A Growth Hormone Receptor Mutation Impairs Growth Hormone Autoregulation in Pituitary Tumors

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Abstract

Pituitary tumors are a diverse group of neoplasms that are classified based on clinical manifestations, hormone excess, and histomorphologic features. Those that cause growth hormone (GH) excess and acromegaly are subdivided into morphologic variants that have not yet been shown to have pathogenetic significance or predictive value for therapy and outcome. Here, we identify a selective somatic histidine-to-leucine substitution in codon 49 of the extracellular domain of the GH receptor (GHR) in a morphologic subtype of human GH-producing pituitary tumors that is characterized by the presence of cytoskeletal aggresomes. This GHR mutation significantly impairs glycosylation-mediated receptor processing, maturation, ligand binding, and signaling. Pharmacologic GH antagonism recapitulates the morphologic phenotype of pituitary tumors from which this mutation was identified, inducing the formation of cytoskeletal keratin aggresomes. This novel GHR mutation provides evidence for impaired hormone autoregulation in the pathogenesis of these pituitary tumors. It explains the lack of responsiveness to somatostatin analogues that reverse this intracellular environment by a cyclic AMP response element (10, 11), and respond to somatostatin analogues that reverse this intracellular environment (12, 13). In contrast, the pathogenetic mechanisms underlying the other pituitary tumor associated with GH hypersecretion and acromegaly, the sparsely granulated somatotroph adenoma, remain unclear.

Indeed, the first molecular alteration identified in pituitary tumors was activating mutation of a G protein that transduces growth hormone (GH)–releasing hormone receptor signaling (7, 8). These gsp mutations were identified in densely granulated somatotroph adenomas (9) that have high adenylyl cyclase levels (9), express the glycoprotein hormone α-subunit that is regulated by a cyclic AMP response element (10, 11), and respond to somatostatin analogues that reverse this intracellular environment (12, 13). In contrast, the pathogenetic mechanisms underlying the other pituitary tumor associated with GH hypersecretion and acromegaly, the sparsely granulated somatotroph adenoma, remain unclear.

We showed that mice with GH receptor (GHR) disruption develop somatotroph hyperplasia (14). We hypothesized that GHR mediates autoregulation of GH. We now provide evidence that sparsely granulated somatotroph tumors, which exhibit characteristic aggregation of cytoskeletal keratin filaments (2, 11, 15), harbor a somatic mutation in the GHR that interferes with posttranslational processing, maturation, ligand binding, and signaling of the GHR. This finding has implications for the pathogenesis of these tumors, for the formation of keratin aggresomes in other tissues (16), and for the design of treatment paradigms for patients with GH-producing tumors.

Materials and Methods

Human pituitary tumors. Human somatotroph adenomas obtained at surgery were classified using accepted criteria (2, 17). WBC were obtained from buffy coat of peripheral blood of the same patients. Frozen samples were stored at –80°C for DNA extraction. The project received institutional approval from the Research Ethics Board of the University Health Network (Toronto, Ontario, Canada) and Toranomon Hospital (Tokyo, Japan).

DNA extraction and sequencing. PCR primer sets were generated to surround each exon of the human GHR except exon 10, which, because of its size, was amplified using multiple primers (Supplemental Table 1). PCR reactions consisted of 100 ng DNA, 2.5 units of Taq DNA polymerase (Perkin-Elmer), 2.0 mmol/L deoxynucleotide triphosphate, 40 mmol/L KCl, 8 mmol/L Tris-HCl (pH 8.3), and 0.4 μmol/L primer. MgCl₂ concentrations varied from 1.0 to 2.0 mmol/L. Exon 8 of GNAS was also sequenced (9).

