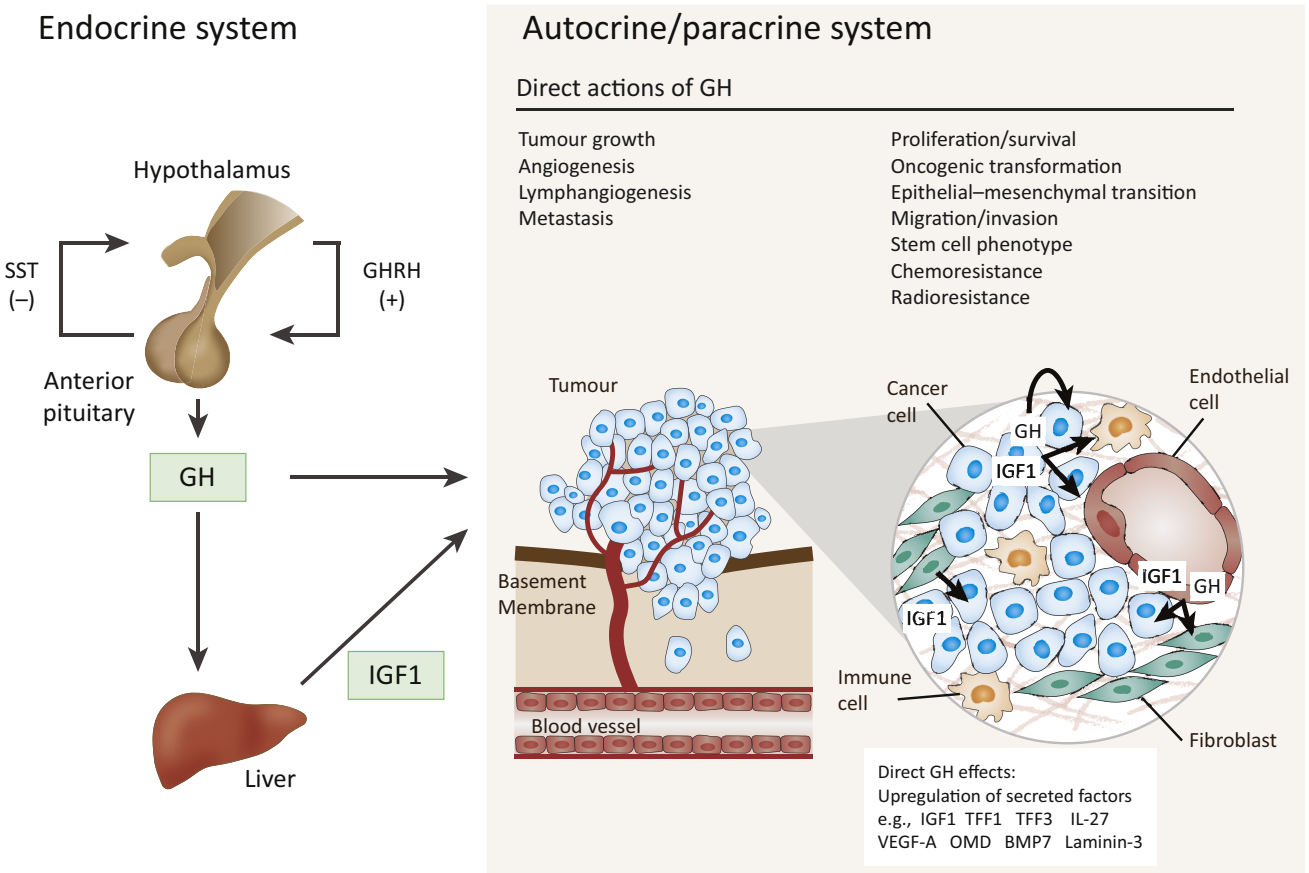

Figure 1. Evidence Supports the Effects of Systemic/Endocrine Growth Hormone (GH), and Also Autocrine/Paracrine Effects on Cancer Cells, through Localised Tumour Expression. In the endocrine system, GH is secreted in a pulsatile fashion from the somatotroph cells of the anterior pituitary. GH release is positively regulated by the secretion of GH releasing hormone (GHRH) and negatively regulated by somatostatin (SST), which are released from the hypothalamus. Additional regulation is provided by a feedback mechanism (not shown). GH stimulates the secondary secretion of insulin-like growth factor 1 (IGF1) from the liver. GH and IGF1 are also secreted by tumour cells (blue cells), as well as by endothelial (red) and potentially immune cells (yellow) and fibroblasts (green), and act locally in an autocrine/paracrine manner to promote the secretion of soluble growth factors such as IGF1, trefoil factor 1 (TFF1), trefoil factor 3 (TFF3), interleukin 27 (IL-27), vascular endothelial growth factor-A (VEGF-A), osteomodulin (OMD), bone morphogenetic protein 7 (BMP7), and laminin-3. Endothelial cells also secrete GH and IGF1 while fibroblasts secrete IGF1.

[66,67]. However, in some cases autocrine expression of GH can apparently enhance the sensitivity of cancer cells to treatments such as arsenic trioxide [68]. GH (both exogenous and autocrine) mediates these actions through coordinated changes in the transcription of genes, microRNAs, and long non-coding RNAs [5,24,69–72]. Changes in transcriptional profiles are mediated through activation of GHR or PRL receptor signal transduction, heterodimerisation of the GHR and PRL receptors, and potentially through transport of the GHR to the nucleus [9,73–76]. Interestingly, expression profiling demonstrates that autocrine and exogenous GH differentially regulate gene expression in breast cancer cells, which is consistent with the differential effects of these two modes of GH delivery discussed above [71]. This may relate to the autocrine activation of oncogenic signalling pathways by GH as opposed to transient activation of the pathway by exogenous GH.

