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Review

Growth Hormone Action as a Target in Cancer: Significance, Mechanisms, and Possible Therapies

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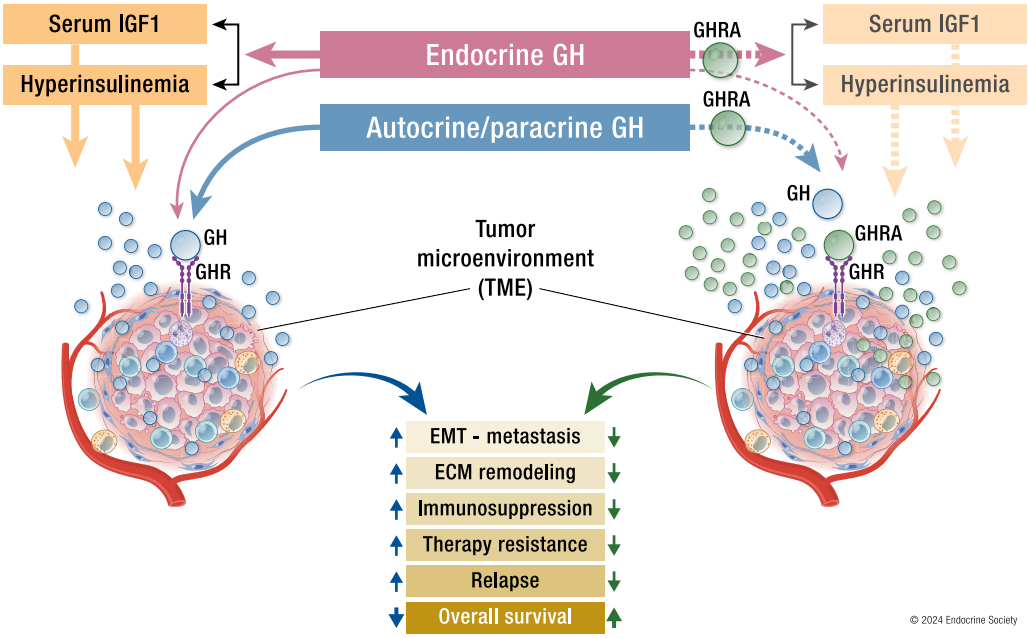
Abstract

Growth hormone (GH) is a pituitary-derived endocrine hormone required for normal postnatal growth and development. Hypo- or hypersecretion of endocrine GH results in 2 pathologic conditions, namely GH deficiency (GHD) and acromegaly. Additionally, GH is also produced in nonpituitary and tumoral tissues, where it acts rather as a cellular growth factor with an autocrine/paracrine mode of action. An increasingly persuasive and large body of evidence over the last 70 years concurs that GH action is implicit in escalating several cancer-associated events, locally and systemically. This pleiotropy of GH's effects is puzzling, but the association with cancer risk automatically raises a concern for patients with acromegaly and for individuals treated with GH. By careful assessment of the available knowledge on the fundamental concepts of cancer, suggestions from epidemiological and clinical studies, and the evidence from specific reports, in this review we aimed to help clarify the distinction of endocrine vs autocrine/paracrine GH in promoting cancer and to reconcile the discrepancies between experimental and clinical data. Along this discourse, we critically weigh the targetability of GH action in cancer—first by detailing the molecular mechanisms which posit GH as a critical node in tumor circuitry; and second, by enumerating the currently available therapeutic options targeting GH action. On the basis of our discussion, we infer that a targeted intervention on GH action in the appropriate patient population can benefit a sizable subset of current cancer prognoses.

Graphical Abstract



Key Words: growth hormone (GH), growth hormone receptor (GHR), cancer, tumor microenvironment, insulin-like growth factor 1 (IGF1)

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Abbreviations: ABC, ATP-binding cassette containing; AT, adipose tissue; ATM, ataxia telangiectasia mutated; BC, breast cancer; CNS, central nervous system; COX, cyclooxygenase; CRC, colorectal carcinoma; CSC, cancer stem cell; DDR, DNA damage repair; DMBA, dimethylbenzanthracene; DTC, differentiated thyroid carcinoma; EC, endometrial cancer; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ER, estrogen receptor; FFA, free fatty acids; FMD, fasting mimicking diet; GBM, glioblastoma; GC, gastric cancer; GH, growth hormone; GHA mice, mice transgenic for growth hormone receptor antagonist; GHD, growth hormone deficiency; GHR, growth hormone receptor; GHRA, growth hormone receptor antagonist; GHRH, growth hormone releasing hormone; GHRHR, growth hormone releasing hormone receptor; HCC, hepatocellular carcinoma; IGF1, insulin-like growth factor 1; IGF1R, insulin-like growth factor 1 receptor; IGFBP, insulin-like growth factor binding protein; LS, Laron syndrome; mAb, monoclonal antibody; MDSC, myeloid-derived suppressor cell; MMC, mitomycin C; MNU, N-methyl-N-nitrosourea; MSC, mesenchymal stem cell; OS, overall survival; PC, prostate cancer; PD1, programmed cell death protein 1; PDL1, programmed cell death ligand 1; PLCγ, phospholipase C-gamma; PR, progesterone receptor; PRL, prolactin; PRLR, prolactin receptor; RFS, relapse-free survival; rhGH, recombinant human growth hormone; SDR, Sprague Dawley rats; SIR, standardized incidence ratio; SNP, single nucleotide polymorphism; SOCS, suppressor of cytokine signaling; SST, somatostatin; SSTR, somatostatin receptor; SVF, stromal vascular fraction; T2DM, type 2 diabetes mellitus; TCGA, the Cancer Genome Atlas; TGFβ, transforming growth factor β; TME, tumor microenvironment; TNBC, triple-negative breast cancer; Treg, regulatory T cells; VEGFA, vascular endothelial growth factor A.

ESSENTIAL POINTS

- Hundreds of studies across > 20 different cancer types over the last 70 years have amassed a persuasive body of evidence implicating GH and cancer
- Autocrine/paracrine, rather than endocrine, GH action promotes age-associated cancer development (*does this reconcile incongruity between empirical and clinical data?*)
- GH signaling **directly** orchestrates several hallmark tumor-supportive mechanisms at/in the tumor microenvironment
- Systemically blocking GH action can also have **indirect** benefits via reduction of IGF1 and improving insulin sensitivity
- GHR antagonism is an effective and highly feasible approach to enhance the efficacy of multiple types of anticancer therapies

Growth hormone (GH) is a 191-amino-acid polypeptide hormone, which was first identified by Evans and colleagues in 1921 in bovine pituitary extracts as a growth-promoting factor when administered to rats (1) and was subsequently purified in 1944 from oxen anterior pituitary lobe (2). Prior to that, the term "*hormone of growth*" was preemptively coined by the American neurosurgeon Harvey Cushing in 1909, annotating an unknown growth-regulating pituitary-derived factor that could underlie pituitary dysfunctions, leading to growth deficits, that had been reported since the late nineteenth century (3, 4). Treatment with cadaveric extracts of human GH started in the early 1950s but because of safety and scalability issues, it was replaced completely by recombinant human GH after the first drug—Protropin (somatrem for injection), a recombinant human GH grown in *Escherichia coli*—was approved in 1985 by the United States Food and Drug Administration (US FDA) for the treatment of GH deficiency (GHD) in children (5). Almost 30 years since their identification of GH, it was Evans and colleagues in the early 1950s who presented the first association of GH and cancer through reports describing occurrence of spontaneous neoplasms in multiple tissues of GH-sufficient normal rats following treatment with pituitary-derived GH (6-8). Since then, over the last 70 years, a large amount of scientific interest and effort have revealed significant details and rekindled pharmaceutical interest in the role of GH in cancer, as well as some concern around the use of GH as a treatment in clinical GHD.

Cancer initiation is preceded by progressive accumulation of mutational changes in the genome (modulated by intrinsic and extrinsic factors like lifestyle and exposure to carcinogens) which turns causal when one or more proto-oncogenes or tumor suppressor genes are mutated (*driver mutation*) to elicit cellular transformation. Classically, cellular growth factors come into play to impart tumoral growth factor autonomy and promote rapid clonal expansion of the transformed cell into a colony of cells, then to a heterogenous self-sufficient tumor mass along with rapid disease progression (9). However, growth factors can also participate in oncogenesis by upregulating the rate of progressive accumulation of mutations and downregulating mutational repair pathways, thus causing genomic instability. GH secreted from the anterior pituitary and distributed via blood throughout the body to exert endocrine effects, functions as a *classical hormone*. In contrast, GH produced in nonpituitary and tumoral tissues is not distributed systemically through blood but rather used in and around the site of origin (autocrine/paracrine effects), acting functionally as a *cellular growth factor* rather than a classical endocrine hormone. As we discuss throughout this review and shown in Fig. 1, this distinction is vital, since the effect of ***GH as a cellular growth factor (autocrine/paracrine action)*** in cancer is more relevant than that of ***GH as a hormone (endocrine action)***, especially in vivo.

Pituitary/Central/Endocrine GH

Mature 22-kDa human GH is encoded by the *GH1* gene, with anterior pituitary gland as the central site of GH production. Pituitary GH, distributed throughout the body via circulation, acts as a classical endocrine hormone exerting its action in tissues which express its cognate receptor—the GH receptor (GHR) (10)). GH secretion by the somatotroph cells of the anterior pituitary gland is under the positive regulatory effects of hypothalamic GH-releasing hormone (GHRH, binds to GHRH receptor GHRHR) and gastric ghrelin (binds to GH secretagogue receptor GHSR), and the negative regulatory effects of hypothalamic somatostatin (SST, binds to SST receptors SSTRs), the latter setting the pulsatile pattern of pituitary GH secretion. Additional regulators of GH production at the hypothalamic and pituitary levels include negative feedback inhibition by GH (an autocrine/paracrine effect via GHR expressed in the pituitary and hypothalamus (11)) and its downstream effector, insulin-like growth factor 1 (IGF1), and multiple other physiologic and lifestyle factors (12). Endocrine GH largely directs postnatal cellular differentiation and proliferation of bone, cartilage, and muscle, which

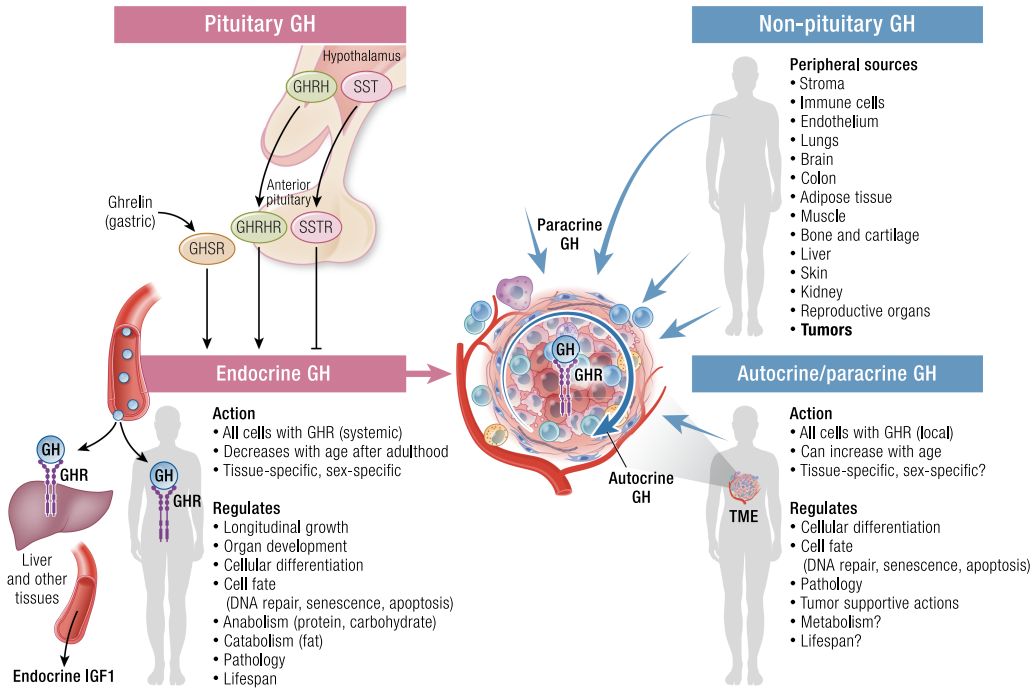

Figure 1. Pituitary (endocrine) and nonpituitary (autocrine/paracrine) GH and cancer: GH produced from the pituitary (induced by hypothalamic GH-releasing hormone [GHRH] and gastric GHRL and suppressed by somatostatin [SST]) acts as an endocrine hormone and affects all cells that express functional GHR exerting listed functions, the major one being inducing most of the circulating IGF1 from the liver (and some other organs). This pituitary/endocrine GH has several known tissue-specific and sex-specific effects and generally decreases with age after adulthood (so called *somatopause*), questioning the importance of its direct effect on a predominantly age-associated disease like cancer. On the other hand, several nonpituitary sites (listed) also secrete GH which does not contribute to circulating levels and has peripheral autocrine/paracrine actions on the tissue of origin, as a local growth factor. This nonpituitary/autocrine/paracrine GH has multiple tissue-specific effects and known effects in pathology, such as in cancer, and can increase with age in some tissues. Therefore, at the tumor microenvironment (TME) in an adult, GH action promoting cancer constitutes primarily the autocrine/paracrine effect of nonpituitary GH and possibly some endocrine effects of the pituitary GH.

establishes normal longitudinal growth. Moreover, GHR has highest expression at the liver hepatocytes, where GH is the principal inducer (>75%) of circulating IGF1, which in turn amplifies and renders several growth-promoting effects of GH. Importantly GH and IGF1 present several mutually exclusive and overlapping physiologic effects in a tissue-specific manner, including in cancer (13, 14). The GHR is also differentially expressed in several other cell types, including adipose tissue (AT), immune system, brain, kidney, bladder, spleen, thymus, stomach, intestines, pancreas, lungs, heart, skin, and the reproductive organs, where endocrine GH helps regulate normal organ development and maintenance of function in a tissue and sex-specific manner. Endocrine GH also plays a distinct role in whole body metabolic homeostasis, by exerting a catabolic effect on lipids (fat breakdown to release free fatty acids and glycerol) and anabolic effects on carbohydrate (increases glucose production via glycogenolysis and gluconeogenesis) and proteins (increases protein production and decreases protein turnover). Pituitary GH production peaks shortly after puberty when adulthood and sexual maturity is reached, and thereafter steadily decreases with age to low levels, accompanied by a concomitant decrease in serum IGF1—a seemingly protective phenomenon collectively described as *somatopause*—often misinterpreted as a condition to be treated with GH replacement therapy due to some phenotypic overlaps with GHD (15). The phenomenon of somatopause itself argues against endocrine GH's role in promoting cancer, as > 90% of cancer diagnosis is in the age group of > 50 years and less than 2% of the cancer deaths are in the age group of < 40 years (16-18).

Extrapituitary/Nonpituitary/Peripheral/Autocrine/Paracrine GH

In 1980, Sporn and Todaro introduced the concept of "autocrine" growth factors in addition to the previously known concepts of endocrine and paracrine factors (19). They used the term to define the polypeptides that are produced and utilized by cells during malignant transformation, and which enable uncoupling of a normal cell from the homeostatic growth signaling program by imparting autonomy in growth signaling. This "self-sufficiency in growth signaling" is essential for rapid mitogenic proliferation necessary for tumor formation and was described as a classical "hallmark" of cancer (20). Mol et al, as early as 1995, reported the first instances of GH acting in an autocrine/paracrine manner in normal mammary tissue (mostly progestin dependent) as well as in mammary tumors (mostly progestin-independent) of dogs and cats (21, 22). Extrapituitary GH production and abundant expression of GHR in several nonpituitary tissues set up a putative autocrine/paracrine loop defined by Harvey and colleagues in 1997 (11, 23). Lobie and colleagues, in 1997, first engineered an autocrine/paracrine production of GH by transiently transfecting rat liver fibroblast cells with human or bovine GH expression plasmids (24). Unlike in pituitary GH expression regulation, GHRH- or SST-mediated regulation of expression is not consistently observed in case of nonpituitary GH (25-27). Moreover, extrapituitary GH is mostly secreted in much lower amounts (no significant contribution to circulating GH levels) (28) but yields a more pronounced local (autocrine/paracrine) effect due to a higher

localized concentration (not diluted in circulation). Importantly, unlike pituitary GH, extrapituitary GH has been reported to increase with age in multiple tissues, including colon, breast, and splenic lymphocytes (29), and has been shown to drive neoplastic events in the aging colon (28, 30, 31) and mammary tissues (32). Furthermore, GH-induced IGF1 production is primarily observed in the hepatocytes of the liver, as well as in the adipose, bone, cartilage, and muscle tissues. In tumor cells, expression of GHR and IGF1 are not always correlated and vary with cancer types, species, and nature of treatment (33). Importantly, age-associated, or DNA-damaging-therapy-induced nonpituitary GH production in the periphery can itself enrich metastatic colonization. This was observed during increased pulmonary metastasis of GHR-positive B16F10 melanoma cells guided by increased GH production in the lungs of DJ1-knockout mice (34). Moreover, aside from the primary tumor site, tumoral GH production is maintained at secondary sites as well (35-37).

Beginning and End of GH Signaling

The GH molecule is an asymmetric ligand (2 different receptor binding sites: 1 and 2) that binds to a preformed GHR homodimer in a 1:2 stoichiometry. A series of seminal work by Waters and colleagues have provided crucial details of GH-mediated GHR signaling (38). The human GHR (638 amino acids) is a class I cytokine receptor with no intrinsic kinase activity. However, kinases like JAK2 (39, 40) and SRC family kinase (SFK) LYN, associated at specific sites in the cytoplasmic domain of GHR, propagate kinase-dependent intracellular signaling following successful GHR activation by GH (41). Interestingly, JAK2 and LYN compete for GHR association and activation in a tissue-specific manner (42). GH-GHR-mediated JAK2 activation is known to trigger the STAT5 pathway as well as the PI3K/AKT/mTOR pathway via IRS1/2, the ERK1/2 pathway via the adaptors SHC-GRB2-SOS, and the 2 scaffold proteins signal regulatory protein α (SIRPα) and SH2B1 (43-46). However, GH-GHR mediated LYN activation does not phosphorylate the GHR, but rather activates the ERK1/2 pathway via phospholipase C-gamma (PLCγ) and phosphokinase C (PKC) (47) and also promotes nano-clustering of GHR at discrete areas of the membrane surface (42).

Termination of these GH promoting signaling cascades is tightly regulated by either deactivation or removal of the activated GHR. For GHR deactivation, a number of protein tyrosine phosphatases (PTPs), suppressor of cytokine signaling (SOCS) family proteins, and protein inhibitors of activated STATs (PIAS) work in tandem to remove the phosphate groups from GHR, JAK, STAT, and other phosphorylated signal transducers (45), resulting in a rapid termination and desensitization of GH action, often via a PLCγ-dependent pathway (48). In the absence of GH, the cell-surface half-life of GHR is ~30 to 60 minutes and is either: (i) cleaved (10% of total GHR) by metalloproteases; or (ii) internalized (75% of total GHR, truncated or intact, +/-ligand) following ubiquitination and degraded. In the absence of GH, constitutive GHR ubiquitination is driven by the E3-ligase βTRCP, which is promoted significantly in the presence of GH by SOCS2 (46, 49). Thus, the surface residence time of ligand-bound GHR is relatively short—approximately 5 to 10 minutes—prior to cleavage or endocytosis. Ligand-bound GHR is endocytosed, shunted toward lysosomal degradation, and in some cases, nuclear localized as well. This pattern of GHR turnover is relevant to consider, as excess ligand may not simply lead to increased GH action. Higher ligand concentrations can lead to temporary suppression of action due to lower membrane retention of ligand-bound receptors. We encourage our readers to refer to recent excellent reviews for additional details of activation, intracellular mediators, variation of surface expression of GHR depending on association with JAK2 vs LYN association (49), deactivation, and termination of GH signaling distilled from hundreds of studies over the last 40 years since the identification of the GH-bound GHR crystal structure (44, 45, 49, 50).

Nuclear GHR Signaling—Intracrine Effect?

Multiple cancer-associated growth factors have shown ligand-activated nuclear translocation and possible intracrine effects, namely, epidermal growth factor receptor (EGFR) (51), IGF1R and insulin receptor (52) which promote tumor proliferation and metastasis. Subcellular GHR immunoreactivity at the nucleus was first reported in cultured cells by Lobie and Waters in 1991 for rabbit GHR (53). Since then, a growing number of studies over the last > 30 years have provided traction to this concept. Nuclear GHR expression has been reported in a variety of cultured cells from rats, rabbits, and humans (54, 55), normal and pathological breast tissue (mastitis, fibroadenoma, papilloma, adenosis, epitheliosis, gynecomastia, carcinoma in situ, and invasive carcinoma) (56), human mesenchymal stem cells (57), as well as in multiple tissues from other cellular and animal models (58-62), and it commonly associates with elevated cell proliferation rates. Nuclear localization of GHR is also supported by the fact that almost half of intracellular JAK2 is nuclear localized (63). Furthermore, it was demonstrated that nuclear targeting of GHR in mouse pro-B Ba/F3 cells generated aggressive metastatic tumors when implanted onto nude mice (64). In a recent elegant study, Perry and colleagues have shown that in human mammary and endometrial cancer cell lines, GH treatment leads to translocation of cell-surface GHR to the nucleus within 5 to 10 minutes (65). Moreover, the nuclear GHR physically interacted with 40 novel proteins to regulate gene transcriptional patterns (65). Interestingly, multiple studies have reported that the GHR antagonist (GHRA) pegvisomant (the only FDA-approved peptide antagonist of GHR) discovered and developed in our laboratory (66) can attenuate nuclear translocation of the GHR in transfected CHO cells (62, 67), and in human gastric cancer cells and xenografts (68). Overall, it is apparent that nuclear localized GHR is indicative of potent cellular GH signaling, although it is unconfirmed whether GH acts here in an intracrine manner or if it is mostly nuclear transfer of surface GH-GHR. Further understanding of the commonality, specificity, and physiological and pathological impact of this phenomenon is crucial.

Promiscuity in GH Signaling

Interactions with prolactin receptor

Across species, mammalian GHs share 70% to 80% homology and marked amino acid sequence similarity. It is important to note that although human (and primate) GH can activate GHR across all mammalian species, non-primate GH cannot activate primate GHR. This species specificity has been traced to a single amino acid interaction in primates—Arg43 of GHR with

Asp171 of GH. In the non-primates, Arg at position-43 of GHR is replaced by Leu, and Asp at position-171 of GH is replaced by His (69). This species specificity is an important factor when designing in vitro and in vivo studies involving treatments of different species of cells treated with different species of GH. This factor is further important as human tumors with human GHR xenografted in nude mice are not expected to respond to mouse GH, and in such cases either exogenously added (injections or pumps) or tumor-derived autocrine hGH can only activate the GHR. The human GHRA pegvisomant also binds poorly to the mouse GHR, and therefore must be used at much higher doses in mice (5-250 mg/kg/day) (70) than prescribed in humans (10-30 mg/day = ~0.1-0.4 mg/kg/day). Additionally, human GH displays receptor promiscuity by acting as a potent activator of the prolactin (PRL) receptor (PRLR) (71, 72). This hGH-PRLR interaction is of paramount importance in highly incident and lethal types of cancers (eg, of breast and prostate), which have overexpression of PRLR, alongside an extrapituitary production of hGH in the epithelium—thus setting up an autocrine/paracrine axis (73-76). Furthermore, Frank and colleagues have put forward evidence of hybrid multimeric receptors formed by homodimers of GHR and PRLR (77, 78). Such hybrid multimers can potentially set up a hyper-signaling cascade with hGH and have encouraged development of bispecific antibodies targeting such interactions in breast cancer (79).

Interactions with EGFR

An additional level of promiscuity in GH signaling is added by the interaction of activated GHR-associated kinases with other tumor-promoting signaling pathways (50). For example, in liver (in vivo and in vitro), following GH binding to GHR and transactivation, the GHR Box-1 associated kinase, JAK2, promotes phosphorylation of EGFR/ERBB1 putatively at Tyr-1068, independent of the intrinsic receptor tyrosine kinase activity of EGFR. This phosphorylated site on EGFR allows docking of Grb2 leading to p42/44-MAPK (ERK1/2) pathway activation (80, 81). Frank and colleagues reported similar GHR-JAK2-mediated EGFR phosphorylation at specific serine/threonine residues (ERBB1 and ERBB2), causing reduction of EGF binding affinity. These receptor interactions appear to protect EGFR from "EGF induced degradation and downregulation, thereby potentiating EGFR signaling" (82-84). The GHR-JAK2-EGFR can lead to ERK1/2 activation and can be attenuated by MEK1 inhibitors (85). A similar role for PRLR in modulating EGFR signaling in normal and transformed cells is also known and could be induced by hGH (86-91). In agreement, total EGFR expression and downstream signaling were markedly suppressed in GHR knockout (GHRKO) mouse liver, while contrastingly, EGFR overexpression and higher basal signaling activity was observed in the livers of bGH mice (92). The bGH mouse liver show increased Ser-1046/1047 phosphorylation, suppressing EGF-induced EGFR activation and possibly preventing EGFR internalization similar to that observed in preadipocytes (93). Also, 5 weeks of GH treatment in young mice can increase hepatic EGFR expression compared to untreated controls (94). This hepatic EGFR modulation by chronic GH exposure coincides with increased spontaneous/carcinogen-induced hepatic neoplasms observed in bGH mice and provides insight to GH's contribution to the etiology of some cancers, especially liver cancer (95, 96).

Interactions with IGF1R

Frank and colleagues have also proposed the existence of an active GHR-JAK2-IGF1R complex in prostate cancer cell line, which can be disrupted by anti-GHR monoclonal antibodies (mAb) (97). Recent elegant studies have further described a complex of GHR-JAK2-STAT5B with the ephrin receptor tyrosine kinase EPH4 culminating in STAT5B activation and IGF1 production (98, 99). In fact, *Eph4* deficiency in mice results in short stature and suppressed circulating IGF1 levels despite GH and GHR expression (99).

Implicating GH in cancer automatically concerns the end-users of GH in treatments of endocrine deficits of GH. A recollection of the earliest work on GH and cancer by Evans and colleagues might offer a very important clue. They showed that following treatment with pituitary-derived GH, although numerous spontaneous neoplasms appeared in multiple tissues of GH-sufficient normal rats (6-8), there was a lack of increased tumors in the treated hypophysectomized rats (100). This might indicate that restoration of pituitary-derived/endocrine GH in GHD subjects may not promote tumorigenesis, unlike in a condition of GH excess.

Epidemiological Data on GH Deficiency/Resistance/Excess and Cancer: Guilty by Association?

GH Deficiency and Resistance and Cancer

From the clinical point of view, one of the strongest indications for the involvement of the GH-IGF1 axis in carcinogenesis comes from the observation that individuals with total resistance to GH action due to inactivating mutations in the GHR (Laron syndrome, LS) and who also have high levels of inactive hGH and low to undetectable circulating levels of IGF1, do not develop cancer (101, 102). The high serum GH in LS, acting on the PRLR, does not lead to tumor development, thus supporting that PRL action alone may not be oncogenic. Across 2 independent studies, Laron and colleagues have reported a complete absence of malignancies in a cohort of 169 and another of 230 LS patients, while an Ecuadorian cohort of 99 LS patients have reported only one death due to an ovarian malignancy compared to > 17% cancer-related deaths in relatives in long-term follow-up studies (reviewed in (102-104)). Multiple studies using lymphoblastoid cells obtained from LS individuals, show a reduction of IGF1-RNA and proteins associated with cell proliferation, progression, and motility, along with enhanced expression of genes associated with protection from oxidative and genotoxic insults (101). Importantly, despite concomitant presence of short stature and obesity, short individuals with LS have greater sensitivity to insulin than their unaffected relatives due to a lack of GH's diabetogenic effect (105). Moreover, their cells, or normal cells treated with LS serum, exhibited reduced DNA breaks and increased pro-apoptotic activities (102)—altogether revealing indications of an intrinsic resistance to mechanisms conducive to cellular transformation due to congenital absence of GH action in human subjects.