Cell culture and transfection. Human somatotrophs obtained surgically were cultured (18) for GHR antagonist studies. It is not possible to obtain sufficient cells in primary culture to carry out dose-response and replicate analyses. In the absence of a human GH-producing cell line, we used the rat pituitary GH cell line (American Type Culture Collection). γ2A is a Janus-activated kinase 2 (JAK2)–deficient human fibrosarcoma line (Dr. G. Stark, Cleveland Clinic, Cleveland, OH; ref. 19). A γ2A cell line stably expressing murine JAK2 in pCDNA3.1 was selected in DMEM supplemented with 400 μg/mL zeocin and screened by blotting with anti-JAK2 antibody; a rabbit serum directed at residues 746 to 1,129 of murine JAK2 (20). γ2A-JAK2 cells were maintained in DMEM (1 g/L glucose) with...
10% fetal bovine serum (FBS), 50 μg/mL gentamicin sulfate, 100 units/mL penicillin, 100 μg/mL streptomycin, 200 μg/mL G418, and 100 μg/mL zeocin. H49L GHR in pcDNA was transfected into γ2A-JAK2 cells with hygromycin-resistant plasmid for selection (21).

**Site-directed mutagenesis.** To create the H49L GHR, we used site-directed mutagenesis (Clontech) and mutagenic PCR primers (forward, 5′-GAGACTTTTTCATGCCTCTGACAGTGGGTCT-3′; reverse, 5′-GAA-CCTATCGTGCGAGGCGCTAGAAAAGTCTCT-3′). The resulting mutation (italics) was confirmed by restriction digestion and nucleotide sequencing.

**Immunoprecipitation and Western blotting.** Protein was extracted from pellets solubilized for 30 min at 4°C in lysis buffer [1% (v/v) Triton X-100, 150 mmol/L NaCl, 10% (v/v) glyceral, 50 mmol/L Tris-HCl (pH 8.0), 100 mmol/L NaF, 2 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate, 10 mmol/L benzamide, 10 μg/mL aprotinin]. After centrifugation at 15,000 × g for 15 min at 4°C, detergent extracts were electrophoresed under reducing conditions or subjected to immunoprecipitation. Equal amounts of protein from total cell lysates, cytosolic and membrane fractions, or media were solubilized in 2× SDS-sample buffer, separated on SDS-polyacrylamide gels, and transferred to nitrocellulose. GH protein levels were determined using an antiserum to rat GH [National Institutes of Diabetest, Digestive and Kidney Diseases (NIDDK)] at 1:50,000; actin was identified using a monoclonal antibody (mAb; Sigma) at 1:500. GHR was identified with anti-GHRmAb, a rabbit antibody against a bacterially expressed N-terminally His-tagged fusion protein of kGHR 271–620 (22) at 1:1,000. Immunoprecipitation was done with anti-GHRmAb-α, a mouse mAb (immunoglobulin G1) directed against bacterially expressed glutathione S-transferase (GST)-rabbit GHR 1–246 (23–27).

For assessment of GH-induced signaling, −p-JAK2 antibody reactive with JAK2 phosphorylated at residues Y1007 and Y1008 (UBI) and rabbit anti–phosphoTyr-STAT5 polyclonal antibody against a phosphopeptide surrounding phosphorylated Tyr694 of murine STAT5A and STAT5B (Zymed) were used for immunoblotting (23–27). Blots were scanned for densitometric analyses.

**125I-hGH binding.** γ2A-JAK2 cells expressing each receptor were equally divided into multiple wells of a six-well plate. Replicate samples of serum-starved cells were incubated in 1-mL binding buffer with 125I-hGH [87,500 counts per minute (cpm) – 34.5 pmol/L] in the presence or absence of 2 μg/mL (–91 nmol/L) unlabeled hGH for 1 h at 25°C. Cells were washed with PBS, solubilized in 0.5-mL 1% SDS/0.1 M NaOH, and lysate was subjected to gamma counting (28). Data are expressed as 125I-GH binding in counts per minute.

**Enzymatic GHR deglycosylation.** γ2A-JAK2 cells were serum starved overnight and solubilized, then supernatants were immunoprecipitated with anti-GHRmAb (25), eluted with 0.5% SDS and 1% glycosidase F (both from New England Biolabs; 500 units) and then digested with endoglycosidase H or N-glycosidase F (both from New England Biolabs; 500 units) and neuraminidase (New England Biolabs; 50 units; combination referred to as F/N) in deglycosylation buffer at 37°C for 16 h. SDS sample buffer eluates were resolved by SDS-PAGE and immunoblotted (26, 29).

