G_s Protein Mutations and Pituitary Tumors: Functional Correlates and Possible Therapeutic Implications

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In more than one third of growth hormone (GH)-secreting pituitary adenomas, a point mutation in the gene for the α-chain of the G stimulatory protein (*gsp* oncogene) causes the constitutive activation of the membrane adenylyl cyclase (AC) resulting in uncontrolled cyclic adenosine monophosphate (cAMP) elevation and GH hypersecretion. Tumors expressing *gsp* are characterized by high membrane AC activity, elevated intracellular cAMP content, and high rates of GH release in culture medium. The AC activity is not further stimulated by GH-releasing hormone (GHRH) and other specific and non-specific agents, while it is lowered by somatostatin, as the G inhibitory protein (G_i) is normally working. Acromegalic patients bearing adenomas with the *gsp* mutation do not present with any obvious clinical or epidemiological distinctive features. However, they have smaller tumors in relation to their circulating GH levels, suggesting that the *gsp* oncogene maintains a high rate of secretory activity in vivo. Most of these patients show paradoxical GH increases to thyrotropin-releasing hormone (TRH), but none to gonadotropin-releasing hormone (GnRH) or an oral glucose tolerance test (OGTT). As with the in vitro data, these patients are not very sensitive to GHRH administration, but are sensitive to the inhibitory action of somatostatin. In our experience, only three of six patients with non-*gsp*-mutated tumors had lowered serum GH levels during the administration of octreotide (100 μg thrice daily for 4 years), while all of six patients with *gsp*-mutated tumors had serum GH levels suppressed by octreotide treatment. Such a good GH suppressibility by somatostatin makes patients with *gsp*-mutated tumors the best candidates for medical treatment with somatostatin analogs.

IT HAS RECENTLY emerged that in more than one third of growth hormone (GH)-secreting pituitary tumors, GH uncontrolled hypersecretion is due to point mutations in the α-chain of G stimulatory protein constitutively activating adenylyl cyclase (AC).[1,2] This discovery has prompted the investigation of functional correlates. As cyclic adenosine monophosphate (cAMP) is also involved in cell replication, the mutated G_s α-chain gene has been recognized as an oncogene termed *gsp*.

FUNCTIONAL CORRELATES IN PITUITARY TUMORS CARRYING *gsp* ONCOGENE

In vitro studies of functional correlates in GH-secreting pituitary tumors have shown that the phenotype of tumors carrying the *gsp* oncogene is consistent with the underlying autonomous AC activation. In fact, this subset of tumors was characterized, in basal conditions, by high membrane AC activity, elevated intracellular cAMP content, and high rates of GH release in culture medium. Adenylyl cyclase activity was not further stimulated by specific and nonspecific agents, so that GH-releasing hormone (GHRH), vasoactive intestinal peptide (VIP), and pituitary adenylate-cyclase–activating polypeptide (PACAP) on the one hand, and guanosine triphosphate (GTP), fluoride, and cholera toxin on the other hand, were unable to significantly increase the enzyme activity.[1,3] On the contrary, as the G inhibitory protein (G_i) is normally working, AC activity was inhibited by somatostatin (SRIH) as expected.[1] Accordingly, exposure to the somatostatin analog octreotide showed that *gsp*-positive tumors exhibited a more marked inhibition of GH secretion than *gsp*-negative tumors, many of which failed to respond.[4] The effects of GHRH on GH release are still debated. The mean GH response was reported to be higher in *gsp*-negative tumors, although, unexpectedly, some *gsp*-positive tumors were shown to respond as well,[4] suggesting that GHRH could also act through cAMP-independent mechanisms.

In somatotroph tumors, GH and glycoprotein hormone α-subunit (α-SU) cosecretion is a frequent finding that might reflect the degree of dedifferentiation. Although the level of α-SU spontaneously released in culture medium was shown to be greater in *gsp*-positive tumors, its immunocytochemical detection was similar in *gsp*-positive and *gsp*-negative tumors, indicating that the presence of the *gsp* oncogene is not influential with respect to the degree of differentiation, but only affects the secretory rate. The frequency of tumors immunoreactive for prolactin was also similar in the two groups. At electron microscopy, the majority of tumors with the *gsp* mutation showed morphological features consistent with a hypersecretory state, appearing densely granulated and with well-developed secretory apparatus.[6]

The presence of the *gsp* mutation in other types of pituitary tumors seems to be uncommon.[7-9] Up to now, only four nonfunctioning adenomas (NFPA), one gonadotropinoma, and three adrenocorticotropic hormone–secreting adenomas (ACTHomas)[10-14] harboring *gsp* mutations have been described. It is surprising that the *gsp* mutation, which causes the constitutive activation of AC, an enzyme involved in secretory processes, is expressed in nonfunctioning tumors. In this respect, the coexistence of a mutation of G_i is of interest. Unfortunately, there are no data available concerning AC activity, cAMP, or other intracellular effectors in NFPA. No phenotypic characteristics distinguishing tumors with the mutation from the wild type have been described.

