MECHANISMS IN ENDOCRINOLOGY

Lessons from growth hormone receptor gene-disrupted mice: are there benefits of endocrine defects?

Reetobrata Basu1,*, Yanrong Qian1,*, and John J Kopchick1,2

1Edison Biotechnology Institute, Ohio University, Athens, Ohio, USA and 2Ohio University Heritage College of Osteopathic Medicine, Ohio University, Athens, Ohio, USA

*(R Basu and Y Qian contributed equally to this work)

Abstract

Growth hormone (GH) is produced primarily by anterior pituitary somatotroph cells. Numerous acute human (h) GH treatment and long-term follow-up studies and extensive use of animal models of GH action have shaped the body of GH research over the past 70 years. Work on the GH receptor (R)-knockout (GHRKO) mice and results of studies on GH-resistant Laron Syndrome (LS) patients have helped define many physiological actions of GH including those dealing with metabolism, obesity, cancer, diabetes, cognition and aging/longevity. In this review, we have discussed several issues dealing with these biological effects of GH and attempt to answer the question of whether decreased GH action may be beneficial.

Introduction

An extensive body of basic and clinical research over the last 70 years focusing on growth hormone (GH) and its cognate receptor (GHR) has yielded a tremendous amount of animal and human data. In the process, GH has acquired a truer identity of not just a hormone responsible for longitudinal growth and organ development, but one with distinct catabolic and anabolic roles across many tissue types and throughout the lifespan of an individual. The clinical conditions of GH excess usually due to a hypersecreting pituitary adenoma (acromegaly) (1) or of GH resistance/insensitivity due to multiple inactivating mutations on the GHR gene (Laron syndrome; LS) (2, 3, 4)

Invited Author’s profile

John J Kopchick has since 1987 been the Goll-Ohio Eminent Scholar and Distinguished Prof. of Molecular Biology in The Edison Biotechnology Institute and the Heritage College of Osteopathic Medicine at Ohio University in Athens, Ohio. He is an internationally recognized leader in the growth hormone field. Dr Kopchick and his group were the first to discover and characterize GH receptor antagonists, an accomplishment for which he and Ohio University were awarded several US and European patents. He also was instrumental in finding a company, Sensus, which applied his research to the development of an FDA-approved drug called Somavert (pegvisomant for injection), which is marketed for patients with acromegaly. Dr Kopchick has published more than 350 scientific articles and serves or has served on the Editorial Boards of many prestigious journals.
or congenital or adult-onset or acquired GH deficiency (GHD) (5, 6, 7, 8) have been and are of critical importance to study the spectrum of GH actions. Laron’s seminal work with the Israeli cohort of LS patients (3, 4, 9, 10) and Guevara’s subsequent 22-year follow-up study on the Ecuadorian cohort (11, 12) of LS patients along with extensive work with GHR-knockout (GHRKO) mice developed 21 years ago in our laboratory (13) have been instrumental in helping to define GH and insulin-like growth factor-1 (IGF-1) activities. Both human LS patients and GHRKO mice share several physical, metabolic and cognitive similarities that will be described below (14, 15, 16, 17).

Growth of GHRKO mice and LS patients is significantly suppressed compared to control individuals (4, 11, 15) largely due to suppression of GH-induced intracellular signaling and depressed IGF-1 production. IGF-1 is one of the primary mediators of GH-regulated longitudinal growth and organ development (18, 19). Therefore, GH as well as IGF-1 deficiency affects body length and weight, body composition and energy metabolism. GH has a catabolic effect on adipose tissues, promoting lipolysis, while possessing an anabolic effect on muscle and bones (20, 21, 22). As expected, GHRKO mice and LS patients have reduced IGF-1 and insulin levels, are insulin sensitive, have shorter bones, less muscle and are obese with a relatively high-fat percentage (15). On the other hand, individuals with elevated levels of GH and IGF-1, as seen in patients with acromegaly, have thicker skin and bones, larger muscles, increased lean mass and are insulin resistant. If this condition is left untreated during childhood, gigantism can occur (23, 24) with several associated metabolic dysfunctions ultimately resulting in a decrease in lifespan (25). Additionally, we, as well as our colleagues throughout the world, have studied mice transgenic for the bovine (b) GH gene (the bGH mouse) (26) and mice transgenic for a GH-analog acting as a competitive antagonist of GHR (the GHA mouse) (27, 28). These mice are dwarf and partially recapitulate the human conditions of acromegaly and GHD, respectively. Finally, GH is intricately associated with several of the top-ranking pathological challenges of the modern world – obesity, diabetes, cancer, memory, cognition and aging. In the following sections of this review, we focus on the recent advances in this field of study and try to distill the lessons on GH’s role in physiology derived from mice and humans who are insensitive/resistant to the action of GH.

### Obesity and diabetes

GH has catabolic effects on adipose tissues while anabolic effects on muscle (20, 21). Therefore, bGH mice or patients with acromegaly have increased lean mass and decreased fat mass and are insulin resistant. In contrast, GHRKO mice as well as Ecuadorian LS patients are resistant to GH and obese yet insulin sensitive (3, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41). Interestingly, the coexistence of insulin sensitivity and obesity are hallmark features of the GHRKO mice and have prompted multiple studies concerning the regulation of glucose and lipid metabolism in these animals.

Decades ago, GH was shown to be a diabetogenic molecule (42). It inhibits insulin action or has anti-insulin activity (42, 43). However, the anti-insulin mechanisms of GH are still not entirely clear as GH perturbs the regulatory subunit of PI3K in mouse adipose and muscle tissues (44, 45, 46) while increased lipolysis and free fatty acids (20, 47) and reduced pyruvate dehydrogenase activity in skeletal muscle are thought to be the mechanism of GH-induced insulin resistance in humans (48). Whatever the mechanism, GHRKO mice and LS patients generally are insulin sensitive and have low insulin levels (3, 17, 27, 29, 30, 31, 32, 33, 37, 38, 39, 40, 41, 49, 50, 51).

Fasting and non-fasting blood glucose levels in young GHRKO mice are significantly lower than wild-type (WT) controls (29, 31, 32, 33, 34, 35, 36). However, in older GHRKO mice, glucose levels increase gradually until they are similar to those of WT controls. Similarly, Israeli LS patients have lower fasting and postprandial glucose levels compared to controls and also have a similar trend of glucose increasing as a function of age (51). With aging, diabetes and associated complications have been reported in the Israeli cohort of patients (52). In contrast, Ecuadorian LS individuals have very low insulin levels (about one third of controls) (37). During glucose tolerance tests or after a high calorie meal, insulin level remains low in the LS subjects as the blood glucose levels are similar to controls, suggesting that LS subjects are insulin sensitive. In fact, though these LS subjects are obese, they have enhanced insulin sensitivity and lower incidence of diabetes than their non-LS relatives (3, 37, 38, 39, 40, 41). The obesity of LS patients is not associated with excessive nutritional intake (53).