**Immunohistochemistry.** For immunohistochemistry, anti-GHRmAb (24) was ammonium sulfate precipitated and affinity purified against a GST fusion protein incorporating the human GHR cytoplasmic domain (24). Immunohistochemistry was done after microwave antigen retrieval using the antigen diluted 1:1,000. Reactions were detected with the ABC Elite kit (Vector Laboratories) and dianaminobenzidine (DAB).

**Morphologic evaluation of GHR antagonism on human pituitary somatotrophs.** To determine if morphologic features of somatotroph adenomas could be reproduced by blocking GH signaling, primary human densely granulated somatotroph tumors were collected sterile in DMEM with antibiotics. Cells were dispersed by mechanical agitation and incubated in 1 mg/mL collagenase for 30 min at 37°C, pelleted, and resuspended in serum-free DMEM with 30 μg/mL putrescine, 1 × 10−6 M hydrocortisone, 1 × 10−11 mol/L T3, 0.01 mg/mL insulin, transferrin, and 0.375% albumin bovine factor V. Cells were plated in 0.01% poly-L-lysine–treated chamber slides and treated for 96 h with 200 ng/mL pegvisomant. This dose was selected on the basis of earlier dose and time course studies. Cells were fixed in 10% formalin for 15 min, then immunohistochemical localization of cytokeratin 7/cytokeratin 8 was done using a mAb (Carn 5.2, Becton Dickinson) diluted 1:50. Reactions were detected with the ABC Elite kit and DAB. For ultrastructural analysis, cells were plated in 24-well plates, treated with pegvisomant, harvested, pelleted in 2% agar, and the solidified agar pellet was fixed in glutaraldehyde and processed for electron microscopy.

**Functional effects of GHR antagonism on pituitary somatotrophs.** GH4 cells were grown in 100-mm plates to 90% confluence and incubated with pegvisomant (Sensus Corp.) at the indicated concentrations.

**Metabolic cell labeling.** To determine the effects of GHR antagonism on GH synthesis and secretion, GH4 cells were grown to 90% confluence before treatment with antagonist for 24 h. Cells were incubated with (Cys-Met)DMEM (ICN Biomedicals, Inc.) for 1 h, followed by metabolic labeling with 100 μCi/mL tritiated [3H]methionine (ICN Biomedicals) for 1 h. Cells were lysed, immunoprecipitated with antibodies to GH (NIDDK) or actin (Sigma), separated on SDS-12% polyacrylamide gel, vacuum dried, and autoradiographed for scanning densitometric analysis.

**Growth in soft agar.** The effect of GH antagonism on pituitary cell proliferation was determined by measuring colony formation in soft agar. GH4 cells were grown to 90% confluence in 100-mm plates, synchronized at G0–G1, then treated with pegvisomant for 24 h. Cells (1 × 104) were harvested and suspended in 0.3% soft agar containing 12% FBS, 10% horse serum, and antibiotics, then plated on a 1-cm dish and incubated at 37°C for 14 days in the presence of the GH antagonist. Growth was assessed by measuring colony number under low magnification (×100). Image analysis was done using MicroComputer Imaging Device Elite version software (Imaging Research, Inc.).

**Statistical analyses.** Data are expressed as mean ± SE. Differences were examined by one-way ANOVA with significance level defined at a threshold of 0.05.

**Results**

**Pituitary somatotroph adenomas harbor somatic GHR mutations.** DNA was obtained from normal tissue, 14 sparsely granulated and 12 densely granulated somatotroph pituitary tumors, and peripheral blood cells of all 26 acromegalic patients. Sequencing of GHR revealed no mutations in 5 normal pituitaries, 5 normal thyroids, and peripheral blood cells of all 26 acromegalic patients. No GHR substitutions were identified in 12 densely granulated somatotroph adenomas. Five of 12 densely granulated adenomas harbored gsp activating mutations (30).