Adding to the wide range of effects in cancer cells, recent studies indicate an autocrine role of GH in regulating Wingless and Int-related protein (WNT) signalling [77], and in promoting a cancer stem cell-like phenotype [40]. GH has been implicated in the activation, differentiation, and maintenance of neural and chondrocyte stem cells [1]. Mammary stem/progenitor cells grown as mammospheres express significantly higher levels of the GHR before differentiation down an epithelial lineage [78]. The GHR is also enriched in hematopoietic stem cells, neurospheres, and embryonic stem cells [78]. In a recent study, GH was shown to be secreted by normal mammary epithelial cells following progesterone stimulation, and to stimulate the proliferation of breast stem and early progenitor cells [79] (Figure 2). This study also identified a subset of GHR-positive mammary stem/progenitor cells in normal human breast tissue with self-renewal properties and the capacity to differentiate into multiple lineages *in vitro* [79]. Chen *et al*. have also demonstrated that autocrine GH is sufficient to promote a cancer stem cell-like phenotype of estrogen receptor-negative mammary carcinoma cells and to promote their tumour-initiating capacity and metastasis *in vivo* [40] (Figure 2).

As described above, GH has also been implicated in colon cancer. In an elegant study, Chesnokova *et al.* were able to demonstrate, by crossing GH-deficient *Prop1*$^{-/-}$ mice with a strain of mouse which normally develops intestinal and colon tumours, that the GH-deficient double-mutant mice had a significantly decreased tumour number and size [80]. Increased expression of p53 was observed in the colon of these mice. Although *Prop1*$^{-/-}$ mice are devoid of pituitary GH expression, they also have dramatically reduced *GH* mRNA expression in colon tissue, and it is therefore difficult to determine whether this effect can be attributed to reduced pituitary or local GH expression. In colon cancer cells and intestinal organoids derived from induced human pluripotent stem cells, GH suppressed the expression of p53 and p21. Furthermore, administration of a GHR antagonist to acromegaly patients increased p53 and adenomatous polyposis coli (APC) expression in the colon [80], while increased p53 was observed in skin fibroblasts derived from individuals with Laron syndrome. This suggests that GH may act within the tumour microenvironment in colon cancer to reduce tumour-suppressor proteins and sustain neoplastic growth [80].