In the well-studied Brazilian cohort of short stature subjects from Itabaianinha who carry mutations in the *GHRHR* gene and produce minimal amounts of GH and IGF1, the incidence of tumors is lower than in the general population, but not absent as in individuals with LS (106). GH treatment to restore

the endocrine deficit did not elevate the risk of cancer compared to that in the general population—indicating limited role of endocrine GH in oncogenesis. Furthermore, a 20-year follow-up study with another cohort of 25 patients (in the remote island of Krk in the Adriatic Sea) with congenital combined pituitary hormone deficiency arising due to loss-of-function mutations of the *PROP1* gene (regulator of cellular differentiation during pituitary development) reported no cases of malignancy along with absence of diabetes despite obesity (107). Similar reduction, but not complete absence of tumors, has been also observed in multiple other populations with isolated or combined congenital GHD due to genetic or structural defects (103), suggesting that in susceptible individuals, reduction of endocrine GH can lower the risks of tumor occurrence (106). These landmark clinical studies are compelling examples of suppressed malignancy stemming from suppression of GH action and collectively imply that GH might be a critical factor in determining cancer risk.

Therefore, it is pertinent to ask whether patients with acromegaly do have an increased risk of cancer due to GH excess? And how is the cancer risk in GH-treated patients with GHD?

Acromegaly and Cancer

Assessment of the studies over the last 50 years on acromegaly and cancer lacks consensus. Therefore, we deem it critical to consider the following mechanistic reasons for the lack of a clear association between cancer incidence in patients with acromegaly. For example: (i) excessive endocrine GH simultaneously stimulates the hepatic production of IGF1 and insulin-like growth factor binding protein 3 (IGFBP-3), 2 forces that may act antagonistically in the mechanisms of tumor development and progression; however, it is now known that in the tumor microenvironment (TME), GH-induced matrix metalloproteases can cleave IGFBPs to increase the availability of free IGF (108, 109); (ii) normal pulsatile insulin signaling, which is required to maintain GHR expression levels in tissues (110-112), could be disrupted due to sustained diabetogenic action of chronically elevated GH (113, 114), leading to changes in GHR expression; and (iii) sustained presence of excess of GH is expected to decrease the membrane retention of GHR, which is internalized 2- to 3-fold faster in a ligand-bound state than when unbound (49). An additional caveat is that the assessment of cancer incidence in patients with acromegaly, most of whom may have undergone IGF1-normalizing treatments, may not be directly comparable to an untreated GH-transgenic laboratory animal. Untreated pediatric acromegaly patients have limited lifespan and often do not survive (due to cardiovascular, diabetic, and renal comorbidities) beyond middle age when cancer is more common. For example, as early as 1966, mortality rate in acromegaly was 50% by the age of 50 years and 89% by 60 years (115, 116). But in recent years, a decline has been observed in the overall mortality in acromegaly related to better management of coexisting morbidities and more effective therapies, resulting in higher frequency of patients with biochemically controlled disease (117, 118). Consequently, patients with controlled acromegaly now live longer and the increased longevity has been associated with a higher number of deaths due to age-related (but not GH-related) malignancies, as observed in individuals without acromegaly (118). Interestingly, a recent nationwide study from Italy, with 811 patients and a 15-year follow-up, infers that disease control does not prevent the increased mortality in the long term, and also reported cancer outweighing cardiovascular events as the leading cause of mortality (119). Most studies quantifying cancer rates in acromegaly include patients who have undergone some form of corrective treatment (eg, transsphenoidal surgery, radiation, somatostatin analogs, GHRA) toward the management of the GH excess. Therefore, despite differences in the degree of responses of the patients to IGF1-normalizing therapies, it may not be surprising that standardized incidence ratios (SIR) of cancers in treated acromegaly patients are comparable to that of the general population.

Despite all the aforementioned caveats, age-related cancers are currently one of the leading causes of mortality in patients with biochemically controlled acromegaly (103, 118). We have earlier reviewed that from 23 studies published in the 60 years between 1957 and 2018, the mean cancer incidence in the acromegaly patients was found to be 9.6%, with reported elevated SIR in a cancer type and cohort/country specific manner (reviewed in (103)). More recent studies, especially with long-term follow-up periods, do largely report increased SIR for multiple cancer types. For example, a nationwide study from Sweden, which included 1296 patients with a median follow-up time of 11.7 years, found a 2-fold increase in the risk of benign tumors and increased SIRs for overall and colorectal, anal, renal, and ureteral cancers (120). A more recent meta-analysis of 19 studies including 11 494 patients also reported elevated SIR for overall and colorectal, anal, thyroid, brain, gastric, urinary, hematologic, pancreatic, and intestinal cancers (121).

It is important to indicate that the association of acromegaly and cancer is more controversial particularly for 2 types of tumors—differentiated thyroid carcinoma (DTC) and colorectal (CRC) carcinomas. In a published document from the Pituitary Society on acromegaly management, it was concluded that the rate of DTC is not greater among acromegaly patients (117). In agreement with this statement, in a prospective, cross-sectional study involving 71 patients with acromegaly and 57 with other pituitary tumors (control group) who were subjected to thyroid ultrasound and fine needle aspiration biopsy when indicated, 2 cases of DTC were found in the control group and none in patients with acromegaly (122). Thus, the high prevalence of DTC observed in some studies may not be related to GH excess, but rather due to intensity of screening and detection of small, asymptomatic, low-risk, thyroid malignant nodules (117, 123). Regarding CRC, the recommendation from the Pituitary Society was to perform screening colonoscopy at diagnosis, with further testing done in the same way as indicated for the general population (117). It is important, however, to note that this statement was classified in the document as mainly based on a large series of small uncontrolled studies (117). In experimental models and as discussed later, the local GH rather than endocrine GH shows an IGF1-independent permissive role in changing the TME (124). Interestingly, CRC was reported as the predominant malignancy and main cause of death in adult patients with untreated GHD. Although this argues against participation of endocrine GH in colorectal carcinogenesis (125), nonpituitary colorectal GH production status was not measured in these studies and, therefore, a role of autocrine/paracrine GH in CRC cannot be eliminated. In addition, differences in age, as well as genetic/epigenetic, ethnic, economic, environmental, and dietary backgrounds, the presence of comorbidities, and more intense surveillance with colonoscopy, also

contribute to the conflicting findings among the studies (126). It is worth noting that the frequency of colonoscopy in patients with acromegaly does not seem to impact colorectal mortality rates (123).

Safety of GH Treatment

The wide availability of recombinant human GH (rhGH) has expanded its clinical prescriptions beyond replacement therapy in children with GHD to those in which pharmacological dosages are used in non-GHD short children of different etiologies, with approved indications varying from country to country. In adults, rhGH is indicated in persons with hypopituitarism and severe GHD, which in many cases is caused by tumors in the hypothalamic-pituitary region. Of particular concern is the use of rhGH in patients harboring conditions associated with inherited risk of malignancies and neoplasms. With advances in oncological treatment, there has been a substantial increase in the number of cancer survivors who develop GHD related to the malignancy or as an adverse consequence of surgeries, radiation, and immuno- and/or chemotherapies (103, 127).

In 2021, the Growth Hormone Research Society organized a virtual workshop with 55 international key opinion leaders representing 10 professional societies to address the safety of rhGH therapy in survivors of cancer and intracranial tumors and in patients with cancer predisposition syndromes (128). The group concluded that associations between rhGH replacement therapy with primary tumor, cancer recurrence, and mortality from cancer in GHD survivors are not supported by present data and that the effects of rhGH replacement on the risk of secondary neoplasia are of lesser magnitude compared to factors related to the host and the treatment of the adjacent tumor. In adult cancer survivors with GHD (either with childhood- or adult-onset cancer in remission), rhGH should only be considered after careful individual risk-benefit analysis and in agreement with the oncologist. The timing to start rhGH therapy following completion of oncological treatment should also be individualized and may be as early as 3 months in children with stable craniopharyngiomas who have significant growth failure and metabolic disturbances, and up to 5 years in adults with a history of solid malignancy, such as breast cancer. In addition, it was stated that rhGH is usually contraindicated in GHD children with cancer predisposition syndromes, but it may be cautiously considered in particular cases (128).

However, conflicting data have recently been reported on the mortality risk related to rhGH therapy in high-risk populations. Two noninterventional studies from Novo Nordisk (NordiNet International Outcome Study [IOS]) and the American Norditropin Studies: Web-Enabled Research [ANSWER]) with 37 702 patients did not observe increased mortality related to rhGH treatment in patients categorized in low-, intermediate-, or high-risk groups (129). In contrast, the Safety and Appropriateness of Growth Hormone Treatments in Europe (SAGhE) study, with 24 232 patients treated with rhGH during childhood with up to 25 years of follow-up, observed an increased mortality associated with rhGH therapy in certain groups of patients with an inherent risk related to the underlying diagnosis (130). The treatment was considered safe in children with isolated GHD, idiopathic short stature, mild skeletal dysplasia, or born small for gestational age, but increased all-cause mortality was seen in patients classified as being at high risk, including individuals with severe cerebral and extracerebral malformation, severe chronic pediatric diseases, genetic disorders (neurofibromatosis type 1, Turner syndrome, Noonan syndrome, and Prader-Willi syndrome), malignancies, and syndromes with known increased risk for cancer (Bloom syndrome, Fanconi syndrome, and Down syndrome and chromosomal breakage) (130). In elderly people with GHD, initial observations from the KIMS Database showed that adverse events related to glucose metabolism, cardiovascular diseases, and neoplasms had higher prevalence in those older than 65 years (131). In contrast, more recent data collected from 2 observational, noninterventional, multicenter registry studies of Novo Nordisk did not identify increased prevalence of cancer in GHD patients older than 60 years treated with rhGH, which was referred as a safe replacement therapy in these individuals (132). Of note, rhGH is contraindicated in any patient with GHD and active malignancy and should be discontinued if any clinically significant tumor progression or relapse is observed (128).

The above collection of data establishes a *circumstantial* association between GH and cancer. Overall, the clinical evidence concurs that cancer risks are marginally elevated in treated acromegaly patients, and not elevated in GH-treated GHD patients. We point out that the "treatments" in both cases are aimed at restoring serum hormones (IGF1 and/or GH) to age-adjusted normal ranges which should be physiologic rather than supraphysiologic in action and could well be the underlying reason. A careful analysis of the evidence thus raises the question whether the autocrine/paracrine action of GH might be more relevant in the pathology of most of the cancers and is necessary to be distinguished from endocrine GH. To clarify this association further, in the following sections we will assess the empirical evidence and prognostic outcomes of GH signaling or its inhibition as studied directly in multiple human cancer types over time.

Empirical Data on GH and Cancer: Guilty by Evidence

In multiple different types of cancers which affect tens of millions of patients worldwide every year, the effects of primarily autocrine/paracrine and in some cases exogenous GH as well as the antagonism of the GHR, have been systematically studied using cultured cells, rodent models, human tissues, human patient transcriptomic data, and in some cases even in human patients (Fig. 2). In this regard, some of the most-studied are the cancers of breast, colorectum, liver, endometrium, and prostate, while compelling evidence has been accumulating in melanoma, stomach, lung, pancreas, bladder, central nervous system (CNS), and multiple other cancer types, indicating that GH is a potent growth factor locally available in the TME with a consistent and versatile range of effects. We briefly discuss these human cancers below, assimilating current information while indicating gaps in knowledge in provocative research areas.

Breast Cancer

The highest number of empirical studies in GH and cancer exist in the field of breast cancer (BC), which is reinforced by the fact that an analysis of the GWAS data from the National Cancer Institute (NCI) Cancer Genetic Markers of Susceptibility project (1145 cases, 1142 controls) published

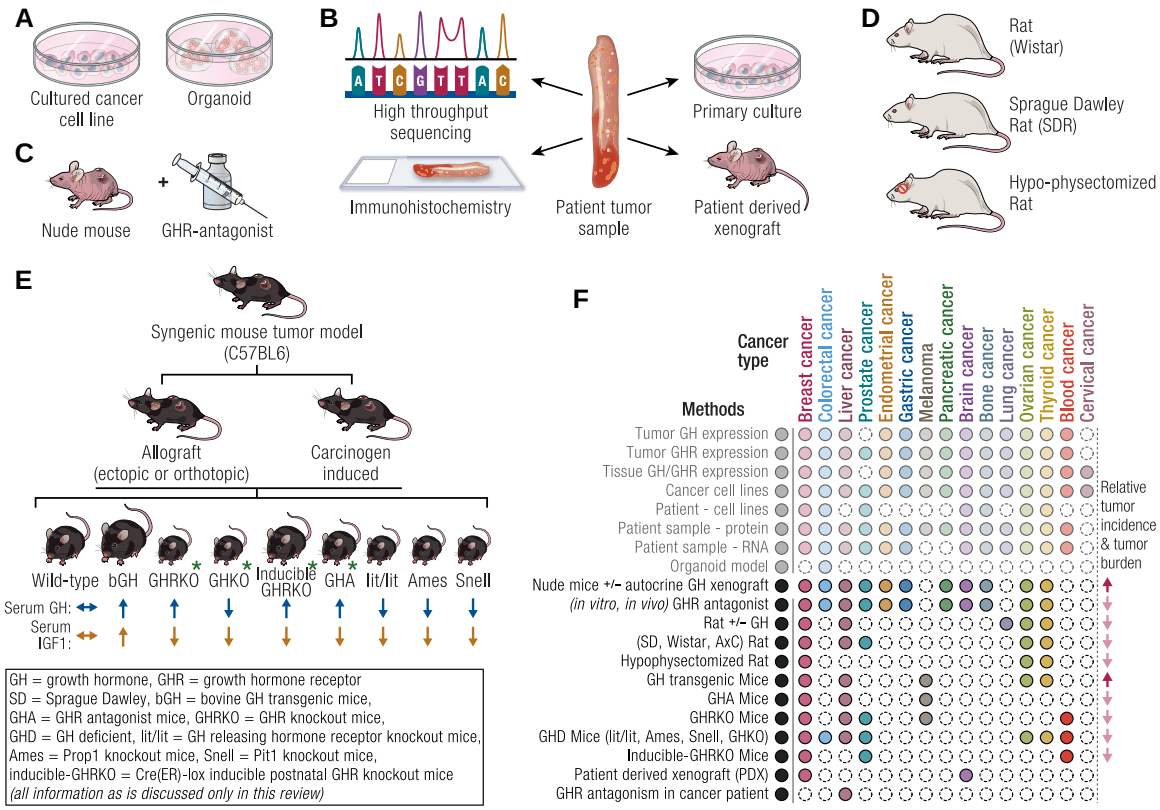

Figure 2. Materials and methods in the study of GH action in cancer. Collectively, the numerous studies in GH and cancer have made use of the following approaches: (A) human and mouse cancer cell lines and organoids; (B) patient-derived tumor biopsy/dissected tumor sample either subjected to high-throughput sequencing (mutations, copy-number, or RNA transcripts) or immunohistochemistry (protein expression) or primary culture or organoids (or can be xenografted in immunocompromised mice); (C) human tumors xenografted onto the flanks of athymic nude mice and treated with blockers of GH action (eg, GHR antagonist); (D) GH-deficient dwarf Sprague Dawley rat (SDR) or hypophysectomized vs corresponding control rats of Wistar type; (E) Syngeneic (same species) mouse tumors (either allografted or carcinogen-induced) in the C57BL6 strain of laboratory mice. This strain and model can be employed for assessment of multiple mouse models of GH action with varying levels of serum GH and IGF1. (F) Distribution of published studies implicating GH in different cancer types using the different methodologies. **Mouse models generated in Kopchick laboratory, Edison Biotechnology Institute, Ohio University, Athens, Ohio.*

Abbreviations: Ames, PROP1 null (deficient in pituitary GH, PRL, TSH); bGH, bovine GH transgenic; GHA, bovine GH antagonist transgenic; GHRKO, GHR knockout; GHKO, GH knockout; inducible GHRKO, postnatal GHR knockout by tamoxifen induction at 6 weeks or 6 months of age; lit/lit, GHRHR knockout; Snell = PIT1 null (deficient in pituitary GH, PRL, TSH).

in 2010, ranked the GH signaling pathway as one of the top 3 pathways (out of 421 involving 3962 genes) associated with BC (133). Alongside ovarian-derived estrogen and progesterone, pituitary hormones like GH and PRL are known to orchestrate normal breast tissue differentiation, development, and also lactogenic functionalities (22, 134-140). Interestingly, hypophysectomy was used as a treatment for BC beginning in the early 1950s, and impressive remission rates were reported (134, 141, 142). Numerous reports confirm high expression of GHR in normal mammary tissue and BC cell lines (56, 143, 144). GHR expression is prominently observed in both epithelial and stromal components of BC lymph node metastases (144), in estrogen receptor (ER)-negative BC cell lines, BC tissue samples, and patient-derived primary cell lines (145). Additionally, *GH* mRNA expression is found in both the normal mammary gland (luminal epithelial cells, ductal myoepithelial cells, stromal fibroblasts) and in both the stromal and epithelial compartments in progressive proliferative disorders of human mammary gland: benign fibroadenoma, pre-invasive intraductal carcinoma, and invasive ductal carcinoma (35). Additionally, patient-derived human mammary cancer cells in culture were found to express GHR in 100% and GH in 52% of the total isolates (146). The co-expression of GH and GHR in normal mammary tissues and upregulation during development of proliferative lesions and in malignancy suggests a putative causal role of autocrine/paracrine rather than endocrine GH action in mammary carcinogenesis (147).

The synthesis and action of GH in mammary cells was outlined in dogs, cats, and humans by Rijnberk and colleagues in 1996. This nonpituitary GH is under progesterone induction in normal mammary cells (148, 149), although following malignancy, GH production in BC cells can be progestin-independent, because GH mRNA has been detected in progesterone receptor (PR)-negative canine (150) and human BC tissue samples (21). Moreover, progesterone treatment does not induce GH production in canine mammary tumor cells (151), although some PR-positive human breast explants can reportedly produce GH (as well as PRL and IGF1) under progesterone stimulation (152, 153). Lobie's group exemplified this by expressing human GH in immortalized human mammary epithelial cells, which was sufficient for oncogenic transformation and in vivo tumorigenic capacity (154) and confirmed by additional independent studies to be oncogenic in abetting neoplastic transformation of mammary ductal cells (32, 155). Autocrine GH expression in several ER-positive, ER-negative, and triple-negative BC cells have shown similar phenotypes of invasive growth, increased proliferation, therapy (chemo- and radiation) resistance, and inhibition of apoptosis (145, 156-165). The GHR antagonist B2036

(core peptide of pegvisomant) successfully attenuated autocrine GH-induced anti-apoptotic effects in BC cells (156-158). Also, the role of PRL in human BC has been known since the 1960s (166), and although PRLR is expressed in 70% of BC biopsies and cell lines (167), the GH-PRLR interaction in BC (74) still remains highly understudied. In response to human GH and/or PRL, specific morphological changes associated with epithelial-to-mesenchymal transition (EMT), along with a 2-fold increased accumulation of "intracytoplasmic lipid droplets," was observed in human T47D BC cells (168). Notably, the PRL-PRLR signaling is important in BC due to high PRLR in the mammary tissue and has been well-described by expert reviews (169-174).

Childbearing or parity affects breast cancer risks in rodents (175, 176), and also results in decreased circulating GH levels compared to virgin control animals (175), which appears to protect these animals from chemically induced mammary tumor development (177-179). Moreover, treatment of parous rats with 17-β-estradiol, but not progesterone, increased circulating GH levels, and increased N-methyl-N-nitrosourea (MNU)-induced mammary tumor incidence from 10% in untreated to 67% in estradiol-treated animals (180). In mice, transgenic expression of both bGH and hGH led to mammary hyperplasia, which proceeded to neoplasia only in case of hGH transgenic animals (155). Multiple other rodent models, including mouse models of varying GH/IGF1 signaling show a consistent tumor-promoting action of GH, which can be suppressed by attenuation by either discontinuation of GH administration, or congenital absence of GH or GHR, or use of GHR antagonists and have been extensively reviewed (33, 70, 103, 134, 155, 181, 182). Table 1 provides a comprehensive listing of the empirical evidence in this area. We highlight some notable examples of GH's more recent association with therapy resistance. For example, a cohort of MNU-treated Sprague Dawley rats (SDR) were also treated with GH and following tumor establishment, GH treatment was withdrawn in one group, and doxorubicin treatment was initiated. The GH-treated SDR were found to be markedly less responsive to the chemotherapy compared to GH-withdrawn SDR (198), indicating an association of GH with chemoresistance in cancer therapy, which has also been reported in multiple studies in other cancers by our group and others (204).

Mouse models of congenital GH insensitivity (GHRKO mice) developed by our laboratory (205), modeling conditions of LS, were crossed with C3(1)/Tag mice which spontaneously develop mammary neoplasms due to expression of the SV40 large T-antigen (206). The tumor burden in the Tag/GHRKO mice was 3 per animal, compared to 10 in the Tag/wild-type counterparts (199). Moreover, tumor sizes in the Tag/GHRKO females were 10 times smaller than that in the Tag/wild-type females. In a similar Tag/ind-GHRKO model, developed by crossing C3(1)/Tag mice with tamoxifen-inducible-GHRKO mice (developed at our lab (207),), *Ghr* was ablated after tumor development (200). *Ghr* null mice showed regression of tumor growth and reduced cell proliferation in tumors compared to control animals. Another unique mouse model, GHRA transgenic mice (GHA) also developed in our laboratory (208), as well as the adult-onset GH-deficient (GHD) mice, when treated with dimethylbenzanthracine (DMBA), resulted in significantly lower mammary tumor frequency, size, and burden compared to wild-type littermates (201, 202). Arumugam et al implanted patient-derived ER-negative primary BC cells with shRNA-mediated GHR knockdown in nude mice. These GHR-deficient xenografts had a markedly reduced growth rate and improved response to docetaxel, again validating the chemosensitizing effect of GHR attenuation (145).

Does the collection of the above studies align with prognoses of actual patients with BC in the clinic? Assessment of results in human BC samples seem to affirm this supposition. For example, histopathological analysis of 157 mammary cancer patients and 33 benign breast disease patients shows that, in the cancer samples, 53% were positive for GH and 68% were positive for PRL expression, compared to 30% and 27%, respectively in noncancer samples (183). Additionally, Kaplan-Meier (KM) and Cox regression analyses showed that tumor GH expression was significantly associated with poorer relapse-free survival (RFS) and overall survival (OS) in the BC patients, with the worse correlation for patients with tumors expressing both GH and PRL (183). Histochemical analysis of 47 BC patient samples inferred that GHR was enriched in ER-negative subtypes (145). In validation, in 671 ER-negative BC patients, higher GHR mRNA expression correlated with significantly poorer survival probability compared to patients with lower GHR expression (145). The GHR-associated poor survival probability further deteriorated in the chemotherapy-treated group, validating the chemorefractory effect of GH (145). We also used the KMplotter platform to assess GHR expression association with OS and RFS in > 5000 patient samples spanning 55 datasets (209). Our analysis additionally found that GHR expression significantly correlates with poorer OS and RFS in patients with triple-negative BC (TNBC) with almost 1.5-fold difference in survival (in months), and with worse outcomes in chemotherapy-treated groups (Fig. 3A-3D). Therefore, GHR can be posited as a clinically relevant target in TNBC, which is provocative given the high mortality and paucity of therapeutic targets in TNBC, and thus demands necessary attention for therapeutic development. The GHR-associated poor survival outcome is also observed in HER2-enriched BC (Fig. 3E), but no significant correlation is observed for GHR expression and survival in ER-positive or PR-positive BC groups (not shown here). Interestingly, GHR as a prognostic marker in HER2 enriched BC patient group appears to support the concept that crosstalk of GHR and EGF receptors described by Frank and colleagues (discussed above) may be clinically relevant.

Liver Cancer

The normal liver presents the highest surface expression of GHR between different organ systems, where GH action on hepatocytes induces the production of > 75% of the circulating IGF1. Notably, although the normal liver has very low IGF1R expression, in hepatocellular carcinoma (HCC), the major form of liver cancer, overexpression of IGF1R is consistently observed (210). Therefore, endocrine GH–driven IGF1 production renders a robust autocrine/paracrine effect on liver tumor cells (210, 211). Interestingly, GHR expression in HCC is less consistent, as several studies have reported a lower GHR expression in human HCC tumor vs normal liver tissues, particularly in hepatitis C virus (HCV)-related HCC, whereas survival studies in patients indicate that lower GHR expression correlates with poorer prognoses (212-221). However, this does not diminish the fact that autocrine/

Table 1. Studies implicating growth hormone in breast cancer

Material	Method	Study summary	Reference
normal mammary epithelial cell	autocrine GH	oncogenic transformation and *in vivo* tumor growth	(32, 154, 155)
BC cell lines, patient samples	RNA and protein	GH and GHR expression	(21, 35, 56, 143-146, 150-153)
patient samples	IHC for GHR	enriched in ER– BC samples	(145)
patient samples	IHC for GH, clinical correlation	GH overexpression in cancer tissue, correlates with poorer survival	(183)
patient sample	protein expression	tumor GHR expression correlates with aggressive metastasis	(144)
patient sample—IHC	correlation with tumor GH expression	tumor GH expression correlates with lymph node metastasis, tumor grade	(183)
autocrine GH in cell line, patient sample-RNA	RNA expression	autocrine GH expression intact in metastatic tumors	(35, 36)
patient data	GWAS data analyses	GH signaling pathway—one of top 3 pathways associated with BC	(133)
human patients	hypophysectomy	high rate of remission	(134, 141, 142)
BC cell lines	autocrine GH	increases proliferation	(146, 156, 158)
MCF7 cell line	autocrine GH	inhibits growth suppressors	(184)
MCF7 cell line	autocrine GH	resistance to chemotherapy (doxorubicin) induced apoptosis	(185)
MCF7 cell line	autocrine GH	increases DDR and resistance to starvation induced apoptosis	(156, 157, 186)
MCF7-hGH cells	GHRA (B2036) treatment	increases starvation induced apoptosis	(157)
BC cells	autocrine GH	resistance to starvation induced apoptosis	(187)
BC cells	autocrine GH	resistance to radiotherapy induced apoptosis	(188)
BC cells	autocrine GH	resistance to chemotherapy (MMC, doxorubicin, cisplatin) induced apoptosis	(162, 189)
BC cells	autocrine GH	resistance to oxidative stress induced apoptosis	(190)
ER+ and TNBC cells	autocrine GH	resistance to chemotherapy (MMC) induced apoptosis	(162)
ER+ and TNBC cells	autocrine GH + GHRA (B2036) treatment	sensitized to radiotherapy induced apoptosis	(188)
ER+ and TNBC cells	exogenous GH + GHRA (pegvisomant) treatment	sensitized to chemotherapy (doxorubicin) induced apoptosis	(160, 163)
patient-derived primary cells	anti-GHR shRNA	decreases cell viability, drug efflux, sensitized to chemotherapy (docetaxel) induced apoptosis	(145)
multiple BC cell types	autocrine GH	induces EMT, promotes migration-invasion	(36, 159, 161, 168)
BC cell line	autocrine GH	promotes DNA methylation, EMT induction	(191)
multiple BC cell types	orthotopic xenograft—nude mice	EMT induction, metastasis, ECM remodeling, stromal fibrosis	(36, 159, 161)
ER– BC cell xenograft in nude mice	autocrine GH	higher rate of invasion and metastases	(162, 188)
nude mouse xenograft	GHRA (B2036) treatment	sensitized to MMC induced apoptosis	(162)
T47D xenografts—nude mice	treated with GH	tumor proliferation, potentiation of estrogen effect	(192, 193)
TNBC cell lines	autocrine GH	resistance to tamoxifen, curcumin induced apoptosis	(164, 165)
estradiol-treated parous rats + MNU	increased serum GH	increased rate of tumorigenesis	(180)
control vs SDR	DMBA or MNU treatment	lower tumor incidence and size in SDR	(194)
SDR + GH	DMBA treatment	increased tumor incidence	(195, 196)
tumor bearing SDR + GH	GH withdrawal	tumor regression	(197)
SDR + GH + doxorubicin	GH withdrawal	sensitized to doxorubicin induced apoptosis	(198)
hGH/bGH transgenic mice	changes in mammary tissue	neoplasia (only for hGH transgenic, not oGH or bGH)	(155)
C3(1)/Tag mice	crossed with GHRKO mice	GHRKO-Tag mice—suppressed tumor incidence and tumor size	(199)
C3(1)/Tag mice	crossed with inducible-GHRKO mice	ind-GHRKO-Tag mice—tumor growth regression	(200)
DMBA treatment	GHA vs control mice	lower tumor incidence, size, and burden	(201)
DMBA treatment	AOiGHD vs control mice	lower tumor incidence, size, and burden	(202)

(*continued*)

Table 1. Continued

Material	Method	Study summary	Reference
MCF7 xenograft in nude mice	GHRA (pegvisomant) treatment	reduced proliferation, increased apoptosis, reduced tumor growth	(162, 163, 203)
ER− primary cell xenograft in nude mice	GHR knockdown cells	reduced tumor growth, sensitized to docetaxel induced apoptosis	(145)

Abbreviations: AOiGHD, adult-onset isolated growth hormone deficiency; BC, breast cancer; DDR, DNA damage repair; DMBA, dimethylbenzanthracene; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; ER, estrogen receptor; GH, growth hormone; GHA mice, mice transgenic for growth hormone receptor antagonist; GHR, growth hormone receptor; GHRA, growth hormone receptor antagonist; GWAS, genome-wide association study; IHC, immunohistochemistry; MMC, mitomycin C; MNU, N-methyl-N-nitrosourea; SDR, Sprague Dawley rats; TNBC, triple-negative breast cancer.

