G_s Protein Mutations and the Pathogenesis and Function of Pituitary Tumors

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Hypothalamic growth hormone-releasing hormone (GHRH) stimulates growth hormone (GH) production and somatotroph proliferation by binding to a seven transmembrane-domain receptor, linked to G_s. G_s stimulates production of cyclic adenosine 3′-5′-monophosphate (cAMP) and hence activation of protein kinase A (PKA). A subgroup of pituitary somatotroph adenomas has been demonstrated, which has constitutive activation of G_s, with reduced in vitro responsiveness to agents that stimulate G_s. Subsequently, somatotroph adenomas have been identified, which have activating mutations of G_s (*gsp*). However, there are no clear clinical or biochemical phenotypic characteristics that enable *gsp*-positive and *gsp*-negative tumors to be differentiated from one another. *Gsp* mutations occur in 35% to 40% of somatotroph adenomas in caucasians, but have a much lower reported prevalence of 4% to 9% in the Japanese population. G-protein mutations also occur in clinically nonfunctioning pituitary tumors and, rarely, in corticotroph adenomas. There is little direct evidence at present to suggest that the *gsp* mutation has a primary oncogenic role in the pathogenesis and function of pituitary tumors. Further functional studies are needed. The *gsp* mutation is probably one of several oncogenic mutations required for pituitary tumor development.

HUMAN SOMATOTROPH adenomas are under stimulatory hypothalamic control by growth hormone-releasing hormone (GHRH) and inhibitory control by somatostatin. Both GHRH and somatostatin bind to seven transmembrane-domain protein receptors, linked to G_s and G_i respectively. When GHRH binds to its receptor, G_s is converted from an inactive heterotrimer, consisting of α, β, and γ subunits bound to guanosine diphosphate (GDP), to an active guanosine triphosphate (GTP)-bound α subunit. In this activated form, $G_s\alpha$ stimulates adenylyl cyclase (AC) activity, resulting in the production of cyclic adenosine-3′-5′-monophosphate (cAMP) and consequent activation of protein kinase A (PKA). This results in the stimulation of growth hormone (GH) production and somatotroph proliferation (Fig 1).

The first indication of a possible dysregulation of this pathway in pituitary tumors was the observation of altered G_s and AC activity in a subgroup of somatotroph adenomas. In comparison with normal rat somatotrophs and other somatotroph adenomas, this group of tumors was found to have constitutive activation of G_s, resulting in marked elevation of AC activity, which failed to respond normally to stimulatory agents.[1] On the basis of this work, eight somatotroph adenomas were examined for $G_s\alpha$ mutations. Four tumors with high levels of AC activity were found to have activating mutations of $G_s\alpha$ (*gsp*) either at codon 201, replacing arginine for cysteine (R201 C) or histidine (R201H), or at codon 227, replacing glutamine for arginine (Q227R).[2] R201C is the site of ADP ribosylation by cholera toxin (CT), which results in loss of intrinsic GTPase activity of $G_s\alpha$, with the result that G_s remains in its activated $G_s\alpha$-GTP–bound form. Q227 is homologous to Q61 in $p21^{ras}$, and mutations at this codon have a similar constitutive activating effect. A subsequent study of 80 somatotroph adenomas reported the presence of elevated adenylyl cyclase activity in 36% of tumors.[3] These data suggested that a group of somatotroph adenomas have constitutive activation of G_s, which would be expected to mimic the effects of GHRH, resulting in increased GH production and cell proliferation. However, a recent study has reported a considerable degree of heterogeneity between *gsp*-positive and *gsp*-negative somatotroph adenomas, in terms of their responses to GHRH in vitro. Octreotide was found to have a variable inhibitory effect in *gsp*-negative tumors, but an inhibitory effect was observed in all *gsp*-positive tumors in vitro.[4] In a recent study of 15 somatotroph adenomas, all tumors were found to contain elevated levels of Ser^{133} phosphorylated, and hence activated, cAMP recognition element binding protein (CREB). Four of these tumors (20%) had *gsp* mutations and two had increased G_s protein expression. In consequence, despite CREB phosphorylation, 73% of these tumors did not have evidence of increased G_s activity,[5] suggesting distal activation of the pathway.