None of 14 sparsely granulated somatotroph adenomas had gsp mutations; however, 6 adenomas showed heterogeneous exon 4 mutation of GHR that predicts substitution of codon 49 from His to Leu in five and to Arg in one. Exon 4 encodes an extracellular cysteine-rich immunoglobulin-like loop (Fig. 1). Substitutions at codons 42 and 44 result in 10- to 100-fold reduction in GH-binding affinity. Amino acids encoded by codons 30 to 53 are important for three-dimensional conformational structure of this region of the receptor (31).

**The GHR codon H49L mutation impairs receptor processing, activation, and GH binding.** To determine how the H49L mutation affects GHR function, we used the rabbit GHR (23–27) because the human GHR cDNA is difficult to propagate in bacteria (32–34). Rabbit and human GHR are both 620 residues in length, 84% identical in amino acid sequence, and completely identical in residues 44 to 52. By site-directed mutagenesis, we introduced a histidine-to-leucine substitution in residue 49. Cells were transfected into fibrosarcoma cells that lack expression of GHR (γ2A-JAK2 cells; refs. 27, 29), extracts were resolved by SDS-PAGE and...
immunoblotted with anti-GHRcyt-AL47, a serum reactive with the receptor cytoplasmic domain (ref. 22; Fig. 2A, left). This revealed two forms of WT and mutant GHR, designated mature and precursor. The abundance of mature form was substantially reduced for mutant GHR.

WT and mutant receptors were immunoprecipitated and subjected to deglycosylation before immunoblotting. GHR is a glycoprotein that undergoes processing after synthesis in endoplasmic reticulum and during Golgi transport to cell surface. Precursor (high-mannose) GHRs have yet to traverse the trans-Golgi, whereas the mature carbohydrate pattern is present in receptors that have exited the Golgi. Precursor GHR is sensitive to deglycosylation with endoglycosidase H, whereas mature GHR at the cell surface is endoglycosidase H resistant (29). The upper band, much less prominent in mutant GHR, was resistant to deglycosylation by endoglycosidase H, whereas the lower band, more prominent in the mutant, was endoglycosidase H sensitive (Fig. 2A). This defines the lower band as precursor and the upper band as mature GHR. Both forms were sensitive to deglycosylation by a combination of N-glycosidase F and neuraminidase (F/N), which removes carbohydrate chains independent of their content and is thus a control to indicate full deglycosylation. Surface immunoprecipitation from intact cells (as in ref. 27) indicated that mature WT and mutant GHRs were detected on cell surface (not shown). Thus, both WT and mutant GHRs can attain maturity and cell-surface localization, but processing of the mutant is reduced, suggesting that the H49L substitution impairs GHR processing and/or stability.

Using immunohistochemical examination, 9 of 10 primary human densely granulated adenomas exhibited GHR staining at...
the cell surface and in the cytoplasmic compartment of the cells (Fig. 2B, left). In contrast, all but one of 10 sparsely granulated adenomas showed significantly diminished GHR staining with only a peculiar and focal cytoplasmic accumulation (Fig. 2B, right). Five of the tumors with reduced GHR staining exhibited the H49L GHR mutation and one had the H49A mutation. The other three tumors with reduced GHR immunoreactivity did not harbor an identified GHR mutation, raising the possibility of alternative mechanisms of GHR destabilization.

To study trafficking and signaling of the H49L GHR, we stably transfected Y2A-JAK2 cells and screened multiple clones by anti-GHRcyt-AL47 immunoblotting for comparison with previously described C14 cells (27, 29) that stably express WT GHR. Two clones, C12 and C21, express mature mutated GHR at levels similar to WT GHR in C14 cells (Fig. 3A). The level of precursor GHR was greater in both mutant clones than that observed in C14 cells. Endoglycosidase digestion of immunoprecipitated receptors verified the identities of stably expressed mature and precursor mutant GHRs (Fig. 3B). Immunolocalization of GHR revealed a pattern of membrane staining with focal cytoplasmic reactivity in C14 cells, consistent with production in the endoplasmic reticulum and maturation to the cell surface (Fig. 2C, left); in contrast, and consistent with the pattern seen in primary tumors, C12 and C21 cells expressing the mutant receptor showed prominent accumulation of immunoreactive receptor in cytoplasmic globules, consistent with the endoplasmic reticulum (Fig. 2C, right).