Nonalcoholic fatty liver disease (NAFLD) is a frequent complication in LS patients and adult GHD patients with a lowered GH and IGF1 levels (54, 55, 56, 57, 58). Six-month
GH replacement therapy in GHD patients was found to improve hepatic lipid profile and oxidative stress, as well as liver enzymes including serum aspartate aminotransferase and alanine aminotransferase and γ-GTP concentrations. Histological studies of liver biopsy specimens showed that GH replacement therapy did decrease steatosis, fibrosis, inflammation and ballooning necrosis, which could be a reflection of the lipolytic properties of GH. Another study with 24-month GH replacement therapy in adult GHD patients showed improving serum liver enzyme levels and decreasing fibrosis (59). Also, low serum IGF-1 levels have been associated with increased histologic severity of NAFLD (60). IGF-I inactivates hepatic stellate cells and induces cellular senescence, therefore ameliorating fibrosis. IGF-I treatment has been shown to improve nonalcoholic steatohepatitis (NASH) and cirrhosis in animal models, suggesting potential clinical applications of IGF-I in these conditions (57). A mechanistic study using a liver-specific GHR ablation mouse line to distinguish the effect of GH and IGF-1 suggested that GHR ablation in the liver leads to increase in lipid uptake, de novo lipogenesis, hyperinsulinemia and hyperglycemia, and the restoration of IGF-1 was insufficient to protect against steatosis-induced hepatic inflammation or oxidative stress (61).

In the following sections, the effects of lack of GH action on insulin-sensitive tissues, including adipose tissue, muscle, liver and pancreatic islets will be discussed.

Adipose tissue of GHRKO mice

Total adipose weight is significantly increased in GHRKO mice (29, 62, 63, 64, 65). Interestingly, there are significant differences among different adipose tissue depots in these dwarf animals. For example, the subcutaneous depot is significantly increased relative to controls. Because the GHRKO mice have much smaller body size than normal littermates, the absolute subcutaneous adipose tissues weight is more than those in WT mice. Also, the retroperitoneal adipose tissues (fat pads next to the kidneys) are increased in male mice compared with controls (62, 63) but not changed when normalized to body weight (29, 35). However, the epididymal depot, which is known to be correlated with cardiovascular or metabolic diseases (66, 67), is smaller or similar than those in WT mice when normalized to body weight (65) implying that the GHRKO mice are obese but healthy. The brown adipose tissues are also increased in GHRKO mice (64, 68).

GH affects multiple aspects of adipose tissues including immune cell infiltration, senescence and fibrosis (65). For example, there is less NLRP3 inflammasome-mediated inflammation in bone marrow and epididymal macrophages in the GHRKO mice relative to controls (69). Also, their epididymal and perinephric adipose tissue have reduced IL-6 levels (70), suggesting that lack of GH action appears to generate an anti-inflammatory state. Senescence is a state of irreversible cell growth arrest and resistant to apoptosis (71). In GHRKO mice, there are less senescent cells in most adipose depots (72). However, dwarf GHA mice have similar levels of senescent cells compared with WT controls (73). Fibrosis is correlated with obesity. It is increased in bGH mice while decreased in GHA or GHRKO mice (65, 74); thus, GH action significantly affects the extracellular matrix in adipose tissues. LS patients also have impressively and preferentially increased subcutaneous adipose tissues, while the other adipose tissue depots are increased to a lesser extent (75).

Muscle of GHRKO mice

GH has anabolic effects on muscle. Thus, GHRKO mice have decreased muscle mass (13, 31, 63) due to smaller size but conserved numbers of muscle fibers (76, 77). In hind limb muscle cells from 4-week-old WT mice, GH increases myofiber size by stimulating the fusion of myoblasts to myotubes, but not the proliferation or differentiation of myoblast precursor cells (77). In contrast, the increased fusion induced by GH was not observed in the cells from GHRKO mice due to absence of GHR (77). There are two subtypes of muscle fibers: type I and type II. Type I fibers produce endurance but low force due to the age difference. Interestingly, GHRKO mice performed better on the Rotarod test compared with WT, bGH or GHA mice at 6-month-old (unpublished data), suggesting better endurance, balance and strength in these dwarf mice. In fact, bGH mice with long-term GH/IGF-1 excess have recently been found to have decreased protein synthesis in the skeletal muscle (78). Clinically, recombinant hGH treatment is known to increase muscle mass in GH-deficient patients (79), while LS patients have decreased muscle mass similar to that seen in GHRKO mice.
promotes the proliferation of pancreatic β cells and insulin secretion (85) implying that in an acute GH stimulation scenario, the insulin level will be increased; however, whether long-term GH stimulation would damage the pancreatic β cells and lead to diabetes is not known.

The influence of diets on GHRKO mice

Due to the unique profile of adipose tissue in the obese but healthy GHRKO mice, multiple types of diets have been fed to these mice to study insulin sensitivity and longevity.

High-fat diet

Generally, high-fat (HF) diets lead to diet-induced obesity and type 2 diabetes (86, 87, 88, 89). When on HF diets for 12 weeks starting at 10 weeks of age, both GHRKO mice and WT mice have significantly increased adipose tissue weights compared with mice in low-fat diet group (35). Interestingly, GHRKO mice show a specific increase in their subcutaneous depot while WT mice have a greater increase in their epididymal and retroperitoneal depots. Another study of male GHRKO mice on a HF diet for 17 weeks starting at 14 weeks of age showed similar trends of different fat depot increases (83). However, glucose homeostasis in these dwarf mice was not significantly altered while WT mice became insulin resistant. Similarly, GHA mice are also resistant to diet-induced type 2 diabetes on HF diet (90, 91), implying that the absence of GH action or GH's diabetogenic effect may maintain glucose homeostasis and protect the animal from diabetes.

Caloric restriction

Caloric restriction (CR) has been widely used to study glucose homeostasis and longevity though its specific mechanisms are not completely understood. CR leads to decreased circulating insulin and IGF-1, which plays an important role in the improved insulin sensitivity and extended lifespan from worms to mammals (92, 93). GHRKO mice have increased lifespan (discussed below). However, CR does not further increase the extended lifespan or improved insulin sensitivity of GHRKO mice (19, 29, 94, 99) suggesting that the molecular mechanism(s) responsible for this ‘lack of effect’ may be overlapping. However, other findings suggest that CR and GHR deficiency use non-overlapping mechanisms regulating insulin sensitivity (17, 82, 95). As discussed earlier, in the skeletal muscle of GHRKO mice, Foxo1 and Foxo3 protein

Liver of GHRKO mice

In the livers of young GHRKO mice, levels of insulin receptor (IR) and tyrosine-phosphorylated IR were both increased compared with WT controls suggesting increased insulin sensitivity (27, 34, 83). However, the downstream insulin signaling cascade was not affected. In addition, the MAPK signaling pathway and levels of Src homology 2 domain-containing-transforming protein C1 (SHC) were not altered in GHRKO mice compared with WT controls. Consistent with increased blood glucose levels in GHRKO mice as they age, the activation of insulin signaling cascades were also reduced compared with young controls (17). In addition, increased phosphorylation of AMPK and cAMP response element-binding protein (p-CREB) in older GHRKO mice further indicates a shift toward gluconeogenesis and lipid oxidation in the liver. However, at all ages tested, GHRKO mice are more insulin sensitive than WT controls (17, 27, 34, 83). Finally, two-year-old (~50% of overall lifespan) GHRKO mice have lower levels of steatosis and inflammation in liver compared withagematched littermate controls, which may contribute to their extended longevity (84).