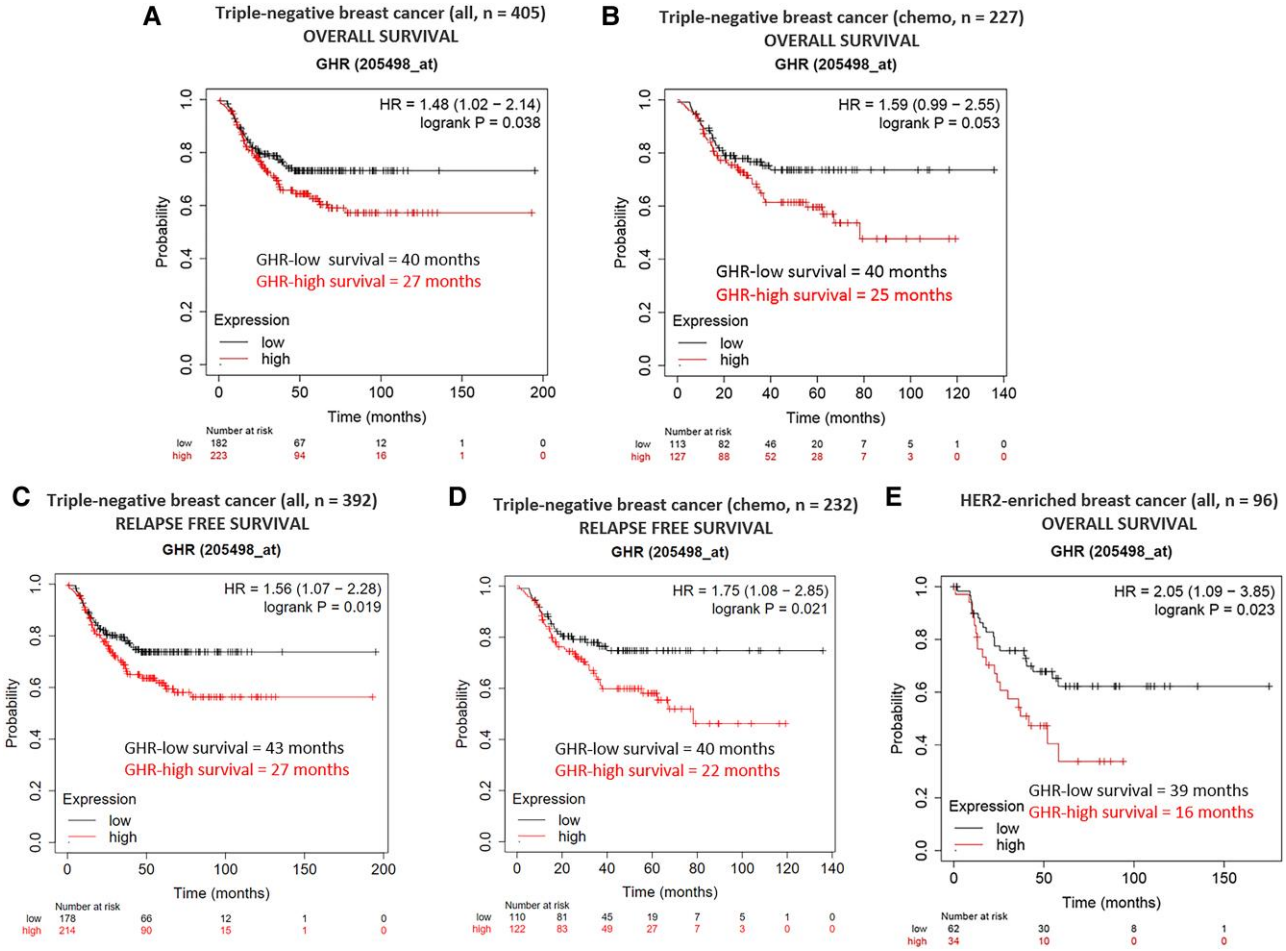

Figure 3. GHR expression negatively correlates with overall survival (OS) and relapse-free survival (RFS) in triple-negative and HER2-enriched breast cancer. KMplotter generated survival probability of breast cancer (BC) patients with low or high GHR expression. (A) OS in patients with triple-negative (ER-PR-HER2-) BC (n = 405); (B) OS in patients with triple-negative BC who underwent chemotherapy (n = 227); (C) RFS in patients with triple-negative BC (n = 392); (D) RFS patients with HER2-enriched (ER-PR-HER2+) BC who underwent chemotherapy (n = 232); (E) OS in patients with HER2-enriched (ER-PR-HER2+) BC (n = 96).

paracrine GH action in the liver tissues is a major contributor to neoplastic development in the liver, as indicated by the following findings.

Parallel to a decrease in GHR, the expression of nonpituitary GH in the liver is significantly ramped up in HCC, as corroborated repeatedly by studies since the 1960s. For example, tumoral expression (mRNA and protein) of hGH alone or hGH and PRL both were significantly associated with poorer RFS in HCC patients and expression of both genes were significantly increased in neoplastic vs normal tissues (222). Unlike in case of PRL, GH being a ligand for both GHR and PRLR, appeared to have a more consistent correlation in patients and models of HCC. Moreover, G120K-hGH which can antagonize both GHR and PRLR activation, was successful in blocking the STAT3 signaling mediated growth of xenograft tumors with autocrine GH and PRL expression in mice. Moreover, human

Table 2. Studies implicating growth hormone in liver cancer

Material	Method	Study summary	Reference
normal liver tissue	RNA and protein	High GHR expression, very low GH expression	(210)
patient samples	RNA and protein	GH overexpression in ~50% samples,	(228)
HCC cell lines, patient samples	RNA and protein, clinical correlations	GH expression increased compared to normal liver, higher GH correlates with poorer survival	(222)
HCC cell lines, patient samples	RNA and protein, clinical correlations	GHR expression is decreased compared to normal liver, lower GHR correlates with poorer survival	(212-218, 220)
human cell lines	autocrine GH	increased proliferation	(95, 237)
cell line with autocrine GH	nude mice xenograft	increased tumor growth *in vivo*	(222)
human cell lines	autocrine GH	induces EMT, promotes migration-invasion, metastasis	(237)
human and mouse cell lines	exogenous GH +/− GHR knockdown	increased ABC transporters and drug efflux, resistance to doxorubicin and sorafenib	(211)
AxC rats + hepatocarcinogen	GH administration	increase in liver tumorigenesis	(223)
Wistar rats + hepatocarcinogen	GH infusion	increase in liver tumorigenesis	(224)
GH transgenic animals	liver pathology	hyperplasia, hypertrophy, age-associated hepatocarcinogenesis	(95, 229-236)
ovine GH transgenic vs control mice	+ hepatocarcinogen	increase in liver tumorigenesis	(225)
lit/lit vs control mice	+ hepatocarcinogen	decrease in liver tumorigenesis and growth	(226)
GHRKO vs control mice	+ hepatocarcinogen	decrease in liver tumorigenesis and growth	(227)
liver-GHRKO vs control mice	+ hepatocarcinogen	decrease in liver tumorigenesis and growth	(227)
GHA mice	syngeneic allograft	increased ABC transporters, resistance to targeted therapy (sorafenib)	(211)
cell line with autocrine GH in nude mice	GHRA (hGH-G120R) treatment	suppressed tumor growth *in vivo*	(222)
human xenograft in nude mice + sorafenib	+ pegvisomant	pegvisomant improves sorafenib efficacy	(228)
mouse allograft in GHA vs control mice	+ sorafenib	transgenic GHR antagonist improves sorafenib efficacy	(211)
human HCC patients + sorafenib	+ pegvisomant	disease stabilization	(228)
human HCC patients + immunotherapy	correlation of serum GH—treatment response	high serum GH correlates with poorer immunotherapy response and survival	(238)

Abbreviations: GH, growth hormone; GHA mice, mice transgenic for growth hormone receptor antagonist; GHR, growth hormone receptor; GHRA, growth hormone receptor antagonist; HCC, hepatocellular carcinoma.

HCC cell lines transfected with GH led to increased IGF1 and IGF2 expression and promoted xenograft growth in nude mice, and this was inhibited by hGH-G120R treatment. GH overexpression also correlates with tumor size, grade, and worse OS and RFS in HCC patients (222). In rodent models, increased GH (exogenous or congenital) promotes chemically induced hepato-carcinogenesis, exemplified by: (i) GH administration in N-2-fluorenyldiactemaide-treated AxC rats (223); (ii) GH infusion in diethylnitrosamine (DEN)-treated Wistar rats (224); and (iii) DEN treatment in neonatal ovine GH transgenic mice compared to nontransgenic controls (225). Contrastingly, mouse models with attenuated GH action show compelling evidence of suppression of chemically induced hepatocarcinogenesis as seen in (i) DEN treatment in the GH-deficient lit/lit (GHRHR mutant) mice (226); (ii) DEN treatment in GHRKO vs littermate controls (tumor incidence 5.6% vs 93.5%) (227); and (iii) DEN treatment in liver-specific GHR null mice compared to controls (227).

In humans, 49.5% (380/767) of HCC patients had increased tumoral GH expression, which correlated with significantly shorter OS in both males and females (228). Additionally, several studies in GH (ovine or bovine or mouse) transgenic mice have shown that hyperplasia and hypertrophy precede age-associated hepatocarcinogenesis in these animals (more in male than female mice, similar to incidence rates observed in humans) (95, 229-236) and are listed in Table 2. This hepatomegaly in GH transgenic mice is mostly a function of amplified GH and associated inflammatory changes (discussed later), so high incidence of hepatocarcinogenesis is not observed in the IGF1 transgenic mice (230). Additional factors may include GH-regulated sustained activation of inflammatory pathways in the liver (discussed below), stabilization of hepatic EGFR levels and signaling (92, 96) (discussed above), as well as activation of protumorigenic factors like galectin-1 (239), c-myc, c-jun, c-fos, AKT, NFκB, GSK3β, PCNA, cyclin-D1, and cyclin-E (95, 236). In comparison, congenital as well as inducible GHRKO mice show markedly decreased hepatocarcinogenesis compared to respective controls (J.K., personal communication). Notably, while several studies have reported that excess GH action increases fibrosis-associated amino acid hydroxyproline in the AT, the same in liver has not yet been determined, while upregulated fibrosis has been reported in small intestine, heart, kidney, and tibial articular cartilages of bGH transgenic mice (240). This should be investigated, as hydroxyproline levels measured in liver biopsies correlate positively with HCC in

Table 3. Studies implicating growth hormone in colorectal cancer

Material	Method	Study summary	Reference
patient samples	IHC	GHR overexpression in tumor vs normal colon tissue	(243-246)
patient sample—IHC	correlation with tumor GH expression	tumor GH expression correlates with lymph node metastasis, tumor grade	(253)
patient samples (pre- and post-radiotherapy)	RNA and protein	GHR levels increased post-treatment, pretreatment GHR correlate with poorer therapy response	(254, 255)
SNP: T1663A in *GH1* gene	correlation study	reduced risk of CRC	(248-250)
colon cell lines, PSC-derived organoids	Exogenous or autocrine GH	reduces apoptosis, inhibits growth suppressors (PTEN, APC), induces EMT	(30)
colon cell lines, PSC-derived organoids	autocrine/paracrine GH	promotes oncogenic transformation	(31, 256, 257)
cell lines	autocrine GH	increased proliferation	(253)
CRC cell lines	GH treatment	resistance to radiotherapy induced apoptosis	(258)
CRC cell lines	exogenous GH	GH attenuates PPARγ-induced apoptosis	(251)
CRC cell lines	autocrine GH	induces EMT, promotes migration-invasion	(253)
nude mice xenograft	anti-GHR siRNA	reduced tumor volume, decreased metastasis, increased response to 5FU	(259)
APC(min+/−) mice	crossed with GHD Ames (Prop1 −/−) mice	Ames-APC(min+/−) cohort: reduced tumor incidence, burden, growth	(30)
patient serum sample	radioimmunoassay for GH	serum GH significantly higher than normal	(260)

Abbreviations: 5FU, 5-fluorouracil; CRC, colorectal carcinoma; EMT, epithelial-to-mesenchymal transition; GH, growth hormone; GHD, growth hormone deficient; GHR, growth hormone receptor; IHC, immunohistochemistry; PSC, pluripotent stem cell.

nonalcoholic steatohepatitis (NASH) mouse models (241). Furthermore, in case of disease prognoses, the clinical relevance of targeting GH action was highlighted by multiple independent studies by us and others, where either pegvisomant treatment markedly increases sorafenib efficacy in nude mice with HCC xenografts (228), or GHA mice with syngeneic Hepa1-6 allografts show significantly higher response to sorafenib compared to wild-type controls (211). Given that IGF1R signaling is activated robustly in HCC, it is relevant to mention here that pegvisomant decreases circulating levels of both ligands of IGF1R—IGF1 and IGF2—to the same extent, as observed in healthy human volunteers as found in a Pfizer clinical trial (242). Recently, Amin and colleagues at the MD Anderson Cancer Center highlighted the real-world therapeutic potential of attenuating GH action when they treated 2 HCC patients, who presented with high plasma GH and sorafenib resistance, with pegvisomant (228). Treatment with 10 mg/day of pegvisomant along with sorafenib induced tumor stability in both, along with 40% reduction in the tumor prognosis marker α-fetoprotein (AFP) levels in one patient. The same group also hinted at the association of upregulated serum GH levels with significantly poorer response to the atezolizumab (anti-PDL1 [programmed cell death ligand 1])-bevacizumab (anti-VEGFA [vascular endothelial growth factor A]) regimen and correlates with decreased OS in 37 HCC patients (238).

Colorectal Cancer

GHR is overexpressed in colorectal neoplasms compared to adjacent nonneoplastic cells. Peripheral GH action was reported to be implicated in colorectal malignancies as early as 2000 (243). Assessments from studies using patient samples of colorectal carcinoma (CRC) and normal colorectal mucous membrane show that GHR protein and RNA is present in 83% to 100% and overexpressed in > 69% of the CRC samples compared to significantly weaker expression in the normal mucosa (244-246). Moreover, the single nucleotide polymorphism (SNP) T1663A; rs2665802 in the human *GH1* gene, which is associated with reduced circulating GH and IGF1 (247), also correlates with a significantly reduced risk of CRC (248-250). In the laboratory, early in vitro studies report that both GH and IGF1 attenuates PPARγ-induced apoptosis in human colon cancer cell lines (251), and IGF1 also has a well-studied promoting effect in CRC (252). However, multiple rodent models (Table 3) affirm that GH can promote CRC incidence and progression in an IGF1-independent manner. For example, in response to treatment with the colon-specific carcinogen azoxymethane, male LID mice (serum IGF1 reduced by 70% due to liver-specific *Igf1* deletion) had no difference in the development of aberrant crypt foci (ACF), tumor incidence, or tumor multiplicity, but they had a 25% reduction in tumor volume compared to wild-type animals (261). This highlighted that IGF1 could be a promoter of proliferative growth in CRC, rather than a causative factor. On the other hand, treatment with GHR-targeted siRNA in nude mice bearing human SW480 cell xenografts suppressed postinoculation increase in tumor volume (262) and also reduced the rate of liver metastasis (259), while improving the efficacy of chemotherapeutic 5-fluorouracil (5FU) treatment. In cell-based studies, autocrine/paracrine GH action has been shown to promote colonic neoplasms, in an IGF1-independent manner (30, 124).

CRC is one of the best validated examples of the oncogenic role of peripheral GH with autocrine/paracrine action and highlights the nonsignificant role of endocrine GH in this cancer. In that regard, an extensive series of studies by Chesnokova and Melmed has highlighted the genomic instability promoted by colon-derived GH on the etiology of neoplasms in the aging colon (30). Unlike endocrine GH, the local production of nonpituitary GH was found to increase in the aging colon and promoted accumulation of

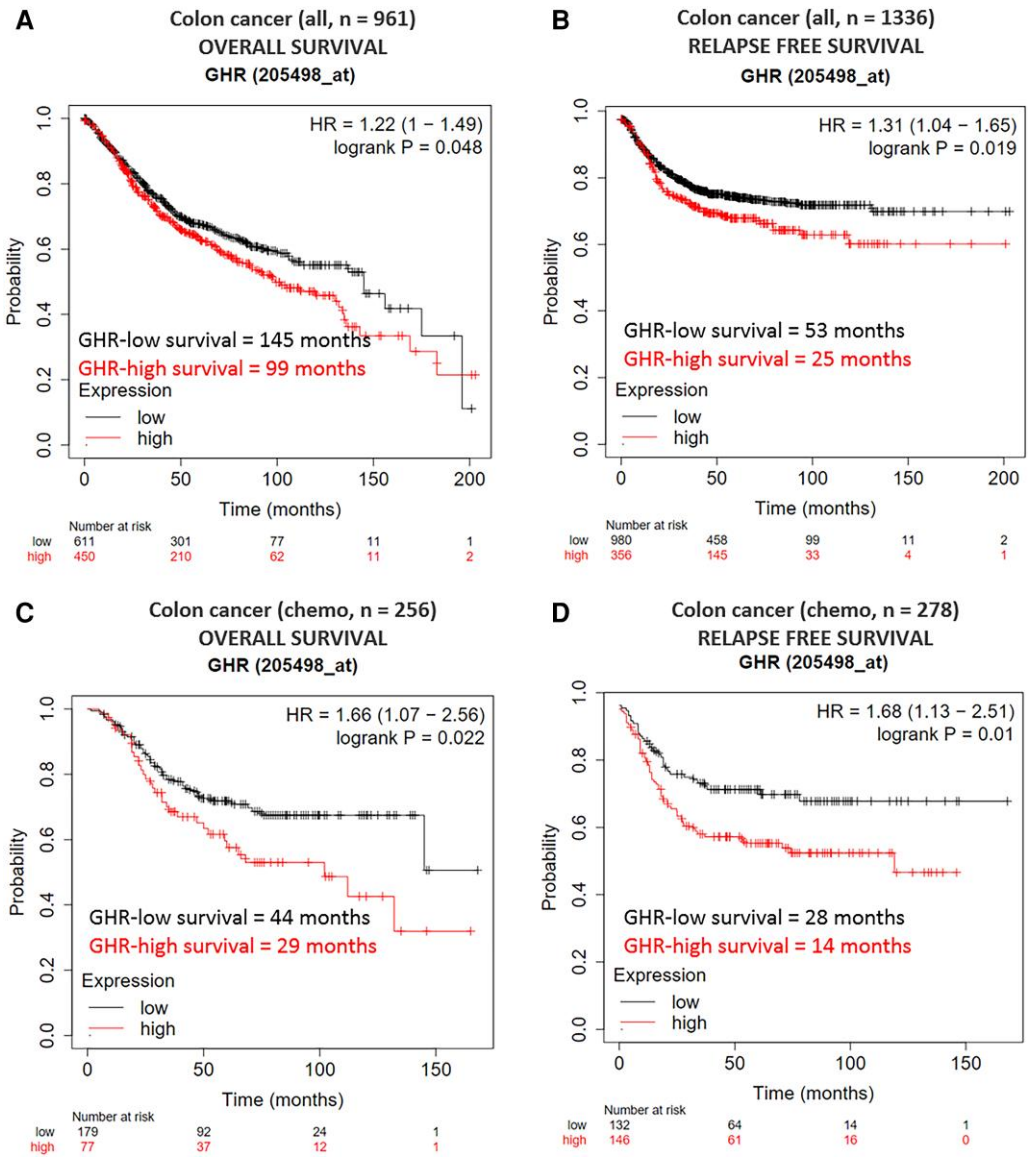

Figure 4. GHR expression negatively correlates with overall survival (OS) and relapse-free survival (RFS) in colon cancer. KMplotter generated survival probability of colon cancer (CC) patients with low or high GHR expression. (A) OS in all patients with CC (n = 961); (B) RFS in patients with CC (n = 1336); (C) OS in patients with CC who underwent chemotherapy (n = 256); (D) RFS in patients with CC who underwent chemotherapy (n = 278).

DNA damage in the normal cells, eliciting an effect which promotes neoplastic transformation (31, 256, 257). However, following transformation, both GH and IGF1 signaling in tumor cells appears to contrastingly promote DNA damage repair (DDR) pathways, ensuring tumor recovery and survival from DNA-damaging treatments like radiation and chemotherapy (263). In agreement, 9-month-old progeny of GHD Ames mice crossed with intestinal tumor-prone APC(min+/-) mice, had significantly reduced tumor incidence, burden, and growth compared to GH-sufficient APC(min+/−) controls (30). Provocative associations of GH action with clinical outcomes in CRC are also numerous. Notably, an early comparison of pre- and post-irradiation specimens of 98 rectal cancer patients show marked increase in tumoral GHR expression following radiotherapy as well as poorer response to irradiation correlating with higher pretreatment GHR levels (254,255). Similar observations were noted more recently for tumoral IGF1R expression as well (264). Recent reviews have additionally discussed the current knowledge in GH-associated tumor promotion and therapy-refractoriness in human CRC (102, 182, 204). Analyzing human patient survival data using the KMplotter platform, we find that a tumoral GHR expression does indeed correlate significantly with poorer OS and RFS in patients with colon cancer. The hazard ratios increased in the groups of patients treated with chemotherapy, with an almost 2-fold difference in survival (in months) between high and low GH expression groups (Fig. 4). Studies in GH and CRC are exemplary in the importance of focusing on age-associated peripheral GH production and its implications in cancer in different tissues and organ systems, as well as in other organ-specific pathologies.

Prostate Cancer

More than 20 years ago, Barkey and colleagues had demonstrated expression of GHR in human prostate cancer (PC; primary tissues and cell lines), with almost 80% higher GHR expression level in the carcinoma tissues than in the benign

Table 4. Studies implicating growth hormone in prostate cancer

Material	Method	Study summary	Reference
primary tissues, cell lines	RNA/protein	GHR overexpressed in neoplastic than normal prostate	(265)
cells and patient samples	GHR expression	readily detectable expression, increases proliferation	(275)
primary tissues, cell lines	GH treatment	acute increase in prostatic androgen receptor	(265)
cell lines	GH treatment	increases IGF1 and IGF1R differentially based in androgen-dependent manner	(268)
tumor-prone C3(1)-Tag mice	crossed with GHRKO mice	progeny have highly reduced tumor incidence, burden, and volume, reduced proliferation, increased apoptosis	(269)
tumor-prone C3(1)-Tag mice	prostate-specific inducible GHRKO mice	progeny have reduced tumor incidence, burden, reduced proliferation, increased apoptosis	(270)
mouse tumor cell lines in nude mice	GHR overexpression	increased xenograft size and weight	(270)
PTEN-null CRC cells in nude mice	GHRA (pegvisomant) treatment, RNA-seq	suppressed tumor growth, several DEGs	(274)
tumor-prone Probasin/Tag rat	crossed with SDR	progeny has markedly reduced tumor incidence	(272)

Abbreviations: GH, growth hormone; GHR, growth hormone receptor; GHRA, growth hormone receptor antagonist; SDR, Sprague Dawley rats.

hyperplasia (265), while case-control studies note a marginal (< 10%) rise in serum IGF1 in PC patients (266). In PC, the GHR aside from classical signaling pathways also shared intracellular crosstalk with androgen signaling. For example, GH affected a rapid but transient (2-12 hours) upregulation (~5-fold) of the prostatic androgen receptors (265), while prostatic GHR isoform expression appeared to be androgen-dependent (267). Moreover, in androgen-dependent LNCaP cells, GH stimulated IGF1 (and IGF1R) expression, while in androgen-insensitive PC3 cells, GH stimulated IGF2 (and inhibited IGF1R) expression (268). Swanson and colleagues employed the C3(1)/Tag mouse crossed with the GHRKO mouse in PC studies (269). The Tag expression is driven by the regulatory region of rat prostate-specific steroid binding protein C3(1) gene, which lead to all male mice spontaneously developing prostate cancer. Importantly, in the Tag/GHRKO progenies dissected at 9 months of age, only 1/7 mice had prostatic intraepithelial neoplasms, compared to 7/8 in the GHR-sufficient control Tag mice. Moreover, the prostate epithelium in the Tag/GHRKO animals present reduced proliferation and increased apoptosis. Likewise, experiments conducted using a tamoxifen-inducible prostate-specific GHR-deleted mice strain yielded similar results, showing markedly reduced prostatic intraepithelial neoplasms and reduced epithelial stratification and proliferation in the 6-month-old animals (270). On the other hand, orthotopic implant of GHR overexpressed murine PC showed an increased xenograft size and weight (270). In another animal model, Probasin/Tag rats, which develop PC by 15 weeks of age (271), were crossed with SDR animals. At 25 weeks of age, despite identical expression of the T-antigen in the prostate, the majority of the Tag/SDR rats lacked invasive carcinomas, compared with 100% in Tag controls (272). The results appear to be IGF1-independent, as in the GH-sufficient rats, prostate IGF1R protein levels decreased and GHR protein levels increased with progressive carcinogenesis, with no significant changes in either serum or prostate IGF1 levels (272). In addition to promoting prostate tumor growth, both GH and IGF1 have been implicated in driving refractoriness to radio- and chemotherapy in PC (204, 273). Expectedly, administration of pegvisomant suppresses xenograft tumor growth along with extensive dysregulation of gene expression (1765 upregulated and 953 downregulated genes) in the xenograft tumors (274). Table 4 compiles most of the empirical evidence in GH and PC studies. Overall, the majority of the studies in PC have modeled endocrine effects of GH, while the autocrine/paracrine actions are unknown and may well be a critical factor. More importantly, PC is rare in men younger than 40 years, and first diagnosis in most of the cases is at 67 years of age. This puts into question the role of endocrine GH in PC, and in light of the evidence of GH in PC discussed above, demands a more careful assessment.