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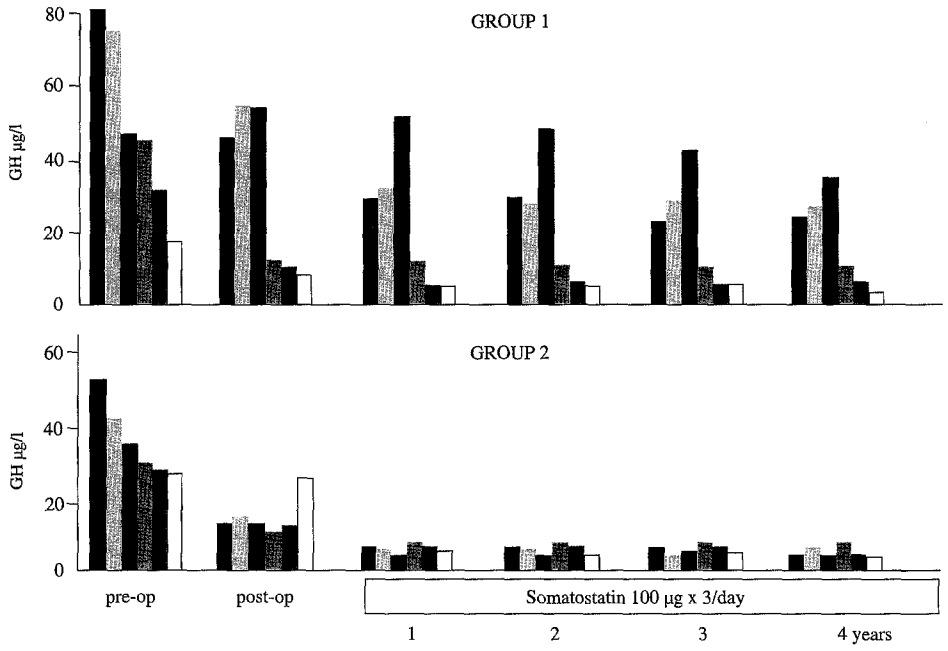

Fig 1. Effect of octreotide administration (100 µg thrice daily, subcutaneously) on serum GH levels in 6 acromegalic patients with non-*gsp*-mutated tumors (group 1) and in 6 other patients with *gsp*-mutated tumors (group 2).

FUNCTIONAL CORRELATES IN PATIENTS WITH *gsp*-MUTATED PITUITARY TUMORS

Due to the small number of patients bearing *gsp*-mutated pituitary tumors other than somatotrophinomas, only acromegaly will be considered here. Several studies[5,6,15,16] have shown that patients bearing GH adenomas with the *gsp* mutation do not present with different clinical features, sex distribution, age, and estimated duration of the disease with respect to patients without this alteration. On the contrary, there is some discrepancy about tumor size and circulating GH levels. The tumor size was reported to be smaller[5,6,9,15] or similar,[8,16] and circulating GH levels to be higher,[6] lower,[15,16] or similar[5,8,9] in the two groups of patients. The observation that serum insulin-like growth factor-1 (IGF-1) concentrations are superimposable in the two groups of patients supports the view that no striking difference in serum GH levels exists.

In our experience, acromegalic patients with constitutively active AC activity have small tumors in relation to their circulating GH levels, suggesting that the *gsp* oncogene maintains a high rate of secretory activity not only in vitro, but also in vivo. In agreement with the in vitro data, patients with constitutively active AC were poorly sensitive to GHRH administration, while they were extremely sensitive to the inhibitory action of both somatostatin and dopamine. This suggests that the expression of *gsp* oncogenic mutations might be partially counteracted in vivo by inhibitory agents, thus accounting for the small tumor size. Thyrotropin-releasing hormone (TRH) was effective in inducing a marked GH increase in many of these patients, at variance with gonadotropin-releasing hormone (GnRH), which was never able to stimulate GH secretion in patients with constitutively active AC. This last finding is of particular interest, as it has been shown that in responsive somatotroph adenomas, GnRH activates AC instead of phospholipase C, as physiologically occurs.[17] Although the initial observation that, in patients with *gsp* mutations, serum GH was partially suppressed by an oral glucose tolerance test (OGTT) has not received further support, it is of interest that none of the patients with mutant tumor so far reported has shown a paradoxical GH increase after a glucose load.[15,16]

THERAPEUTIC IMPLICATIONS

The results of pituitary surgery in acromegalic patients grouped according to the presence or absence of constitutively activated AC, have not shown any difference in success and recurrence rates.[6,16] Also, local invasiveness, judged by the neurosurgeon, did not differ in the two tumor groups.[15,16] With regard to medical therapy, although the finding that GH secretion is well suppressed by somatostatin and dopamine in *gsp*-positive tumors[6] should have prompted a comparison of the effects of these drugs in acromegalic patients with or without the *gsp* mutation, no data are in fact available. Yang et al[18] reported on the effects of acute octreotide administration in 10 patients analyzed for the *gsp* oncogene. All three *gsp*-positive patients showed good suppression of serum GH, while five of seven *gsp*-negative patients were poorly responsive.

In our experience, octreotide treatment greatly suppressed serum GH levels in six of six acromegalic patients with *gsp*-mutated tumors, while this was not the case in six other patients with non-*gsp*-mutated tumors. In fact, only three of them responded to therapy, while the remaining three patients were partially resistant (Fig 1). Thus, patients with *gsp*-mutated tumors seem to be the best candidates for treatment with somatostatin analogs.

CONCLUSIONS

The discovery of the *gsp* mutation has provided evidence of a common mechanism involved in both tumor formation and hormone hypersecretion, and may lead to better targeting of therapy. As it has been demonstrated that the degree of responsiveness to octreotide correlates with the expression of SRIH receptors, it is tempting to speculate that SRIH receptors are highly expressed in *gsp*-positive tumors and that this may represent a sort of compensatory mechanism. If this hypothesis is confirmed, patients harboring *gsp*-mutated tumors will be the best candidates for medical treatment with somatostatin analogs as the first choice therapy. However, a prerequisite is the ability to differentiate patients with *gsp*-mutated tumors on clinical and/or biochemical grounds. Further efforts are necessary to achieve this goal.

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