Pancreatic islets of GHRKO mice

Consistent with lower circulating insulin levels, GHRKO mice display smaller islets, smaller cell size, smaller beta cells mass, as well as decreased insulin content (29, 50). These features suggest lower insulin production capability (50). In fact, GHRKO mice are not able to produce large amounts of insulin in a short period of time to handle the high level of glucose found in the glucose tolerance tests. Therefore, they are glucose intolerant (50). Also, GH

References

(15, 33, 65, 80), which can be rescued partially by IGF-1 treatment (80). Though there is a significant decrease in circulating IGF-1, there is no significant changes in protein IGF-1 levels in the muscles of GHRKO mice, suggesting that endocrine IGF-1 is important for muscle size (81). The muscles of GHRKO mice are more insulin sensitive than those in control mice (27). Compared with WT mice, levels of p85/IRS-1 phosphorylation, p-AKT and GLUT4 levels were increased in the muscle of 14-month-old GHRKO mice (27) suggesting increased insulin induced intracellular signaling. Also, levels of Foxo1 and Foxo3, transcription factors involved in the regulation of gluconeogenesis and glycogenolysis, were lower in the skeletal muscle in GHRKO mice (82) again suggesting increased insulin sensitivity in muscles.
levels were reduced (see ‘Muscle of GHRKO mouse’ section); however, CR did not significantly affect Foxo1 or Foxo3 protein levels in either GHRKO or WT mice (82). Also, peroxisome proliferator-activated receptors α (PPARα) is a transcription factor highly expressed in the liver where it participates in the regulation of fatty acid (FA) oxidation and decreases circulating fatty acids and triglycerides to improve insulin sensitivity (96). The protein levels of PPARα in the liver of GHRKO mice is elevated compared with WT mice; however, CR has no effect on the level of PPARα (95) indicating that different mechanisms are involved in improved insulin sensitivity and extended lifespan through GHR deficiency and CR.

Cancer

The reports of remarkably low cancer incidence and progression in the GHRKO mouse (97) as well as in LS individuals (14, 102, 103) coupled with human epidemiological studies reporting an increased risk for specific cancers (colorectal, thyroid) in patients with acromegaly (100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111) collectively offer an indication of the anticancer effects of attenuating GH action. In GH-treated patients with childhood- or adult-onset GHD, no association of GH treatment and development of primary cancer was found, based on available data (112, 113). Several excellent reviews have discussed the topic in recent years (24, 114, 115, 116, 117, 118). A recent genome wide association study (GWAS) had reported the GH-induced intracellular signaling network to be the third highest pathway associated with enhanced susceptibility to breast cancer (119). Increased local GH levels and a dysregulated or higher than normal GHR expression have also been reported in cancers of breast (120), prostate (121, 122), colon (123), pelvis (124), stomach (125), lung (126), pancreas (127), endometrium (128), skin (129, 130, 131, 132), liver (133, 134, 135, 136), kidney (137), nerve (138), meninges (139) and glia (140) including in vivo xenograft studies. We have summarized the collective data in Table 1. Below, we focus on the recent work done with the GHRKO, GHA and bGH mice, studies using GHR antagonist pegvisomant and summarize the known and newfound roles of the GH–GHR axis in cancer.

Cancer studies using GHR antagonist pegvisomant

Several in vitro and in vivo xenograft studies have highlighted the potential of the GHR antagonist, B2036, or pegvisomant (pegylated B2036) in human cancers. The GHR antagonist was found to markedly inhibit the growth and progression of breast (151) and prostate cancers (152). Xenografts of COLO-205 human colon cancer cells in athymic nude mice, when treated with pegvisomant, had a 39% decrease in tumor-volume or pegvisomant (pegylated B2036) in human cancers. The GHR antagonist was found to markedly inhibit the growth and progression of breast (151) and prostate cancers (152). Xenografts of COLO-205 human colon cancer cells in athymic nude mice, when treated with pegvisomant, had a 39% decrease in tumor-volume and increased levels in bGH mice (149). Consistent with this fact, liver carcinogenesis was preceded by a markedly higher hepatocellular proliferation and inflammation in bGH mice (133), while the carcinogen diethylnitrosamine (DEN) induced 2.6-fold and 4-fold higher rates of hepatic tumor proliferation in the liver of male and female bGH animals, respectively, compared to their WT littermates (134). Interestingly, peripubertal exposure to GH in Snell mice and Lewis dwarf rats was found to be a potential contributor in oncogenesis through dysregulation of DNA repair genes including Gadd45α and Bbc3 (150). How early life GH exposure followed by the absence of GH action compares in the adult-onset GHRKO models remains to be determined.

Cancer studies with the GHRKO mouse

The GHRKO mouse, along with the GHA, bGH and other mouse models of modified GH action, have been valuable for studying the implications of GH–GHR interaction in cancer (24, 141). Swanson and colleagues had crossed the GHRKO mice (13) with the C3(1)/Tag mice (142), which spontaneously develop mammary neoplasms in females and prostate neoplasms in males due to the expression of SV40 large T-antigen (143). The Tag/GHRKO mice were found to develop only three tumors per mouse compared to ten in Tag/WT mice (143) with tumor sizes in Tag/ GHRKO animals being ten times smaller than the same in Tag/WT counterparts (143). Similar studies with male offspring of the same mouse model found only one in eight Tag/GHRKO animals developing a prostate neoplasm compared to eight in ten Tag/WT mice (144). Likewise, Pollak and coworkers had shown the GHA mice to have significantly lower mammary tumor formation induced by the carcinogen, dimethylbenz[a]anthracene (DMBA) (145), which was also observed in identical studies with adult-onset isolated GHD mice (146). Recently young adult bGH mice livers have shown enlargement of hepatocytes and increased levels of PCNA, Cyclin-D1 and c-Jun and upregulated activation states of STAT3, c-SRC, AKT and mTOR (147, 148). Additionally, GH increases hepatic EGFR expression and downstream signaling, confirmed by diminished EGFR levels in GHRKO mice and increased levels in bGH mice (149). Consistent with this fact, liver carcinogenesis was preceded by a markedly higher hepatocellular proliferation and inflammation in bGH mice (133), while the carcinogen diethylnitrosamine (DEN) induced 2.6-fold and 4-fold higher rates of hepatic tumor proliferation in the liver of male and female bGH animals, respectively, compared to their WT littermates (134). Interestingly, peripubertal exposure to GH in Snell mice and Lewis dwarf rats was found to be a potential contributor in oncogenesis through dysregulation of DNA repair genes including Gadd45α and Bbc3 (150). How early life GH exposure followed by the absence of GH action compares in the adult-onset GHRKO models remains to be determined.
Table 1  List of studies on GHR expression and function in human cancers.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Type of study</th>
<th>Type of effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>In vitro</td>
<td>Autocrine GH and GHR expression drives proliferation, migration, invasion, angiogenesis, reduces oxidative stress and apoptosis</td>
<td>(120, 158, 318)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Autocrine GH increases hTERT stability through αCP1, αCP2</td>
<td>(164)</td>
</tr>
<tr>
<td></td>
<td>In vitro</td>
<td>Pegvisomant increased doxorubicin efficacy on cells</td>
<td>(166)</td>
</tr>
<tr>
<td></td>
<td>In vitro</td>
<td>GH increases resistance to mitomycin-C and radiotherapy</td>
<td>(181)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Pegvisomant attenuated xenograft growth in nude mice</td>
<td>(160, 319)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Reduced DMBA-induced tumorigenesis in GHA and GHD mice</td>
<td>(151)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Female C3(1)/Tag-GHRKO mice had 3x fewer and 10x smaller mammary neoplasms than C3(1)/Tag-wildtype mice</td>
<td>(145, 146)</td>
</tr>
<tr>
<td></td>
<td>In vitro, In vivo</td>
<td>Autocrine GH-stimulated migration, invasion, tumorigenesis and metastasis in culture and in nude mice</td>
<td>(165)</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>In vivo</td>
<td>bGH mice had increased number of liver neoplasms than WT</td>
<td>(133)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>DEN-induced hepatoma rates were significantly lower in lit/lit and significantly higher in both sexes of bGH mice</td>
<td>(134, 320)</td>
</tr>
<tr>
<td></td>
<td>In vitro, In vivo</td>
<td>Autocrine GH increased oncogenicity and xenograft growth in nude mice; HGH-G120R expression reversed the effects</td>
<td>(159)</td>
</tr>
<tr>
<td></td>
<td>In vitro</td>
<td>Autocrine GH-stimulated cancer stem cell properties by STAT3-mediated claudin-1 suppression</td>
<td>(136)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>In vitro</td>
<td>Autocrine GH-GHR-driven proliferation of prostate tumors</td>
<td>(121, 122)</td>
</tr>
<tr>
<td></td>
<td>In vitro</td>
<td>GH-stimulated IGF and estradiol-driven tumor proliferation</td>
<td>(321)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Male C3(1)/Tag-GHRKO mice had 8X fewer and smaller prostate neoplasms than C3(1)/Tag-wildtype mice</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Xenograft growth attenuated in GH-deficient lit/lit mice</td>
<td>(322)</td>
</tr>
<tr>
<td></td>
<td>In vitro, In vivo</td>
<td>Increased proliferation, migration, invasion, clonogenicity, MMP expression: Pegvisomant treatment reversed the effects</td>
<td>(152)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>In vitro</td>
<td>GH de-sensitizes to radiotherapy by blocking DNA damage</td>
<td>(183)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Pegvisomant suppressed xenograft growth in nude mice</td>
<td>(153)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>siRNA-mediated targeting of GHR decreased xenograft growth, volume, and size in nude mice</td>
<td>(323)</td>
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<tr>
<td>Melanoma</td>
<td>In vitro</td>
<td>GH suppressed tumor p53 and apoptosis and promoted EMT and oncogenesis; tumor growth reduced in GH-deficient mice</td>
<td>(168)</td>
</tr>
<tr>
<td></td>
<td>In vitro</td>
<td>High level of GHR expression in human melanoma</td>
<td>(129, 132, 324)</td>
</tr>
<tr>
<td></td>
<td>In vitro</td>
<td>siRNA mediated GHR knockdown inhibits proliferation, migration, invasion, EMT, GH treatment has reverse effect</td>
<td>(130)</td>
</tr>
<tr>
<td></td>
<td>In vitro</td>
<td>GHRKD decreases multidrug transporter expressions, increases drug retention, lowers drug EC_{50}</td>
<td>(131)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>High melanoma GHR expression and GH production in lungs drives metastases of mouse melanoma in DJ1-KO mice</td>
<td>(325, 326)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>In vitro</td>
<td>siRNA-mediated GHR knockdown inhibit proliferation, migration, invasion, clonogenicity, EMT</td>
<td>(127)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>In vitro</td>
<td>Autocrine GH increased viability, proliferation, survival, and clonogenicity in culture; B2036 expression reversed it</td>
<td>(128)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>GH increases radiotherapy resistance</td>
<td>(160)</td>
</tr>
<tr>
<td>Meningioma</td>
<td>In vitro</td>
<td>GH action on GHR-expressing meningiomas increased cell growth; B2036 inhibited the same</td>
<td>(139)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Pegvisomant significantly reduced volume, weight and growth of primary tumor xenografts in nude mice</td>
<td>(154)</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>In vitro</td>
<td>High level of expression of GH and GHR in cells</td>
<td>(138)</td>
</tr>
<tr>
<td>Glioma</td>
<td>In vitro</td>
<td>High level of expression of GH and GHR in cells; GHR is cytoplasmic, not nuclear</td>
<td>(140)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>In vitro</td>
<td>P495T SNP in GHR markedly enhances GH signaling</td>
<td>(126)</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>In vitro, Patient tissue samples</td>
<td>Significantly higher GHR expression in majority of gastric adenocarcinoma than surrounding gastric mucosa</td>
<td>(125)</td>
</tr>
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</table>