Endometrial Cancer

Multiple reports confirm that GH action promotes endometrial cancer (EC) cell proliferation, invasive growth, and protection from several antineoplastic approaches. The normal human endometrium preferentially expresses GH in the glandular cells of the decidua (rather than the glandular secretory cells and stromal cells), and mostly between the mid and late luteal phase (276) and is known to drive epithelial proliferation (277-279). Numerous studies show that this autocrine/paracrine GH continues to play major role in endometrial pathologies including cancer (Table 5), although age-associated changes in endometrial GH production are yet unknown. This is hinted in histopathological analyses of healthy and diseased endometrial tissues from an Australian biobank, where GH expression was upregulated by 3.4-fold in endometriosis and 3.8-fold in endometrial cancer compared to normal uterine tissue (280). A series of studies by Lobie and colleagues described additional details of autocrine GH in endometrial cancer, which closely followed the results of similar experiments in mammary cancer. For example, forced expression of human GH in EC cells increased proliferative growth, rescue from serum-withdrawal induced apoptosis, anchorage independent cell growth, induction of EMT, and enhanced tumorigenicity in nude mice (187, 283). Additionally, autocrine GH was protective against several inducers of DNA damage-inducing anticancer therapies,

Table 5. Studies implicating growth hormone in endometrial cancer

Material	Method	Study summary	Reference
normal tissue expression	GH RNA/protein	expressed in glandular epithelia, drives cell proliferation	(276-279)
patient sample—IHC	autocrine GH	increased proliferation	(187)
patient sample—IHC	correlation with tumor GH expression	tumor GH expression correlates with ovarian metastasis, tumor grade	(183)
patient sample—IHC	GH protein expression	upregulated 3.8-fold in cancer samples	(280)
patient data	survival correlation analysis	tumor GH (or GH + PRL) levels correlate with poorer survival	(281, 282)
EC cells	autocrine GH	resistance to starvation induced apoptosis	(187)
EC cells	autocrine GH	resistance to radiotherapy induced apoptosis	(188)
EC cells	autocrine GH	resistance to chemotherapy (MMC, doxorubicin, cisplatin) induced apoptosis	(162, 189)
EC cells	autocrine GH	resistance to oxidative stress induced apoptosis	(190)
EC cells	autocrine GH	induces EMT, promotes migration-invasion, metastasis	(187, 283)
EC cells	GHRA (B2036) treatment	blocks inhibition of growth suppression	(187)
EC cells	GHRA (B2036) treatment	increases starvation induced apoptosis	(187)
EC cells	autocrine GH + GHRA (B2036) treatment	increases chemotherapy (MMC) induced apoptosis	(162)
EC cells	autocrine GH + GHRA (B2036) treatment	increases radiotherapy induced apoptosis	(188)
EC cell nude mice xenograft	autocrine GH	increased tumorigenicity and tumor growth	(187)

Abbreviations: EC, endometrial cancer; EMT, epithelial-to-mesenchymal transition; GH, growth hormone; GHRA, growth hormone receptor antagonist; IHC, immunohistochemistry; MMC, mitomycin C; PRL, prolactin.

including radiation and chemotherapy (mitomycin C [MMC], doxorubicin, cisplatin) (162, 188, 189). Notably, all the above effects of autocrine GH were attenuated significantly by GHRA (either B2036 or pegvisomant). To ascertain if this empirical evidence has clinical correlates, we used the KMplotter platform to assess GHR or GH1 expression associated OS and RFS in endometrial cancer patients (Fig. 5). We saw that a tumoral GHR expression does indeed correlate significantly with poorer OS and RFS in patients with EC. The results are similar for increased tumoral expression of GH in the same group of patients, with almost 3-fold difference in survival (in months) between high and low GH expression groups (Fig. 5). Our findings corroborate earlier reports that higher tumoral GH or both GH and PRL protein levels correlate inversely with 5-year survival of EC patients (183). Therefore, future studies aiming at sensitizing EC to antineoplastic regimens by combining GHR antagonism can pave the way for improved prognoses in patients.

Melanoma

GHR is expressed in the skin, preferentially in the dermal fibroblasts where it increases fibroblast cell numbers. IGF1 is also detected in dermal fibroblasts and increases proliferation of keratinocytes, which have abundant IGF1R levels (284), whereas both GH and IGF1 drive melanocyte proliferation (285). Several in vitro and in vivo studies have implicated GH's role in melanoma (Table 6). The relative upregulation of GHR in melanoma cells compared to normal cutaneous cells observed from a histopathologic analysis of 126 paraffin-embedded tissue samples was the first implication of GH in melanoma pathology (292). Autocrine GH RNA and protein are both detected in the human melanoma cells (287, 288) and show active downstream signaling via STAT5 and STAT3, as well as SRC, AKT-mTOR, and ERK1/2 pathways. Moreover, intriguing clinical reports of 2 patients, above the age of 50, were diagnosed with melanoma following 3 months of recombinant GH treatment (293). By assessing GHR expression levels in all 60 cancer cell lines in the National Cancer Institute's (NCI60) panel covering 9 different cancer types, we found that the metastatic melanoma cell lines have the highest level of GHR RNA expression, and mean GHR expression in melanoma cell lines was 50 times higher than the mean GHR expression for the entire NCI60 panel (286). A series of subsequent in vitro studies show that GH drives multiple oncogenic processes in melanoma including proliferation, induction of EMT, anchorage independent growth, migration-invasion, multidrug efflux, melanosomal drug sequestration, altered cellular metabolism, and chemoresistance (211, 288, 290, 291). In vivo, an excess of serum GH increases the intrinsic drug resistance in human melanoma cells, which was exacerbated with introduction of chemotherapy and was IGF1 independent (291). Moreover, both GH-knockout (GHKO) and GHA mice showed reduced implanted murine melanoma tumor growth. Interestingly, the GHA mice, unlike the GHKO mice, responded significantly better to cisplatin treatment compared to wild-type mice. This indicates that not the mere absence/suppression of circulating GH is sufficient to curb chemotherapy-induced drug resistance, which may be a function of autocrine GH that the GHRA can effectively block (211). Interestingly, a pathogenic splice variant of GHRHR has been observed in 61% of the melanoma samples compared to only 8% of the dysplastic nevi, but unlike the GHRHR found in the pituitary, this variant does not appear to control GH production in the melanoma cells (294). Moreover, some somatostatin receptors are also expressed in melanoma cells, but they are unable to regulate GH production. In contrast to GHR antagonism, pasireotide trials in malignant melanoma patients have not presented good efficacy (295). The strong corroboration of gene

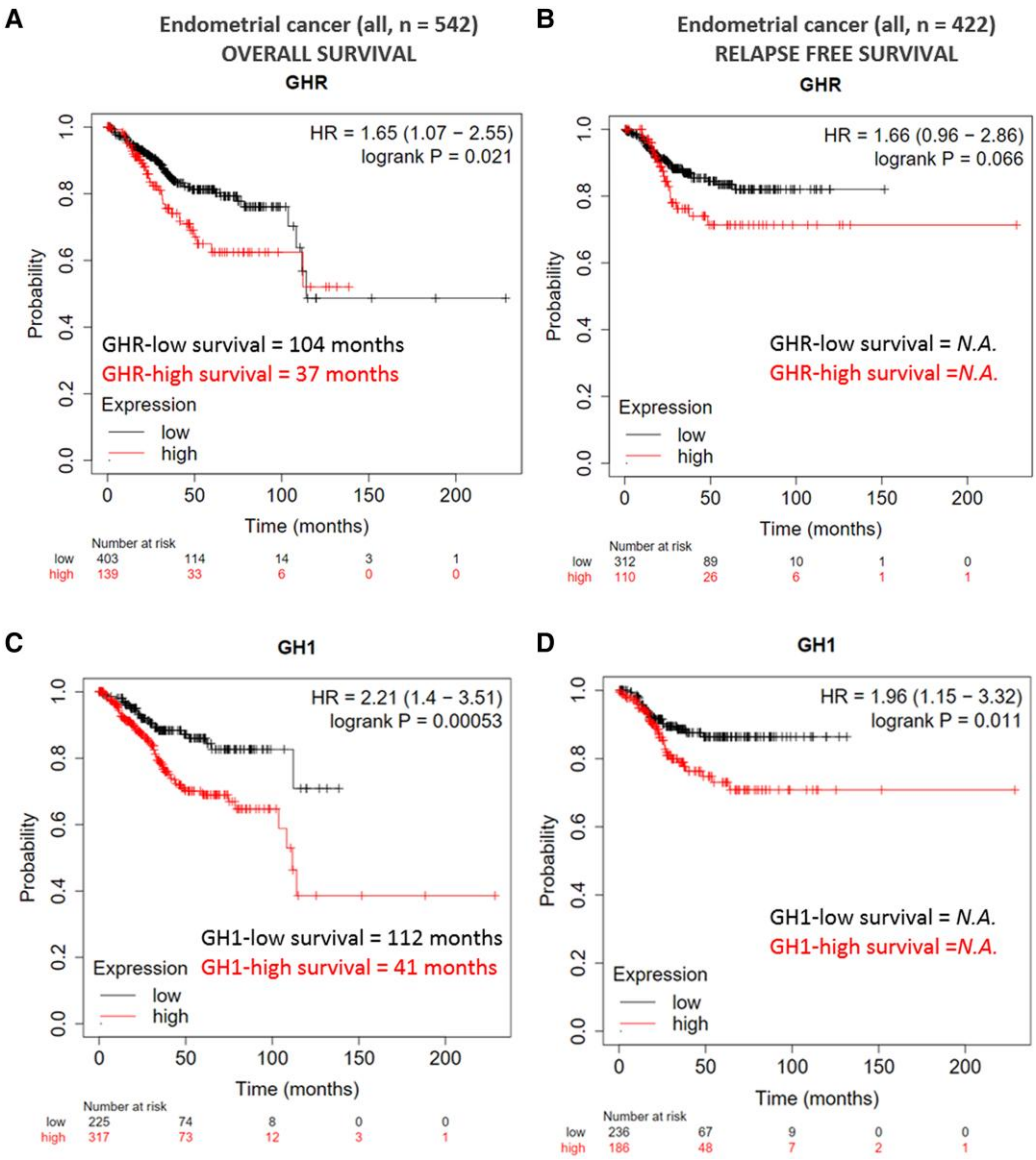

Figure 5. GH and GHR expression negatively correlate with overall survival (OS) and relapse-free survival (RFS) in endometrial cancer. KMplotter generated survival probability of endometrial cancer (EC) patients with low or high GHR expression (A, B) or GH1 expression (C, D). (A) OS correlation with GHR in all patients with EC (n = 542); (B) RFS correlation with GHR expression in patients with EC (n = 422); (C) OS correlation with GH1 in all patients with EC (n = 542); (D) RFS correlation with GH1 expression in patients with EC (n = 422).

expression patterns identified from in vitro experiments with that found in the Cancer Genome Atlas (TCGA) transcriptome of patients with melanoma, and the efficacy of GHR antagonism against both autocrine and exogenous GH, indicate that GHR antagonism may boost chemotherapy, targeted therapy, or immunotherapy approaches currently used in melanoma.

Gastric Cancer

Gastric cancer (GC) is one of the top 5 common and lethal cancer types globally. Both GHR and PRLR are detected throughout the normal gastric mucosal and glandular epithelium, as well as in all stages of GC progression from neoplasm to metastasis, indicating consistent GH action in this organ (296). An increase in GH action during oncogenic transformation in GC can be inferred from the fact that GHR is overexpressed in most primary GC samples compared to adjacent normal stomach tissue and correlates with worsening tumor grade and stage (297, 298). Moreover, supra-normal serum GH levels are often observed in GC patients and this correlates with poorer response to therapy (260, 299). Intriguingly, while the Ecuadorian patients with LS have almost zero cases of cancer, GC is responsible for as much as 30% of the cancer-associated deaths in their first-degree relatives (300). This does not, however, implicate endocrine GH in GC, due to lack of incidence in younger populations and no variation in incidence in acromegaly or in GHD patients treated with GH. Mechanistically, multiple in vitro studies implicate GH in promoting GC tumor proliferation, invasive growth, and suppression of apoptosis via a JAK2-STAT5 and PI3K-AKT pathway activation (297, 298, 301). In vivo, nude mice xenografts of GC cells transfected with GHR-targeting shRNA show significant suppression of tumor growth compared to GHR-sufficient xenografts (298), confirming the cell culture results. In fact, GHR is one of the top 5 biomarkers in GC

Table 6. Studies implicating growth hormone in melanoma

Material	Method	Study summary	Reference
patient sample	IHC for GHR	upregulated GHR expression in melanoma	(285)
different cancer cell lines (NCI-60)	GHR expression	melanoma has highest GHR expression among other cancer types	(286)
melanoma cells	conditioned media	spontaneous autocrine GH production	(287, 288)
melanoma cells	exogenous GH	increased proliferation	(287, 289)
human and mouse melanoma cells	exogenous GH	drug efflux, drug sequestration, resistance to chemotherapy (doxorubicin, cisplatin, paclitaxel, vemurafenib)	(288, 290)
cell line	exogenous GH	increases cell proliferation, induces EMT, promotes migration-invasion	(287, 289, 291)
cell line + GH	GHR knockdown	decreases ABC transporter expression, decreases drug efflux, reduces cell proliferation, blocks EMT, decreases migration-invasion	(289, 290)
cell line + GH	anti-GHR mAb	decreases migration-invasion	(287)
bGH, GHRKO mice	syngeneic allograft	high GH drives increased ABC transporter expression, increased EMT	(211, 291)
GHA, GHKO mice	syngeneic allograft	reduced ABC transporters, sensitive to chemotherapy (cisplatin)	(211, 291)

Abbreviations: EMT, epithelial-to-mesenchymal transition; GH, growth hormone; GHR, growth hormone receptor; IHC, immunohistochemistry.

with GH binding protein levels upregulated by 2.8-fold in the serum of GC patients compared to healthy subjects (302). Our survival analysis of 371 GC patient samples (TCGA dataset) using the KMplotter platform confirms the inverse correlation of GHR and GH expression with poorer patient survival in GC (Fig. 6). Increasing levels of both GHR and GH1 RNA levels in the tumor samples correlate with significantly higher hazard ratios for both OS and RFS in the patients (Fig. 6). Additionally, among 75 patients of advanced GC undergoing anti-PD1-antibody therapy, higher serum GH positively correlates with lower PFS, OS, and disease control rate (299), similar to observations in patients with HCC (discussed above). Recent studies indicate that in GC, GH's proliferative capacity is associated with a nuclear localization of the GHR and can be successfully antagonized by pegvisomant both in cultured cells and mouse xenografts (68). These results (summarized in Table 7) provide a preliminary but provocative indication that GHR abrogation may boost immunotherapy outcomes in cancer patients and necessitates immediate preclinical validation. Despite clear indications of autocrine GH expression, most of the above studies considered only the endocrine or exogenous GH administration. Therefore, future studies need to delineate the contributions of endocrine vs autocrine/paracrine GH action in GC for successful targeting and improvement of prognoses.

Brain Cancer

The effect of targeting the GHR on the prognoses of cancers of the CNS are sparse. As CNS tumors are enriched in pediatric patients, we re-emphasize that GH treatment in GHD pediatric patients even with prior cases of CNS tumor does not elevate risks of cancer compared to that in the GH-sufficient population (128). Nonetheless, several persuasive reports implicate GH action in the development and pathology of the CNS, including in cancers (listed in Table 8). For example, in meningioma, a common type of brain cancer, GHR is widely expressed in all samples tested and treatment with B2036 reduced cell proliferation of primary meningioma cultures (303). The efficacy of GHRA was further corroborated in slowing down tumor growth by > 40% in 15 different human patient-derived meningioma primary cell xenografts in nude mice and treated with pegvisomant for 8 weeks (304). In another very common brain cancer, glioma, an entire panel of glioma cell lines were positive for GHR and IGF1R expression (305). Subsequent studies in glioblastoma (GBM), which is the most common type of brain cancer, show GHR overexpression in one-third of the patients and enriched in tumors with suppressed EGFR levels, and often associated with a reduced expression of SOCS2 due to SOCS2 promoter/enhancer hypermethylation (309). Moreover, patient-derived GBM tumors in culture showed endogenous GH production and also showed aggravated xenograft growth in nude mice (309). In vitro assays with GBM cell lines show that GHRA (hGH-G120K) can successfully block GH-promoted cell migration and invasion (309). Additionally, STAT5B was identified as a marker of poor prognosis in GBM by correlation analysis of tissue microarray data with survival in 167 WHO 2016 GBM patient samples (312). Studies in brain gliomas have additionally addressed the distinction between the effects of pituitary (endocrine) and tumor-derived (autocrine) sources of GH. On one hand, the success of GHRH antagonists (MZ-5-156) in suppressing the growth of human glioma cells in vitro and in nude mice (306, 307) ascribe a role of endocrine GH in glioma, although GHRH can induce GH release from some extrapituitary neural tissues like retinal ganglion cells as well (313). On the other hand, a subsequent assessment of 25 glioma samples from cancer patients showed GH immunoreactivity in 100% of the samples along with colocalization with GHR (predominantly cytoplasmic than nuclear) (308), confirming a robust autocrine/paracrine GH action in brain tumors. Cyst fluids from GBM patients also show an enrichment of GH and positive correlation with tumor volume (310). Therefore, GHR remains as an untapped potential drug target in glioma. Importantly, IGF1 has a profound tumor-promoting and therapy-refractory role in glioma and has been enthusiastically investigated (314-317). By virtue of suppressive effects on both IGF1 and IGF2 (ligands of IGF1R) serum levels, pegvisomant and similar GHRAs can potentially have a significant effect in brain tumor therapy.

Bone Cancer and Metastases

In addition to the primary sites of osteosarcomas, bones are also a principal site for metastasis, quiescence, and relapse

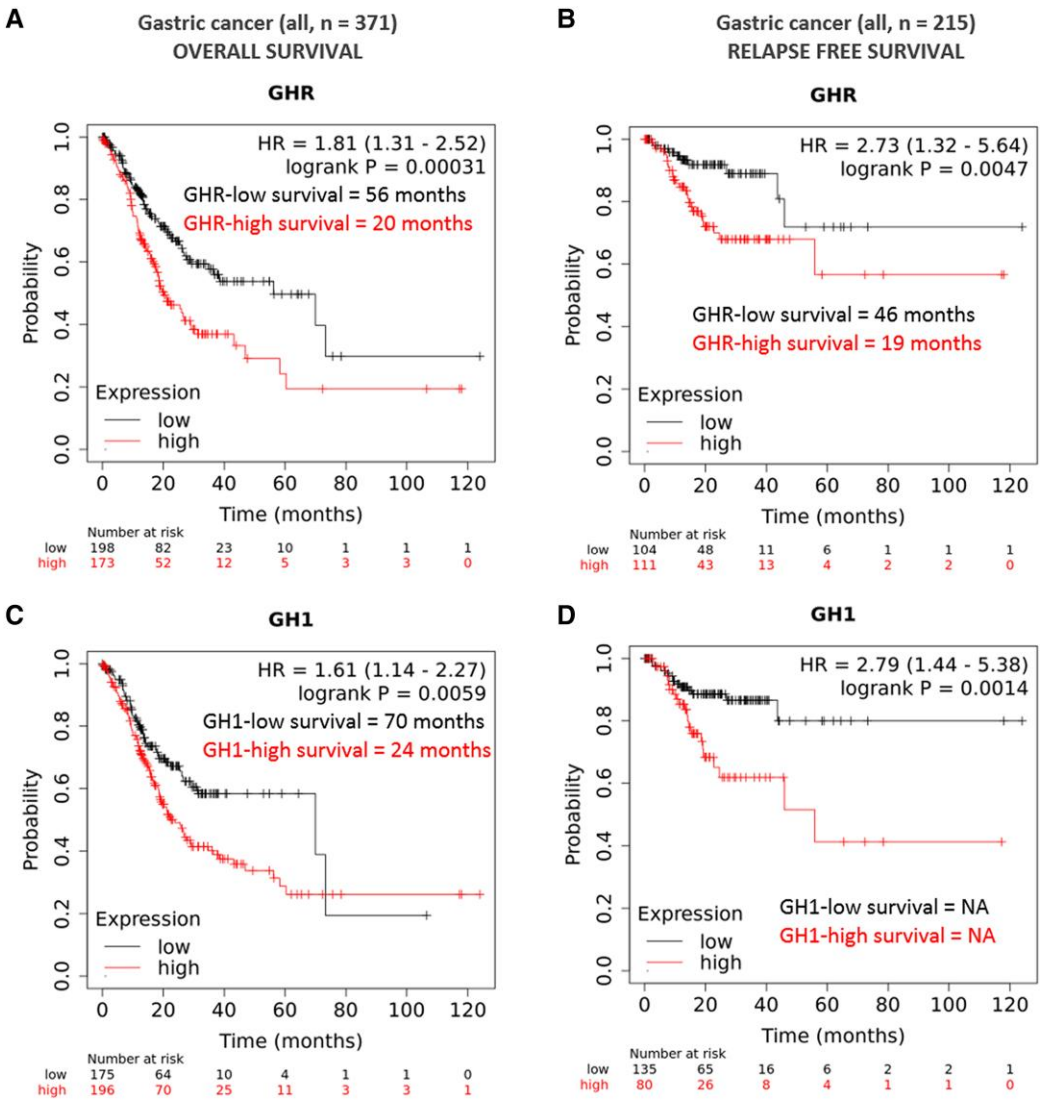

Figure 6. GH and GHR expression negatively correlate with overall survival (OS) and relapse-free survival (RFS) in gastric cancer. KMplotter generated survival probability of gastric cancer (GC) patients with low or high GHR expression (A, B) or GH1 expression (C, D). (A) OS correlation with GHR in all patients with GC (n = 371); (B) RFS correlation with GHR expression in patients with GC (n = 215); (C) OS correlation with GH1 in all patients with GC (n = 371); (D) RFS correlation with GH1 expression in patients with GC (n = 215).

for several cancers, including those of the breast and prostate (318), and are devoid of any effective treatment options. GH drives mesenchymal stem cell (MSC) differentiation into osteoblasts (319, 320) and has critical functions in the bone and cartilage tissues (321), including several IGF1-independent effects (322, 323). In bones, the importance of GH action in the context of cancer (listed in Table 9) is thus two-fold: in promoting bone tumors and also in promoting bone as a site for tumor metastasis. GH expression has been reported in canine metaphyseal growth plates as well as in 25% of studied canine osteosarcoma specimens (324) and, therefore, presents the premise for autocrine/paracrine GH action, abetting metastasis. GH signaling via JAK2/STAT5 (A and B) has also been shown in rat osteosarcoma cells (UMR106) that express endogenous GHR (325). Recently, Cheng et al have provided the first direct study of GH and human osteosarcoma (326). The authors showed that in human osteosarcoma samples, GHR expression was markedly higher than that in osteofibrous dysplasia, while in human osteosarcoma cell lines (143B and U20S), GHR knockdown promoted apoptosis, suppressed invasive phenotypes, and also suppressed xenograft growth in nude mice, primarily by suppression of the PI3K/AKT pathway (326). In patients with osteosarcoma and Ewing sarcoma, serum IGF1 and IGFBP3, downstream targets of hepatic GHR activation, are elevated and correlate with tumor grade and recurrence (327). Moreover, multiple recent studies using mouse models of congenital excess GH as well as GHR antagonism have revealed exciting molecular details implicating the GH-GHR pair in upregulating inflammation and loss of bone and cartilage in the joints (328-330). As inflammation is a hallmark of cancer (discussed below), it can be reasonably postulated that more studies targeting GH in bone tumor and/or metastasis posits promising results. Lastly, muscle is also a contributor of endocrine GH-induced IGF1 synthesis, and the proximal structural association of muscles and bones presents a rather enriched source of paracrine GH/IGF action in the TME (331).

Table 7. Studies implicating growth hormone in gastric cancer

Material	Method	Study summary	Reference
patient samples	RT-qPCR and Southern blot	GHR and PRLR expression in normal gastric epithelia, all stages of GC from neoplasm to metastasis	(296)
patient samples	RT-qPCR and IHC	GHR overexpressed in cancer vs adjacent normal tissue, correlates with worse tumor grade and stage	(297, 298)
patient serum sample	radioimmunoassay for GH	serum GH significantly higher than normal	(260)
patient serum	GH immunoassay and clinical correlation	higher serum GH correlates with poorer response to anti-PD1 immunotherapy	(299)
patient serum biomarker profiling	antibody microarray, ELISA	serum GHR increased by 2.8-fold in GC patients, GHR a biomarker of GC	(302)
patient samples, GC cell lines	GHR level, GH treatment	increased proliferation, increased EMT, decreased apoptosis, nuclear GHR	(68, 297, 301)
GC cells	GH treatment	inhibits growth suppression	(298)
GC cells	GHR knockdown	increases apoptosis	(298)
GC cells, nude mouse xenograft	GHR knockout cells	suppressed tumor growth	(298)
GC cells, nude mice xenograft	GHRA (pegvisomant) treatment	inhibits cell proliferation and tumor growth, blocks nuclear localization of GHR	(68)

Abbreviations: EMT, epithelial-to-mesenchymal transition; GC, gastric cancer; GH, growth hormone; GHR, growth hormone receptor; GHRA, growth hormone receptor antagonist; IHC, immunohistochemistry; PRLR, prolactin receptor.

Table 8. Studies implicating growth hormone in brain cancer

Material	Method	Study summary	Reference
meningioma—patient samples	RT-PCR	GHR expressed in all samples	(303)
meningioma—primary cell culture	GHRA-B2036 treatment	suppressed meningioma cell growth	(303)
meningioma—patient-derived xenograft in nude mice	GHRA (pegvisomant) treatment/8 weeks	suppressed *in vivo* tumor growth	(304)
glioma—cell lines	RT-PCR	GHR, IGF1R expressed in all samples	(305)
glioma—cell lines and nude mice xenograft	GHRH antagonist treatment	suppressed growth *in vitro* and *in vivo*	(306, 307)
glioma—patient samples	radioimmunoassay	GH and GHR present in all samples and colocalize	(308)
glioblastoma—patient samples	gene expression analysis	GHR expression in 1/3 of samples	(309)
glioblastoma—patient-derived cells in culture and nude mice xenograft	cell/tumor growth	endogenous GH production, aggressive growth *in vivo*	(309)
glioblastoma—patient sample	proteomic analysis	enrichment of GH, correlates with patient tumor volume	(310)
multiple brain tumor patient samples	IHC for GHR	73% of craniopharyngiomas, 59% of pituitary adenomas, and 23% in germ cell tumors express GHR	(311)

Abbreviations: GH, growth hormone; GHR, growth hormone receptor; GHRH, growth hormone releasing hormone; IGF1R, insulin-like growth factor 1 receptor; IHC, immunohistochemistry.

Table 9. Studies implicating growth hormone in bone cancer and metastases

Material	Method	Study summary	Reference
canine osteosarcoma samples	RT-PCR	GH present in 25% of samples	(324)
rat osteosarcoma cells + GH	immunoprecipitation, Western blot	GH activates JAK2-STAT5	(325)
patient samples	IHC	GHR overexpression in osteosarcoma samples	(326)
OS cell lines, nude mice xenograft	GHR knockdown	attenuated cell growth, increased apoptosis, suppressed xenograft growth	(326)
patient samples (OS and Ewing sarcoma)	RT-PCR and ELISA, clinical correlation	increased serum IGF1, IGFBP3 correlate with tumor grade and relapse	(327)

Abbreviations: GH, growth hormone; GHR, growth hormone receptor; IGF1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein.