X chromosome inactivation studies in pituitary adenomas from female patients have demonstrated monoclonal patterns for somatotroph adenomas, clinically nonfunctioning pituitary adenomas, corticotroph adenomas, and prolactinomas.[6-8] Monoclonality implies an underlying somatic mutation, resulting in tumor formation. The *gsp* mutation could fulfill such a function. A number of studies have now confirmed the presence of *gsp* mutations in somatotroph adenomas (Table 1).[2,4,9-14] The most frequent mutation is R201C, with mutations at Q227 occurring rarely. It appears that in the caucasian population, *gsp* mutations occur in 35% to 40% of somatotroph adenomas. Interestingly, these mutations appear to have a much lower prevalence in the Japanese population, with reported prevalences of 9%[13] and 4%.[14]

In addition to somatotroph adenomas, G-protein gene mutations have also been described in other pituitary adenomas. In a series of 21 clinically nonfunctioning pituitary tumors (NFTs), two tumors (10%) were found to have the *gsp* mutations R201C and Q227L.[15] In another study of NFTs, the *gsp* mutations R201C and Q227R were identified in two of 22 tumors (9%). In addition, activating mutations of $G_i2\alpha$ (gip) Q205R, were also identified in three tumors. Two of these tumors harbored what at first

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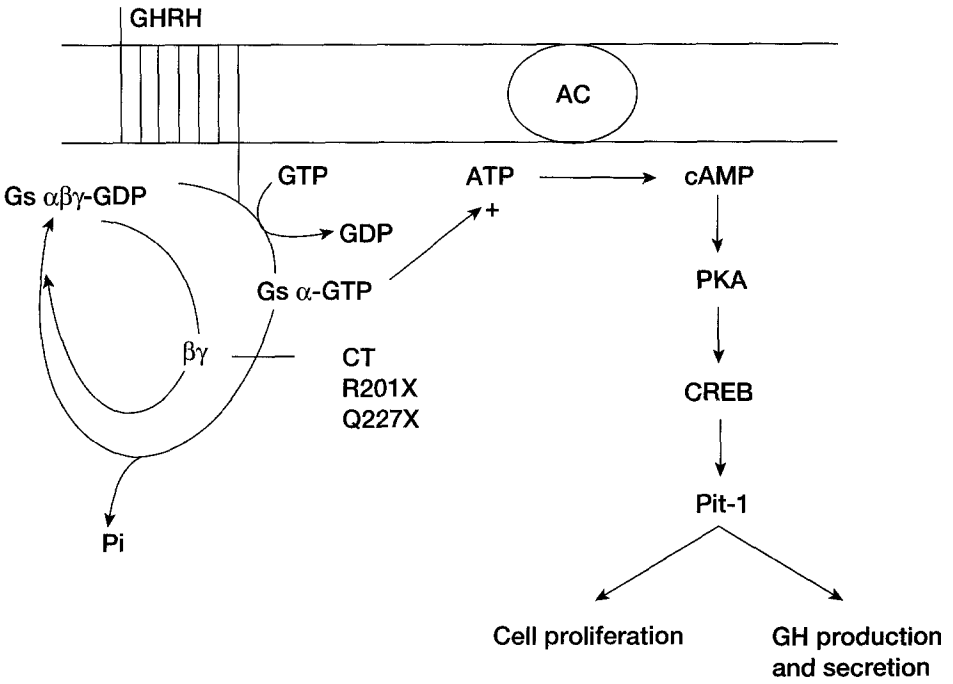

Fig 1. Activation of G_s occurs when GHRH interacts with its 7 transmembrane receptor. This results in the activation of AC, with consequent stimulation of cAMP production, and ultimately cell proliferation and stimulation of GH production and secretion. Constitutive activation of G_s by inhibition of intrinsic GTPase results from ADP ribosylation by CT or point mutations at codons 201 or 227 of $G_s\alpha$-encoding amino acid X.