untreated controls (153). B2036 also abrogated growth of GH-GHR-expressing human meningioma cultures in vitro (139) and in vivo (154). A similar study with GH and GHR-expressing human endometrial cancer cell lines, AN3 and RL95-2, reported marked suppression of cell proliferation and clonogenic capacity following treatment with B2036 (128). Lobie’s group also demonstrated that induced expression of B2036 blocked...
STAT3-mediated oncogenicity of autocrine GH in Bel-7404 human hepatoma cells in vitro (135). Appropriately designed clinical trials would be necessary to evaluate the efficacy of a combination of GHR antagonist and anticancer therapeutic agents in human cancer patients (155). One such large-scale clinical trial was launched in 2009 by Pfizer, across US, Canada, Finland and Germany, for a combination therapy of pegvisomant and figitumumab, an IGF-1R-specific monoclonal antibody directed against solid tumors (breast, lung, colorectal, prostate, sarcoma) (156). Unfortunately, the study was terminated ‘due to inability to recruit patients on a timely basis as well as business reasons’ as stated by the study sponsors (156).

Mechanisms of GH–GHR action in cancer

A significant body of work, especially by Lobie and colleagues have underlined the role of autocrine/paracrine GH in different cancer types (128, 136, 138, 157, 158, 159, 160). Tumor-secreted autocrine GH was found to be a potent inducer of oncogenic signaling in tumor cells than endocrine GH (157) partly due to enhanced binding to nuclear GHRs (135, 161, 162). The oncogenic processes regulated by GH include direct as well as IGF-mediated mitogenic and proliferative effects as well as a number of IGF-independent (114, 118, 141) oncoprotective pathways. Below are listed the GH-dependent cancer-supporting mechanisms that have been studied and reported by different groups worldwide affecting incidence, proliferation, response to treatment and relapse of tumors:

1. Activation of JAK2, STATs 1, 3 and 5, SRC, MAPK (p44/42 and p38) and PI3K-AKT-mTOR pathways (130, 141, 162, 163).
2. Induction of hepatic as well as peripheral IGF-1 production, which in itself increase cancer incidence and adverse prognosis (98).
3. ERK1/2-mediated resistance to oxidative stress in turn reducing apoptosis (99, 127, 164).
4. Enhanced stemness due to GH action (136, 165).
5. Increased stability of human telomerase reverse transcriptase (hTERT) mRNA via induction of αCP1 and αCP2 (166).
6. Direct upregulation of epithelial-mesenchymal transition (EMT) (reviewed in (167)) possibly by a p53-dependent manner (168, 169).
7. Driving the oncogenic HGF-cMET loop and EGF receptor levels (130, 149).
8. Driving drug resistance, through increasing drug efflux via upregulation of ATP-binding cassette (ABC)-transporter expression and EMT on tumors (130, 131, 167).