Table 10. Studies implicating growth hormone in other cancers

Material	Method	Study summary	Reference
Lung cancer			
patient samples, cell lines	RNA, protein	GH and GHR widely expressed in normal lung tissue, promotes IGF1 production	(332-335)
patient sample	high-throughput sequencing	P495T mutation in GHR leads to decreased GHR deactivation, promotes oncogenesis	(336-338)
lung epithelial cells	P495T-GHR expression	induces EMT, promotes migration-invasion	(339)
Pancreatic cancer			
patient samples, human cell lines	GHR—Western blot, IHC	GHR is overexpressed in pancreatic tumor cells and tissue	(340)
human cell lines	GH treatment (+GHR knockdown)	increased proliferation (blocked by GHRA)	(340)
human cell lines	GH treatment (+GHR knockdown)	increased EMT (blocked by GHRA)	(340)
human and mouse cell lines	GH +/− pegvisomant	increased response to chemotherapy (gemcitabine, doxorubicin)	(341)
Nude mice +/− gemcitabine	GHRA (pegvisomant or comp-G) treatment	increased response to gemcitabine	(342)
cell line + GH	exogenous GH	induces EMT, promotes migration-invasion, (blocks EMT)	(340)
Lymphoma			
patient samples, cells	radioimmunoassay, Northern blot	GH and PRL expression	(343)
Burkitt lymphoma cell with GHR overexpression	DNA microarray, Western blot	resistance to methyl methanesulfonate-induced apoptosis	(344)
Burkitt lymphoma cell with GHR overexpression	anti-GH oligonucleotide	increased DNA damage	(344)
Cervical cancer			
CC cells	exogenous GH	induces EMT, promotes migration-invasion	(345)
Liposarcoma			
patient samples	RT-qPCR	GHR expressed in tumor samples	(346)

Abbreviations: EMT, epithelial-to-mesenchymal transition; GH, growth hormone; GHR, growth hormone receptor; GHRA, growth hormone receptor antagonist; IGF1, insulin-like growth factor 1; IHC, immunohistochemistry; PRL, prolactin.

Other Cancers

The cancer types discussed above share a relatively higher volume of empirical studies implicating GH action in different parts of tumor development and therapeutic response. In addition to these, numerous other excellent studies spanning multiple other cancer types (listed in Table 10) have affirmed the role of GH in promoting cancer.

Human lung cancer is one of the most incident and fatal cancer types. GH and GHR are expressed in the lungs, where autocrine/paracrine GH action is known to regulate a proteome signature which includes IGF1 (332-335). Three independent studies from the UK, China, and USA have reported that the *GHR*-P495T SNP in the *GHR* gene (C to A on GHR1526; rs6183), resulting in an amino acid change at peptide position 495 from proline to threonine, is associated with a markedly higher odds ratio for both small cell and non-small cell lung cancer (336-338). Chhabra et al have described that the P495T mutation impairs the SOCS2 binding site along with a phosphorylated Y487 at the intracellular domain of the activated GHR leading to a sustained activation of GHR (339). This oncogenic role of GHR in lung cancer is indeed an excellent example of the important position of GH action as a target in cancer.

Pancreatic cancer is a highly common, lethal, and therapy-resistant malignancy, due to difficulties in early diagnoses and an intrinsic antineoplastic resistance. Compared to its low expression in the normal acinar and ductal cells, GHR is overexpressed in pancreatic ductal adenocarcinoma (> 90% of pancreatic cancer cases). Pancreatic cancer cell lines and human pancreatic ductal adenocarcinoma samples show significant overexpression of GHR, positively correlating with worsening tumor stage (340). In vitro experiments with pancreatic cancer cell lines show exogenous and autocrine GH-driven resistance to apoptosis, induction of EMT, and resistance to chemotherapy (340-342). In a male and female nude mice xenograft study, cotreatment with GHRA and gemcitabine significantly improved the efficacy of the chemotherapy, resulting in multiple tumor-free mice at the end of the study (341, 342). Chemotherapies such as gemcitabine, paclitaxel, and folfironox, which are currently the leading treatment approaches in pancreatic ductal adenocarcinoma, are substrates of ATP-binding cassette (ABC) transporters upregulated by GH action. Therefore, GHR targeting should be explored to restore cytotoxic capacities of existing chemotherapeutic options, using relevant mouse models.

GH is produced by the ovary (347), cervix (348), endometrium, and breast tissues as discussed above (135), with known autocrine/paracrine functions via consistent GHR expression also observed in each of these tissues (348-351). Moreover, it is well-studied that estrogen exerts an inhibitory effect on GH action in peripheral tissues (352-354). Despite distinct roles of GH in the pathophysiology of these organs (355), the effects of GH signaling or the antagonism thereof in the cancers of ovary and cervix are so far overlooked compared to the large volume of studies in breast and endometrial cancers. A single study in cervical cancer showed that exogenously added GH preferentially induces an epithelial-mesenchymal hybrid phenotype (increased cell-scattering, migration, EMT marker

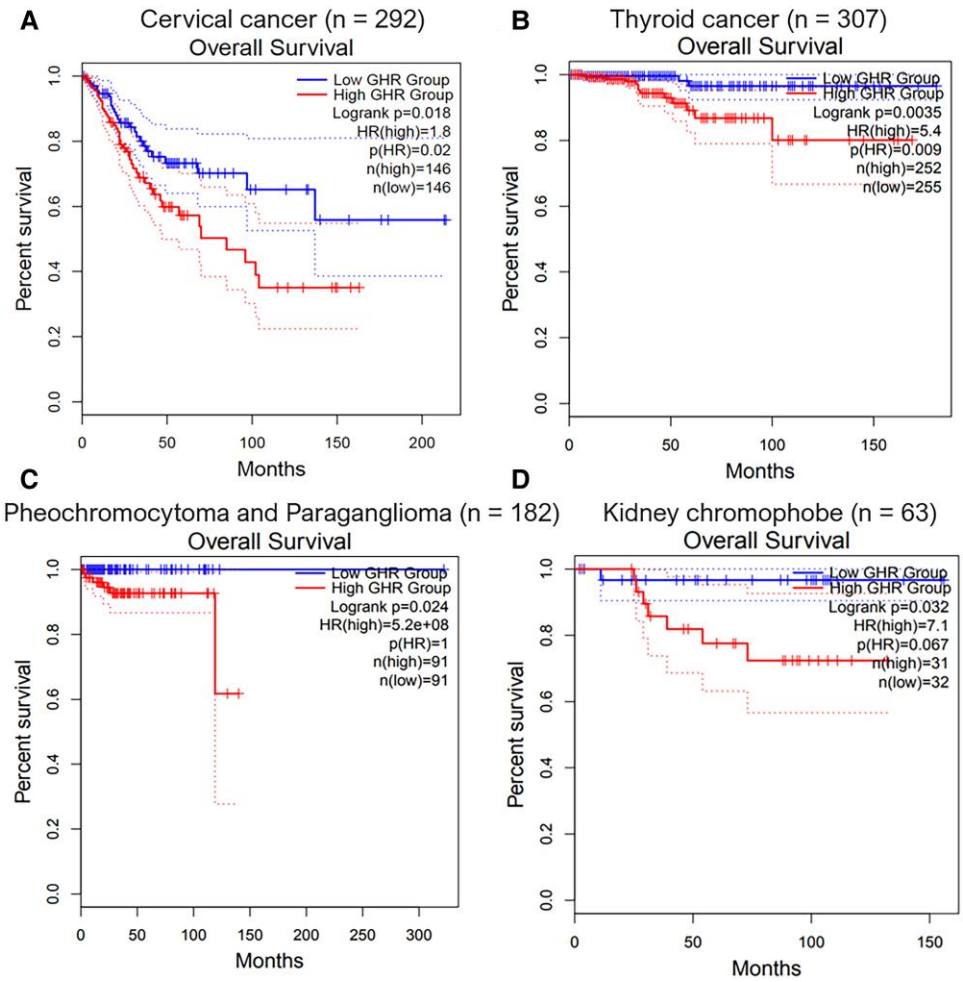

Figure 7. GHR expression negatively correlates with overall survival (OS) in different cancer types. GEPIA2 platform generated survival probability in cancer patients with low or high GHR expression for (A) cervical cancer (n = 292); (B) thyroid cancer (n = 307); (C) pheochromocytoma and paraganglioma (n = 182); and (D) kidney chromophobe (n = 63) from The Cancer Genome Atlas (TCGA) database.

expression) in cervical cancer cells but not in noncancer cells (345). In our analysis of the TCGA database for patients with cervical cancer, a significant correlation of GHR expression with poorer OS and higher hazard ratio was observed (Fig. 7A), corroborating the validity of the single study and highlighting the importance of GH action in cervical cancer. The age-associated nonpituitary GH production in these tissues is unknown and needs to be ascertained. Moreover, the status and impact of GH-induced PRLR activation is critical yet mostly unexplored in any of these cancer types which have high PRLR expression, since PRLR activation is known to be detrimental in all of these cases (169, 356-359).

A number of cancers, despite known GH and/or GHR expression and function in the normal physiological state, lack any studies in their role in the corresponding malignancy. One example is thyroid cancer, where in our assessment of thyroid cancer patients in the TCGA database, we observed a clear inverse association of patient survival with increasing GHR expression (Fig. 7B). Similarly in the rare neuroendocrine cancers pheochromocytoma and paraganglioma, developing from chromaffin cells of the neural crest, IGF1 signaling has been well-studied (360), but GH action has not been checked. We found that tumoral GHR expression strongly correlates with markedly poorer patient survival (TCGA dataset) in this cancer type (Fig. 7C). Similarly, in chromophobe renal cell carcinoma, a rare kidney tumor of low malignancy and high survival rate, GHR expression significantly correlates with poorer patient survival (Fig. 7D). Tumor-promoting actions of IGF1 have however been well-studied in renal cancers (361, 362) and validates a space for application of GHR antagonism to lower IGF1R ligands in renal cancers, Human GH is expressed by several lymphocytes and leukocytes (reviewed later), while autocrine GH production has also been observed in some lymphomas, such as Burkitt lymphoma (343, 363), where autocrine GH conferred resistance to methyl methanesulfonate-induced apoptosis (344). Lastly, consistent with extensively studied role of GH-GHR pair in the AT, the presence of GHR has been reported by a single report in lipoma (8/12 samples) and liposarcomas (3/10 samples), especially in the vascular endothelium of the liposarcomas (346), although no additional follow-up studies in this area are reported.

We must mention here that overexpression of *GH1* or *GHR* in the tumor does not automatically correlate with poorer overall survival in all cancer types. In some cancers (eg, liver cancer), tumoral GHR expression is in fact positively correlated with survival in several patient groups (discussed above). Yet, in vitro, in vivo, and even in human patients a clear

anticancer benefit using GHRAs in liver cancer is reported (discussed above). Resultantly, as with any particular gene in cancer, it would not be prudent to draw a universal correlation of *GH1* or *GHR* expression with decreased survival merely based on the cancer type and is highly dependent on different characteristics of the cohort (age, sex, race, treatment type, tumor grade and stage, clinical history) under consideration. Additionally, as now confirmed by single-cell sequencing, expression of a given gene has an astounding degree of inter- and intra-tumor heterogeneity (364, 365) and, therefore, each tumor in individual cancer patients is unique, and biopsy samples can help capture only a fraction of it. Therefore, inclusion of high-resolution methods like single-cell analyses in preclinical and clinical studies is key to ascertaining the extent of GH action in the TME of a particular cancer and is critical in determining the subsequent applicability of GHR antagonism in a cancer patient.

In essence, the above series of clinical and empirical findings strongly support and validate a definite role of GH/GHR expression and action in the genesis and progression of different human cancers and constitutes a body of evidence which is more than associative. These studies and others have in detail also elucidated the molecular mechanisms underlying the observations and are essential in adequately understanding the clinical value of targeting GH action in cancer. Below we summarize the details of numerous mechanistic findings of GH's versatile effects in cancer, which further emphasize the limited role of endocrine GH in age-associated cancer development and vindicates the autocrine/paracrine action of nonpituitary (peripheral tissues, tumor) GH in cancer.

Mechanisms of Action of GH in Cancer

With time, our understanding of cancer as a disease has graduated from an initial reductionist view of a tumor being a homogeneous mass of aberrant transformed cells, to the current single-cell resolution view of a tumor being a highly heterotypic and efficient organ system with a multitude of distinct cell types working in tandem to ensure survival and expansion (20). Starting in year 2000, Hanahan and Weinberg introduced a landmark treatise following the evolving understanding of cancer as a disease (20, 366, 367) titled "Hallmarks of Cancer." It provides the conceptual scaffold to understand the functional capabilities acquired for neoplastic transformation and maintenance of malignancy. The *basic features* of a tumor cell, as introduced in 2000 are sustained proliferative signaling, evading growth suppressors, enabling replicative immortality, resisting cell death, activating invasion and metastasis, and inducing or accessing vasculature (20). In the following years, additional *emerging hallmarks* (ie, deregulating cellular metabolism, avoiding immune destruction, nonmutational epigenetic reprogramming, unlocking phenotypic plasticity) and *enabling characteristics* (ie, tumor-promoting inflammation, genome instability and mutation, senescent cells, polymorphic microbiomes) were added (366, 367). Together these functional requisites are defining features of the TME, composed of tumor and associated nontumor cell milieu. A plethora of existing and rapidly emerging evidence mechanistically implicates GH in nearly all these hallmarks of cancer. Both the tumor and the nontumor components of the TME can express GH and GHR, which can be potentiated by the DNA-damaging antineoplastic therapies (chemotherapy, radiation) and also aging. Therefore, for the brevity of discussion here, we partition the mechanisms of *direct GH action* in the TME into: (i) direct actions on tumor cells of TME; and (ii) direct actions on nontumor cells of TME.

Additionally, 2 *indirect GH actions* are of paramount importance from the therapeutic perspective and often deteriorates prognoses in several cancers: (i) production of IGF1; and (ii) promoting insulin resistance and hyperinsulinemia. Below, we briefly summarize only the relevant information on these indirect actions of GH to deliberately bring into context the putative "indirect" effects of GHR antagonism in cancer.

Direct Action of GH on Tumor Cells of the TME

The direct actions of GH (mostly autocrine/paracrine) on the tumor cells of the TME (Fig. 8), with reported mechanistic details, are summarized below.

Sustained proliferative signaling

A large body of studies on GH and cancer collectively affirm that GH action drives proliferative signaling, exemplified by GH-induced increase and GHR antagonism (siRNA, shRNA, GHRA)-induced attenuation of proliferative growth of tumor cells in culture (in vitro) and xenograft tumor volume increase in mice (in vivo) (see Tables 1-10). GH-mediated activation of GHR has been reported to promote tumor cell proliferation in multiple models of several human and rodent cancers. Mechanistically, in addition to the endocrine effect of GH in producing another highly potent cellular growth factor IGF1, there are multiple signaling circuits underlying this direct GH-mediated effect: (i) direct induction of proliferative intracellular signaling via tumoral GHR activation; (ii) direct induction of proliferative intracellular signaling via tumoral PRLR activation; (iii) nuclear localized GHR signaling; (iv) GHR-associated JAK2 mediated cross-phosphorylation of other tyrosine kinase receptors (eg, EGFR, IGF1R); (v) increasing circulating levels of a potent tumor cell growth promoters like insulin due to excess GH-induced hyperinsulinemia; (vi) suppression of some mechanisms of inactivation, such as SOCS-2, perpetuating GH action and tumor proliferation (339); and (vii) tumor cell directed induction of GH production by nontumor cells of TME and/or increase of the surface expression of GHR (366). Specific SNPs of *GH1* are also known to increase risks for breast cancer (368) and colon cancer (248, 369), although multiple related genes are frequently affected by SNPs in the *GH1* locus.

Importantly, in most human tumors, a repertoire of growth factors capable of driving proliferative signaling exists, and the tumors attain growth factor autonomy by switching on ectopic production of these growth factors into an autocrine/paracrine route. GH is one of these many growth factors and is produced by tumor and tumor-associated nontumor cells. Similar to most successfully targeted growth factors in cancer, tumor cell proliferation does not seem to be GH obligatory, as several tumors lacking GHR and/or GH do show uninterrupted growth in vitro and in vivo. Moreover, in almost every xenograft model tested (discussed above), attenuation of GHR signaling alone decelerates but does not completely stop the tumor growth. Therefore, despite its proliferative potential and abundant production at the tumor site, it is plausible that the role of GH in the TME is more specialized than driving sheer cellular multiplication, especially given

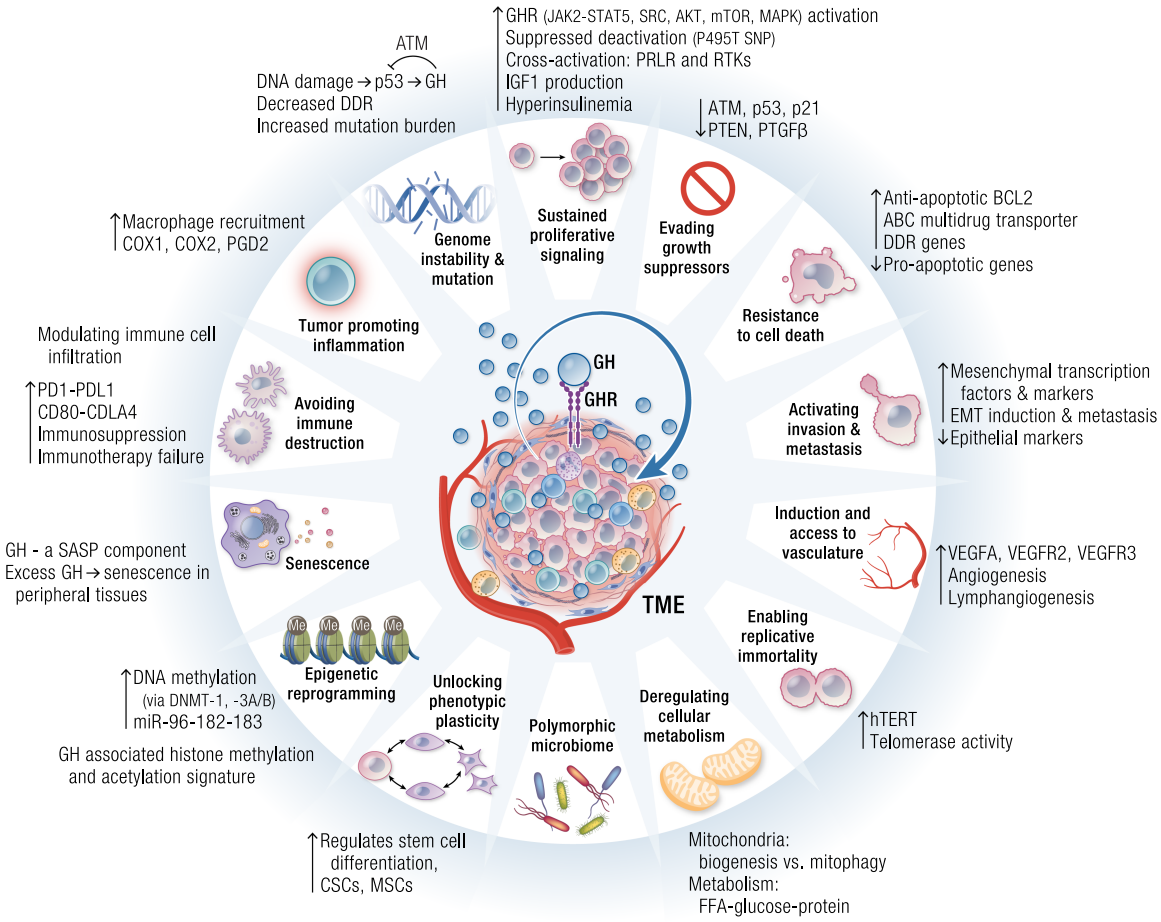

Figure 8. Direct tumor-promoting actions of GH on the cancer/tumor cells of tumor microenvironment (TME): multiple cells in the TME, including the cancer/tumor cells, can produce GH and express GHR (and PRLR), thereby enabling an autocrine/paracrine GH action in the TME. The direct effect of GH on the cancer/tumor cells at the TME include almost all the "Hallmarks of Cancer" (as defined by Hanahan, 2022), reported across hundreds of studies in GH and cancer to date. While the implications of GH-regulated variations in "polymorphic microbiomes" in cancer are still at a nascent stage, a compelling volume of evidence continues to accumulate detailing autocrine/paracrine GH's contribution in several hallmarks of cancer.

the known versatility, range, and tissue-specific exclusivity of GH's normal physiological actions.

Evasion from growth suppressors

Strategies for evasion from cellular growth suppression in tumors include downregulation and/or loss-of-function mutations of tumor suppressor genes like *TP53*, *PTEN*, and others. Example of GH-regulated evasion from growth suppression is reported in MCF7-hGH cells where autocrine GH represses transcription of placental transforming growth factor β (PTGFβ; a mediator of cell cycle arrest and apoptosis) and concomitantly increases mitogenic factor cyclin-D1 (184), also observed in GC cells (298). More recently, GH has been described as a p53 target and in turn a negative feedback regulator of p53 to inhibit apoptotic cell fate. In agreement, treatment of human endometrial cancer cells with a GHRA (B2036), increased the levels of tumor suppressor genes *ATM* and *TP53*, as well as multiple pro-apoptotic genes (187). Along such actions, local GH does indeed appear to promote a "field cancerization" amenable to neoplastic transformation, by additionally attenuating another tumor suppressor gene, phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*), in human colon cell lines and organoids (30).

Resistance to cell death

GH promotes resistance to cell death via multiple mechanisms (like attenuation of apoptosis, upregulation of DDR, and promotion of cytotoxic drug efflux) which have been identified and consistently observed across a number of human cancers. GH-GHR downstream signaling regulated cell fate determination via apoptosis is fairly consistent in normal and tumor cells. For example, human GH-mediated STAT5 activation protects pancreatic beta cells from IL1β-, IFNγ-, and TNFα-induced apoptosis in a NO-independent manner, by increasing expression of anti-apoptotic *BCLxL* gene, which harbors STAT5 promoter/enhancer binding sites (370). Moreover, forced expression of GH in immortalized mammary epithelial cells induced oncogenic transformation and conferred anti-apoptotic effects via BCL2 upregulation (154). This GH-mediated increase of anti-apoptotic *BCL2* genes and suppression of pro-apoptotic genes has been observed across multiple cancer studies as well. In cancer cells, autocrine GH conferred protection from apoptosis induced by serum starvation (187), ionizing radiation (188), the alkylating agent mitomycin C (MMC) (162), hydrogen peroxide-induced oxidative stress (190), or chemotherapies like doxorubicin and cisplatin (189). A series of studies with MCF7-hGH cells have revealed some of the mechanisms by

which autocrine GH (but not exogenous GH) promotes anti-apoptotic rather than mitogenic pathways. Examples of this include (i) increase in expression of homeobox domain containing protein HOXA1 and the anti-apoptotic protein BCL2 conferring doxorubicin resistance (185); and (ii) increase in the expression of DDR genes like DNA repair helicase (ERCC3), ATP-dependent helicase II, DNA repair protein XRCC1, superoxide dismutase, UV excision repair protein RAD23, and growth arrest and DNA-damage inducible protein (GADD) 45 and GADD153 (CHOP), which protects the cells from apoptosis induced by serum withdrawal in a JAK2/p38-MAPK dependent manner (156, 157, 186). GH dose-dependent upregulation of GADD45 and APEN proteins also protects CRC cells from ionizing radiation-induced DNA damage (258).

GHRAs (B2036 or pegvisomant) have shown efficacy in blocking anti-apoptotic effects of GH in several studies (157, 298) and sensitized cancer cells to the apoptotic effects of MMC (162), doxorubicin, and ionizing radiation therapy (160, 163, 188). The effects of exogenous GH in conferring chemoresistance were found to be independent of IGF1 or estrogen signaling (160). Importantly, in contrast to its role in DNA damage accumulation in normal cells, GH in tumor cells actively drives DDR, ensuring protection from apoptosis (263), which could be a function of altered downstream signaling intermediates (STAT5 vs SRC). This tumor-supportive role of GH is particularly important in resistance against anticancer radiation therapy, which has been extensively reviewed (204, 273, 371). Interestingly, promoting the DDR pathway in a tumor cell also decreases the tumor mutation burden, which in turn suppresses the expression of mutation-associated neoantigens (MANA), which are critical for determining efficacy of approved and developing targeted therapies in cancer. It needs to be investigated whether GH action, as a corollary of promoting DDR in tumor cells, upregulates suppression of MANA levels in tumor cells.

Most tumor cells overexpress (especially following initiation of chemotherapy) ATP-binding cassette containing transporter (ABC transporter) proteins, which are intrinsic methods of cellular detoxification exploited by tumor cells to remove chemotherapy (372). There are 48 different types of vertebrate ABC transporters with a very broad range of antineoplastics as substrates and significant substrate overlap, which makes it challenging to therapeutically target individual transporters (373). ABC transporters decrease intracellular drug retention, enabling protection from induction of drug-induced DNA damage and activation of the apoptotic program (374, 375). GH exerts both IGF1-dependent and IGF1-independent regulation of ABC transporter gene expression, as demonstrated in melanoma (211, 288, 290, 291), liver cancer (211, 237), ER-negative breast cancer (145), and pancreatic cancer (J.J.K., *manuscript under review*) in vitro and in vivo where GHR attenuation significantly improved the therapeutic efficacies of sorafenib, docetaxel, and gemcitabine, respectively. We further compared the effects of cisplatin treatment in the GH-knockout mice (GHKO—*low GH, low IGF1*) and GHA mice (transgenic for GHRA, *high GH, low IGF1*). These experiments clarified that although absence of GH in GHKO animals decelerated tumor growth compared to wild-type mice in vivo, it did not further improve chemotherapy efficacy (211). However, the presence of a circulating GHRA (as in GHA mice) was able to reduce cisplatin-induced ABC transporter levels and further improve cisplatin efficacy (211). These findings appear to have a clinical relevance, as we indeed find a high degree of correlation between RNA expression of *GHR* and major ABC transporters implicated in cancer drug resistance in human patient tumor transcriptomic data across 40 different cancer types (Fig. 9A). Recent reviews have assimilated the details of GH-mediated resistance to the apoptotic effects of cytotoxic agents (radiation and chemotherapy) in normal and cancer cells (240, 273, 371) and present a persuasive argument for implementing GHR antagonism for enhancing/restoring the clinical efficacy of these types of anticancer agents.