Targeting Different Facets of GH Secretion – Therapeutic Considerations

With an increasing number of studies implicating GH signalling in cancer, there has been a great deal of interest in antagonising this axis for therapeutic purposes. In an oncology setting, antagonism of GH signalling would be expected to have the added benefit of suppressing IGF1-mediated effects on cancer progression.

The GHR is a challenging therapeutic target because it has no intrinsic kinase activity and relies on the recruitment of non-receptor tyrosine kinases for activity. A detailed mechanism of activation of this receptor has recently been described [81–83]. The only clinically available GHR antagonist is pegvisomant, which was discovered by the laboratory of John Kopchick over 20

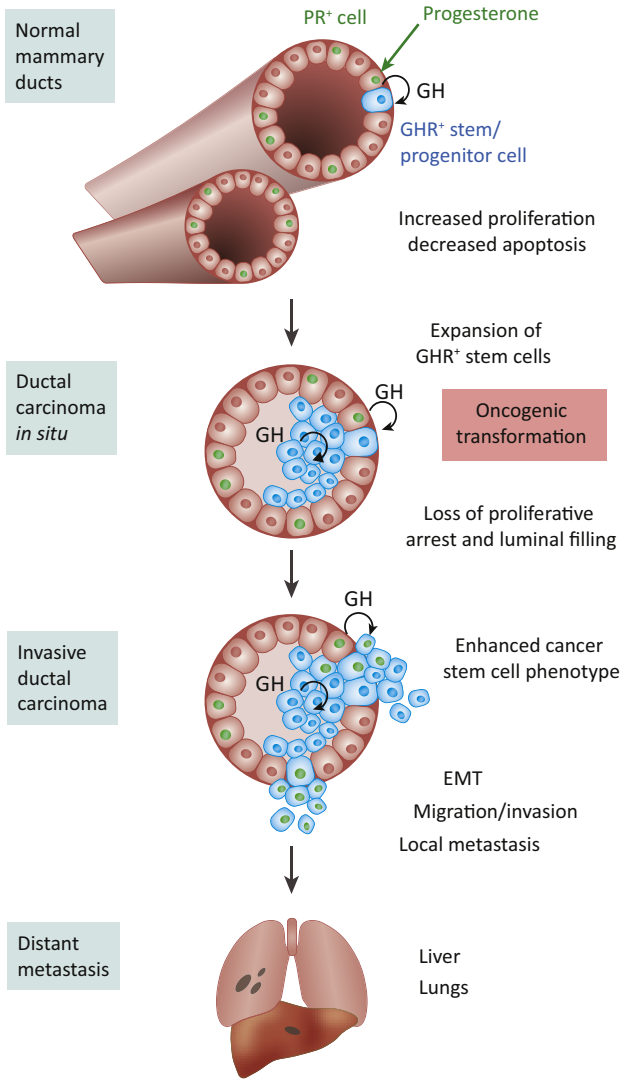

Figure 2. Growth Hormone (GH) Increases Mammary Progenitor/Stem Cell Proliferation, Enhances Oncogenic Transformation, and Promotes a Cancer Stem Cell-Like Phenotype and Distant Metastasis [79]. Oncogenic transformation and loss of proliferative arrest leads to luminal filling [57]. Increased tumour expression of GH regulates signal transduction and promotes a transcriptional profile consistent with epithelial to mesenchymal transition (EMT) and increased mammary carcinoma cell migration and invasion [55]. Autocrine GH also enhances a cancer stem cell phenotype, leading to liver and lung metastases in xenograft studies [40].