Role of GH–GHR in cancer therapy resistance

Despite the discovery of new cancer therapeutics (170, 171, 172, 173, 174, 175, 176, 177), there are existing limitations to the targeted- and immune-therapy approaches (178, 179) including the development of drug resistance (180). Data depicting an association of GH action and chemotherapy and radiotherapy refractoriness in human cancers have been scarce (141). Zatelli and coworkers had reported endocrine GH-stimulated doxorubicin resistance in MCF7 cells in culture that was ameliorated by treatment with pegvisomant (181). The group inferred that the drug resistance in MCF7 cells was possibly due to GH-induced IGF-1 expression as IGF-1 independently also increased the number of viable cells in doxorubicin treatment (181). Zatelli’s group also demonstrated a protective effect of GH in inhibiting doxorubicin-induced apoptosis in estrogen receptor-negative breast cancer cells, which was significantly abrogated using pegvisomant (182). In an in vitro study, Lobie and colleagues had identified autocrine GH causing increased resistance of breast cancer cells (MCF7, T47D, and MDA-MB-231) to mitomycin-C (MMC)-induced apoptosis by protecting against DNA damage (160). The identical protective effect of GH was also seen in HCT-8 colorectal cancer cells where recombinant human GH treatment protected the tumor cells against radiation in a dose-dependent manner (183). Mechanisms of chemotherapeutic resistance in cancer includes but are not limited to (i) abundant expression of a repertoire of drug efflux pumps (184, 185, 186, 187), (ii) sequestration of drugs in melanosomes during melanogenesis (188, 189) and (iii) upregulation of EMT markers (159, 190, 191, 192, 193, 194), a critical mechanism of phenotype switch and chemotherapy evasion in several types of cancers (130, 131). Recently, we reported a GH–GHR-dependent mechanism of drug resistance in human melanoma (130, 131), where we attenuated GHR in human melanoma cells leading to direct downregulation of specific drug efflux pumps of the ABC family leading to increased drug efficacy at a lower dose (131). In response to treatment with anticancer drugs like doxorubicin, paclitaxel, cisplatin or vemurafenib, knocking down GHR on human melanoma cells potentially
blocked the increase of expression of ABCB1, ABCC1, ABCC2, ABCG1 and ABCG2 transporters irrespective of the presence of GH. As a result of siRNA-mediated GHR downregulation in melanoma, intracellular drug retention increased markedly and cell viability reduced significantly, thereby increasing the efficacy of same doses of drugs due to reduced drug efflux (131). Treatment with GH alone had a reverse effect (131). In fact, a chronic (3-week) treatment with GH alone increased the drug resistance in human melanoma cells, increasing the EC₅₀ values for vemurafenib and upregulating EMT (131). We also reported that GH upregulates the process of EMT in melanoma (130, 131) and refer the readers to our recent review of GH and EMT in cancer and normal tissues (167). The combination of the above results may help to define the mechanism(s) underlying the development of drug resistance in several types of cancers overexpressing GHR and the earlier observations of GH-associated therapy resistance.

**Aging and longevity**

In mice and men, the somatotropic axis occupy a central position in the study of aging, healthspan and longevity (195, 196, 197, 198, 199, 200, 201). GHD mouse models have consistently shown an extended lifespan, while the results are inconsistent in GHD humans (200, 202, 203). In fact, the GHRKO mouse currently holds the Methuselah Mouse Prize as the world’s longest-lived laboratory mouse, a title awarded by the Methuselah Foundation (http://reason.com/archives/2004/08/18/methuselah-mouse). Results of several studies are summarized in Table 2. GHRKO mice live 21% (females) to 40% (males) longer

### Table 2  List of studies showing changes in lifespan due to direct changes in GH-GHR action.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Type of change in GH-GHR action</th>
<th>Causes of change in GH/GHR action</th>
<th>Effect on lifespan</th>
<th>References</th>
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<tbody>
<tr>
<td>Human subjects</td>
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<tr>
<td>Acromegaly patients</td>
<td>GH excess</td>
<td>Hypersecreting pituitary adenoma</td>
<td>Shorter (untreated)</td>
<td>(102, 108, 200)</td>
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<tr>
<td>Offspring of Leiden Longevity Study</td>
<td>GH deficiency</td>
<td>Lower GH secretion, tighter feedback inhibition</td>
<td>Longer</td>
<td>(327)</td>
</tr>
<tr>
<td>Laron Syndrome – Israeli cohort</td>
<td>GH resistance</td>
<td>Mutated non-functional GHR</td>
<td>Shorter/normal</td>
<td>(3, 4, 9, 200, 328)</td>
</tr>
<tr>
<td>Laron Syndrome – Ecuadorian cohort</td>
<td>GH resistance</td>
<td>Mutated non-functional GHR</td>
<td>Longer/normal</td>
<td>(12, 200)</td>
</tr>
<tr>
<td>Itabaianinha cohort</td>
<td>GH deficiency</td>
<td>GHRHR mutation</td>
<td>Longer/normal</td>
<td>(200, 329, 330)</td>
</tr>
<tr>
<td>Sindh cohort</td>
<td>GH deficiency</td>
<td>GHRHR mutation</td>
<td>-Unknown-</td>
<td>(200, 333)</td>
</tr>
<tr>
<td>Swiss cohort</td>
<td>GH deficiency</td>
<td>Gh1 mutation</td>
<td>Shorter</td>
<td>(200, 334)</td>
</tr>
<tr>
<td>African pygmies</td>
<td>Differential</td>
<td>SNPs in GHR, IGF1 locus</td>
<td>Shorter</td>
<td>(335, 336, 337, 338, 339, 340)</td>
</tr>
<tr>
<td>Ashkenazi Jews Centenarian cohort, Old Order Amish (AFCS), Cardiovascular Health Study, French Long-lived Study</td>
<td>Differential</td>
<td>Exon-3-deleted GHR isoform</td>
<td>Longer in males; normal in women</td>
<td>(228, 341)</td>
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<tr>
<td>Mouse lines</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>bGH</td>
<td>GH excess</td>
<td>Transgenic expression of bovine GH</td>
<td>Shorter</td>
<td>(26, 211, 249, 342)</td>
</tr>
<tr>
<td>GHRKO</td>
<td>GH resistance</td>
<td>Mutated non-functional GHR</td>
<td>Longer</td>
<td>(15, 200, 201, 343, 344)</td>
</tr>
<tr>
<td>Adult-onset-GHRKO</td>
<td>GH resistance starting at 6-week age</td>
<td>Tamoxifen-inducible global GHRKO starting at 6-weeks age</td>
<td>Longer (female)</td>
<td>(249)</td>
</tr>
<tr>
<td>GHA</td>
<td>GH deficiency</td>
<td>Transgenic mutant GH acting as a GHR antagonist</td>
<td>Normal</td>
<td>(28, 200)</td>
</tr>
<tr>
<td>Ames</td>
<td>GH deficiency</td>
<td>PRO1 mutant; lacks GH, PRL, TSH</td>
<td>Longer</td>
<td>(200, 345, 346, 347)</td>
</tr>
<tr>
<td>Snell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lit/lit</td>
<td>GH deficiency</td>
<td>PIT1 mutation</td>
<td>Longer</td>
<td>(200, 348, 349)</td>
</tr>
<tr>
<td>GHRKHO</td>
<td>GH deficiency</td>
<td>GHRHR mutation</td>
<td>Longer</td>
<td>(200, 350)</td>
</tr>
<tr>
<td></td>
<td>GH deficiency</td>
<td>GHRH mutation</td>
<td>Longer</td>
<td>(351)</td>
</tr>
</tbody>
</table>
than their wild-type counterparts, when maintained in a C57BL/6J background (204). Interestingly, when GHRKO mice were generated and maintained in a mixed genetic background, they lived 38% (females) to 55% (males) longer than their WT counterparts (15). These observed gender-specific differences in GHRKO mouse models stem from gender-specific differences in the wild-type C57BL/6J mice. While both male and female GHRKO mice have similar lifespan, the WT female mice live significantly longer than the WT male mice. The underlying reasons are not completely understood, but could be a result of estrogen-mediated antagonism of GH action at the level of GHR expression and SOCS-mediated downregulation of GHR-JAK2-STAT5 activation (205, 206, 207). The estrogen modulation of GH action in liver also highlights the sexually dimorphic nature of GH-regulated hepatic genes expression (208, 209, 210). Importantly, the observation of increased lifespan in dwarf GHRKO mice does not extend to the dwarf GHA mice, which have a normal lifespan (28). This may be ascribed to an increase in adiposity, leptin and insulin levels with age in GHA mice (15). On the other hand, giant bGH mice live significantly shorter than their wild-type counterparts (211). In humans, isolated studies have reported a higher than normal mortality in acromegaly patients (108), although several interventions like trans-sphenoidal surgery, somatostatin analogs or pegvisomant treatment, to normalize serum IGF-1 levels, often normalize lifespan (200). In this regard, a 20-year follow-up of 333 acromegaly patients diagnosed between 1980 and 1999 in Finland, 87% of whom underwent surgery or radiotherapy, found a significantly higher mortality rate, especially in females, with the major risk factor shifting toward cancer rather than cardiovascular diseases along the course of study (102).