Table 1. *Gsp* Mutations Reported in Pituitary Tumors

Reference	Tumor	No.	*Gsp*-Positive	Mutations
Landis et al, 1989	Somatotroph	8	4	R201C (2)
				R201H (1)
				Q227R (1)
Landis et al, 1990	Somatotroph	25	10 (40%)	R201C (8)
				R201H (1)
				Q227R (1)
Lyons et al, 1990	Somatotroph	42	18 (43%)	R201C (14)
				R201H (2)
				Q227R (2)
	Lactotroph	12	0	
	Thyrotroph	2	0	
	Corticotroph	7	0	
	NFT	3	0	
Harris et al, 1992	Somatotroph	26	9 (35%)	R201C (7)
				R201H (2)
Drews et al, 1992	Somatotroph	13	4 (31%)	R201C (3)
				R201S (1)
	Somatotroph/ lactotroph	2	1	R201H
Yoshimoto et al, 1993	Somatotroph	43	4 (9%)	R201C
Hosoi et al, 1993	Somatotroph	45	2 (4%)	R201C
Tordjman et al, 1993	NFT	21	2 (10%)	R201C (1)
				Q227L (1)
	Lactotroph	4	0	
Williamson et al, 1994	NFT	22	2 (9%)	R201C (1)
				Q227R (1)
Adams et al, 1995	Somatotroph	30	10 (33%)	R201C (9)
				R201H (1)
Williamson et al, 1995	Corticotroph	32	2 (6%)	Q227R (1)
				Q227H (1)

Abbreviation: NFT, nonfunctioning pituitary tumor.

sight appeared to be paradoxical double mutations of both *gsp* and *gip*.[16]

Corticotropin (ACTH) is under stimulatory hypothalamic control by corticotrophin-releasing hormone (CRH). CRH acts via the G_s-AC system, and has been shown to promote the in vitro proliferation of corticotrophs.[17] Although G-protein gene mutations have been identified in corticotroph adenomas, they appear to occur infrequently. Two *gsp* mutations, Q227R and Q227H, have been described in a series of 32 tumors (6%). In addition, one *gip* mutation, R179G, was also identified (Table 1).[18]

These, largely observational, data do not provide a clear picture of the role(s) of mutant G_s proteins in the pathogenesis and function of pituitary tumors. Indirect evidence for a possible role of G_s overactivity has been provided by transgenic animal models. Mice transgenic for a rat GH promoter-CT transgene developed somatotroph hyperplasia and gigantism, although none exhibited tumor formation up to 13 months of age.[19] In a subsequent study, mice aged ≥ 10 months, transgenic for GHRH, were reported to develop pituitary adenomas in association with hyperplasia of somatotrophs, lactotrophs, and mammosomatotrophs. Apart from immunostaining for GH, some of these tumors also immunostained for prolactin, thyroid-stimulating hormone β-subunit, and glycoprotein hormone α-subunit.[20] More direct evidence for the possible role(s) of the *gsp* mutation in tumor pathogenesis and function has been obtained from in vitro transfection studies. Transient expression of the $G_s\alpha$ mutant Q227L in rat GH3 pituitary cells has been reported to stimulate prolactin promoter activity, although no stimulation of GH promoter activity could be demonstrated.[21] Transient transfections of GH3 cells and AtT20 pituitary cells with R201, R201H, and Q227L were found to result in increased transcription of the immediate early gene, c-*fos*.[22]

In summary, although there is evidence of increased G_s activity in subgroups of pituitary tumors, particularly somatotrophinomas, the role(s) of $G_s\alpha$ in terms of pituitary tumor pathogenesis and function remains unclear. Although in vitro studies have suggested that somatotroph adenomas can be clearly separated into two groups on the basis of *gsp* mutations and consequent activation of recent data have demonstrated considerable overlap between the two groups, particularly in terms of their responses to GHRH.[4]

More particularly, from the clinical point of view, there is a lack of clear-cut phenotypic characteristics between *gsp*-positive and *gsp*-negative tumors. It is possible that a degree of cellular adaptation to the *gsp* mutation occurs, as has been demonstrated in the pancreatic islets of *gsp* transgenic mice.[23] Alternatively, there may be a compensatory inhibitory drive on the cell, possibly from somatostatin, which would not be evident during in vitro experiments. The concept of the *gsp* mutation being oncogenic fits in well with the central role of the AC system in the control of somatotroph proliferation and function. However, there has been no clear demonstration to date that the *gsp* mutation is oncogenic in pituitary cells. Further functional studies of the *gsp* mutation in pituitary cells need to be performed to clearly characterize its role as an oncogene in pituitary tumor development. It is likely that the *gsp* mutation will turn out to be but one example of a number of oncogenic mutations that are required for pituitary tumor development.

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