Activation of invasion and metastasis

Consistent data found in multiple human cancer types indicates that GH's ability to effect cellular differentiation as observed in normal cells appears to be harnessed by the malignant tumor cells in driving expression of genes that induce phenotypic plasticity—for example, the EMT program (367, 376). GH is a potent inducer of a subtype of EMT in normal cells in cases like wound healing, tissue repair, and organ fibrosis and has been consistently found in cancer types to induce tumoral EMT as well (377). In this case, multiple studies using the MCF7-hGH and additional cell lines of ER+, ER−, and TNBC subtypes with forced expression of GH undergo extensive rearrangement of gene expression under autocrine/paracrine GH effect (36, 159, 161, 162) and differentially involve both the SRC and the JAK2-STAT5, and STAT3 signaling pathways. Relatively consistent across cancer types (187, 237, 253, 283), the events include a suppression of cell-cell adhesion factors (eg, plakoglobin, E-cadherin, claudin-1, occludin), with concomitant upregulation of EMT-inducing transcription factors (eg, Snail, Slug, ZEB1/2, Twist1) and appearance of mesenchymal markers (like vimentin, N-cadherin) (reviewed in (240, 371, 377)). In these cells, autocrine GH also induces the microRNA cluster miR-96-182-183, which targets breast cancer metastasis suppressor 1-like (BRMS1L) protein and drives invasive xenograft growth in nude mice (36). Importantly, exogenously added GH in cultured cells have elicited very similar responses in different cancer cells as well as in mice. Using the same rationale, transfection of normal cells with the P495T SNP variant of GHR (deficient in SOCS2-mediated receptor deactivation) induced sustained EMT (339). In several of the above studies, attenuation of GH signaling using either RNA-interference or GHRA have successfully suppressed EMT induction and invasive tumor phenotypes. An additional effect of the EMT initiation is the extensive production of extracellular matrix (ECM) rearrangement components (eg, collagen) and factors (eg, matrix metalloproteases), which are strongly promoted by GH in non-tumor tissues (240). Autocrine GH action has been found to drive this desmoplastic event (378) as indicated by the pronounced fibroblastic stroma surrounding MCF7-hGH orthotopic xenografts in nude mice, compared to corresponding controls (159).

In corroboration of the above, histopathological analyses of different human cancer patient samples show a significant association of tumoral GH expression with lymph node and proximal tissue metastasis (183, 253). Furthermore, even after metastasis, GH production of primary tumors was maintained in the secondary sites as well (35-37), whereas GHR expression (both epithelial and stromal compartments) was found to be remarkably increased in axillary lymph node

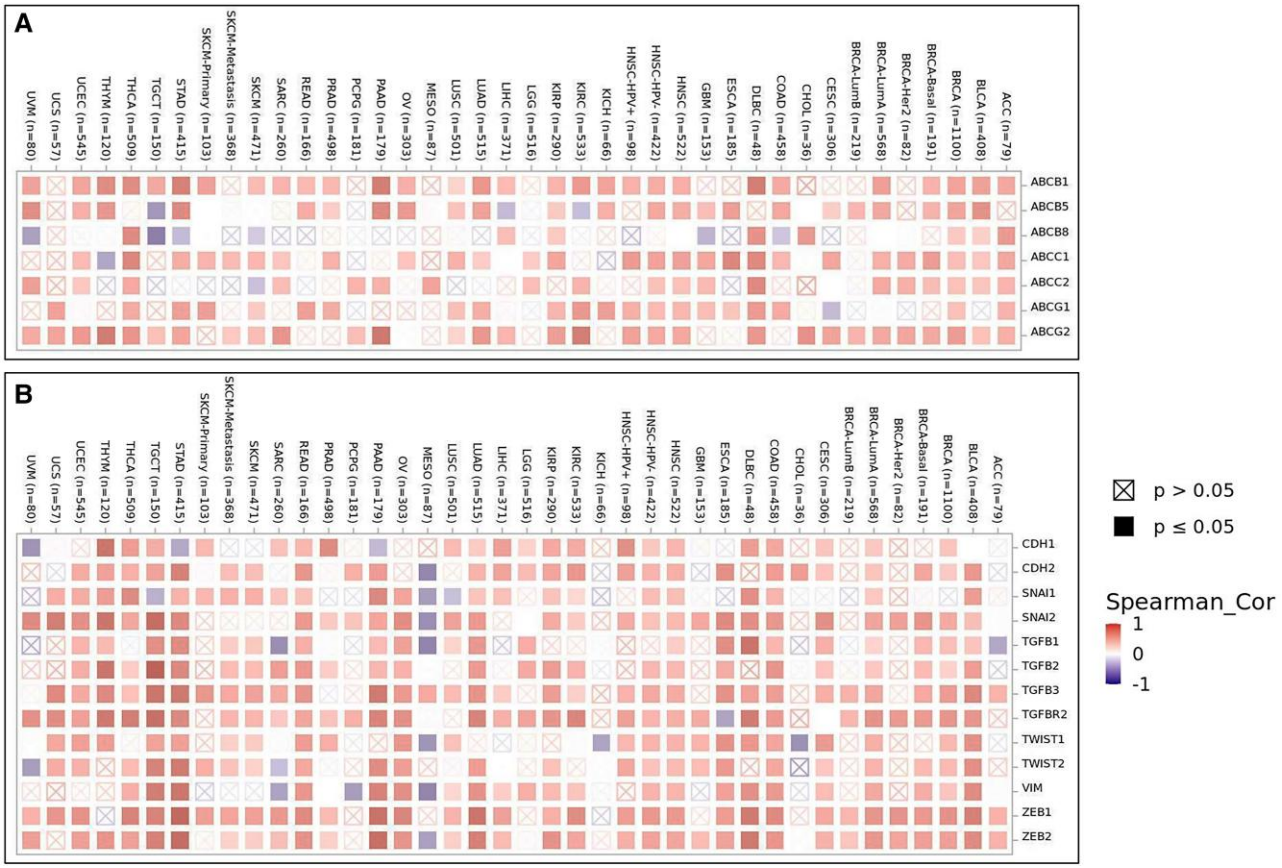

Figure 9. Pan-cancer correlation of (A) GHR and ABC transporters; and (B) GHR and epithelial-to-mesenchymal transition (EMT) mediators across 40 different cancer types (TCGA datasets).

metastasized ductal invasive mammary tumor cells, compared to nonmetastatic controls (144). These findings from in vitro and in vivo studies are consistent in human patients (TCGA dataset) where RNA levels of *GHR* and several markers of EMT show a robust correlation, as observed in 40 different cancer types (Fig. 9B).

Induction and access to vasculature

The ability of GH treatment to ameliorate vascular insufficiencies in GHD children, and identification of several vascular dysfunctions under a state of GH/IGF1 excess in the case of acromegaly while endothelial functions are unperturbed in patients with LS (379), suggests a distinct role of excess GH action in the endothelium (380). Importantly, both GH and GHR are expressed in the blood vascular endothelial cells (BVECs) (381). Significant GH expression has been reported in cultured human microvascular endothelial cells HMEC1 (382), in bovine brain capillary endothelial cells (383), and in vascular endothelium of blood vessels infiltrating tumors in human BC and EC patient samples (183). The GHR is also expressed by the blood vascular endothelial cells in culture and in patient samples, more frequently than GH. As early as the 1950s, Snell and Ticoll demonstrated that prolonged GH treatment in rats lead to increased neoplasms in the lymphatic tissues than that found in untreated controls (384), while GHR is frequently upregulated in vascular pathologies (385). Subsequently, it was shown that forced GH expression in MCF7-hGH cells promotes survival, proliferation, migration, and invasion of co-cultured HMEC1 cells and stimulates tube formation in vitro via an increase in VEGFA expression (382). GHR expression is 5-30-fold higher in the lymphatic endothelial cells compared to blood vascular endothelial cells, co-localizes with markers of lymphangiogenesis (386) and appears to be under the regulation of the lymphatic master transcription factor PROX1 (387). Moreover, GH drives lymphangiogenesis in a VEGFR2 or VEGFR3 independent manner and GH action does not induce IGF1 in the lymphatic endothelial cells (386). Tumoral GH can thus be an autocrine/paracrine trophic factor for tumor neighboring endothelium. Overall, GH's ability to promote angiogenesis and lymphangiogenesis in normal cells is exploited by the tumor cells to ensure tumor survival, possible systemic crosstalk, and metastatic dissemination, potentially being another effective target that would inhibit cancer progression. Therefore, inhibition of GH action has the ability to potentiate the efficacy of anti-VEGF treatments.

Enabling replicative immortality

GH has been implicated in enabling replicative immortality in tumor cells, by upregulating telomerase activity. In the MCF7-hGH cells, autocrine GH, via JAK2, increased the expression of RNA-binding alpha complex proteins αCP1 and αCP2, which in turn bind to CU-rich cis-regulatory elements in the 3′-UTR of human reverse transcriptase catalytic subunit (hTERT) mRNA and stabilizes the latter (388). hTERT is a critical functional component of the telomerase complex and sustained hTERT mRNA stabilization restores telomerase activity in cells, allowing replicative immortality. This remains a prominent but solitary example of GH-mediated telomerase promotion in cancer. It is possible, though, that

excess GH action might affect telomere shortening differently in nontransformed cells, as skin fibroblasts from patients with acromegaly have shorter telomere lengths (389). Thus, additional studies are required to corroborate these findings and validate the results in vivo as well as in other types of cancers with GH and/or GHR overexpression.

Deregulating cellular metabolism

Endocrine GH has an important role in maintaining metabolic homeostasis, with well-defined actions on glucose, lipid, and protein metabolism (390) as well as in the mitochondria (391, 392). Alterations in normal metabolic pathways are a hallmark feature of cancer, essential to provide mechanisms of supply of cellular building blocks for supporting the excessive demands of tumor proliferation. In the 1930s, Warburg pointed out that unlike normal cells, cancer cells largely prefer glycolysis irrespective of oxygen availability, as glycolysis can generate more intermediates for cell building macromolecules. A steady supply of glucose is required to sustain this aerobic glycolysis and is mediated by increasing GLUT1 and GLUT4 in cancer cells. GH also increases cellular glucose content via increasing de novo gluconeogenesis and glycogenolysis, a situation perfect for tumor glycolytic fueling. We have indeed observed that in human melanoma cells, exogenous GH increases extracellular acidification rate (ECAR, an indicator of glycolysis) in a dose-dependent manner (288), although GH can suppress glycolysis in inflammatory macrophages (393). In AT, GH normally promotes lipid breakdown and release of free fatty acids (FFA) in circulation. Cancer cells are now known to mobilize lipolysis in neighboring adipocytes to release FFA, which are imported via specialized membrane transporters like fatty acid translocase (FAT or CD36), and the fatty acid transporter protein (FATP or SLC27) family and can either be stored (as lipid droplets) for subsequent NADPH and acetyl-CoA production via β-oxidation or used for membrane biosynthesis (394). Therefore, autocrine/paracrine GH in the TME has a definite potential to recapitulate the lipolytic functions of endocrine GH and promote tumor growth via FFA supply. GH's role in mitochondrial biogenesis has shown both promoting and suppressing effects while mitophagy has not been studied (392). Altogether, the status and bearing of age-associated occurrence of tissue-specific GH increase and metabolic shifts in cancer etiology possess ample merit to warrant further study.

Avoiding immune destruction

The persistence of tumor growth and/or relapse is highly dependent on successful evasion of the constant surveillance and elimination performed by the body's immune system. Understanding these mechanisms has been the pillar of the transformative immunotherapy approach in cancer, ushering in approaches like the immune checkpoint inhibitors and the chimeric antigen receptor T-cell (CAR-T) therapies. Accumulating evidence has begun to indicate that GH action is associated with the success of immune checkpoint inhibitors, as observed in patients with HCC at the MD Anderson Cancer Center in Texas, USA and patients with GC in China (discussed above). In the first case, out of 37 patients with HCC followed for 1 year post-treatment with atezolizumab (anti-PDL1 mAb; immune checkpoint inhibitor) and bevacizumab (anti-VEGF), the cohort with higher serum GH levels had only a 33% survival rate (median OS 9.3 months), compared to 70% in the group with lower serum GH (median OS 18.9 months) (238). In the second case, among 75 patients with advanced GC treated with anti-PD1 mAbs (alone or in combination with chemotherapy), the group with higher serum GH had a disease control rate of only 30% compared to 53% in the group with lower serum GH; the group with higher serum GH had a concomitant shorter PFS and OS (299). Furthermore, increased serum GH levels appear to drive pathways that can interfere with anti-PD1/PDL1 and anti-CTLA4 immunotherapy approaches. First, treatment with GH or GH-inducing ghrelin in 22-month-old male Fischer rats with sepsis decreases PD1 expression in splenocytes (395, 396). It is relevant to mention that GH is also locally produced in the lymphocytes and increases with age in the splenic lymphocytes of live rats (29). Second, bGH mouse kidneys show 2- to 4-fold upregulation of CD80 antigen (397) which binds to the T-cell CTLA4 and leads to T-cell inactivation (398). The above observations seem to point at endocrine GH, while it is unknown if ectopic GH secretion from the tumor contributes to a higher serum GH. Multiple reports of ectopic acromegaly (supra-normal serum GH due to excessive production of GH or GHRH from a nonpituitary site, ~1% of acromegaly cases) do report several neuroendocrine tumors and other types of tumor tissues (lung carcinoid and adenoid cystic tumors, pancreatic cell tumors, gastrointestinal tract tumors, pheochromocytomas, thymic carcinomas) as sites of origin of excess serum GH (399, 400). But does this happen in a tumor under autocrine/paracrine action? Almost on cue, a recent comparative analysis of GH-related immunotherapy response and tumoral immune landscape across several cancer types and databases appears to answer that. The authors found that GH overexpression is associated with an immune-deficient TME, designated as an "immune-desert" (401), characterized by suppressed infiltration of activated cytotoxic immune cells and suppressed expression of immunotherapy targets like PD1 or PDL1. Their study also identified increased association of microsatellite instability and tumor mutation burden—2 key determinants of immunotherapy response—with human *GH1* expression and confirmed a suppressed response to immune checkpoint inhibitors in patients with higher serum GH levels in independent study cohorts (401). Moreover, several reports suggest a therapeutic role of GH administration in management of autoimmune diseases such as inflammatory bowel disease (402), pediatric Crohn's disease (403), autoimmune diabetes (404), and collagen-induced arthritis (405), which altogether suggest an immunosuppressive function of GH. Overall, much deserved attention should be directed at understanding the interaction of GH and immunotherapy targets in normal and cancer tissues to identify improved antineoplastic combination regimens. While the mechanistic details underlying these provocative observations are yet to be described, reviewing the literature in GH-immune cell interactions can provide ample indications of a role of GH in suppressing antitumor immunity in the TME. These are discussed later under "Immunosuppression."

Tumor-promoting inflammation

Inflammatory signaling from the TME elicits innate and adaptive immune responses leading to immune cell infiltration in the TME—long confirmed by pathologists. Although this association of tumor cells and immune cells is apparently paradoxical, it is now understood that it might be a case of a

calculated risk. Multiple components of the innate immune system can be adapted by tumoral cytokines to be tumor-supportive, and inflammatory responses can enable several tumor hallmark properties. Infiltration of cytotoxic immune cells on the other hand, might be an unwanted pitfall of inflammation in the TME, managed by immune-suppressive mechanisms. Primarily, mouse models of GHD (Ames, Snell) and GH insensitivity (GHRKO) have reduced age-associated inflammation partly due to increased levels of serum adiponectin (406)—an anti-inflammatory adipokine with well-studied anticancer effects (407). Moreover, pro-inflammatory cytokine IL6 levels were suppressed in the GHRKO animals (408), while mediators of the inflammatory pathway, like cyclooxygenase 2 (COX2) and prostaglandin (PGD2) in the testes were decreased (also in GHRH-KO mice) relative to wild-type controls (409). Moreover, GHRKO mouse AT depots had altered stromal vascular fraction (SVF) with modified T-cell population, which protects the animals from obesity-related white AT inflammation (410). In contrast, GH transgenic mice have inflammatory liver cancer (411) concurrent with increased immune-suppressive cell (macrophage and T-regulatory cells) infiltration in peripheral tissues (409, 412), and elevated levels of inflammatory markers (COX1, COX2, PGD2, cytosolic phospholipase A2α [cPLA2α]) (409, 413). Moreover, GH administration at a dose of 1 mg/kg body weight using an osmotic mini-pump in Swiss-Webster mice increases hepatic COX1 in the male mice (413), whereas knocking down GHR in human pancreatic cancer cells decreases cellular COX2 levels (340). Additional studies are rapidly emerging portraying the details of GH-regulated inflammatory reactions in the aging skeletal system (329, 330), in the hypothalamus (414), and in macrophages (415), all supporting a microenvironmental contribution of GH-regulated inflammation in cancer. Although no studies have so far directly queried GH's role in promoting inflammatory pathways in cancer, it may underlie the net outcome of GHR antagonism or GH excess observed in vivo. Overall, it is evident that autocrine/paracrine GH can potentially drive the cancer hallmark of local inflammation at the TME and needs to be systematically studied.

Genome instability and mutation

Alterations in the genes that are involved in DNA damage response, repair, and decision of cell fate are fundamental to the process of oncogenesis and replicative immortality observed in tumor cells, especially withstanding DNA-damaging anticancer treatments. In untransformed human colon cells, exogenous GH is found to increase chemotherapy (etoposide)-induced DNA damage and suppress DDR via nonhomologous end-joining, leading to accumulation of mutated unrepaired DNA in the cells, as a prelude to oncogenesis (256). The *TP53* gene is central to the cellular decisions of post-damage repair or apoptosis or senescence and is called the "caretaker of the genome" (366). Recent studies by Chesnokova and Melmed indicate that GH and p53 share a negative feedback regulation in normal cells via reduced phosphorylation of the primary responder to DNA damage—the ataxia telangiectasia mutated (ATM) kinase. Exogenous or age-associated DNA damage induces p53, which in turn induces the expression of multiple other genes necessary for cell fate determination, including the *GH1* gene in both humans and mice. The mechanism of this effect has 2 components: first, GH increases the expression of the transcription factor tripartite-motif protein 29 (TRIM29) which suppresses histone acetyltransferase TIP60 expression. This abrogates the autophosphorylation of ATM kinase, leading to reduced phosphorylation and stabilization of ATM downstream of p53 and other DDR proteins (256). Pegvisomant treatment rescues the ATM inhibition, indicating that this is a direct effect of GH via the GHR (256). Also, GH induces wild-type p53-inducible phosphatase 1 (WIP1) protein, which in turn dephosphorylates ATM kinase and thereby suppresses ATM downstream p53 (257), enabling reduced DDR response and accumulation of mutations.

In agreement to this fascinating finding, normal skin fibroblasts from LS patients show increased baseline expression of p53 and decreased levels of proliferation, identical to observations in colon tissues of Ames and GHRKO mice (30). Forced expression of GHR in the GHR-deficient fibroblasts from LS patients decreases p53 protein levels in a dose-dependent manner, while suppression of GHR expression in wild-type fibroblasts increases p53 levels (30). Similarly, colonic mucosal biopsies from patients with acromegaly show marked restoration of p53 levels following 8-week pegvisomant treatment (30). Interestingly, these effects of GH were confirmed to be IGF1 independent, as disruption of IGF1R expression or signaling did not have any effect on increased colon DNA damage either in vitro or in vivo (124). A significant suppression of p53 under conditions of GH excess has also been reported in other tissues, as the synovium in the bGH knee-joints compared to wild-type controls (330), and therefore can be postulated to be highly permissive of oncogenesis.

Senescent cells

GH-secreting pituitary adenomas also harbor high amounts of senescent cells. Investigations in these adenomas and subsequently in nonpituitary cells, including BC and CRC cells, now affirm that GH is secreted by the senescent cells and is an active part of the senescence associated secretory phenotype (SASP) (416). However, limited autocrine effect of GH in the senescent cell can be expected, as peroxide-induced senescent human mesenchymal stem cells show reduced GH internalization and poor activation of JAK/STAT pathways following GH treatment (416). However, Chesnokova et al have shown that nutlin-induced senescence in normal and tumor cells triggers GH production under direct p53 regulation (26, 27), which aligns with increased senescence in the skin fibroblasts from patients with acromegaly (389). Contrastingly, senescence burden was significantly lower in the white AT of GHD Snell mice and in GHRKO mice (417), as well as in the GHRKO pigs (418). Additionally, sustained IGF1 stimulation can also lead to premature cellular senescence via the mitochondrial protein thioredoxin-interacting protein (TXNIP) (419), particularly relevant given that GH is the primary inducer of circulating IGF1. Radiation and chemotherapy are cornerstones of anticancer treatment, and both induce extensive DNA damage in the entire TME. An interesting hypothesis is that DNA damage induces GH production (263) and GH in turn promotes resistance to apoptosis and can potentially drive senescence in the DNA-damaged nontumor cells in the TME. Further experimental evidence is required as to whether suppression of senescence can be an additional therapeutic benefit of GHR antagonism in cancer.

Nonmutational epigenetic reprogramming

Over the last decade, tremendous advancements in epigenetics research have culminated in multiple epigenetic modulators acquiring regulatory approvals and steadily populating anticancer drug discovery pipelines (420, 421). Although GH action in cancer epigenetics is heavily understudied, some exciting observations have been reported. Chia and Rotwein have described GH-induced rapid (30-60 mins post-treatment) increase of core histone acetylation and chromatin opening preceding STAT5b binding to promoter/enhancer elements upstream of *Igf1*, *Socs2*, *Cish*, *Igfals*, and *Spi2.1* genes in the liver of adult male GH-deficient rats (422), while GH treatment suppresses miR-29a via IGF1 in lit/lit mice to promote insulin resistance and fibrosis (423). An important finding that can have useful implications on sex-specific etiology and therapeutic outcome in cancer patients is that the pulsatility of plasma GH levels regulates sex-specific hepatic chromatin accessibility via dynamic binding of STAT5 and BCL2 in mice (424-427). The histone methylation and acetylation signature from Ames mice are highly consistent (428) and while the implications of this epigenetic patterning in cancer are yet unknown, Ames mice do have delayed occurrence and reduced number of neoplasms (429). In cancer, autocrine GH in MCF7-hGH cells has shown modulation of critical epigenetic mechanisms that promote EMT, such as DNA methylation, via DNMT regulation (191) and upregulation of miR-96-182-183 cluster (36). In addition, several miRNAs and long noncoding RNAs (lncRNAs) have been shown to regulate the GH/IGF axis in cancer and have been reviewed elsewhere (430-432). Together these studies provide impetus for future investigations in epigenetic modulations orchestrated by GH, especially underlying disease states.

Unlocking phenotypic plasticity

GH drives stem cell differentiation in both normal and tumor cells. Autocrine GH expressed in mammary epithelial cells can promote oncogenic transformation, preceded by an acinar to luminal phenotypic switch (154), while GHR-positive stem/progenitor cells in mammary tissues can form mammospheres unlike the GHR-negative cells. As a paracrine effect, progesterone-stimulated GH in the mammary epithelia can induce progenitor cell differentiation along luminal and myoepithelial lineages, while GHR-negative cells mostly differentiated along luminal lineage (32). In samples of human ductal carcinoma in situ (DCIS) lesions, GHR was present in > 50% of 176 samples, in which it is co-expressed with the stem cell marker ALDH1A1 (32% of 75 samples) and ALDH1A3 (24% of 67 samples) and represented a cell population that was expanded in the DCIS lesions (32). Autocrine GH expression in ER-negative BC cell lines MDA-MB-453 and SKBR3 also stimulated an increase in cancer stem cell (CSC)-like properties in vitro and enhanced tumorigenicity in xenograft studies (161). Furthermore, in CRC cell lines (DLD1 and Caco2), autocrine GH expression led to a 2- to 3-fold increase in ALDH1 levels, while multiple markers of stemness (CD24, CD44, NANOG, SAL4, POU5F1) were preferentially observed in GH-expressing CRC cells (253). Induction of the EMT program and increase in CSC populace in the TME are interconnected. GH is a potent inducer of EMT and GH-driven stemness in cancer is indeed accompanied by a GH-driven induction of the EMT program. Another characteristic of CSC is marked upregulation of ABC transporters, allowing not only minimal intracellular drug residency but also accumulation of tumor-supportive biomolecules in the TME, like IL1β, prostaglandins, and glutathiones (433). In fact, in Huh7 and HepG2 cells with forced expression of hGH, appearance of CSC phenotypes included upregulated ABCG2 expression (237). As CSCs predict poor prognosis and are essential in cancer relapse, GH targeting might improve disease prognosis.

GH is a potent regulator of cellular differentiation, although specific expression and the role of GH signaling in cancer-associated extensive de-differentiation, transdifferentiation, or blocked differentiation has not been systematically studied, despite several compelling actions in normal tissues. Human GH can singularly drive cellular differentiation in the progenitor mesenchymal stem cells (MSCs) into myocytes, adipocytes, or osteocytes (434-438). GH's capacity to affect MSC differentiation is IGF1-independent, wherein IGF1 preferentially promotes clonal expansion of the GH-differentiated cells (434). GH can also induce transdifferentiation (439-441) partly by differential inductions of the proto-oncogenes c-fos and c-jun (439, 442, 443), while recent work have also shown examples of "blocked differentiation" by GH in determining MSC differentiation by blocking adipogenic and favoring myogenic lineage in a context-dependent manner (437, 444, 445). In cancer, MSCs are a common part of the TME and can have extensive participation in tumorigenesis acting as cells of origin, providing tumor-supportive cytokines and growth factors, promoting chemoresistance, maintaining CSCs, promoting EMT, mediating immunosuppression, and can also transdifferentiate into stromal fibroblasts (446). Therefore, the overt role of GH in MSCs can be a critical factor in cancer propagation and prognosis.

Polymorphic microbiomes

Recent evidence shows that in addition to well-known direct oncogenic effects of pathogenic *H. pylori* and hepatitis C virus, our resident microbiota, even as a part of the TME (447, 448), can profoundly influence cancer by affecting cell proliferation, inflammation, genomic instability, cellular metabolism, antitumor immunity, and therapeutic response (448-450), and have promising biomarker properties (451). Although no work has as yet connected the GH-microbiome-cancer axis, recent work from our laboratory has made promising inroads by showing that excess or lack of endocrine GH does lead to dysbiosis in bGH and GHKO mice compared to corresponding controls (452-454). Consistent variations in multiple phyla and genera of gut microbiomes with congenital GH status, and also in an age-dependent manner, were observed in these mice. Multiple recent reports further affirm distinct relative variations in the gut microbiome of patients with acromegaly (455-457) as well as pediatric GHD patients (458). The ramifications of these exciting findings in cancer will be forthcoming.