To probe the basis for accumulation of precursor mutant GHR, we carried out experiments in which steady-state GHR levels were monitored after inhibiting new protein synthesis with cycloheximide (Fig. 3C). As expected, precursor GHR relative to mature

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**Figure 3.** Pituitary tumor-derived H49L mutation impairs GHR signaling. A, cells stably expressing WT receptor (C14) or mutant (C12 and C21 clones) were detergent extracted and GHR was immunoblotted. Mature and precursor GHR are indicated. B, endoglycosidase treatment was done as in Fig. 2. C, C14 and C12 cells were treated with cycloheximide (CHX) for durations indicated and GHR was immunoblotted. Relative abundance of mature and precursor GHR was determined densitometrically from three separate experiments. Points, mean of receptor remaining at each time point relative to that present in untreated cells; bars, SE. Note relative stability of the mutant precursor form. D, serum-starved C14 and C21 cells were treated for 15 min with GH at concentrations indicated before harvest. Detergent extracts resolved by SDS-PAGE were immunoblotted with antibodies that detect the tyrosine-phosphorylated and total JAK2 and STAT5. Note diminished GH-induced JAK2 and STAT5 tyrosine phosphorylation in cells expressing mutant GHR. Representative experiments for each cell line. E, quantitation of GH-induced STAT5 tyrosine phosphorylation. Experiments including those in (D) were evaluated by densitometry, in each case considering the maximal phospho-STAT5 signal elicited by GH in cells expressing WT GHR as 100%. Points, mean (n = 3); bars, SE. F, serum-starved C14 and C21 cells subjected to radiolabeled GH binding studies, as detailed in Materials and Methods. Equal aliquots of identically prepared cells were also evaluated by whole-lysate immunoblotting to indicate that mature GHR abundance (inset) was similar between the two cells. Columns, mean of triplicate; bars, SE. Representative of two independent experiments with these cells.
GHR was greater in C12 versus C14 cells. With increasing duration of cycloheximide treatment, precursor GHR amount dropped and mature GHR was more stable (35). Mature mutant GHR was as stable as mature WT GHR; in contrast, precursor mutant GHR was dramatically more stable than precursor WT GHR. These data suggest that diminished processing of mutant GHR is accompanied by accumulation and resistance to degradation of its precursor; however, mutant receptors that do achieve maturity have a similar half-life as mature WT GHR. Similar results were seen with C21 cells (not shown).

Signaling mediated by mature cell-surface mutant and WT GHRs was compared (Fig. 3D). Serum-starved C21 or C12 and C14 cells were exposed to GH (0–250 ng/mL) for assessment of JAK2 and STAT5 phosphorylation. Despite similar JAK2 and STAT5 levels, the response to GH was markedly blunted in C21 and C12 cells compared with C14 cells (Fig. 3E), suggesting that the mutant receptor that achieves maturity is also wounded with regard to GH-induced signaling.

We tested GH-binding capacity in the mutant receptor-bearing cells. Binding of radiolabeled GH to cell surface was significantly reduced in C12 cells compared with C14 cells (Fig. 3F). Similar results were obtained with C21 cells (not shown). These data indicate that the mutation affects GH binding capacity and this may account for at least part of the relative resistance of the mutant receptor to activation of intracellular signaling by GH.