Factors for extended lifespan of GHRKO mice

Reduction of GH/IGF1 action has been found to be responsible for lifespan extension in humans and mouse models (196, 212). Low serum IGF-1 levels is undoubtedly an important factor for increased lifespan of the GHRKO mice as IGF-1 deficiency alone is known to extend maximum but not mean lifespan (213). Additional factors that lead to the GHRKO mice being the longest-lived mouse in the world include the following –

1. increased stress resistance resulting from superior redox homeostasis due to upregulated expression and activity of glutaredoxin and thioredoxin detoxification systems (214, 215, 216, 217) rather than changes in free-radical scavenging (218),
2. reduced activity of TORC1 kinase complex (219, 220, 221),
3. resistance to neoplastic incidence and growth (24, 97),
4. suppressed hypothalamic inflammation (222) – an end-goal of anti-aging drugs (223),
5. an anti-inflammatory adipokine profile, inverted liver-WAT lipid distribution and reduced senescent cell burden in WAT (62, 68, 70, 72),
6. increased markers of mitochondrial biogenesis including higher levels of AMPK, SIRT1 and SIRT3 in heart and increased eNOS and PGC1α levels in skeletal muscle and kidney (224),
7. age-related changes in DNA methylation patterns lead to increased sensitivity to circulating GH, IGF1 and insulin with concomitant depletion of very small embryonic-like stem cell pool and impaired tissue regeneration (225, 226),
8. resistance to diabetogenic effects of a HF diet (14), and
9. improved insulin sensitivity (15).

Lifespan in human GHR deficiency

In humans, the Israeli cohort of LS patients appear to have a normal lifespan with some instances of diabetes mellitus in later years (3). It is important to note that most of these LS patients received recombinant IGF-1 treatment, which can be a critical determinant in subsequent relevant observations. The Ecuadorian LS patients have a long but not exceptional lifespan, yet seem to undergo delayed aging, compared to their normal GH-responsive relatives (12). Primary causes of shortened lifespan in the Ecuadorian LS patients were high childhood mortality, and death due to accidents, convulsion or alcohol-related but not cancer or diabetes (12). This marked protection from cancer was also consistent in the Israeli cohort of LS patients (99) and, therefore, display one of the most prominent factors underlying lifespan extension due to GHR deficiency in humans (227). For now, a conclusive answer to the question whether the link between GHR dysfunction and extended longevity observed in GHRKO mice holds in GH-resistant humans remains unknown (198, 201).

Lifespan in d3-GHR variants

A recent report of exceptional longevity in males associated with enrichment of an exon-3-deleted isoform of the GHR (d3-GHR) provides a new perspective of GHR isoforms and lifespan extension (228). In-frame splicing of exon-2 to exon-4 of the GHR precursor results in d3-GHR splice
variant, with 22 amino acids less at the N-terminal than the full-length GHR. Individuals with d3-GHR have low serum IGF-1 and IGFBP1 levels yet surprisingly reduced levels of GH-, GHR- and GH-binding protein (GHBP). The d3-GHR male offspring of centenarians were relatively tall and, had a significantly increased lifespan indicating augmented GH–GHR signaling (228). It is worth inquiring whether a reduced activity of metalloprotease TACE/ADAM17 action in cleaving the GH-bound extracellular domain of the GHR homodimer generating GHBP or regulators of it like TIMP3 (229, 230) are causal links or if there is a modified kinetics of interaction of the GH molecule with a 22-amino acid deleted extracellular domain of the d3-GHR giving rise to a GHBP analog. Additionally, SOCS2 polymorphisms, which increase the tone of GH-induced signaling intermediates, can also allow for an extended GHR activity (231). On the other hand, gender-specific variations of d3-GHR are highlighted by normal lifespan, higher than normal IGF-1 levels, yet shorter stature in female offspring of Ashkenazi centenarians (232). Multiple studies have reported the effects of homozygous d3-GHR in acromegaly patients. A retrospective study in 2010 with 76 acromegaly patients including seven with a homozygous d3-GHR polymorphism reported a lower BMI in the d3-GHR homozygous patients (233). Later studies from Korea (234) and Turkey (235) have reported higher BMIs in d3-GHR polymorphic acromegaly patients as the only consistent difference due to this particular GHR isoform. Interestingly, the d3-GHR isoform was not found to be a determinant in response to pegvisomant treatment in acromegaly patients (236). The d3-GHR isoform and its sexually dimorphic phenotypes provide an additional opportunity of dissecting the nature of association of the GH/IGF axis in determining lifespan.

Future directions of lifespan studies on GHRKO mice

Contemporary studies in GHD mouse models present an enriched set of queries related to aging. Some are listed below:

1. GH-deficient mouse models have shown improved proteostasis with reduced protein as well as DNA synthesis with sex-and tissue-specific differences (237, 238). Most recent aging research in yeast (239, 240) and Caenorhabditis elegans (241) implicate transcription factors like Gcn4 or Aft4 in maintenance of proteostasis and are worth inquiring into the long-lived GHRKO mice.

2. Bartke and colleagues have intensively studied various conditions of GH deficits and aging (198, 242) and recently reported that a short (6 weeks) phase of early exposure to GH (starting at 2 weeks age) reduced lifespan and cellular stress resistance in Ames dwarf mice (243). A parallel thyroxine treatment had no effect on lifespan (243, 244, 245), whereas thyroxine and GH combined treatment reduced maximal lifespan in only female Ames mice (246).

3. Extensive work by Brown-Borg and colleagues have described epigenetic signatures in DNA methylation patterns in Ames mice compared to their WT littermates (247, 248). This indicates that a tissue- and sex-specific systematic interrogation of the epigenetic landscape in GHRKO mice as well as in Israeli and Ecuadorian cohorts of LS patients can be valuable.

In 2013 in Erice, Italy, a workshop with leading experts in aging studies discussed prospects and feasibility of interventions in slowing aging in humans. Based on validated evidences of safety and efficacy of known drugs, the panel of experts listed the following six approaches as the most promising pro-longevity strategies: (1) pharmacological inhibition of the GH/IGF axis, (2) inhibition of the mTOR-S6K pathway or inflammation, (3) chronic treatment with metformin, (4) activation of sirtuins or AMPK, (5) use of epigenetic modulators and (6) intermittent or prolonged fasting (92). Of these approaches, ‘reducing the activity of the GH/IGF-I somatotropic axis is perhaps the most validated and consistent genetic intervention to extend mouse lifespan’ and targeting the GH/IGF axis was voted as the top adult life intervention toward extending longevity or treating age-related pathologies. The GHR antagonist, pegvisomant, use in humans was particularly mentioned for its ‘reassuring’ safety profiles with few long-term serious adverse events (92).

Meanwhile, we have recently reported that knocking out GHR expression in adult mice (at 6 weeks of age) can result in females with increased maximal longevity and several healthy traits. Thus, temporal regulation of the GH/IGF-1 axis may translate the health benefits observed in experimental models and humans with congenital GH resistance (249). Whether the same extrapolates to humans, defining the underlying mechanisms of the observed sex specificity, as well as what would be the limiting adult age of meaningful intervention in GH/IGF axis toward increasing lifespan, remains unknown.
Cognition

Aging is associated with a progressive decline in memory and cognition. As increased longevity with absence of GH/IGF-1 action is fundamentally important, the quality of the extended lifespan must be evaluated.