Direct Action of GH on Nontumor Cells of the TME

GH action has a profound influence in orchestrating a tumor-supportive TME by distinct actions on the nontumor cells of the milieu (28) (Fig. 10). The action of endocrine GH in normal cellular physiology has been well-studied over the last century and includes several cell populations which are also active components of a TME. The effect of GH on these

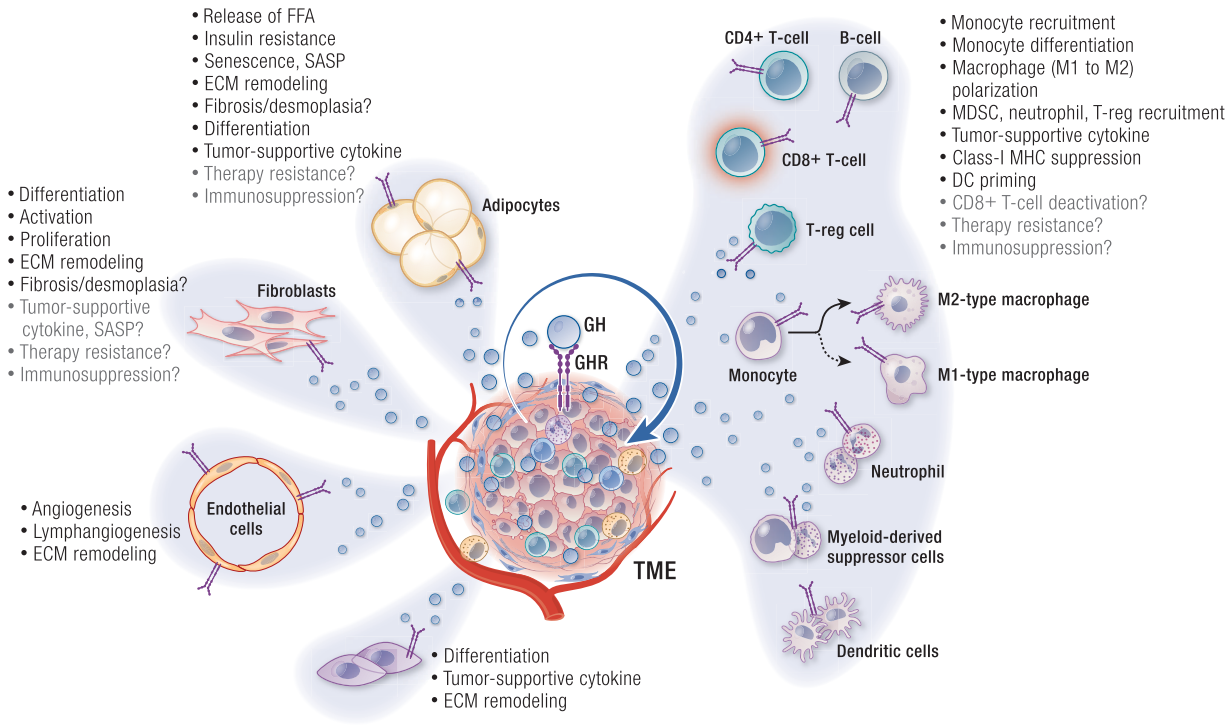

Figure 10. Direct tumor-promoting actions of GH on the nontumor cells of the tumor microenvironment (TME): different cells in the TME, including the tumor proximal nontumor cells, can produce GH and also express GHR (and PRLR), thereby enabling an autocrine/paracrine GH action in the TME. The GH action on the nontumor components (immune cells, fibroblasts, adipocytes, stem cells, endothelial cells) can exert several tumor-promoting effects in the TME (as mentioned and discussed in this review) but this awaits direct experimental validation.

normal cells elicits outcomes which can constitute well-studied tumor-promoting effects in the TME of mammalian cancers. Examples include GH's action on the endothelial cells (see "Induction and access to vasculature") and stem cells (see "Unlocking phenotypic plasticity"), discussed above. Additionally, critical components of the milieu are immune cells, stromal fibroblasts, and AT, where GH (pituitary or nonpituitary) exerts profound effects, which in the context of the TME, can be designated as highly tumor-supportive. Below, we will highlight the additional covert actions of GH resulting from interactions with these cell types, although there are phenotypic and functional overlaps: (i) ECM remodeling/fibrosis which fuels the desmoplastic TME; (ii) regulating the nature of immune cell infiltration in the TME to escape antitumor immunity; and (iii) dysregulation of the AT, which in the TME is mobilized for versatile tumor support. Currently, it is unknown whether and to what proportion/extent these functionalities of GH are exerted by endocrine vs autocrine/paracrine GH produced in the TME, and thus this presents a truly fascinating and relatively unexplored area of research involving GH attenuation in anticancer therapy.

Extracellular matrix remodeling/fibrosis

Desmoplasia, or the stromal remodeling in the TME, characterized by myofibroblast activation and increased deposition of the ECM, is commonly found in almost all cancers, and consistently correlates with worse prognoses. This fibrotic event in the TME is one of the most challenging obstacles for antitumor immunity (459), leading to current enthusiasm in development of innovative approaches targeting the tumor stromal components (460). A major contributor to tumor desmoplasia is activation of fibroblast and myofibroblast cell population in the TME (461-464). GH and GHR expression levels in dermal cells are highest in the fibroblast cells (284, 465-467), where GH drives fibroblast proliferation (284, 468-470). In tumors, endogenous GH expression has been confirmed in histopathological analyses in tumor-associated stromal fibroblast cells and in human BC and EC tumor samples (183). A principal inducer of fibrosis in normal and cancer cells is the transforming growth factor β (TGFβ) pathway (471) and TGFβ is upregulated in the serum of untreated, but not in treated, patients with acromegaly (472). GH overexpression in EL4 mouse lymphoma cells increases TGFβ production (473), while GH induces TGFβ production (474) and intracellular signaling (475, 476) in glomerular podocytes contributing to renal pathologies (477, 478). In agreement, Tgfb1 transcripts are also found to be significantly upregulated in microdissected glomeruli from the bGH mice (479), although the AT fibrosis in bGH mice appears to be TGFβ-independent (480). Moreover, GH is an active component of SASP (see "Senescent cells"), which is a potent inducer of fibrosis in both tumor and nontumor settings (481).

Although no studies have yet investigated GH action in cancer desmoplasia, compelling evidence of GH in promoting fibrosis is steadily accumulating (summarized in Table 11) and this has recently been designated as a covert action of GH with deterministic contributions in aging and age-associated morbidities such as cardiovascular disease and cancer (240). For example, subpopulations of patients with acromegaly suffer from myocardial and hepatic fibrosis, and also present with increased collagen turnover with elevated serum levels of type I collagen, collagen-specific amino acid hydroxyproline,

Table 11. GH action in fibrosis

In vivo	Tissue	Effect of GH action	Reference
healthy young individual	Once-daily GH injection; 1 day	4-fold increase in tendon collagen I protein	(482)
acromegaly vs normal patients	serum	increased type I collagen, collagen-specific amino acid hydroxyproline, and procollagen III amino terminal pro-peptide	(240)
acromegaly vs normal patients	urine	increased total and nondialyzable hydroxyproline	(483)
acromegaly vs normal patients	serum	increased serum TGFβ in untreated patients (not in treated)	(483)
bGH vs wild-type mice	adipose tissue	increased AT fibrosis	(484)
GHKO vs wild-type mice	adipose tissue	decreased AT fibrosis, collagen content	(485)
liver-GHRKO vs wild-type mice	adipose tissue	increased AT fibrosis, collagen content	(486)
fat- or adipose-GHRKO vs wild-type mice	adipose tissue	decreased AT fibrosis, collagen content	(484, 487, 488)
bGH vs wild-type mice	small intestine, heart, kidney, and tibial cartilage	increased fibrosis, collagen deposition	(240)
bGH vs wild-type mice	plasma	increased hydroxyproline level	(489)
GHRKO vs wild-type mice	plasma	decreased hydroxyproline level	(489)
bGH vs wild-type mice	microdissected glomeruli	increased TGFβ transcript	(479)
multiple mouse tissues	skin, intestinal myofibroblasts, kidneys, tendons, ischemic neural tissues, and rat jejunum	increased collagen deposition	(240)
glomerular podocytes	kidney	increased TGFβ production and signaling	(474-476)

Abbreviations: AT, adipose tissue; GH, growth hormone; GHR, growth hormone receptor; TGFβ, transforming growth factor β.

and procollagen III amino terminal pro-peptide (PIIINP) (240). In healthy young individuals, a GH injection of 0.03 to 0.05 mg/kg/day for 14 days increased tendon collagen I protein synthesis by almost 4-fold (482). In fact, an increase in circulating levels of collagen-specific nonessential amino acid hydroxyproline is known to be increased in adult patients with cancer (490, 491) and is a known marker of cancer desmoplasia/fibrosis (492, 493) and chemoresistance (494). Additionally, abnormal urinary excretion of hydroxyproline is observed prior to bone metastasis of breast and lung cancers (495). In patients with acromegaly, both total and nondialyzable urinary hydroxyproline output is elevated (483), while recent metabolomic profiling of plasma in bGH and GHRKO mice show significantly increased and decreased hydroxyproline levels, respectively (489). Moreover, Berryman and colleagues have enumerated AT fibrosis across multiple mouse models of GH action, wherein fibrosis was measured using collagen-specific picrosirius-red staining and measurement of hydroxyproline content. Such evaluations show that subcutaneous and perigonadal AT depots of bGH mice have increased fibrosis (484), whereas collagen content was decreased in the subcutaneous AT depot of GHKO mice (485). Importantly, the variations in AT fibrosis observed in bGH or GHKO mice, may not be IGF1 dependent, as the liver-specific GHRKO mice with increased serum GH (486) had increased AT collagen content despite markedly lower serum and hepatic IGF1 (484), whereas the attenuation of GH reception in the AT using a fat-specific (aP2 promote/enhancer (484, 487),) or adipose-specific (adiponectin promoter/enhancer (488)) GHRKO mice had lower subcutaneous AT collagen content, despite unchanged serum GH/IGF1 levels. Furthermore, GH-induced fibrosis is not restricted to the AT, as bGH mice show increased fibrosis additionally in small intestine, heart, kidney, and tibial cartilage (240). Other studies have also reported increased collagen synthesis induced by acute or chronic GH administration in several non-AT tissues, like skin, intestinal myofibroblasts, kidneys, tendons, ischemic neural tissues, and rat jejunum, covered by a recent review (240). In conclusion, GH action in desmoplasia awaits systematic investigation, especially in the backdrop of galloping advances in current research targeting the TME for cancer treatment.

Immunosuppression

Collective information about GH action and immune cell interaction (summarized in Table 12) appears to indicate a prominent role of GH in the tumor *immune* microenvironment (TIME) (28, 529). Over the last 30 years, GH gene expression has been shown in the immune tissues of thymus, bone marrow, and spleen (530), as well as in the lymphoid tissues (531). Moreover, GH with autocrine/paracrine action is produced by the lymphocytes (496-501) and leukocytes (501-503). Autocrine GH promotes lymphocyte proliferation (505) and drives autocrine/paracrine IGF1 production in leukocytes (508, 509). Interestingly, lymphocyte GH production increases with age (29), while hypoxia, a common feature of most solid tumors (532), induces GH production via HIF1 binding sites at GH gene promoter/enhancer region −176 to −172 bp in lymphocytes (more in T cells than B cells) and is predicted to have a profound implication in the TME (504).

In tumors, subtypes of innate and adaptive immune cells, like the T-regulatory cells (Treg) (533, 534), anti-inflammatory (M2-type) macrophages (535, 536), myeloid-derived suppressor cells (MDSCs) (537-539), and neutrophils (540-542) drive intrinsic and acquired resistance to antitumor

Table 12. Actions of GH on nontumor cells: putative role at the TME

Cell type	Tissue	Observation	Reference
lymphocytes, leucocytes	blood	GH expression	(496-503)
lymphocytes	blood	GH production increases with age	(29)
lymphocytes (more in T cells, also in B cells)	blood	hypoxia (HIF1a) induces GH expression	(504)
lymphocytes	blood	GH promotes proliferation	(505)
leucocytes	blood	GH expression suppressed by stress at older age	(506, 507)
leucocytes (PBMC)	blood	GH drives autocrine/paracrine IGF1 production	(508, 509)
Macrophage and Treg cells	AT stromal vascular fraction (SVF) (bGH mice)	GH recruits immunosuppressive cells at AT	(412)
Macrophage	AT SVF (bGH mice), *in vitro*	GH induces M2-type macrophage polarization	(393, 412, 510, 511)
Macrophage	intestine, serum (bGH mice)	GH induces M2-type macrophage polarization	(511, 512)
Monocyte	multiple tissues	GH promotes monocyte recruitment	(513)
Monocyte, macrophage	multiple tissues	GH promotes macrophage activation	(514)
Macrophage	serum (Snell, GHRKO mice)	higher M2-type macrophage in GH-deficient and -insensitive mice	(515)
polymorphonuclear MDSC	serum and TME (CRC patients)	increased GH expression and upregulated JAK/STAT pathway activation, increased DDR—autocrine loop	(516)
Neutrophil-to-lymphocyte ratio	serum of untreated acromegaly patients (before and after treatment)	elevated in acromegaly patients, partial decrease after corrective treatments	(517)
Neutrophil	serum (Wistar rat)	GH induces neutrophil priming in sepsis model	(518, 519)
Neutrophil	serum (human subjects)	neutrophils express GH	(520, 521)
Multiple immune cells	serum (acute GH treatment in GHD patients)	GH increased monocytes, neutrophils, dendritic cells (DCs), decreased activated B cells, cytotoxic- and helper-T cells	(522)
B cells	serum (after 12-mo GH treatment in GHD children)	reduced B-cell counts	(523)
lymphocyte and leukocyte	serum (after GH treatment in idiopathic short stature children)	decreased lymphocyte and leucocyte counts	(524)
CD8+ T cells	serum (after GH treatment in idiopathic short stature children)	decreased CD8+ T cells, increase in CD4+ T cells	(525)
endothelial cells	vascular, lymphatic endothelium	GH promotes angiogenesis, lymphangiogenesis	(382, 386)
mesenchymal stem cell (MSC)	*in vitro*, *in vivo*	differentiation	(434-441)
adipocyte	adipose tissue (AT)	GH drives lipolysis, FFA release	(526-528)
3T3L1 adipocyte	cell culture	increases collagen gene RNA	(484)
fibroblasts	stroma (skin), tumor sample (BC, EC)	GH expression	(183)
fibroblasts	stroma (skin)	fibroblast proliferation	(284, 465-470)
glomerular podocytes	kidney	increases TGFβ production and signaling	(474-476)

Abbreviations: AT, adipose tissue; BC, breast cancer; CRC, colorectal carcinoma; EC, endometrial cancer; FFA, free fatty acids; GH, growth hormone; GHR, growth hormone receptor; TGFβ, transforming growth factor β; TME, tumor microenvironment.

immune cell infiltration and immunotherapy resistance. In the immune subpopulations of AT depots in bGH mice there is increased Treg and macrophage infiltration in the subcutaneous and mesenteric AT compared to that in wild-type controls (412), while the relative levels of M2-macrophage were upregulated in all AT depots in the bGH mice (412). Notably, GH can act as a chemoattractant to recruit monocytes (513) and can induce monocyte differentiation and macrophage activation (514). GH also often induces polarization of activated antitumor pro-inflammatory M1-type macrophages to protumor anti-inflammatory M2 types (510-512). Mechanisms underlying this role of GH include metabolic reprogramming of macrophage via mitochondrial restructuring (393), and a PI3K-dependent upregulation of a macrophage anti-inflammatory transcription factor MAFB and concomitant suppression of pro-inflammatory activin-A (511). Interestingly, M2-type macrophages are known to actively secrete IGF1 (543) but is unknown if it occurs in a GH-dependent manner. However, congenital deficiency/absence of GH action globally or in macrophages appears to promote M2-type macrophage infiltration in healthy tissues in mice (415, 515).

On the other hand, a recent study presented the transcriptomic profiling of different MDSC subsets in circulation and TME of CRC patients, wherein polymorphonuclear/granulocytic MDSCs (PMN-MDSCs) were most abundant (544)

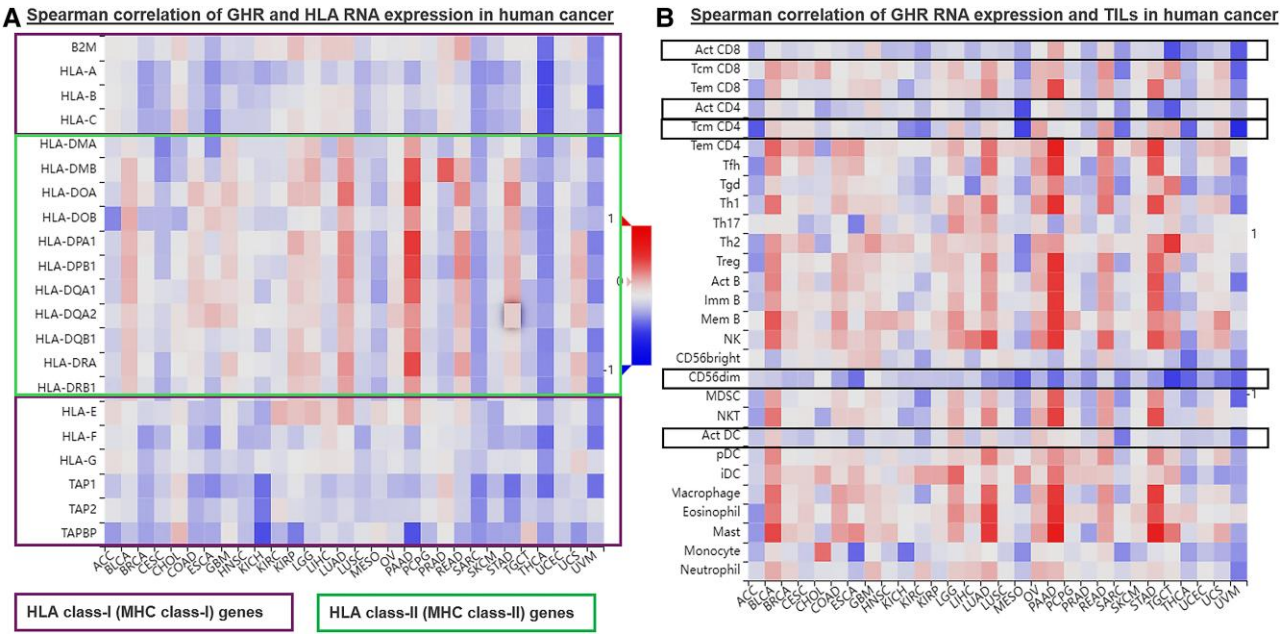

Figure 11. Pan-cancer correlation of GHR RNA expression with (A) human leucocyte antigen [HLA], the human MHC class I and class II gene, RNA expression; and (B) tumor-infiltrating lymphocytes (TILs) in patient tumor samples across 30 different cancer types (TCGA datasets; generated using TISIDB platform).

with a marked upregulation of *GH1* expression along with that of the JAK/STAT pathway and DDR genes (516), indicating an autocrine/paracrine GH action with MDSCs as a source of GH in the TME. This finding further supports the series of work on autocrine/paracrine GH and its role in DDR in colon cancer conducted by Chesnokova and colleagues. Additionally, IGF1R and INSR overexpression and signaling are also major drivers of MDSC-mediated immunosuppression in the TME (545, 546). Moreover, tumor-associated neutrophils (TANs) exert protumorigenic effects (547) and higher TAN presence is a biomarker of poorer prognoses in cancer patients (548). In this regard, a recent study from Poland involving 62 patients with acromegaly (58 re-evaluated after therapy), 134 nonfunctioning pituitary adenoma patients, and 120 healthy subjects, have reported a markedly higher neutrophil-to-lymphocyte ratio and systemic-immune-inflammation index along with lower lymphocyte counts than in acromegaly patients who were partly biochemically "cured" by corrective surgery (517). Moreover, neutrophils also produce both the 22-kDa and 20-kDa GH isoforms (520, 521) and can be a source of tumoral GH.

The class I HLA genes (classical: HLA-A, B, C, nonclassical: HLA-E, F, G, H, J, K, L) along with essential components like the β-2-microglobulin (B2M) associate with ATP-dependent transporters associated with antigen presenting (TAP1, TAP2), tapasin (TAPBP), and multiple chaperone proteins constituting the class I MHC complex, which is ubiquitous in all cells and generally present endogenous antigens to CD8+ T cells for detection and elimination of aberrant cells. The antigen presenting cells (APCs) (eg, macrophages, dendritic cells, and B cells) can also cross-present extracellular proteins via the MHC-I complex after phagocytosis/internalization. To avoid this method of immune detection and elimination, tumor cells adopt several methods, including: (i) suppressing T-cell recruitment by modulating the expression of immune-inhibitory surface proteins (eg, PDL1 and PDL2 binds to receptor PD1) (549); (ii) suppression of the HLA class I genes (550, 551); and (iii) suppressing the infiltration of antitumor immune cells from entering the TME. Of these, GH-regulated immunosuppression modalities have been discussed in "Avoiding immune destruction" above. From analyzing the human tumor transcriptomic data for > 10 000 cancer patients for 30 different cancer types in the TCGA dataset, we found that GHR expression consistently correlated inversely with a pan-cancer expression of all components of the HLA class I system in patient tumor samples of 25/30 cancer types (Fig. 11A), implicating GH action with suppressed tumoral MHC class I presentation. Further, we also see markedly consistent pan-cancer inverse correlation of GHR expression with reduced tumor infiltration levels of multiple major antitumor immune cell types, such as activated CD8+ T cells, activated and central memory CD4+ T cells, CD50-dim and CD56-bright natural killer (NK) cells, and activated dendritic cells (DCs) (Fig. 11B), each of which associate with poorer therapy response and prognoses in several human cancers (552-554). A recent landmark study by Gujral et al, from the Icahn School of Medicine at Mt. Sinai, reports the acute effect of GH in 54 GHD pediatric patients (22 tested positive for GHD) at baseline and over the course of 3 hours after a GH stimulation test (522). A significant increase in circulating monocyte, DCs, and granulocytes (especially neutrophils) and significant decreases in cytotoxic and helper T cells and activated B cells over the course of 3 hours following GH stimulation, was observed. In fact, a significant suppression of B-lymphocytes by GH has been reported in multiple prior studies (523-525).

It is to be noted that in the above cases of patients with GHD treated with GH, despite reported changes in lymphocyte composition, no supra-normal risk of infections was observed within the follow-up periods. Importantly, no differences in infectious diseases or immune response were observed in the lifetime of untreated congenital GHD patients, compared to GH-sufficient control subjects (555), while patients with isolated congenital GHD in the Itabaianinha cohort (Brazil) are

less sensitive to highly endemic parasitic infection from *Leishmania amazonensis* (556) and also had lower confirmed cases and disease progression with SARS-CoV-2 infection than controls (557). Similarly, no cases of endemic Chagas disease resulting from *Trypanosoma cruzi* parasitic infection have been reported in the Ecuadorian cohort of individuals with LS, despite high prevalence in relatives, wherein high serum GH levels appear to be protective (558). These observations provide a robust impetus to the investigation of GH-immune interactions and modulation of the same in cancer.

Putting together the multiple aspects of GH's interaction with the immune cell types known to date, it can be reasonably postulated that presence of GH in the TME may promote an immunologically "cold" tumor state which is resistant to immunotherapy (559). Therefore, a thorough investigation of the extent and details of GH's ability to modulate immunotherapy outcomes in cancer should be pursued.

Adipose tissue dysregulation

Tumor proximal AT has now been established to have a prominent role in tumor progression, invasive growth, metastases, immunosuppression, drug resistance, and ECM remodeling/fibrosis/desmoplasia in the TME (560, 561). Tumors often tutor proximal AT to include adipocytes in the TME, dubbed *cancer-associated adipocytes*. These cancer-associated adipocytes undergo lipolysis and contribute a supply of FFAs, which allow several tumor-supporting roles like metabolic reprogramming via upregulation of fatty acid oxidation and supply of growth factors and immune modulating cytokines, as well as transdifferentiate into stromal components like fibroblasts and myofibroblasts to drive desmoplasia. Due to the rapidly advancing understanding of AT biology and the abundant expression of GH and GHR and their prominent endocrine actions in the AT, a significant body of studies on how GH affects the overall health of AT is available, of which GH-regulated AT fibrosis has been mentioned in "Extracellular matrix remodeling/fibrosis" above. Collectively, this work provides ample hints to address the question of how an ectopic and possibly excess production of GH from a tumor site might affect the proximal AT.

Surprisingly, GH and AT in the context of cancer have not yet been approached and thus present an exciting and promising area of investigation. While the physiologic function of endocrine GH is lipolytic, endocrine GH excess or deficiency differentially causes lipodystrophy in AT (526-528). Moreover, reports on the role of autocrine/paracrine GH in AT are sparse. GH primarily promotes breakdown of adipocyte lipids, ultimately releasing FFA that can be taken up by frequently observed upregulation of tumoral fatty acid transporters and act as fuel for altered metabolism in tumor cells (394). It is expected, but unknown, whether and how TME-derived GH exerts this action on cancer-associated adipocytes in a paracrine fashion. Moreover, an increase in FFAs increases insulin resistance in a systematic manner (562), which is consistently associated with poorer therapeutic response, higher recurrence, and poorer OS in almost all cancer types (discussed further later) (563, 564). Insulin resistance/hyperinsulinemia is indeed a frequent characteristic of GH excess and is similarly observed in humans and in mouse models (see "Promoting insulin resistance" below). Furthermore, GH-mediated release of FFA also upregulates fibroblast growth factor 21 (FGF21) expression in the liver tissues (565), while the serum FGF21 levels are known to be elevated in GH transgenic mice (566). This is important, as recent reports show that FGF21 in turn exerts immune exhaustion of CD8+ T cells (567).

In mouse models of suppressed GH action (ie, Ames, Snell, lit/lit, GHRKO, GHKO, and GHA mice) increased adiposity compared to GH-sufficient wild-type littermates has been reported, surprisingly alongside improved insulin sensitivity (568, 569), indicating that despite increase in "quantity," there has been improvements in the "quality" of fat tissue (528, 570), which is an important consideration given that AT quality affects TME. For example, AT from GHD Snell mice or GH-insensitive GHRKO mice both have lower levels of AT-derived tumor necrosis factor α, interleukin-6, monocyte chemoattractant protein 1 (515), and IGF1 (571) and higher levels of adiponectin, which elicit several anticancer benefits (568, 572, 573). Contrastingly, the bGH mice have elevated AT-derived IGF1 (571) and higher senescent cell burden in the AT (417). Importantly, AT houses a large number of nonadipocyte cells in the SVF, which altogether determine AT "quality." Five-month-old bGH mice were found to have decreased adiposity with reduced percent fat mass in adipose and mesenteric (visceral) AT depots, which showed a significant increase in proportion of SVF cells, including significantly higher percentage of macrophages and Treg cells. Additionally, 9/10 of the most significantly altered pathways in the subcutaneous AT depot in bGH vs wild-type mice were immune related (412, 574). In the same study, additional enrichment of tumor-supportive pathways, including increased fatty acid oxidation, high degree of ectopic gene expression, and branched-chain amino acid (BCAA) degradation (an indicator of IGF1 production) (575) are observed in bGH subcutaneous AT (574, 576). Fibrosis, a major corollary of excess GH action, has been exemplified by the AT and discussed in " Extracellular matrix remodeling/fibrosis" above. Overall, it is apparent that excess or ectopic GH action has the potential to modulate the AT into tumor-supportive functionalities; this remains to be empirically validated.

Therefore, it is timely and necessary to clarify if and which of the known actions of GH in immune cells, adipocytes, fibroblasts, and endothelial cells are triggered under the effect of ectopic GH from a GH-secreting tumor of different types of cancers. It is of further value to ascertain what fraction of the GH (and IGF1) at the TME is contributed by tumor and nontumor cells and its localized effects by single-cell analyses methods. Given the remarkable heterogeneity of a tumor, we can expect specific subpopulations of tumor cells (and nontumor cells) in the TME to be expressing either GH or GHR or both. Moreover, it would be valuable to identify the variability and distribution of GH active cells in the TME and trace their origins and clinical implications.

Indirect Actions of GH in Cancer

Two specific and well-studied actions of endocrine GH that have profound effects in cancer are GH-mediated endocrine IGF1 production and systemic insulin resistance, which are briefly summarized below:

IGF1 production

GH binding to the GHR activates the JAK2-STAT5B pathway, which induces *IGF1* gene expression from liver (>75% of serum) and multiple nonhepatic tissues (altogether <25%:

skeletal muscle, AT, kidney, immune cells, cartilage tissue). However, GH-induced IGF1 production, although not consistently observed in human and mouse tumor cells, exerts potent growth-promoting effects in the TME, as IGF1R expression is extensive in the tumor and nontumor cells. The IGF/insulin family has not only been heavily implicated in driving cancer progression, but also has been extensively pursued in pharmaceutical clinical trials. Our current review undertakes a discourse on the exclusive association of GH in cancer. Although no discussion of GH in cancer is complete without acknowledging the role of IGF1, it is beyond the scope of this review to accommodate detailing the role of IGF1 or IGF2 in promoting IGF1R and INSR activation. Moreover, although IGF1 amplifies several of the actions of GH, both GH and IGF1 have mutually exclusive physiologic actions in health and disease, including in cancer. In this regard, we encourage our readers toward excellent reviews on IGF/insulin—cancer association (564, 577-579).