**GHR antagonism results in sparsely granulated phenotype with fibrous bodies.** The fibrous body is unique to human cells and therefore primary human somatotrophs were used for these studies. To determine the morphologic effects of GHR interruption, eight primary human pituitary densely granulated somatotroph tumors were cultured in the presence of the pharmacologic GHR antagonist pegvisomant. Four days after exposure to this agent, cultured cells examined by immunohistochemical localization of cytokeratins revealed a rearrangement of intermediate filaments (Fig. 4A). Compared with the uniform distribution of keratin in control cells from densely granulated adenomas, GHR-antagonized cells displayed juxtanuclear accumulation of keratin filaments into fibrous bodies (Fig. 4A). These data were corroborated by electron microscopy, which confirmed striking intermediate filament rearrangement and fibrous body formation (Fig. 4B). These data indicate that aggresome formation is a result of GHR antagonism in human somatotroph cells. As such, this morphologic hallmark of sparsely granulated, but not densely granulated, somatotroph tumors can be considered a surrogate marker of reduced GHR signaling, consistent with the effect of the GHR mutations identified in sparsely granulated pituitary somatotroph tumors.

**GHR antagonism alters GH synthesis and secretion.** The structural homology of the GH antagonist pegvisomant with GH renders it cross-reactive with many antisera used to detect the native hormone. The extent of GH immunogenicity of pegvisomant was determined by Western blotting of antagonist-spiked serum-free media. Detection with antirat GH under reducing conditions yielded a 22-kDa protein with an intensity that mirrored the concentration of GH antagonist added (Fig. 5A).

To circumvent cross-reactivity and to permit examination of de novo GH synthesis and secretion, we metabolically labeled pegvisomant-treated rodent GH4 pituitary somatotrophs with [35S]methionine. Total cellular protein and corresponding conditioned media were immunoprecipitated with anti-GH and detected for newly incorporated 35S. Cell lysates and media showed an increase in labeled GH in the presence of pegvisomant (Fig. 5B). It should be noted that the addition of GH to culture media failed to alter new GH synthesis (not shown); this is likely due to the large quantities of GH produced by these cells that enrich the media and
saturate GHR to a level that cannot be altered by the addition of exogenous GH. Nevertheless, these data confirm the importance of GH in autoregulating its own production.

**GHR antagonism alters pituitary cell growth.** The effect of GHR antagonism on pituitary GH4 somatotroph cell proliferation was examined in a soft agar assay. Incubation in soft agar containing pegvisomant for 2 weeks resulted in a significant increase in colony number from 50 ± 5 colonies in control cells to 69 ± 3 colonies in cells exposed to 200 ng/mL pegvisomant, 70 ± 3 colonies in cells exposed to 400 ng/mL pegvisomant, and 73 ± 4 colonies in cells treated with 800 ng/mL pegvisomant (P < 0.008 for each of 200, 400, and 800 ng/mL compared with control). Exposure to 100 ng/mL of the GH antagonist did not significantly alter colony numbers.

**Discussion**

Mutations in the GHR have been described as a cause of impaired growth. Germ-line mutations in exon 4 of the GHR result in Laron type dwarfism with GH resistance (36–38). The mutation in GHR exon 4 that we found in sparsely granulated somatotroph tumors is associated with both altered processing and diminished GH binding and signaling of the GHR. In reconstitution studies, we document alteration of the steady-state ratio of precursor and mature forms of the H49L GHR. We verified the identity of these forms by testing their sensitivity to deglycosylation with endoglycosidase H and a combination of N-glycosidase F and neuraminidase. In reconstitution studies, only a small fraction of mutant receptors achieved endoglycosidase H resistance indicative of cell-surface expression. This indicates that one major defect in the mutant receptor is a relatively low expression of mature GHR on the cell surface, leading to ineffective sensing of ambient GH and lack of negative feedback on GH production and growth.

Receptor stability and signaling were investigated in stably transfected clones in which mature mutant GHR levels approximated those in cells expressing WT GHR. Further, the signaling elements JAK2 and STAT5 were also similar in these cells. Treatment with cycloheximide revealed that WT and mutant mature receptors exhibit similar stability, but the mutant precursor GHR was particularly long-lived relative to the WT precursor. These data suggest that the stability and deficient processing of the mutant precursor are related phenomena. The immunolocalization studies are consistent with accumulation of the mutant receptor form in the endoplasmic reticulum; this could possibly lead to a stress response impairing GH trafficking to granules and secretion. The latter is certainly consistent with the sparsely granulated phenotype in which this GHR mutation was identified. Our studies of stable transfectants also indicate that the mutant mature GHR transduces acute GH-induced signals less effectively and binds GH less avidly than the WT mature receptor. This signaling defect, along with the dramatic alteration in detectable mature form of the GHR verified by immunohistochemical analysis in sparsely granulated compared with densely granulated somatotroph adenomas, may contribute to tumor pathology.

