

Novel Therapies for GH-Secreting Pituitary Tumors

BY ANTHONY HEANEY, MD, AND SHLOMO MELMED, MD



rowth-hormone (GH)-producing cells (somatotrophs) are located in the lateral wings of the anterior pituitary gland and comprise 35-45% of pituitary cells. The human pituitary gland contains 5-15 mg of GH. The human GH genome locus contains a cluster of 5 highly conserved genes located on the long arm of human chromosome 17q22-24, of which the hGH-N gene is selectively transcribed in pituitary somatotrophs and codes for a 22-kDa protein.¹

GH synthesis and release are under the control of several hormonal factors, including GH-releasing hormone (GHRH), somatostatin, Ghrelin, insulin growth factor-I (IGF-I), thyroid hormone and glucocorticoids. These hormones bind to specific pituitary receptors and interact respectively to stimulate and inhibit pulsatile pituitary GH secretion. Circulating GH secreted by normal or adenomatous pituitary cells bind to hepatic GH receptors to stimulate IGF-1 production from the liver and other tissues, which in turn mediates GH-directed somatic and metabolic actions.

include enlargement of the extremities, hypertension, cardiovascular and cerebrovascular disease, diabetes mellitus and increased cancer prevalence, resulting in a 2-4 fold higher mortality in acromegalics compared to the normal population (Figure 1).²

Pure GH-cell adenomas either arise insidiously, grow slowly, contain densely staining cytoplasmic GH granules and typically present during middle age, or contain sparse GH-staining granules, grow rapidly and arise in younger subjects with more florid disease. Other mixed GH-cell tumors include the somatomammotroph adenoma that contains distinct populations of GH- and prolactin (PRL)-immunoreactive cells and the monomorphous acidophil stem cell adenoma (which contains giant mitochondria and grows rapidly, invading surrounding tissues).

The pathogenesis of GH-secreting pituitary tumors remains unclear, but both pituitary and hypothalamic factors influence pituitary development and progression (Figure 2).³ Although transgenic GHRH expression in mice leads to somatotroph hyperplasia and human GH-secreting pituitary adenomas express GHRH, Ghrelin and SRIF receptors, activating

SEVERAL CANDIDATE GENES HAVE BEEN IMPLICATED IN GH CELL TUMOR PATHOGENESIS

In 1886, Pierre Marie published the first clinical description of disordered somatic growth and proportion, and proposed the name "acromegaly." Benda later recognized the relationship of this syndrome to a pituitary tumor, and Cushing, Davidoff and Bailey demonstrated clinical remission of acromegaly-related soft tissue signs after adenoma resection. The establishment of the unequivocal link between hyperfunctioning adenoma and acromegaly was the earliest example of a pituitary disorder to be clinically and pathologically recognized and appropriately managed by the surgical excision of a hypersecreting source.

The prevalence of acromegaly is estimated to range from 38-69 cases per million, while the annual incidence of new patients is 3-4 cases per million. Based upon these largely Western European studies, it is apparent that more than 1,000 new cases of acromegaly are diagnosed annually in the United States. In 95% of cases, acromegaly is caused by excessive GH secretion from a pituitary adenoma, but regardless of the etiology, the disease is characterized by elevated GH and IGF-I levels. GH and IGF-I act both independently and dependently to induce the features of somatotrophism, which

mutations of GHRH or SRIF in pituitary adenomas have not been reported. In rare clinical instances, ectopic GHRH from hypothalamic, abdominal or chest neuroendocrine tumors causes somatotroph hyperplasia and occasionally adenoma with resultant unrestrained GH secretion and acromegaly. However, histologic examination reveals no evidence of somatotroph hyperplasia in pituitary tissue surrounding a pituitary GH-cell adenoma in most cases, indicating that intrinsic pituitary factors play a predominant role in GH-secreting pituitary tumor development. In support of this, the monoclonal origin of somatotroph adenomas was confirmed by X-chromosome inactivation analysis. Several candidate genes have been implicated in GH cell tumor pathogenesis. Point mutations in two critical sites of the GTP-binding domain of Gs (α) proteins, preventing GTPase activity and resulting in constitutive adenyl cyclase activation, have been demonstrated in ~40% of GH-secreting pituitary tumors. The recently characterized securin protein, pituitary tumor-derived transforming gene (PTTG), which regulates sister chromatid separation during the cell cycle, is overexpressed in GH-secreting

pituitary tumors and correlates with tumor size and invasiveness.⁴ Therefore the sequence of events leading to somatotroph clonal expansion appears multifactorial, and although oncogene activation may be required to initiate tumorigenesis, tumor growth promotion may involve cooperative interactions between hypothalamic and pituitary growth factors.