Endocrine GH action on the liver induces the production of IGF1 as well as IGF1 sequestering IGFBP3 and acid-labile subunit (ALS), raising a concern more than 20 years back whether the IGF1-IGFBP3 complexation would negate the IGF1-mediated cancer-promoting effect of GH (580). Updated research findings help address this concern: (i) the endocrine GH action of hepatic IGF1 and IGFBP3 production is markedly reduced by somatopause, which coincides temporally with the vast majority of oncogenic events, thus challenging the role of endocrine GH and emphasizing the role of autocrine/paracrine GH in cancer; (ii) unlike endocrine GH, autocrine/paracrine GH is rarely is not known to induce IGFBP3 in peripheral tissues or the TME; and (iii) GH has been reported in multiple cancers to induce matrix metalloproteinases like MMP9 (as a corollary of switching on the EMT program), which are known to cleave IGFBPs to release sequestrated IGF1 in the TME (109).

IGF1R is a highly expressed tyrosine kinase receptor that is activated almost equipotently by IGF1 and IGF2 and often forms heterodimers with the INSR-A (581-583). Several tumors of epithelial and mesenchymal origin have a robust autocrine IGF2 loop with aggressive and therapy-resistant malignancies, together called "IGF2-omas," and are important in IGF1R action in cancer (reviewed by (583-587)). IGF1 can be produced at the TME irrespective of GH stimulation and can activate highly expressed IGF1R on site, exerting tumor-supportive actions like blocking apoptosis, increasing DDR, promoting migration invasion and anchorage independent growth, inducing EMT, promoting angiogenesis, regulating tumoral glucose uptake and metabolism, and orchestrating immune-suppression (reviewed in (577-579)). IGF1R activation also supports tumoral therapy resistance (reviewed in (204)), while nuclear localization of IGF1R blunts the efficacy of IGF1R mAbs (579). The stunning near-complete protection from cancer observed in patients with LS is ascribed to almost undetectable levels of circulating IGF1, due to complete absence of GH action caused by inactivating mutations of the GHR (101). Multiple studies using cells from the LS patients have revealed multiple tumor-protective and decelerated aging pathways (588-592), which is a function of attenuated endocrine, autocrine, and paracrine reception of GH signal. Overall, attenuating endocrine GH action suppresses endocrine IGF1 production and thereby suppresses the protumor actions of IGF1—observable in human patients with GHD, LS, and numerous mouse models of deficit of GH action.

Promoting insulin resistance

Diabetes, specifically type 2 diabetes mellitus (T2DM), mostly associated with obesity and characterized by hyperinsulinemia and peripheral insulin resistance, is a risk factor for particular cancers including that of pancreas, breast, cervix, prostate, endometrium, ovary, and colon (593-596). Insulin exerts robust mitogenic action and cellular growth-promoting action via INSR downstream PI3K/AKT/mTOR and IRS/MAPK signaling activation. In the case of T2DM, although some of the body's metabolic tissues may acquire unresponsiveness to insulin, tumor cells express functional INSR and respond to the insulin excess as a tumor fuel (564). Moreover, anticancer therapies often induce insulin resistance in patients as an adverse effect (597, 598). Hyperinsulinemia is consistently associated with a 25% to 41% increased risk of mortality from any cancer (563, 564, 595, 599-602), thus making cancer one of the leading cause of mortality in patients with diabetes (602, 603).

As early as 1931, Houssay and Biasotti reported the diabetogenic actions of anterior pituitary extracts, indicating that a high propensity of diabetes in acromegaly is perhaps an outcome of this phenomenon (105). Several subsequent reports have validated this critical observation (604-609), and it is now well established that GH is diabetogenic and promotes insulin resistance. In fact, secondary diabetes mellitus is found in 55% of the patients with acromegaly (610) and is an important aspect of acromegalic comorbidity (611). Notable studies in human patients and in mouse models have further detailed the underlying mechanisms and have been extensively reviewed (390, 612-615). Importantly, in congenital mouse models of GHD (Ames, Snell, Ghrh null, lit/lit, GHRKO, GHA, and the GHKO mouse (569)), and even postnatal attenuation of GH action (inducible-GHRKO and AOiGHD mice (616-618)), all have improved insulin sensitivity compared to age-matched littermates, despite higher liver triglyceride content (reviewed in (569)). Importantly, the improvement in insulin sensitivity in these mouse models of decreases GH action, despite a significantly higher fat mass (and often higher liver triglyceride content), and thus emphasizes the intricate modulatory effect of GH action on AT for promoting insulin resistance. This is similar to the improved insulin sensitivity observed in the Ecuadorian cohort of patients with LS patients, and the 2 cohorts of patients with GHD from Itabaianinha, Brazil and from the island of Krk, Adriatic Sea. On the other hand, early onset insulin resistance leading to hyperinsulinemia and T2DM despite reduced bodyfat, is a metabolic hallmark of untreated acromegaly (619). To reiterate, in mice and human subjects of decreased GH action and increased insulin sensitivity, cancer incidence and progression is also markedly lower.

Pulsatile GH and insulin signaling constitute a delicate metabolic homeostasis in the normal system and have distinct effects on mutual receptor expression. As shown by multiple studies, chronically high GH signaling in the case of GH excess provokes compensatory insulin production, causing hyperinsulinemia. Sustained exposure to insulin downregulates INSR expression, further stabilizing hyperinsulinemia and leading to T2DM (620). Generally, GH is lipolytic in action and increases blood glucose levels by increasing hepatic gluconeogenesis, while insulin is lipogenic and decreases blood glucose levels by glucose uptake and suppression of hepatic gluconeogenesis. GH interferes with insulin signaling and glucose uptake primarily via upregulation of p85α regulatory

subunit of PI3K, and an increase of FFA (390, 562, 613). Moreover, AT cellular senescence is a feature of T2DM with tumor-supportive action (621) and is decreased in mouse models of decreased GH action, while bGH mice have higher susceptibility to develop diabetes but not obesity on high-fat diet (622). Lastly, in HiGH mice, which have elevated GH and IGF1 due to somatotrope-specific ablation of IGF1R and InsR, increased DMBA-induced mammary tumor growth was observed when put on a high-fat rather than a normal chow diet (202). Although this outcome can be secondary to intrinsic insulin resistance in HiGH mice, these animals are a good model to assess whether GHR antagonism mediated improvement in insulin sensitivity counteracts high-fat diet-promoted neoplastic incidence. Several excellent reviews have summarized the empirical and mechanistic details of the diabetogenic actions of GH (114, 613, 614, 623).

Can tumor-derived GH induce hyperinsulinemia? In absence of direct studies, this question can be answered by indicating that changing localized GH action in just one tissue can indeed influence systemic insulin sensitivity, as is observed in the case of visceral fat transplanted from GHRKO to wild-type mice, improves insulin sensitivity and glucose tolerance (624). Moreover, pharmacologic inhibition of GHR with pegvisomant have repeatedly and consistently shown an improvement in insulin sensitivity across multiple studies in patients with acromegaly (625-632), markedly superior to the prescribed peptide analogs of SST, which have frequent hyperglycemic side effects (633, 634). Therefore, in the treatment of cancer, GHR antagonism is a clinically appealing proposition as a method to improve antineoplastic efficacy, with improvement of insulin sensitivity and suppression of IGF1 as putative "side effects" (635).

Promising Therapies Targeting GH Action in Cancer

The presence of an active IGF/insulin system determining lifespan in invertebrates like *C. elegans* and *D. melanogaster*, and appearance of the GH/GHR homologue much later in the basal chordate amphioxus (636), indicates that IGF1/insulin predates GH in evolution as a growth factor. Therefore, modulating GH action can be used as an indirect regulator of physiologic actions of IGF1/insulin, as exemplified by studies from numerous animal models in this area as well as human pathologies and observations from pharmaceutical agents targeting the GH axis. At this point, it is apparent that molecules that can attenuate GH action can also have applications in improving the efficacy of anticancer therapies, including radiation, chemotherapy, targeted therapy, and immunotherapy. There is active pharmaceutical interest in developing potent inhibitors of GH action by virtue of its provocative indications in cancer as well as proven application in acromegaly, and potential uses in glomerulopathies, insulin resistance, diabetic comorbidities like diabetic nephropathy and retinopathy, or in promoting healthy aging (637). These agents can target either GH production (GHRHR inhibitors, SST analogs, dopamine agonists), or GHR synthesis (small molecule, oligonucleotides), or GHR activation (anti-GHR mAb, modified human GH analogs, antisense oligonucleotide, small molecules). A series of timely and exhaustive reviews continue to enlist, detail, and compare these approaches (70, 638, 639). Below, we briefly summarize some of these strategies of inhibition of GH action (Table 13) that are or can be relevant in anticancer applications.

Inhibition of GH Production

GHRHR antagonists

Andrew Schally and colleagues have pioneered the development and use of GHRH (and GHRHR) antagonists (including long-acting isoforms) for more than 30 years, toward possible applications in acromegaly, diabetic retinopathy, and cancer by blocking GH-induced local IGF1 production (640-644). Similar to GH, expression of extrapituitary GHRH (668) and splice variants of GHRHR in multiple cancers, including breast, prostate, lung (669), esophageal (670), endometrial (671), thyroid (672), gastrointestinal (673), and melanoma (674) were observed. Consequently, GHRH or GHRHR antagonists have shown efficacy in a series of human cancer cell lines and rodent xenograft studies (675) in promoting apoptosis and blocking proliferation, EMT induction, and therapy resistance. These include cancers of prostate (676, 677), breast (678, 679), pituitary adenoma (680), lung (681, 682), esophagus (670), thyroid (683), endometrium (684), ovarian (685), gastric (686), melanoma (674), glioblastoma (687), mesothelioma (688), pheochromocytoma (689), osteosarcoma (690), Ewing sarcoma (690), and acute myeloid leukemia (691). However, GHRH-GHRHR regulated GH and/or downstream IGF1 production from tumors is not a consistent feature of any particular cancer type and highly variable and often absent from one cell line to another in the same cancer type.

Somatostatin analogs

A large proportion of neuroendocrine tumors express SST receptors (SSTRs), although SST-induced endocrine GH-mediated IGF1 production is not the exclusive target of SSTR inhibition in cancer. SST-SSTR have also been reported in gliomas (692), melanoma (645), CRCs (693), thyroid cancer (694), and breast cancer (695). Different SST analogs target different SSTRs and although the classical SST peptide analogs octreotide and lanreotide (SSTR2, SSTR5 inhibitors) have shown efficacy in reducing serum IGF1 levels in subsets of patients with acromegaly, their anticancer efficacy as monotherapy has not been promising so far (696). Relatively better treatment responses were observed in some clinical trials in combination with chemo- or targeted therapies, especially for neuroendocrine tumors (696). Second-generation multi-SSTR inhibitors, such as pasireotide (SOM230), have shown better response in systemic IGF1 suppression and treatment of pituitary tumors in Cushing syndrome (646) and in preclinical studies with human meningioma (647), melanoma (295), bronchial carcinoma (648), and corticotroph adenomas (649), although insulin resistance is a frequently observed side effect in pasireotide treatment (697). Recently developed orally active nonpeptide selective SSTR2 agonists against acromegaly, such as paltusotine, have shown some promising data against carcinoid syndrome including neuroendocrine tumors (see https://crinetics.com/pipeline/paltusotine-acromegaly-nets-carcinoid-syndrome/; phase 2). Overall, targeting the SST-SSTR axis for blocking GH action in cancer has not been clinically beneficial.

Dopamine agonists

Cabergoline is an ergot-derived small molecule agonist of the dopamine D2 receptors (698), used to treat hyperprolactinemia. Cabergoline has low efficacy in few patients with acromegaly (699, 700). In a phase 2 pilot study for metastatic

Table 13. Molecules and methods for attenuating human GH action

Molecule	Type	Origin	Tested anticancer effects	Reference
Inhibitors of GH production				
GHRHR antagonists	peptide	Andrew Schally and colleagues	Yes	(640-644)
SST analogs	peptide	Novartis, Ipsen	Yes	(295, 645-649)
Dopamine agonists	small molecule	Par Pharma	Yes	(650)
Inhibition of GHR synthesis				
BM-001	small molecule	University Medical Center, Utrecht, Netherlands	Yes	(651)
ATL oligomers	antisense oligodeoxynucleotide	Antisense Therapeutics, Australia	No	(652)
Inhibition of GHR activation				
G120R-hGH, B2036	peptide	Kopchick and colleagues, Ohio University	Yes	(70, 639, 653)
pegvisomant	peptide	Pfizer Inc.	Yes	(70, 639, 653)
B2036 site-specific pegylations	peptide	Perry, Maynard and colleagues	No	(654, 655)
hGH-G120 K site-specific pegylations (compound-G, compound-D)	peptide (GHR, PRLR dual inhibition)	Kopchick and colleagues, Ohio University and InfinixBio, Columbus, OH	Yes	(656)
S1H (16 amino acid)	peptide (GHR, PRLR dual inhibition)	Kopchick, Holub and colleagues, Ohio University	No	(657)
AZP3813 (16 amino acid)	peptide (bicyclic)	Amolyt Pharma, Netherlands and PeptiDream, Japan	No	(658)
mab18.24	monoclonal antibody (mAb)	Frank and colleagues	No	(659)
GF185	mAb	—	No	(660)
CG86	mAb	—	No	(661)
RN172	mAb	Pfizer Inc.	No	(639)
H53	bispecific (GHR, PRLR) mAb	Chen and colleagues, The First Hospital of Jilin University, China	yes	(79)
BVT-A	small molecule	Swedish Orphan Biovitrum AB (SOBI)	No	(662)
GHR-blockers	small molecules, mAbs	Longo and colleagues	No	patent US10246446B2
Fasting mimicking diet (FMD)	dietary supplement	L-Nutra (Longo and colleagues)	Yes	(663-667)

Abbreviations: GHR, growth hormone receptor; GHRHR, growth hormone releasing hormone receptor; PRLR, prolactin receptor; SST, somatostatin.

breast cancer, cabergoline achieved negligible overall response with disease control in a small subset of patients (650).

Inhibition of GHR Synthesis

Small molecules

A small molecule series (BM-001, -002, -003) has been recently developed by the University Medical Center, Utrecht, Netherlands which targets GHR synthesis (651), from a screening of a 38 480-compound library. Of these, BM001 (injected thrice/week for 3 weeks) suppressed serum IGF1 by 73% (blocking endocrine GH) and caused significant and durable reductions in human breast cancer xenografts in immunocompromised mice (blocking autocrine/paracrine GH) (651).

ATL oligomers

The 2′-O-(2-methoxyethyl)-modified phosphorothioate oligodeoxynucleotides (ATL-227446, -261303, -260120, -1103) targeted against the GHR mRNA (652, 701, 702) have shown IGF1 lowering efficacy in mice. Of these, ATL1103 (a 20-mer oligodeoxynucleotide, now "Atesidorsen"), was tested in a phase 2 clinical trial for acromegaly, where at a once/twice per week dosing for 14 weeks, it achieved a median reduction of 27.8% in serum IGF1 levels (652). No cancer studies with the ATL oligos have been reported and the ATL molecule has been discontinued.

Inhibition of GHR Activation

Modified GH analogs

Although the biochemical structure was identified in 1971, it was not until 1992 that the first crystal structure of human GH bound to GHR was reported (703). Based on the biochemical structure, we were successful in discovering the first antagonist of the mouse GHR in 1990 (704) and the first antagonist of the human GHR soon after (653). This modified GH analog is a competitive inhibitor of the GHR and proceeded to be the first (and to date only) FDA-approved GHR antagonist: pegvisomant (marketed as Somavert by Pfizer Inc.), prescribed for the treatment of acromegaly (reviewed in (66, 705)). B2036, the core peptide of pegvisomant, harbors a total of 9 amino acid substitutions, of which the G120K confers binding interruptions at site-2 of GH-GHR (see "Beginning and End of GH Signaling") while the remaining substitutions allow improved binding at site-1, as well as attenuates binding to PRLR and

non-primate GHRs (70, 639, 706). Meta-analyses of multiple clinical trials including ACROSTUDY (2221 patients) spanning 15 countries across 2004-2017 (707) and subsequent studies (632, 708) over the last 20 years have reported pegvisomant to be highly efficacious in systemically blocking GH action, alongside improving insulin sensitivity, with minimal adverse side effects in human patients. Pegvisomant is pegylated with a variable number of 5-kDa polyethyleneglycol (PEG) per peptide molecule leading to >72-hour half-life in humans (~24-hours in mice).

Multiple recent studies, including by us, have sought to devise improved modified GH analogs as GHRAs by employing different pegylation methods (638). Of these, Perry and Maynard have reported 3 different approaches using B2036 as the core peptide: (i) attaching 4 to 7 amine-reactive 5-kDa methoxy-PEG succinimidyl propionate residues, resulting in a 15-hour half-life in mice (709); (ii) substituting unnatural amino acid propargyl tyrosine at Y35 position to allow site-specific attachment of 20-kDa methoxy-terminated PEG via click chemistry leading to a 12.5-fold improvement over pegvisomant in attenuating Ba/F3-hGHR cell proliferation (654); and (iii) a 40-kDa methoxy-PEG maleimide site-specifically attached to a cysteine substituted at S144 position, leading to a 58.3-hour half-life in mice and reduction of serum IGF1 by > 50% at a low dose of 10 mg/kg/day (655). Our group has developed a library of GH analogs using different combinations of branched and charged/uncharged PEGs of different molecular masses for site-specific attachment at specific substituted cysteine residues on the G120K-hGH as the core peptide (656). Of these, compound-G (2 charged, branched 4.5-kGa PEGs at cysteines incorporated at T142 and H151 positions), at 100 mg/kg/day dosing, reduced mouse serum IGF1 by 28% and IGFBP3 by 50% in 1 week (656). Importantly, unlike B2036, G120K-hGH does bind to the PRLR, as does compound-G, which acts as a dual inhibitor of both GHR and PRLR activation and has shown consistent in vitro and in vivo anticancer effects, prompting further developmental work.

Small peptide inhibitors

A unique approach at disrupting the site-1 binding of GH-GHR was adopted by mimicking the amino acid residues 36-51 in the human GH peptide, which constitutes the mini helix important for the human GH binding to the human GHR and PRLR (657). This 16-amino-acid peptide has shown robust inhibition of GH-GHR, GH-PRLR, and PRL-PRLR inhibition in vitro and is currently under active development.

A second peptide antagonist of GHR has been reported recently from a collaboration between a Dutch and Japanese biopharma. Their candidate compound—AZP3813—a 16-amino acid bicyclic peptide, has shown IGF1 lowering effects in SDR rats (658), as well as in beagle dogs (710). In 2023, AZP3813 entered phase 1 human clinical trials for the treatment of acromegaly.

Monoclonal antibodies

One of the first reports on mAbs against GHR was presented by Frank and colleagues in 2011, where anti-GHR (mab18.24) reportedly blocked GHR signaling by blocking site-2 interaction sites of GH-GHR (659). Subsequently, 3 more GHR-targeting antibodies have been reported (GF185 (660), CG86 (661), and RN172 (639)) with in vitro and in vivo efficacies, but they do not have any follow-up reports or cancer-related results.

Interestingly, an anti-idiotypic bispecific antibody (H53) against GHR and PRLR has also been developed and has shown inhibitory potency against breast cancer models in cells and nude mice xenografts (79).

Of note, in an attempt to create a longer-acting GHRA, a fusion protein of the GH binding protein with an amino acid-substituted human GH (G120R, W104A) was constructed by Wilkinson et al; the molecule showed a half-life of 40.5-hours in rabbits, with a 14% decrease in serum IGF1 over 7 days after a single injection (711).

Small-molecule GHRAs

There are currently 2 reports of small molecule inhibitors of the GHR. One of the first reports was from the Swedish Orphan Biovitrum AB (SOBI), concerning a low molecular weight compound BVT-A, which showed IGF1-lowering effects in SDR (662) and a second from Longo and colleagues who disclosed a number of small molecules aimed at attenuating GHR action in 2019 (patent US10246446B2). To date, follow-up reports or results on cancer-related uses are not available from either.

Fasting Mimicking Diet

Short-term fasting increases serum GH and IGF1 levels, while extended fasting (> 3 days) induces peripheral GH resistance due to lack of insulin action (reviewed in (712)). The clinical success of fasting mimicking diet (FMD, low-protein, low-calorie, plant-based, 5-day dietary intervention) by Longo and colleagues, is another excellent example showing that suppression of IGF1 and insulin can have clinical benefits in multiple pathophysiology including cancer (713, 714). In cancer, FMD supplementation in human patients has synergized with chemotherapy (663) and targeted therapies (664) in leukemia and promoted antitumor immunity (715). FMD has also improved immunotherapy efficacy, with reduced immunotherapy-related adverse events and tumor-associated macrophage activity in multiple mouse models of breast cancer (665-667). Moreover, in relevance to tumor-promoting effects of GH/IGF, in preclinical animal models and clinical trials, FMD appears to exert several broad-spectrum anticancer effects, including an increase in lifespan with major reductions in inflammatory diseases and tumor incidence (716), decrease of visceral fat, rejuvenated immune system, decreased biomarkers of cancer (716), protection from insulin resistance from high-fat high-calorie diet (717), reversal of diabetes (718), improved glycemic control in T2DM patients (719), reduced insulin resistance, reduced prediabetes markers, and improved cardiometabolic and immune age in randomized clinical trials (720).

Lessons in GHR Targeting From IGF1R Targeting in Cancer

A 2023 JAMA report summarized that in the last 2 decades (2000-2021), 183 clinical trials have studied 16 IGF1R candidate inhibitor molecules across 12 000 patients at a cost of $1.6 billion in research and $2.3 billion in development expenses (721, 722). These trials included 9 monoclonal antibodies (including Pfizer's figitimumab, Merck's dalotozumab, Amgen's ganitumab), 6 small-molecule inhibitors (also RTK-inhibitors), and 1 antisense

oligodeoxynucleotide targeted against IGF1R (579, 721). Unfortunately, all of the IGF1R targeting agents failed to live up to the promise of clinical efficacy in any of the late-phase cancer trials (reviewed in (579, 723)). Essential insights, highly relevant to GHR targeting in cancer, can be obtained from the mechanisms underlying this result (723, 724). Some of the prominent mechanisms by which IGF1R inhibition was overridden in target tumors in vivo can be summarized as: (i) compensatory signaling (by IGF1, IGF2, INS) via INSR; (ii) compensatory signaling via increased (endocrine) GH production (de-repression of negative feedback inhibition by IGF1-IGF1R at hypothalamic-pituitary axis); (iii) compensatory signaling by EGFR (an otherwise hetero-dimerization partner of IGF1R); (iv) nuclear localization of IGF1R; (v) lack of biomarkers of disease response beyond serum IGF1; and (vi) poor patient selection.

In the case of GHR antagonism, the clinical efficacy of pegvisomant is an excellent example of FDA-verified clinical safety, tolerability, and effectiveness of systemic GHR antagonism with a highly specific molecule, and possible clinical countermechanisms of compensation. As discussed above, a peptide mimetic GHRA (eg, pegvisomant and other biosimilars), in addition to systemically blocking GH-GHR activation (endocrine, autocrine, paracrine), can mechanistically offer additional significant advantages up front because: (i) Systemic GH-GHR inhibition lowers endocrine IGF1 production; importantly, in the dose-escalation trials, pegvisomant lowered both serum IGF1 and IGF2, thus dampening IGF1R ligand supply; (ii) Systemic inhibition of GH-GHR action lowers development of insulin resistance, which has beneficial effects in cancer prognoses; (iii) Possible signaling from excess GH via binding to PRLR, does not appear to compensate GHR blockade (eg, in case of acromegaly treatment), although responses in oncology could be different. However, dual antagonists of GHR and PRLR are effective suppressors of possible compensatory GH-PRLR signaling; (iv) Pegvisomant can effectively inhibit nuclear localization of GHR as well as surface GHR; and (v) GHR-EGFR or GHR-IGF1R cross-interactions require active GHR-associated JAK2 for effect. In the absence of GHR activation, reduced compensation from these other RTKs can be speculated, but are subject to experimental validation. Additionally, adequate biomarkers of successful GHR antagonism (beyond serum IGF1 suppression) are essential to confirm anticancer benefits. For example, some additional biomarkers of effective GHR blockade are concomitant decrease in hepatic IGFBP3 and ALS, as well as suppression of SOCS2 and CISH expression. Moreover, progressive and dose-dependent lowering of markers of disease progression, like CA-19-9 (for gastrointestinal cancers of liver, pancreas, gastric, colorectal, and some other types) are also clinically valuable. Lastly, appropriate patient selection based on high-resolution quantification of tumor gene expression will be an essential step for effecting clinically durable success in future trials.

Overall, it is apparent that the use of inhibitors of GHR synthesis in cancer are contingent upon tumoral GH synthesis being under the control of the corresponding regulatory circuit. In comparison, inhibitors of GHR synthesis or activation can provide a definite systemic blockade of GH reception, although GH-PRLR interactions are not attenuated by these. Herein, dual inhibitors of GHR and PRLR can be a better fit for use, especially in cancers with high PRLR expression, to block a wider spectrum of GH action including the endocrine, paracrine, and autocrine effects.

Conclusion

In this review, we have put forth the details of how GH promotes cancer and why targeting GH action as a therapeutic option to accompany anticancer approaches may be significantly beneficial to improve prognoses. Having said that, it is vital to understand and consider the differences and overlaps in endocrine vs autocrine/paracrine GH actions in cancer, as that is a definite way that the clinical and epidemiological data appears to reconcile with the empirical data. At this point, we may conclude by acknowledging some clearly emerging questions about the scope of application of GHR antagonism in relevant cancer types: (i) Can GHR antagonism reduce cancer incidence? (ii) Can GHR antagonism reduce cancer relapse? and most importantly: (iii) What is the tissue-specific and sex-specific status of nonpituitary GH with age? While scientific efforts are to be directed toward answering these vital questions, there are early indications. Notably, it is now well-studied that GH promotes aging, and cancer is now understood to be largely an age-associated disease. That is why decelerating the molecular mediators of aging also mechanistically lowers the risks of cancer incidence, as proven by collective literature in this area, including the example of patients with LS and congenital isolated GHD (31, 725). Therefore, one may ask whether lowering GH action at an advanced age might also lower the risks of cancer. Recent studies from our laboratory have already started to provide some intriguing answers to this enigmatic question. For example, given the observed enrichment of hallmarks of healthy aging (reduced cancer incidence, improved insulin sensitivity, reduced musculoskeletal and cognitive decline) in the congenital GHRKO mice (world's longest-lived laboratory mouse (726)), we recently conditionally ablated GHR in the mice at an adult age of 6 months (6mGHRKO mice)—thus attenuating all endocrine, autocrine, and paracrine actions of GH in these animals. Subsequent end-of-life pathology shows that this mid-life abrogation of GH action results in markedly lowering the rates of malignancy in both male and female mice, in addition to sex-specific improvement in insulin sensitivity and extended lifespan (617, 727). These findings suggest that GHR attenuation could serve as an effective maintenance therapy to attenuate high-risk cancer occurrence or recurrence. Finally, GHR antagonism exerts synergistic mechanisms of action in promoting the efficacy of several anticancer approaches including radiotherapy, chemotherapy, and targeted therapies such as immunotherapy. Simply put, in the context of cancer, blocking the GHR renders a *triple whammy* in anticancer effects: (i) antagonism of GH action in an endocrine manner as well as in an autocrine/paracrine scenario; (ii) suppression of IGF1; and (iii) improving systemic insulin sensitivity. This combination could be of great value in anticancer therapy. On the back of extensive preclinical data and availability of approved and/or candidate antagonists of GH action discussed above, well-designed clinical trials in cancer patients are the call-of-the-hour to test whether GH action indeed has the potential to be an Achille's heel of cancer.

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