To determine the effect of impaired GH signaling on GH regulation, we examined the effect of the pharmacologic antagonist on GH synthesis using metabolic GH labeling. GH synthesis and secretion was stimulated in response to disrupted GH signaling. We also show significant GH-like immunogenicity of the antagonist as detected by commonly used antisera. This finding underscores the need for cautious interpretation of GH levels in the circulation of patients treated with this GH antagonist.

The data presented here point to direct alterations of GH autoregulation as a potential pathogenetic mechanism in a subgroup of pituitary somatotroph adenomas. Although others have not found mutations of GHR in somatotroph adenomas, reduced expression of the receptor has been noted (39). We specifically sought evidence to distinguish between the two major types of somatotroph adenomas. We identified a heterozygous GHR substitution specifically in sparsely granulated somatotroph adenomas. This represents an example of a nonoverlapping genotype-phenotype mutation that contrasts with the Gso mutations exclusive to densely granulated somatotroph adenomas. Although not all of the sparsely granulated tumors exhibited GHR mutations, the vast majority showed reduced GHR immunoreactivity, raising the possibility of alternative mechanisms of GHR destabilization to explain this variant of somatotroph adenoma.

Our findings have important therapeutic implications for the medical treatment of patients with GH hypersecretion. Clinical management of acromegalic patients has been strengthened by the introduction of GH antagonists (40). The currently available formulation, pegvisomant, is a polyethylene glycosylated GH analogue with a glycine substitution at GH codon 120 that severely restricts GHR dimerization and signaling (41). The consequence of GH antagonism is potent inhibition of insulin-like growth factor-I (IGF-I) generation that has been consistently noted in clinical trials (40). Studies in normal human subjects have similarly shown a dose-dependent inhibition of circulating IGF-I levels in response to pegvisomant (42). Concomitant with changes in IGF-I, serum GH is significantly increased (42); this may not be due to detection of the antagonist because it has been noted even in studies that used a two-site assay that does not recognize pegvisomant (43). Some investigators have suggested that pegvisomant is not likely to effect pituitary somatotrophs directly (42). However, there is evidence for both direct GH autoregulation in the pituitary (14) and direct vascularization of the adenohypophysis that would circumvent the blood-brain barrier (44) and permit the GH analogue direct access to pituitary somatotrophs. Thus, interruption of GH autoregulation in patients with densely granulated somatotroph adenomas that have intact GHR signaling may be a clinical concern. Fortunately, those patients seem to represent the group that responds to more conventional somatostatin analogue therapy (12, 13) whereas the patients who are more likely to require GH antagonist treatment are those with sparsely granulated somatotroph adenomas that have altered GH signaling due to the mutation we describe or other as yet unidentified mechanisms.

In summary, the profile of molecular alteration identified here is important on several fronts. First, the new mutation accounts for a subset of somatotroph tumors; it provides insight into normal regulation of GH secretion by local GH via the GHR in the pituitary, and it represents a putative mechanism whereby the mutant could alter these effects. From a therapeutic perspective, the finding of this mutation and the pharmacologic interruption studies described here provide a rationale for the distinct responsiveness of these tumors to different forms of pharmacotherapy. Densely granulated adenomas are more likely to harbor Gso mutations, providing an intracellular target for somatostatin analogue inhibition (12, 13). In contrast, the disruption of GH...
autoregulation by GHR mutation in sparsely granulated adenomas renders GHR antagonism a more appropriate therapeutic option less likely to be associated with treatment-induced tumor activation. These data provide the framework for structured therapeutic plans based on morphologic and molecular phenotyping.

References
32. Leung DW, Spencer SA, Cachianes G, et al. Growth hormone receptor and serum binding pro-
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