As tight control of GH and normalization of serum IGF-1 normalizes mortality rates in patients with acromegaly, the primary goal of a comprehensive strategy for treating patients with acromegaly is IGF-1 normalization, which should also aim to manage the pituitary mass. Currently available therapies include transsphenoidal pituitary surgery and radiotherapy. In specialized centers, these treatment modalities offer cure rates of ~70 to 80% for small GH-secreting pituitary tumors, less than 1 cm in diameter, but they are not always effective and are associated with significant morbidity.⁵ Current medical therapies for acromegaly include dopamine agonists and somatostatin analogs. Dopamine agonists, such as bromocriptine and cabergoline, a longer-acting selective D₂-receptor agonist, bind to pituitary dopamine type 2 receptors, but control the GH/IGF-1 axis in only 10 to 40% of patients. Octreotide, an octapeptide analog of somatostatin, has similar affinity for SSTR₂, but exhibits 45-fold higher potency than native somatostatin in inhibiting pituitary GH release. Administered subcutaneously in divided doses of 100-500 µg three times daily or as a long-acting depot analog administered intramuscularly monthly, octreotide normalizes IGF-1 in ~60% or more of acromegalic patients. Although long-term studies using long-acting somatostatin analogs are not yet available, short-term studies show that although depot somatostatin analogs limit disease in the majority of cases, they are not always effective in suppressing GH-action to normal levels, and inadequately suppress GH in ~70% of acromegalic patients. Furthermore, 10 to 15% of acromegalic patients are non-responsive to somatostatin analogs, primarily due to paucity of pituitary tumor somatostatin receptor (SSTR₂) expression, the latter conferring high affinity for octreotide, and facilitating somatostatin-mediated inhibition of GH release.⁶

Peripheral growth hormone receptor (GHR) antagonists are a new class of drugs, based on molecular modeling, the development of which has been facilitated by understanding the molecular interaction between GH and its class 1 cytokine receptor. B2036-PEG (pegvisomant) is the first GHR antagonist that has been developed to treat acromegaly. The development of pegvisomant represents a major advance in managing acromegaly, providing a specific, medical treatment for this condition.⁷

To mediate its action, a single GH molecule associates with

two receptor molecules through two unique receptor-binding sites on GH and a common binding pocket on the extracellular domain of two GH receptors (Figure 3). Due to differences in affinity, it is proposed that a GH-receptor binds to site 1 on GH, followed by recruitment of a second receptor to site 2, and it appears that ligand-driven receptor dimerization is the key event leading to signal activation, and triggering phosphorylation cascades that include the Jak2/

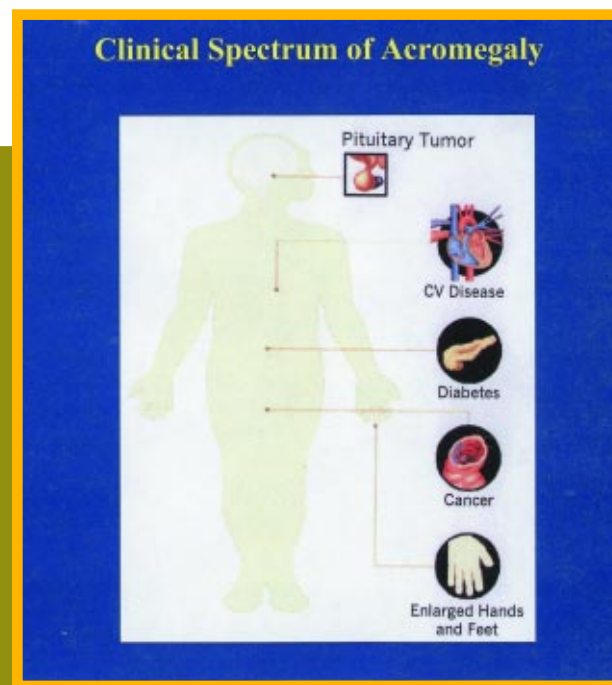


Figure 1. Schematic diagram showing the clinical manifestations of acromegaly. In most cases, pituitary tumor-derived growth hormone excess leads to cardiovascular disease, insulin resistance, enlargement of the extremities and increased cancer growth, via IGF-I dependent and independent mechanisms, accounting for an ~3-fold increase in mortality rates in acromegalic patients.