GHR presence in brain

Early studies in rabbits (250), chickens (251) and human cadavers within 48 h of death (252), showed GH binding in different brain regions. Later, quantitative PCR and immunohistochemical studies confirmed a near ubiquitous expression of GHR throughout the brain of humans as well as rats and mice (reviewed in (253, 254, 255)). Particularly high expression levels of GHR was observed in the choroid plexus, pituitary, hippocampus, thalamus and hypothalamus (256). It was further observed that GH binding in brain tissue decrease as a function of age (257) along with age-related decline in GH production as well as cognition and memory (258). However, it is important to note that the GH affecting the central nervous system at any point of time can also have a peripheral source in any other tissue in the body and not necessarily originate in the brain tissues, as GH is known to have a receptor-mediated crossing across the blood-brain-barrier (BBB) (259). In this context, additional sources of GH could be leucocytes (260) and in increasing amounts from aging splenic lymphocytes (261).

Cognitive studies with GHRKO mice

The apparent dichotomy in the patterns of GH action in affecting cognition and memory is reflected in the decreased brain size in bGH mice and increased brain size in GHA and GHRKO animals relative to total body weight although the rest of the body parts show a more proportionate variation (253). However, no difference in total cell number was reported in the embryonal central nervous system of GHRKO mice and its WT counterparts (262). This not only implicates a critical role of GH in normal brain maturation but also suggests the role of GH in neuronal migration as either an aversive or attractive stimulus to developing neurons. This is especially relevant in GHRKO mice brain as striatal cholinergic neurons and calretinin and calbindin-expressing cortical interneurons were upregulated, parvalbumin neuron population was unchanged while astrocyte levels decreased (262). GHRKO mice have been well studied for different effects of age-related changes. Along with an extended lifespan, they display a reduced speed of neuronal and musculoskeletal deterioration (263), enhanced cognitive performance (264) and increased expression of NMDAR receptors, which are directly involved in potentiating long-term and short-term memory (265) compared to WT littermates. Similar studies in GHRKO (266) and Ames mice (267) showed significantly better memory retention compared to WT controls. An exemplary display of the delayed aging-related cognitive decline in the 12- to 15-month-old GHRKO mice was observed when they outperformed same-aged WT littermates and scored as well as 2–3-month-old WT mice in the Morris Water Maze test for spatial learning and cognitive memory (268).

In contrast, bGH mice performed poorly in cognitive tests as reported by us and others (269, 270). In an inhibitory avoidance test, 6-month-old bGH mice performed to the same level as 25-month-old WT mice indicating an accelerated decline in learning and memory retention (269). We recently showed poor spatial learning and short-term memory retention of bGH mice in Barne’s Maze studies wherein the dwarf GHA mice showed significantly superior results above WT littermates (270). The GHA mice used more direct search strategies and committed significantly less errors than same-aged littermates in the study while bGH mice performed significantly poorer than both GHA as well as WT littermates of same age (270). A study by Schrag and coworkers using organotypic slice system culture of hippocampal tissue of adult mice reported decreased hyperphosphorylation of tau proteins induced by Abeta-25-35 in Ames mice (271). The underlying molecular mechanisms for these set of observations explaining a reduced age-related decline in cognition in the absence and reduction of GH action in mice is valuable and remain to be fully described.

Cognitive studies in humans with GHR deficiency

In humans with congenital GH resistance, the Israeli cohort of LS patients showed a normal to sub-normal IQ score, depending on the type of inactivating amino acid mutation in the GHR (3, 9, 272). However, recently, a small study with 12 members of the Ecuadorian cohort of LS patients reported that they performed significantly better in memory tasks and had significantly lower cognitive impairment compared to their unaffected relatives (273). In human acromegaly patients, several studies have reported cognition deficits, decreased short-term and long-term memory, anxiety and impaired decision making and decreased activities in pre-frontal and mid-temporal
cortices in brain (274, 275, 276, 277). Interestingly, recent reports and multiple meta-analyses directly implicate a higher than normal serum IGF-1 as a disease trait marker in major depressive disorder (MDD) and bipolar disorder (BD) further stimulating the pleiotropy of GH action in cognitive development and maintenance (278, 279, 280, 281).

GH treatment does not appear to affect cognitive decline in normal aging GHD patients, and instead, is known to significantly increase the risk of diabetes, edema, carpal-tunnel syndrome, obstructive sleep apnea, intracranial hypertension, pancreatitis and scoliosis (112, 113, 282). One particular situation in which a transient GH treatment has been found to marginally but significantly improve cognition and memory is growth hormone deficiency (GHD) resulting from traumatic brain injury (TBI) (283), especially treatment beginning after an initial acute phase resolves (284). In childhood or adult-onset GHD because of pituitary dysfunction, a decline in neurocognitive functionalities like cognition, memory, motivation, attention and sleep and overall quality of life (QoL) have been observed. GH replacement therapy showed significant improvements in these variables (254, 285, 286, 287, 288, 289, 290, 291, 292). Similar observations were also made following GH treatment in patients with Prader-Willi syndrome (293). Interestingly, while considering the detrimental effect of lack of GH in GHD patients, an important point to consider is that GHD patients did not have a congenital absence of GH action, but rather had a peri-or post-natal exposure to GH, prior to development of GHD. As recent research indicates, an early life GH treatment in fact decreases lifespan and adversely affects the inflammatory profiles in liver and adipose tissue of the long-lived GHD Ames mice (243, 244). Additionally, high endogenous GH levels in children born very preterm was associated with a reduced spatial memory and an enlarged amygdala (294). Together, the currently available information from humans and mice indicate an improvement in memory and cognition associated with congenital absence of GH action as well as with GH treatment in some cases of GH deficiency. Further clarity in this field can be achieved by analyses in a sex-specific manner, the distinctions between (i) the role of GH in a developing brain with active neurogenesis vs an adult brain with very limited areas of neurogenesis; as well as (ii) the contribution of local GH or peripheral GH crossing the BBB in affecting different brain regions in an autocrine/paracrine manner.

### Advantages and disadvantages of GHR deficiency

The various advantages and disadvantages of GHR deficiency or GH resistance arising due to mutational defects in the GHR in human LS patients and the GHRKO mouse as discussed in the above sections have been represented in Fig. 1.

### Tissue-specific GHR-knockout mouse models

To study the specific effects of GH in various tissues, the GHR in different tissues have been specifically and conditionally knocked down using tissue-specific promoter/enhancers in the Cre-flox system (295, 296). These studies are of value to help understand the impact of GH action in growth, glucose homeostasis, cancer, aging and longevity (Fig. 2).

### Liver-specific GHRKO

GH induces IGF-1 production through binding to GHR on almost all tissues, although ~90% of circulating IGF-1 is produced in the liver. Thus, our laboratory generated liver-specific GHRKO mice (Li-GHRKO) using Cre-flox system under the control of albumin promoter (297). Another study also generated an independent liver-specific GHRKO mouse line, which is called GHRLD (298). Both mouse lines have high GH, low IGF-1 levels, hyperglycemia and hyperinsulinemia, increased lipid accumulation and liver weight compared with control mice. However, several phenotypic differences have been observed in these two mouse lines. First, Li-GHRKO mice have decreased body size, weight, and adiposity compared with control mice at 6 months of age, while 16-week-old GHRLD showed no significant difference at body weight or composition. Second, liver steatosis was presented in both male mouse lines but only in female GHRLD mice, not in the female Li-GHRKOs. Also, GHRLD mice have increased fibrosis and inflammation. It is noteworthy that the study of Li-GHRKO mice was done in a longitudinal order while the GHRLD mice were mostly studied at 16 weeks of age.