years ago [84,85], and subsequently developed by Pfizer Inc. The basis for pegvisomant is a mutated GH molecule; introduction of a single mutation in binding site 2 (G120K) completely disrupts the ability of GH to activate the GHR. Additional mutations at binding site 1 increase the affinity for the receptor, while pegylation increases serum half-life of the drug to 72 h [84,85]. Pegvisomant is a highly efficient blocker of the GHR, effectively normalising circulating IGF1 in >90% of patients with acromegaly, and is safe and well tolerated [86–88]. However, there are some drawbacks, including the high cost of manufacture and the fact that the formulation (Somavert) is self-administered daily by subcutaneous injection, and regular rotation of injection sites is necessary to prevent injection-site reactions [86].

A key hinderance in preclinical development relates to species specificity; although both human GH and the protein component of pegvisomant (B2036) can bind to the mouse GHR, pegylation reduces binding affinity significantly for the mouse receptor. As a result, exogenous administration of the pegylated antagonist to rodents at human dose equivalents has little effect, and much higher concentrations are necessary to reduce serum IGF1 [84]. A slight reduction in binding affinity on pegylation is also seen in the case of the human receptor, but not to the same extent. In addition, because rodent GH is incapable of activating the human GHR, such models do not assess the growth response of human xenografts to systemic GH – and consequently do not assess the effects of antagonising endocrine GH on human tumour cell growth. Given these limitations with animal models, it is possible that GHR antagonism with pegvisomant may be more beneficial in a human clinical setting given both the increased affinity of the antagonist for the receptor and efficient functional antagonism of systemic GH/IGF1 as well as of autocrine/paracrine GH [67].

Although pegvisomant is the only clinically available inhibitor, this landscape is likely to change shortly because others are in clinical trials or preclinical development. Other inhibitory strategies include ATL1103, an antisense therapeutic directed at GHR (Antisense Therapeutics Ltd) [89]; a translational fusion of mutated GH linked to GH binding protein which avoids the need for modifications such as pegylation to extend half-life [90]; and a neutralising GHR antibody [91,92]. Release of GH from the anterior pituitary can also be blocked by antagonists of the GH releasing hormone (GHRH) receptor or somatostatin agonists [24,93]. GHRH receptor antagonists have therapeutic benefit in cancer, not only because of endocrine suppression of GH and IGF1 but also though direct inhibition of autocrine GHRH and hence GH, as well as through inhibition of localised IGF1 or IGF2 secretion from tumours [93].

In vitro studies demonstrate efficacy of GHR antagonism or knockdown in established and primary cancer cell lines. GHR antagonism with B2036 reduces the proliferation of primary human mammary carcinoma cells which secrete GH [94], mammosphere formation by estrogen receptor-negative breast cancer cell line cell lines expressing GH [40], and human microvascular endothelial cell proliferation and tube formation [54]. B2036 also inhibits endometrial cancer cell proliferation and enhances radiation sensitivity [62]. Recent studies have also demonstrated a role for the GHR in pancreatic cancer cells. GHR knockdown with siRNA effectively reduced anchorage-independent growth, *in vitro* cell migration/invasion, and GHR-mediated signal transduction in the pancreatic cell lines PANC1 and HPAC [43]. Demonstrated efficacy for GHR knockdown has also been observed in melanoma cells, in part through suppression of autocrine GH-mediated effects [59].

Only a handful of preclinical studies have used pegvisomant in a preclinical oncology setting. Promising growth-inhibitory effects have been observed in tumour models such as colon, breast, and meningioma xenografts where pegvisomant was used as a monotherapy. As might be expected, not all tumour models responded to pegvisomant treatment (reviewed in [9,67,95]). Intriguingly, combining pegvisomant and ionising radiation delays the regrowth of tumours in an RL95-2 endometrial cell xenograft model, despite pegvisomant being ineffective as a single agent in this particular cancer model. In addition, reduced vascularisation was observed for non-irradiated tumours treated with pegvisomant, suggesting inhibition of tumour angiogenesis [67]. The mechanism contributing to delayed regrowth is unclear, but it will be interesting to see whether combined GHR antagonism and ionising radiation is more effective in cancer models which respond to pegvisomant as a single agent [67].