STAT5 pathway. The importance of GH-receptor dimerization is emphasized in experiments that demonstrate high GH concentrations (which favor the monomeric GH-GHR complex) inhibit GH-signaling, or that truncated GH receptors (lacking the cytoplasmic domain) act as dominant negative inhibitors of signaling by heterodimerization with the full-length GH-receptor. GH-mutants, harboring mutations at site 2, block GH-stimulated cell proliferation, the conformational changes that accompany receptor dimerization, and Jak/STAT signaling. These observations form the basis of the molecular drug design of GH antagonists for clinical application.

Pegvisomant is a GH analog that, like GH, consists of 191 amino acids differing by the presence of a single amino acid substitution at amino acid 120, resulting in total loss of affinity for the second GH receptor, thereby inhibiting dimerization. A further 8 amino acid substitution in the first

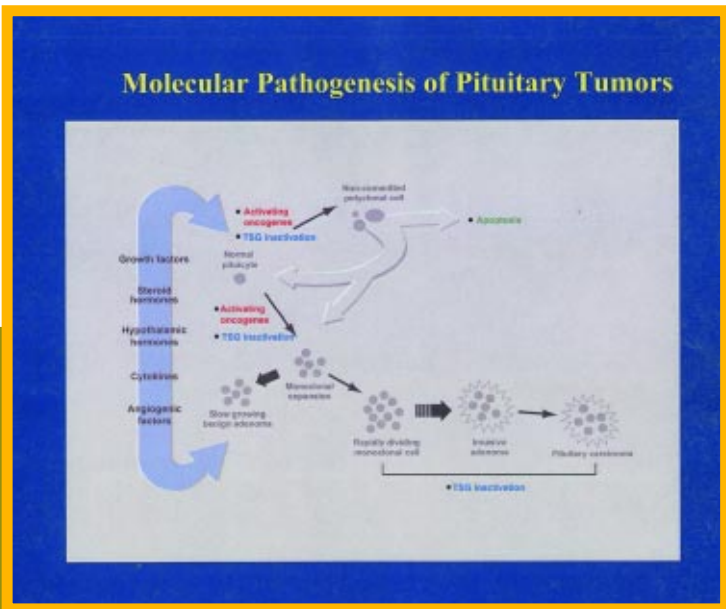


Figure 2. Model of pituitary somatotroph tumorigenesis. Cells responding to endocrine or paracrine stimuli may expand in a polyclonal manner (top sequence). As a consequence of increased proliferation, their susceptibility to acquire activating mutations (red) or loss of inactivating mutations (blue) is increased, prompting the emergence of a rapidly expanding monoclonal cell population (downward shaded arrows). At some point in the polyclonal cell expansion, cells susceptible to acquiring the "hit" develop, which will foster emergence of the monoclonal population. Alternatively, a normal cell may acquire sufficient activating mutations or loss of inactivating events to prompt a rapidly expanding monoclonal cell population from onset (lower sequence). Following additional genetic events, this monoclonal expansion may evolve into an invasive pituitary tumor, with further events promoting the progression to metastatic pituitary carcinoma. The progress of both these pathways will be driven by a variety of hormonal stimuli, growth and angiogenic factors and altered receptor expression (blue shaded arrows, left).

binding site greatly enhances receptor affinity at that locus, thereby giving the antagonist a kinetic advantage over GH in binding to receptors at site 1. In addition to a site 2 mutation, B2036 possesses eight mutations at site 1 that serve to alter GH affinity for GH-binding protein (GHBP) and potentially enhance GH antagonistic potency. A further modification of B2036 is its conjugation to 4-5 moieties of polyethylene glycol (PEG) 5000 to prolong its circulating half-life and lower its immunogenic potential. Despite these modifications to enhance its binding to the GH-receptor and antagonistic potency, in clinical practice, high daily doses of pegvisomant that are 20-40 times the daily production of GH are required to suppress IGF-1 levels in acromegaly. This apparent discrepancy between expected and observed *in vivo* potency may be due to pegylation-related reduction in B2036 affinity for the GHR. However, this reduction in B2036-PEG bioactivity is more than compensated for, *in vivo*, by the positive effect of pegylation on B2036-PEG pharmacokinetics and clearance.