An adult-onset hepatocyte-specific GH knockdown (aLivGHRkd) mouse model was developed through injection at 10- to 12-weeks-age with adeno-associated virus (AAV) bearing a liver-specific thyroxine-binding globulin (TBG) promoter driving a Cre-recombinase transgene (299). Seven days after the injection, hepatic de novo lipogenesis significantly increased and hepatosteatosis...
Lessons from GHR-disrupted mice

Advantages and disadvantages of growth hormone (GH) resistance due to mutations in GH receptor (GHR), as observed in human Laron Syndrome (LS) patients and the global GHR-knockout (GHRKO) mouse.

Developed in male mice and ovariectomized female mice suggesting that hepatic GH actions normally serve to inhibit de novo lipogenesis in the livers. To dissect the mechanisms of GH resistance and IGF-1 deficiency-induced liver steatosis, GHRLD mice that expressed a hepatic IGF-1 transgene was studied and results suggested

Figure 1

Advantages and disadvantages of growth hormone (GH) resistance due to mutations in GH receptor (GHR), as observed in human Laron Syndrome (LS) patients and the global GHR-knockout (GHRKO) mouse.

Figure 2

Global, tissue-specific and time-dependent GHRKO mice and their characters.
that restoration of IGF-1 improved glucose homeostasis and insulin sensitivity; however, it was unable to protect against lipid accumulation completely and was insufficient to inhibit steatosis-induced hepatic inflammation and oxidative stress (61).

Gene expression of key regulators of mitochondrial biogenesis and apoptosis was significantly altered in brains and kidneys in Li-GHRKO mice compared with control mice with different patterns of changes in male and females. However, no differences in the livers were observed (300, 301). The Li-GHRKO mice do not have decreased number of cancers and do not have increased lifespan, suggesting that lowered IGF-1 itself is insufficient to increase lifespan. GHRLD mice have increased incidence of liver cancer probably due to steatosis induced by the absence of hepatic GH action (302). Also, as discussed earlier, improved glucose homeostasis and insulin sensitivity may be one of the keys of longevity.

Adipose tissues-specific GHRKO

Adipose tissues are GH and insulin-sensitive tissues that are responsible for a part of whole-body glucose uptake to maintain glucose homeostasis. To understand the GH action on adipose tissues and its potential impacts, our laboratory used Fabp4 promoter/enhancer to specifically knockdown GHR (FaGHRKO) (303). The FaGHRKO mice have general increase in all fat depots and body weight due to the absence of GH’s lipolytic effects. In contrast, GHRKO mice have depot-specific increase, that is, a unique increase in the proportion of the subcutaneous depot relative to controls. More importantly, the improved glucose homeostasis found in GHRKO mice was not seen in FaGHRKO mice suggesting that different depots may play different roles in glucose homeostasis. Interestingly, the lifespan of FaGHRKO mice was decreased suggesting that the potential significance of improved glucose homeostasis in extended longevity.

Muscle-specific GHRKO

Another important insulin-sensitive tissue is muscle. To study the GH action on muscle, two independent muscle-specific GHRKO mice were generated using different promoters/enhancers. The first used Mef-2c-73k promoter/enhancer to knockout GHR in muscle (304); the second used muscle creatine kinase (MCK) promoter/enhancer (305, 306, 307). Mef-2c-73k is expressed in adult muscle and fetal heart and brain, while MCK is expressed in skeletal and cardiac muscle during all stages of development. This difference may be used to explain the differences between these two mouse lines. That is, the changes of growth, body compositions and insulin sensitivities were opposite in these two lines (304, 305, 306, 307, 308). Importantly, the MCK males increased maximum lifespan along with decreased inflammation (305, 307, 308), and they were protected from HF diet-induced metabolic deterioration (306).

Inducible cardio-specific GHRKO

To clarify the direct effects of GHR on cardiac tissue, our laboratory generated a tamoxifen-inducible cardiogenesis-specific GHR-disrupted (iC-GHRKO) mouse line at 4 months (309, 310). No difference in baseline or in post-dobutamine stress test echocardiography measurements or longitudinal systolic blood pressure measurements was found when compared with controls. However, at age 6.5 months, these mice had decreased fat mass. Furthermore, by 12.5 months, the fat mass of iC-GHRKO mice was the same as controls and the mice developed glucose intolerance and insulin resistance accompanied by decreased circulating IGF-1. Surprisingly, this study indicates that the disruption of cardiomyocyte GH-induced signaling does not affect cardiac function in adult mice, while it does affect metabolic parameters.

Macrophage-specific GHRKO

GHRKO and GHRLD mice have altered levels of inflammatory markers (91, 61). Macrophages play important roles in inflammation. Therefore, macrophage-specific GHRKO mice (MacGHRKO) were generated using LysM promoter/enhancer to better understand the direct effects of GH on the inflammatory responses (311). When on HF diet (45% fat), the MacGHRKO mice have increased epididymal adipose depot and impaired glucose intolerance and insulin resistance as well as increased adipose inflammation.

Pancreatic β cells-specific GHRKO

GH promotes the proliferation of pancreatic β cells (85). To further study the effects of GH on the β cells and glucose homeostasis, GHR in the β cells was specifically knocked down in the mice (βGHRKO) (312). These mice have decreased number of β cells. When on HF diet, they experienced impaired insulin response, which was not seen in normal chow diet, suggesting that GH has a direct effect on promoting pancreatic β cell proliferation.
to increase insulin secretion, and therefore, to prevent impaired glucose homeostasis.

**Time-dependent GHRKO**

GHRKO mice have improved glucose homeostasis, resistance to cancer and extended lifespan. To study the effects of temporal suppression of GH in adults, which may have clinical implications, our laboratory generated 6-week-old adult-onset GHRKO mice (aGHRKO) using tamoxifen-inducible Cre-flox system employing the ROSA26-Cre-ERT locus (249). As stated earlier, we found that these mice have delayed growth, increased adiposity and improved insulin sensitivity, similar to several of the benefits found in GHRKO mice. Surprisingly, maximal lifespan extension was only observed in females.

**Closing comments**

Decades of research has documented a pleiotropic role of GH/IGF-1 in human aging and disease (16). In mice and humans, the global absence of GH action leads to improved glucose homeostasis, cognition, better memory, possibly a longer than normal lifespan and substantial protection from incidence of diabetes, cancer, as well as neuropathologies. A reduction in GH action has also been associated with increased cellular stress resistance and mitochondrial health. However, there are sex-specific differences (90, 313, 314, 315) as well as unavoidable tradeoffs in the process. A reduced stature, obesity and possible reduction in reproductive fitness have been associated with studies in the GH-deficient and resistant dwarf mouse models (16, 316). Further, extrapolating lifespan extension and delayed aging in animal models to humans is being increasingly fine-tuned, as we continue to understand the intrinsic differences in the mechanism(s) of aging as a function of GH action (317). Through intensive studies on single and multiple organ-specific GHR knockouts and adult-onset models of global GHR disruption, it would be possible to further clarify the enigmatic role of GH–GHR in health and disease. The dwarf, obese, cancer-free, diabetes-resistant, more cognizant, longest-lived GHRKO mouse and the numerous of existing and ongoing studies on it, leaves us with a tantalizing question – ‘Is less more?’

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