Other recent studies have used alternative strategies such as siRNA knockdown of the GHR. Exogenous GH increases tumour growth in immunodeficient mice inoculated with the human colon carcinoma cell line SW480, whereas subcutaneous or intraperitoneal delivery of an

expression plasmid containing a *GHR* siRNA expression vector significantly reduced tumour growth and liver metastases [61,96]. Furthermore, forced expression of hGH-G120R, a peptide inhibitor which blocks GHR and PRL receptor signalling reduces liver cancer cell proliferation and anchorage-independent growth *in vitro*, and tumour growth *in vivo* [41].

With increasing interest in this area there will be a need for careful characterisation of the cancer cell lines used in preclinical studies if useful data regarding the efficacy of inhibition are to be obtained, particularly with reference to the expression of relevant receptors, autocrine growth factors, and the signal transduction pathways utilised. For example, human GH can activate the PRL receptor in humans and other mammals. The GHR can heterodimerise with the PRL receptor, and this may impact on the effectiveness of GHR antagonism [75,76]. Furthermore, the GHR interacts with the IGF1 receptor [97], and crosstalks with the epidermal growth factor receptor [98,99]. There are also multiple GHR isoforms, including three truncated isoforms, and, depending on expression levels, these may act as dominant negative inhibitors of signal transduction in some cell lines [100]. Furthermore, proteolytic digestion fragments derived from GH have been reported, although their function is unclear. Different combinations of relevant receptors and isoforms may explain the variation in GH signalling which occurs in different cell lines, and this will require careful consideration.

One of the major issues in this field is that many standard carcinoma cell lines do not express GH when cultured, despite the fact that clinical studies indicate that GH expression is widespread in malignant tumour cells [41,52,94]. For example, in a study by Chiesa *et al.*, 52% of 27 primary human mammary carcinoma cell cultures studied expressed GH, and all expressed GHR [94]. However, GH-negative primary mammary carcinoma cell cultures appeared to be more sensitive to exogenous GH stimulation than GH-positive cultures [94].

Concluding Remarks and Future Perspectives

Recent clinical studies have gone a long way towards supporting target validation, and provide a framework for expanding clinical correlation into larger cohorts. However, despite substantial evidence supporting a causal role for the GH/IGF1 axis in cancer, the potential utility of GH antagonism in cancer therapy is still unclear and further preclinical studies are warranted. Therapeutic use of GH antagonism in cancer need not be restricted to GH-positive tumours, given that GH-negative cancer cells may be more sensitive to exogenous GH stimulation [94]. However, careful characterisation of the cancer cell lines used in preclinical studies will be required. The GHR antagonist pegvisomant is clinically available, and thus a positive outcome in preclinical studies has the potential for rapid translation into the clinic. Other antagonists are in development and are showing promise in preclinical and clinical trials.

Outstanding Questions

Despite the growing body of evidence implicating GH in cancer, preclinical studies investigating the therapeutic potential of GHR antagonism for the purposes of treating cancer are limited. Promising growth-inhibitory effects have been observed in tumour xenograft models such as colon, breast, and meningioma using the GHR antagonist pegvisomant, but further preclinical studies are required.

Will delineation of signal transduction pathways predict which tumours are more likely to respond to GHR antagonism? Human GH interacts with GH and PRL receptor homo- and heterodimers, and activates multiple signal transduction pathways.

Although acromegaly has been associated with an increased risk of thyroid and colon cancer, the link to cancer risk and mortality is remains controversial.

Disclaimer Statement

PEL is an inventor on patent application US2010-0203060A1. TZ and PEL have consulted for Perseis Therapeutics Ltd. TZ and PEL consult for and have equity interest in Wuhan Long Ke Ltd. TZ and PEL are inventors on Chinese patent 20130446539.5.

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