Whereas somatostatin analogs and dopamine agonists bind centrally to specific pituitary tumor receptors and inhibit pituitary GH secretion, pegvisomant acts at the peripheral GH receptor by blocking GH action. An important consequence is that pegvisomant does not lower circulating GH levels. Hence GH cannot be used as a marker of disease activity, but serum IGF-I provides the primary measure of efficacy.

In a double blind, placebo-controlled, phase III study in 112 patients with acromegaly, serum IGF-I fell within the normal age-related reference range in 89% of patients receiving pegvisomant (20 mg/day). Other measures of GH-action, including serum-free IGF-I, IGFBP-3 and acid-labile subunit, also showed a dose-dependent decline which paralleled decreased

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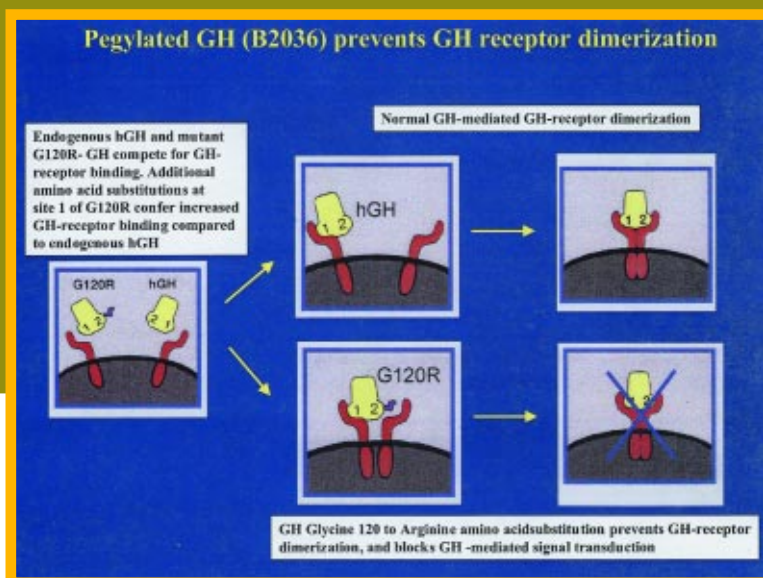


Figure 3. Schematic representation of the mechanism of action of B2036. To mediate its action, a single GH molecule (hGH) associates with two GH-receptor molecules (upper panel), binding the first GH-receptor at site 1, followed by recruitment and binding of a second GH-receptor at site 1. In this manner, ligand-dependent dimerization is the key event leading to GH-mediated signal activation. B2036 is a GH analog harboring specific site 1 mutations, which confer increased binding to the first GH-receptor. A further site 2 Glycine to arginine amino acid substitution (position 120) prevents dimerization of the GH-receptor with the second GH-receptor, thus blocking GH-mediated signal transduction (lower panel). Pegylation prolongs the half-life of the mutant GH allowing it to advantageously compete with endogenous GH (hGH).

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serum IGF-I levels. More importantly, biochemical improvements were accompanied by a significant improvement in patient well-being and a reduction in soft-tissue swelling, reflected by ring size.⁸

In summary, the development of pegvisomant, based on key molecular insights into the interaction between GH and its receptor, represents a major advance in the management of acromegaly, providing a specific and effective medical treatment for this condition. Nevertheless, the pituitary tumor remains untouched by this drug, and bioinactivated GH levels remain persistently elevated.

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Tony Heaney, MD, is a staff physician in the Division of Endocrinology at Cedars-Sinai Medical Center in Los Angeles. Dr. Heaney's research and clinical interests focus on the elucidation of the pathogenesis and management of neuroendocrine tumors.

Shlomo Melmed, MD, is Senior Vice President of Academic Affairs, Director of the Burns and Allen Research Institute and Co-Director of The Pituitary Center, all at Cedars-Sinai Medical Center in Los Angeles. He is an internationally renowned authority on the pathogenesis and management of pituitary tumors.